Targeting neurotransmitter receptors for central nervous system therapeutics: from molecular signalling to behavioural pharmacology

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A thesis submitted by

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Since the creation of the world,
God's invisible qualities,
his eternal power and divine nature,
have been clearly seen,
being understood from what has been made.

The Bible
(Paul's letter to the Romans ch. 1 verse 20)

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Acknowledgements

Since being awarded a Ph.D. 20 years ago, I have had the privilege of working with world-class scientists that generously shared their experience and insights. The work summarised in this thesis would not have been possible without them and I gratefully acknowledge their part in helping me develop the concepts that became central to my research.

Prof. Philip Strange supervised my Ph.D. work at the Biological Laboratory at UKC and initiated me into the study of neuropharmacology. In so doing, he sowed the seed of a passion for neurotransmitter receptor research that motivates me to this day. Dr. Mark Millan welcomed me to the Servier Research Centre and coached me in writing cogently-argued research manuscripts. He was a constant source of innovative ideas and encouraged me to investigate neuropharmacological mechanisms of many kinds. By appointing me as head of Neurobiology Division at Pierre Fabre, Dr. Francis Colpaert gave me the opportunity to make the leap from molecular signalling to behavioural pharmacology studies. His vision for rigorous data-driven research was inspirational and he is sorely missed[a].

Other researchers who provided scientific challenge, as well as humour and close friendship, include Dr.s Didier Cussac, Bernard Vacher and Ronan Depoortère. The many hours we spent talking, debating and joking in each others' offices are a source of happy memories. Other esteemed ex-colleagues from the Psychopharmacology "P10" Division at Servier and the Neurobiology 2 Division at Pierre Fabre are also to be found among the authors of the publications in this thesis.

Several researchers from academic institutions were the source of much-appreciated intellectual input and valued collaboration over the years. In particular, Prof.s Francesc Artigas (Barcelona), Umberto Spampinato (Bordeaux) and Luc Zimmer (Lyon) are acknowledged for their scientific excellence and personal friendship.

Last, but not least, my pursuit of a research career has implied career sacrifices on the part of my spouse, Sue. Through the years, she has shown unfailing support and unselfish willingness to set aside her own professional aspirations in order to allow me to develop my own. She merits my profound gratitude and steadfast love.

[a] Dr. Francis Colpaert passed away on 15 October 2010.

Summary

Although chemically-mediated neuronal signaling was discovered nearly a century ago, it was only relatively recently, in the 1980s, that the study of neurotransmitter receptor pharmacology markedly accelerated, with the widespread use of specific radiotracers capable of labelling receptors in tissue preparations. This led to the discovery of an amazing (at the time) diversity of receptor subtypes with distinct physiological properties and pharmacological profiles. Among the principal neurotransmitter receptors to be investigated were those of the monoamines: serotonin (5-hydroxytryptamine, 5-HT), dopamine and noradrenaline, which act at a combined total of at least 27 receptors. The focus of the present D.Sc. thesis is to review the contribution of my own research to the understanding of the molecular signalling, neurochemistry and behavioural pharmacology of central nervous system monoamine receptors. A somewhat unusual feature of this work is that it was mostly carried out in pharmaceutical industry laboratories. These had the enlightened view that creative (and marketable) applied science requires a commitment to innovative basic science. My work therefore combined exploration of previously-unsuspected mechanisms of receptor function with the identification and characterisation of new drugs for use as exploratory tools and/or as therapeutic candidates. The principal advances made in the course of my work were as follows.

- Serotonin 5-HT_{1A} receptor signalling: the *in vitro* studies I conducted revealed the presence of constitutive activity in both recombinant cell lines and native rat brain tissue. The level of G-protein activation by 5-HT_{1A} receptors varies with receptor expression, receptor:G-protein stoichiometry and interactions at specific G-protein subtypes.
- 5-HT_{1A} receptor subpopulations: I demonstrated the presence of "biased agonism" at 5-HT_{1A} receptors in specific brain regions. This translated to distinct neurochemical, behavioural and therapeutic-like responses. My work led to the identification of a new selective agonist, F15599, which exhibits reinforced anti-depressant-like activity and was taken to early clinical trials.
- 5-HT_{1A} receptor activation in psychotic disorders: the pharmacological studies that I directed showed that compounds possessing 5-HT_{1A} agonist properties exhibit a superior profile of action in models of negative symptoms and cognitive deficits compared with conventional antipsychotics. My work led to the identification of novel drugs, notably F15063.
- 5-HT_{1B}, 5-HT_{1D}, 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors: my research showed that these receptors exhibit diverse profiles of G-protein coupling, biased agonism and constitutive activity. Notably, 5-HT_{1B} receptors exhibited "protean" agonism for the endogenous neurotransmitter, 5-HT.
- Dopamine D_2, D_3 and D_4 receptors: these displayed specific molecular signalling mechanisms. D_4 receptors showed complex signalling and "promiscuous" activation by noradrenaline, in addition to dopamine. My studies contributed to the identification of novel ligands at D_3 and D_4 receptors. Of these, S33138, a D_3 receptor antagonist, was later tested in schizophrenia patients.
- Noradrenergic α2 receptors: I conducted the first multiparametric study of anti-Parkinson's disease agents with differing profiles of action at α2 and other monoamine receptors. The clinical drug, piribedil, was shown to possess α2 receptor antagonism.

Candidate Declaration

- From reference 1 up to reference 54: when named as first author (28 references), I wrote the manuscript and either carried out the experimental work or supervised its execution. When named as second author (16 references) or last author (3 references), I made a major contribution to conceiving and supervising the project and to writing the manuscript. When named as a minor author (7 references), I performed or supervised some of the experimental work necessary to the completion of the paper.

- From reference 55 to reference 103: when named as first author (8 references), I conceived and supervised the project and wrote the manuscript. When named as last author (23 references), I conceived and supervised the project and made a major contribution to writing the manuscript. When named as second author or minor author (18 references), then I had a substantial intellectual input to conceiving the project and writing the manuscript.

- Publications 1 and 2 were based on results included as part of a submission for a Ph.D. thesis to the University of Kent at Canterbury in 1992.

I confirm that this is a true statement and that, subject to any comments above, the submission is my own original work.

25 June 2012

Applicant's Qualifications

Doctor of Philosophy, Neuropharmacology, University of Kent (1992)

Bachelor of Science (with honours), Biochemistry, University ofKent (1988)

Publications included in this Thesis

(Abstracts listed in Part B)

1992 - 1996

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Publication metrics

The Table shows citation and Impact Factor (IF) information concerning the author's publications. These are identified in accordance with the numbering shown in the publications list. Citation information was obtained from Google Scholar (www.scholar.google.com) on 21 May 2012. The 36 publications that have 40 or more citations are shown in bold font. IFs are from 2012 listings and the "Average IF" refers to the mean score of the 103 publications included in this Thesis.
Overall, the author's entire work (including over 130 peer-reviewed publications plus patents and conference proceedings) has been cited 5238 times with an *h-index* of 41 and an *i10-index* of 104.

JOURNAL	No. of ref.s	Publication number and *citations*	IF
Behavioral Brain Research	1	90[2]	3.393
Behavioural Pharmacology	3	76[32], 81[15], 87[8]	2.530
Biochemical J	1	**1**[43]	5.016
Biochemical Pharmacology	1	**2**[44]	4.889
Brain Research	2	42[22], 58[36]	2.623
British J Pharmacology	10	**8**[76], 35[22], 53[11], 67[20], 71[31], 72[25], **73**[41], 88[19], 91[3], 94[7]	4.925
Cellular Signalling	1	100	4.243
Current Opinion Invest Drugs	2	**75**[46], 96[21]	3.395
European J Neuroscience	1	**21**[97]	3.658
Eur J Nuclear Med Mol Imaging	1	93[8]	5.036
European J Pharmacology	17	**5**[50], **7**[44], 11[20], **19**[175], 24[15], 29[16], 39[27], 41[11], **63**[48], **64**[55], 78[18], 80[10], 83[9], 84[21], 85[5], 86[5], 92[3]	2.737
Eur Neuropsychopharmacology	1	95[6]	4.201
Excerpta Medica Int Congress	1	54[4]	**N/A**
Int J Neuropsychopharmacology	3	**56**[88], 82[12], 97[6]	4.699
J Neurochemistry	1	98[3]	4.337
J Nuclear Medicine	1	103	7.022
J Pharmacology Exp Therapeutics	15	**6**[57], 9[31], **12**[48], **14**[119], **18**[51], **27**[61], **33**[93], 38[28], **48**[199], **49**[89], **50**[124], **51**[239], 57[13], **60**[73], 79[9]	4.017
Molecular Pharmacology	6	**20**[73], **23**[84], 31[30], **45**[87], 46[34], 69[25]	4.725
Naunyn-Schmiedeberg's Archives Pharmacology	12	13[33], 16[18], **17**[83], 22[13], 26[10], 30[15], 32[10], 36[4], **47**[55], 52[20], 68[23], 74[7]	2.500
Neuropharmacology	9	3[11], **4**[90], **10**[78], 28[8], 34[23], **59**[56], 61[13], **62**[57], 70[20]	4.677
Neuropsychiatry	1	99[3]	**N/A**
Neuropsychopharmacology	5	**15**[52], 25[29], 37[27], **65**[67], **66**[40]	6.685
Pharmacol Biochem Behaviour	2	43[15], 89[5]	2.624
Psychopharmacology	6	**40**[60], 44[29], 55[13], 77[16], 101[9], 102	3.817
Total: 24 journals	**Tot.: 103**	**Total: 3859 citations**	**Average IF: 3.821**

Abbreviations

AR	Adrenergic receptor
cAMP	Cyclic Adenosine monophospate
CHO	Chinese hamster ovary
CNS	Central Nervous System
5CSRTT	5-choice serial reaction time test
5-CT	5-carboxyamidotryptamine
DA	Dopamine
DNMTP	Delayed non-matching to position
DOI	2,5-Dimethoxy-4-iodo-amphetamine
EEDQ	N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline
EPS	Extrapyramidal Symptoms
ERK	Extracellular signal-regulated kinase
FCX	Frontal Cortex
FGF	Fibroblast Growth Factor
GABA	Gamma-aminobutyric acid
GIRK1	G-protein-regulated inwardly rectifying potassium channels
GPCR	G-protein coupled receptor
GDP	Guanosine-diphosphate
GTP	Guanosine-triphosphate
GTPγS	Guanosine-5'-O-(3-thio)-triphosphate
H	histamine
HEK	Human embryonic kidney cells
5-HT	5-hydroxytryptamine (serotonin)
L-DOPA	L-dihydroxyphenylalanine
LSD	Lysergic acid diethylamide
MAOI	Monoamine Oxidase Inhibitor
MAPK	Mitogen-activated-protein kinase
MDD	Major Depressive Disorder
MDMA	3,4-methylene-dioxymethamphetamine
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NAc	Nucleus Accumbens
NAD	Noradrenaline
NMDA	N-methyl-D-aspartic acid
PCP	Phenylcyclohexylpiperidine
PD	Parkinson's dsease
PET	Positron Emission Tomography
PFC	Prefrontal cortex
PI	Phosphatidylinositol
PKC	Protein kinase C
PLA2	Phospholipase A2
PLC	Phospholipase C
PPI	Pre-pulse inhibition
PTX	Pertussis toxin
RGS	Regulators of G-protein Signalling
SNPC	Substantia Nigra Pars Compacta
SPA	Scintillation proximity assay
SSRI	Selective Serotonin Reuptake Inhibitor
USV	Ultrasonic Vocalization

Part A: Overview of the publications included in this Thesis

Part A is sub-divided into 8 chapters describing the significance of the author's publications on monoamine receptor signaling and function. The work is presented in the context of the knowledge available at the time of publication and in relation to its contribution to studies by other researchers. The thesis covers 103 peer-reviewed publications and the discussion sections concentrate on those publications that made the most notable conceptual advances. A "Key points" section summarises the main messages of each chapter.

Throughout the text, the author's publications are referenced numerically ([**1**], [**2**], [**3**] …etc.) in accordace with the numbering shown in the list of publications.

Abstracts from the author's publications included in this Thesis are shown in Part B.

Publications by other authors and the author's publications that were not peer-reviewed (such as conference proceeedings) are referenced in the text by numbers in superscript [1, 2, 3, …etc.] and listed in the Bibliography at the end of the thesis.

1. Introduction

1.1 Key points

- CNS disorders constitute a major medical need and result in large social burden and financial liability. Each year, an estimated 38% of the European population suffers from a mental disorder.
- Schizophrenia, depression and Parkinson's disease are three of the major CNS disorders and their treatment often relies on medications that are only partially effective or poorly tolerated.
- Many psychotropic agents target the activity of monoamine neurotransmitter systems: dopamine, serotonin and noradrenaline. These neurotransmitters act at several dozen subtypes of receptors, most of which belong to the family of G-protein-coupled receptors (GPCRs).
- The study of the signalling and therapeutic applications of monoamine GPCRs constitutes the main focus of the author's work described in this thesis.

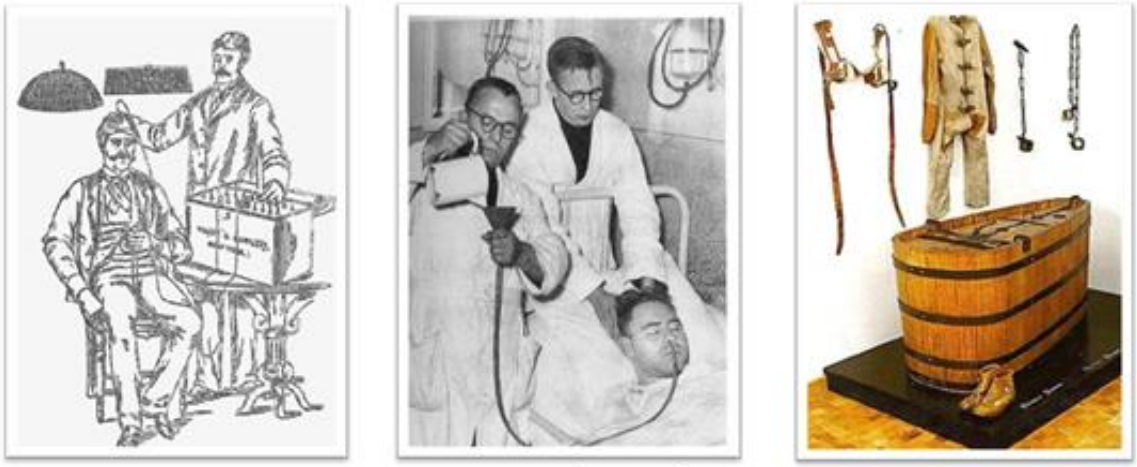
1.2 Social burden of psychiatric and neurological disorders

Whereas physical injury or disorders such as cancer or HIV often attract considerable sympathy and arouse public consciousness, such responses are noticeably less present for central nervous system (CNS) disorders [3,4]. No doubt part of the reason for this is that psychiatric and neurological disorders may be associated with unflattering notions of being "mad" or "senile" and often occur in patient populations, such as the elderly, that have less socioeconomic influence. In addition, the complexity of the CNS and the substantial financial investments necessary to develop novel therapeutics for CNS disorders have recently resulted in an exodus of pharmaceutical research from this field. Over recent years, several large pharmaceutical companies, including AstraZeneca, Pfizer, Novartis, Sanofi and GlaxoSmithKline have ceaased CNS drug discovery operations and/or have de-emphasized CNS research, causing concern that advances in this field will be compromised [5].

However, great medical needs exist in the area of CNS disorders. A recent report published by the European College of Neuropsychopharmacology (ECNP) highlighted the burden of neuropsychiatric and neurological disorders in Europe [6]. The report surveyed the situation in 30 countries covering a population of 514 million people and concluded that, each year, 38.2% of the European population (164.8 million people) suffers from a mental disorder, the most common being anxiety disorders (14%), insomnia (7%), major depression (6.9%), somatoform disorders (6.3%), alcohol and drug dependence (>4%), attention-deficit and hyperactivity disorders (ADHD, 5% in the young), and dementia (1% among those aged 60-65, 30% among those aged 85 and above). In addition, the study reported that only one third of cases receive adequate treatment, and this often occurs with delays of up to several years. The financial costs of mental disorders in Europe are estimated at a colossal

€ 798 billion per year, of which € 296 billion are needed to cover direct medical costs. Only about € 30 billion is spent on medications, the remainder being spent on non-medical and indirect costs, such as lost working time and community care[6]. It is therefore reasonable to surmize that even modest advances in the understanding of mental disorders could bring substantial advances in the care of millions of patients, diminish their suffering and lighten the burden on society. This mission, notably as regards the discovery of improved medications, has been the focus of my research for the past two decades, particularly as regards three of the major mental disorders: schizophrenia, depression and Parkinson's disease.

Figure 1.1 The way things were before the advent of psychopharmacology.
Methods of "treating" patients with psychiatric disorders used in the early and mid-1900s. Left: electroconvulsive shock; Centre: intranasal glucose administration following insulin-induced coma; Right: chains, straight-jacket, covered bath-tub.



Schizophrenia

Schizophrenia was identified as a serious thought disorder in antiquity [7,8] and has a major socio-economic impact not only because of its debilitating influence on social activity [9], but also because of the high costs of treatment associated with chronic pathology. So-called 'positive' symptoms of schizophrenia include hallucinations and delusions. In contrast, 'negative' symptoms consist of behavioral impairments, including social interaction deficits, disorganized speech and blunted affect. In addition, a variety of cognitive disturbances are observed, including working and reference memory deficits, executive function impairments and decreased vigilance. Cognitive impairment has been proposed to constitute a core symptom of schizophrenia in its own right [10,11]. The etiology of schizophrenia is likely to include numerous elements, such as genetic vulnerability, environmental and developmental insults [12,13].

Depression

Major depressive disorder (MDD; commonly called major depression or clinical depression) is a clinical syndrome with a multitude of symptoms, including lowered mood, fatigue and disturbed sleep, inability to experience pleasure from activities usually found enjoyable (anhedonia), low self-

esteem and self-confidence, loss of appetite, and low libido. Depression runs a chronic or recurrent course and suicidal ideation/behavior is a particularly troubling component. According to the World Health Organization [14], depressive disorders (major depression, dysthymia, and other depressive disorders) are one of the leading causes of disability and will affect approximately 17% of the population in their lifetime. Although use of antidepressants has tripled over the past decade and a half, drug discovery efforts have fallen short: less than one-third of major depressive disorder patients administered adequate doses of current antidepressants show significant improvement after months of treatment. At least a third of patients can be described as suffering from treatment-resistant depression, and, as a group, the latter are more likely to suffer a higher symptom burden and a more chronic illness course compared to their non-treatment-resistant counterparts[15,16].

Parkinson's disease

Parkinson's disease is characterized by massive degeneration of dopaminergic cell bodies in the substantia nigra pars compacta (SNPC) and a profound depletion of dopamine (DA) in the striatum [17,18]. This loss of nigrostriatal dopaminergic innervation elicits a spectrum of motor symptoms, including bradykinesia, rigidity, tremor, impaired gait, and postural instability. Parkinsonian patients also display depressed mood and cognitive deficits [19]. Symptomatic treatment with L-dihydroxyphenylalanine (L-DOPA), which is metabolized into DA, still provides the mainstay of symptom management [20,21]. Unfortunately, L-DOPA is poorly effective against symptoms such as cognitive dysfunction and, upon prolonged exposure, its efficacy fluctuates. Moreover, L-DOPA may be neurotoxic through transformation to 6-hydroxydopamine and elicits both autonomic side effects and dyskinesia [22]. Direct dopaminergic agonists provide advantages in terms of potential neuroprotective properties and a lesser propensity to elicit dyskinesia[23]. However, they can elicit psychiatric side effects and their efficacy in long-term monotherapy remains limited.

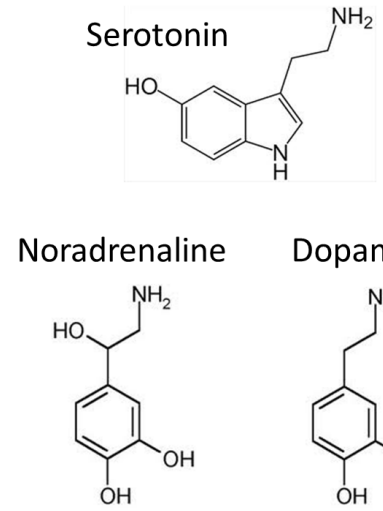
In summary, effective treatment of major neuropsychiatric and neurological disorders represent a large significant unmet medical need: new therapeutic agents are needed that are more effective, have a faster onset of action, and a reduced side-effect profile.

1.3 Rise of psychopharmacology

It is remarkable that the rise of psychopharmacology can be traced to specific events that took place in the early 1950s, with the discovery of psychoactive molecules which led to the identification of underlying mechanisms of neurotransmission, notably of monoamine neurotransmitters. The prototypical drug that led to these advances was the phenothiazine antipsychotic, chlorpromazine. Although originally synthesised as an antihistaminergic sedative, it was tested in schizophrenia patients in 1952 and found to elicit "spectacular results in the most violent states of agitation" [24]. The observation that psychoses could be pharmacotherapeutically-controlled represented a revolutionary advance over practices that (nowadays) appear primitive, such as insulin-induced coma, electroshock therapy or surgical lobotomy (Fig. 1.1) but that were current only a few decades ago [25]. Not surprisingly, the discovery of pharmacological means of controlling psychotic symptoms triggered intense interest in synthesising new drugs and investigating their mechanism of action.

It became apparent that chlorpromazine and other antipsychotics interacted with cell-surface neuronal receptors of monoamine neurotransmitters (Fig. 1.2), notably dopamine, serotonin (5-hydroxytryptamine, 5-HT) and noradrenaline. Other advances from the middle of last century provided further impetus to research on neurotransmitters, including the discovery of monoamine oxidase inhibitors (MAOI) which increased CNS levels of monoamine neurotransmitters by inhibition of the enzymes responsible for their metabolism [26,27], and the synthesis of the tricyclic antidepressants, such as imipramine or amitriptyline, which were among the first molecules to be identified that exert antidepressant activity by inhibiting the reuptake of monoamine neurotransmitters from extracellular compartments [28]. These findings attracted additional attention to the mechanism of action of monoamine neurotransmitters. The identification and characterization of the receptors that mediate the effects of these neurotransmitters has been a focus of major research efforts over recent decades and constitutes the basis for the work presented in this thesis.

Figure 1.2: Structures of monoamine neurotransmitters.



Multiple monoamine receptor subtypes

Despite the discovery of the drugs mentioned above, it was only relatively recently, in the 1970s, that the molecular study of neurotransmitter receptor pharmacology markedly accelerated, with the widespread use of radiotracers capable of specifically labelling receptors in tissue preparations. This led to the discovery of an amazing (at the time) diversity of receptor subtypes with distinct anatomical distributions, pharmacological profiles and physiological properties (see Fig. 1.3). Among the first receptors to be identified were those of serotonin, dopamine and noradrenaline, which act at a combined total of at least 27 receptors, not counting splice variants and receptor isoforms [29]. Almost all of these monoamine neurotransmitter receptors belong to the super-family of G-protein-coupled receptors (GPCRs; the exception is the 5-HT3 receptor, which is an ion channel) and are differentiated by their coupling to distinct G-protein subtypes, second messenger systems and effector responses, as well as their diverse physiological distributions and behavioural responses. The elucidation of this immense diversity has been the objective of many researchers for several decades.

1.4 Contribution of author's work

Two major areas of interest underlie my contribution to the field of monoamine receptor function: (i) the investigation of *in vitro* responses of monoamine receptors to their endogenous neurotransmitters and to synthetic ligands (described in Chapters 2, 5, 6, 7 and 8); and (ii) the

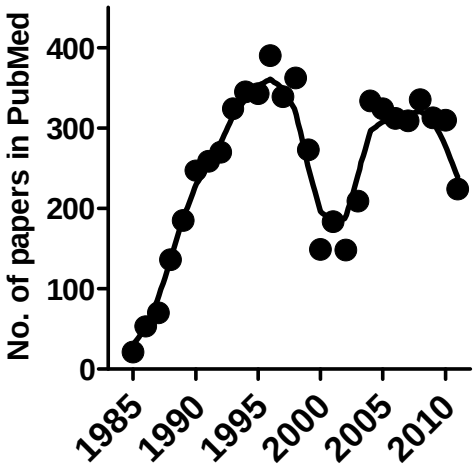
Figure 1.3: Monoamine neuronal circuitry.
Circles represent dopaminergic, noradrenergic, serotonergic and GABAergic cell bodies. The lines and triangles represent neuronal projections to different brain regions, notably the frontal cortex. A feed-back glutamatergic loop from the frontal cortex to GABA neurons controlling serotonergic transmission is also shown. Diagram adapted from [1].

Note: for copyright reasons, this Figure is omitted from the public access version of the thesis.

translation of *in vitro* responses to *in vivo* physiological effects using neurochemical substrates and/or behavioural read-outs (described principally in Chapters 3 and 4).

A substantial proportion of my work (over half the publications in this thesis) has focused on serotonin 5-HT_{1A} receptors. This receptor subtype was identified by pharmacological means [30,31] and attracted much attention as a target for novel anxiolytic drugs, offering an alternative treatment to benzodiazepine anxiolytics that suffer from a range of short-comings, including sedation and dependence liability [32]. Researching the function of 5-HT_{1A} receptors has been a "leitmotif" of my work since my doctoral research at the University of Kent, which was focused on the cloning and characterisation of a Chinese Hamster Ovary (CHO) cell line stably expressing high levels of recombinant human 5-HT_{1A} receptors [**1**]. This cell line was subsequently extensively charachterized in pharmacological assays, notably focusing on the capacity of 5-HT_{1A} receptors to constitutively activate different subtypes of coupled G-proteins (see Chapter 2). In addition, my studies contributed to a growing consensus that distinct sub-populations of 5-HT_{1A} receptors expressed in different brain regions exhibit markedly separate (or even opposing) functions. These sub-populations may be pharmacologically targeted by novel pharmacological tools commonly referred

Figure 1.4: 5-HT$_{1A}$ publications by year
Searches carried out on 25 May 2012 in the National Institutes of Health repository for peer-reviewed research reports in the life sciences (PubMed).



to as "biased" or "functionally-selective" agonists. The discovery of a prototypical 5-HT$_{1A}$ receptor biased agonist (F15599) in the course of my research is described in Chapter 3. Although the number of publications on 5-HT$_{1A}$ receptors diminished in the late 1990s, it rebounded between 2000 and 2005 (Fig. 1.4). Part of the reason for this resurgence of interest was the realization that 5-HT$_{1A}$ receptors are implicated in a variety of clinical disorders in addition to anxious states. Thus, my work contributed to the recognition that activation of 5-HT$_{1A}$ receptors is important for the improved treatment of some aspects of psychotic disorders, notably negative symptoms and cognitive deficits. This work is described in Chapter 4.

As concerns other serotonin receptors, the present thesis describes work that I carried out on 5-HT$_{1B}$, 5-HT$_{1D,}$ 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors. Their diverse intracellular signalling mechanisms were investigated using novel methodologies that were developed and characterised under my supervision. In the case of 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors, this involved developing a method to measure their constitutive activation of coupled G-proteins (see Chapter 5). At 5-HT2 receptor subtypes, exploratory studies showed the presence of functional selectivity (5-HT$_{2A}$), regulation of dopamine release (5-HT$_{2B}$) and "promiscuous" coupling to multiple G-proteins (5-HT$_{2C}$). This work is described in Chapter 6.

Although dopamine D$_2$ receptors are the primary target for antipsychotic activity (discussed principally in Chapter 4), other dopamine receptor subtypes, such as D$_3$ and D$_4$, contribute to the activity of antipsychotic drugs. Chapter 7 describes the work I carried out to characterize D$_2$, D$_3$ and D$_4$ receptor pharmacology. Finally, I investigated the mechanism of action of antiparkinsonian drugs that I had been using as reference dopaminergic agonists. I realized that some of these drugs possessed multireceptor profiles that had only been superficially characterized in a few scattered studies, despite the drugs' extensive clinical usage. I therefore carried out an extensive *in vitro* caracterization of anti-Parkinson's disease drugs, showing that their receptor profiles vary widely. In particular, antagonism of noradrenergic α2 receptors constitutes a distinguishing feature of some antiparkinsonian drugs, such as piribedil. This work is described in Chapter 8.

Overall, my research has demonstrated that the diverse agonist / antagonist actions of drugs at monoamine receptors profoundly influences their physiological effects and therapeutic potential.

2. Serotonin 5-HT$_{1A}$ receptor signalling: G-protein activation

2.1 Key points

- Using agonist ([^{3}H]8-OH-DPAT), partial agonist ([^{3}H]S15535) and antagonist ([^{3}H]WAY100635) radioligands to label human recombinant 5-HT$_{1A}$ receptors, compounds were shown to exhibit differences in binding affinity and binding density depending on receptor / G-protein coupling status [**2,16,35,39**]

- The author developed and optimised novel methods to determine agonist action at 5-HT$_{1A}$ receptors by G-protein activation using [^{35}S]GTPγS binding. These measures were demonstrated to provide useful measures of efficacy at 5-HT$_{1A}$ receptors [**5,13,15**]

- Cloned human and native rat hippocampal 5-HT$_{1A}$ receptors were shown to constitutively activate G-proteins *in vitro* [**8**,**69**]. The capacity of agonists and inverse agonists to elicit G-protein activation was found to depend on receptor-G-protein stoichiometry [**10**].

- Regional variations exist in native brain activation of G-proteins by 5-HT$_{1A}$ receptors, as demonstrated by [^{35}S]GTPγS autoradiography [**24,36,52**]

- A methodology was developed to determine 5-HT$_{1A}$ receptor-mediated activation of G-protein subtypes using antibody capture and scintillation proximity assay. 5-HT$_{1A}$ receptors were shown to modulate different G-protein subtypes with differing levels of constitutive activity [**46**].

2.2 Background to author's work

5-HT$_{1A}$ receptors as targets for CNS disorders

Since the identification of serotonin as a central nervous system neurotransmitter in 1954 [(33)], extensive investigation has been devoted to its complex functions. Indeed, 5-HT interacts with 13 receptor subtypes, divided into 7 families (5-HT$_1$ to 5-HT$_7$) based on amino acid sequence and functional homologies [(29)]. 5-HT$_{1A}$ receptors have attracted particular interest because they exert inhibitory influence on serotonergic tone and are widely distributed in post-synaptic brain regions such as cortex, septum and hippocampus, implicated in the control of mood, cognition and pain [(32,34-36)]. Accordingly, 5-HT$_{1A}$ receptors are targets for pharmacotherapy of a variety of CNS disorders. The partial agonists, buspirone and tandospirone, are clinically-available anxiolytics [(32,37)]. The antidepressant effects of 5-HT$_{1A}$ receptor agonists [(38-40)] have been explored with flesinoxan [(41,42)], and with flibanserin [(43,44)]. 5-HT$_{1A}$ receptor activation is also a prominent feature of several anti-Parkinson's disease (PD) drugs, including bromocriptine, lisuride and pardoprunox (SLV308) [**48,50**]

[45,46] and plays an important role in the action of atypical antipsychotics **[75,96,101]** [47]. Indeed, clozapine, ziprasidone, aripiprazole and lurasidone act as 5-HT_{1A} receptor partial agonists (as well as possessing other pharmacological properties) [**4,56,101**] [48,49] (see Chapter 4). 5-HT_{1A} receptor agonists such as xaliproden and repinotan have been tested for potential neuro-protective activity [**91**] [50-52] and the potent and high-efficacy agonist, befiradol, is active in chronic pain models [**57**] [35,53].

The 5-HT_{1A} receptor was the first human serotonergic receptor to be cloned [54] and was heterologously expressed in various different cellular environments (including COS7, HeLa, CHO, NIH3T3, Sf9 and E. Coli cells) enabling the study of its coupling to G-proteins and second messenger systems [**1**] [55]. Although 5-HT_{1A} receptors interact with potassium channels and calcium channels, inositol phosphate metabolism and arachidonic acid production[55], their best-characterised intracellular functional response was, for many years, the inhibition of adenylyl cyclase activity. This had been the principal read-out used to differentiate the agonist/antagonist activity of serotonergic ligands at this receptor [56,57].

However, it is known that apparent agonist efficacy at central nervous system 5-HT_{1A}

Figure 2.1: complexity of serotonergic function

Note: for copyright reasons, this Figure is omitted from the public access version of the thesis.

receptors can differ depending on the brain region studied. In cortical neurons in primary culture, for example, ipsapirone and buspirone act as antagonists whereas in hippocampal neurons they acted as partial agonists [57]. Further, ipsapirone acts as a full agonist at presynaptic 5-HT_{1A} autoreceptors [58]. These differences in efficacy have been attributed to the presence of significant receptor reserve at presynaptic but not at postsynaptic 5-HT_{1A} receptors [59-61] and highlight the fact that 5-HT_{1A} receptors in different brain regions can have markedly different properties. One cause for such differences is likely to be that 5-HT_{1A} receptors can adopt different G-protein coupling conformations in different brain regions and/or couple to different G-protein subtypes. My initial research in the early 1990s was therefore aimed at elucidating the coupling of 5-HT_{1A} receptors to G-proteins using agonist / antagonist and inverse agonist radiotracers that bind differentially to 5-HT_{1A} receptors.

Subsequently, my interest focussed on measuring the first step in the intracellular activation cascade by measuring the activation of the G-protein itself. Indeed, like other members of the superfamily of heptahelical cell surface receptors, 5-HT_{1A} receptors couple to heterotrimeric intracellular guanine nucleotide binding proteins (G-proteins). Under the influence of agonists, G-protein coupled receptors (GPCRs) promote nucleotide exchange by G-protein α subunits, which release GDP and bind GTP. This exchange triggers subunit dissociation and transmission of signalling to a variety of intracellular second messenger systems. Hydrolysis of GTP by the Gα subunit and re-association with Gβγ subunits re-establishes the initial basal state [62]. The GDP/GTP exchange step is therefore of considerable interest in the investigation of ligand efficacy. Some authors had investigated G-protein activation by measuring GTPase activity in hippocampal tissue [63] but it can also be investigated by use of the hydrolysis-resistant GTP analogue, [^{35}S]GTPγS (Fig. 2.2). This was a key tool in my research and marked an effort to investigate receptor activation at a level that was close to the receptor, rather than using more distal read-outs such as adenylyl cyclase inhibition.

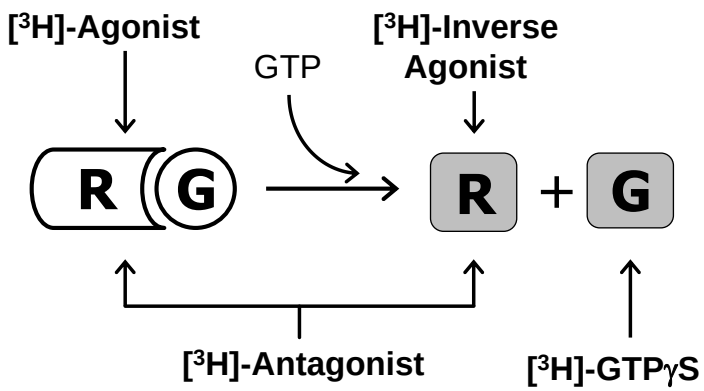
2.3 Contribution of author's work

Investigating agonist and inverse agonist influence on ligand binding.

My interest in the effects of agonists and inverse agonists on serotonin 5-HT_{1A} receptor/G-protein coupling began in studies conducted during my Ph.D. studentship at the University of Kent, under Prof. Philip Strange's supervision. This early work consisted of transfecting Chinese hamster ovary (CHO) cells with a plasmid vector containing the coding sequence (G21) of the human 5-HT_{1A} receptor [**1**] and characterizing the highly-expressed recombinant receptor. The latter showed high affinity for the prototypical 5-HT_{1A} receptor selective agonist radiotracer, [^{3}H]8-OH-DPAT, and exhibited the expected capacity to inhibit forskolin-stimulated adenylyl cyclase activity. At that time, no selective antagonist was available, so the question of distinguishing between G-protein-coupled and –uncoupled receptors was problematic. In an attempt to address this issue, I decided to use [^{3}H]spiperone as an antagonist radiotracer. Spiperone is a potent dopamine D_2 receptor antagonist which has only modest 5-HT_{1A} affinity but I surmised that it might be a useful tool when tested on a cell line which expressed a "pure" population of 5-HT_{1A} receptors. I anticipated that it would label only a small (but measurable) number of 5-HT_{1A} receptors but, surprisingly, [^{3}H]spiperone showed a high level of labelling, its Bmax value in saturation binding experiments being similar to that of

[^{3}H]8-OH-DPAT [**2**]. In addition, [^{3}H]spiperone's Bmax value was almost doubled when incubations were carried out in the presence of GTP, which uncouples GPCRs from coupled G-proteins [**2**] (Fig. 2.2). Conversely, the binding of [^{3}H]8-OH-DPAT was almost eliminated in the presence of GTP [**1**]. These data provided some of the first evidence that reciprocal agonist and inverse agonists "states" of 5-HT$_{1A}$ receptors can be identified and paved the way for a series of more detailed studies that I carried out in my later research. In particular, I investigated the coupling of 5-HT$_{1A}$ receptors to G-proteins by testing the effects of GTP on the affinity of a variety of serotonergic ligands, notably inverse agonists such as spiperone, in competition with selective radiolabels of different efficacies: the agonist [^{3}H]8-OH-DPAT [64], the partial agonist [^{3}H]S15535, which was synthesized and characterised in my laboratory [**5,9,16,24**], and the novel (at the time) antagonist [^{3}H]WAY100635 [65,66]. This compound was the first selective "neutral" antagonist that became available for characterizing 5-HT$_{1A}$ receptors and was therefore an important tool throughout my work in this field. Notably, its "neutral" antagonism was initially confirmed in sensitive *in vitro* assays [**8,41,78**] although it later became apparent that weak agonist or inverse agonist activity may be detected depending on the specific G-protein subtypes present in the assays [**46**] [67,68].

Figure 2.2: Simplified model of receptor/G-protein coupling
The Figure shows an early understanding of the interaction between receptors (R) and G-proteins (G). Agonists preferentially label receptors that are coupled to G-proteins (RG), whereas inverse agonists preferentially label uncoupled receptors. "Neutral" antagonists do not differentiate between G-protein coupling states. GTP elicits RG uncoupling and [^{35}S]GTPγS labels activated G-proteins, providing a measure of agonist efficacy. Later studies led to a more sophisticated model (see Fig. 5.1).



In a study of the same CHO-h5-HT$_{1A}$ cell line cloned during my Ph.D. studentship, [^{3}H]WAY100635 labelled about twice as many sites as the agonist, [^{3}H]8-OH-DPAT [**35**]. These data are consistent with the hypothesis that it labels both G protein-coupled and uncoupled rat 5-HT$_{1A}$ receptors [69] and that the total number of h5 HT$_{1A}$ receptor sites is equivalent to the sum of sites previously detected with [^{3}H]8-OH-DPAT and the inverse agonist, [^{3}H]spiperone [**2**] [70]. [^{3}H]S15535 labelled more sites than [^{3}H]8-OH-DPAT but fewer than [^{3}H]WAY100635, reflecting its partial agonist activity at 5-HT$_{1A}$ receptors [**5,16**]. In competition binding experiments with [^{3}H]WAY100635, a series of ligands showed binding affinity changes induced by GTP-elicited G-protein coupling. The magnitude of the affinity changes at 5-HT$_{1A}$ receptors correlated with agonist or inverse agonist efficacy determined in [^{35}S]GTPγS binding experiments [**35,39**]. Agonist affinities were considerably decreased whereas those of partial agonists were only modestly reduced. WAY100635 was the only ligand which exhibited no sensitivity to GTP-induced receptor uncoupling, consistent with "neutral" antagonist

properties in this system. In comparison, the competition isotherms of methiothepin, (+)butaclamol and spiperone, were modestly, but significantly displaced to the left (higher affinity) consistent with their inverse agonist properties in [^{35}S]GTPγS binding studies (see next section) [**8,10,19**] [71-73].

Two intriguing observations from these studies attracted my attention. Firstly, the correlation between affinity changes and agonist efficacy did not apply to all the ligands tested: two chemically related compounds, S14506 and S14761, exhibited only modest affinity shifts [**35**], despite acting as efficacious agonists in *in vitro* and *in vivo* functional models [**19**] [74]. [^{3}H]S14506 also exhibits 'atypical' binding properties at rat 5-HT$_{1A}$ receptors, being insensitive to G protein uncoupling induced by guanine nucleotides and the alkylating agent, N-ethyl-maleimide (NEM) [75], properties usually associated with antagonists. Further, another study found that S14506 displayed little difference in its affinity for rat 5-HT$_{1A}$ receptors labelled by [^{3}H]8-OH-DPAT or by the antagonist [^{3}H]p-MPPF [76]. Secondly, the atypical antipsychotic, clozapine, also showed little sensitivity to G-protein uncoupling at 5-HT$_{1A}$ receptors [**39**], despite showing clear partial agonism [**4,19**]. These data indicating unusual agonist interactions with 5-HT$_{1A}$ receptors led me to investigate two issues: (i) the influence of different G-protein subtypes on 5-HT$_{1A}$ receptor signalling (see below and Chapter 3); and (ii) the effects of different atypical antipsychotics on 5-HT$_{1A}$ receptor activation (described in Chapter 4).

Investigating agonist and inverse agonist influence on G-protein activation.

Although the majority of *in vitro* studies on 5-HT$_{1A}$ receptors up to the 1990s had used adenylyl cyclase as a measure of receptor activation, I was interested to use another measure which reflected molecular events which lie closer to the receptor itself and, specifically, its G-protein coupling status. The use of [^{35}S]GTPγS binding , a non-hydrolysable analogue of GTP that binds to agonist-activated G-proteins, had been described at other receptor subtypes, notably muscarinic m1 to m4 and serotonin 5-HT$_{1D}$ [77,78]. I therefore adapted the methodology to 5-HT$_{1A}$ receptors and rapidly found that it constitutes a powerful tool to investigate receptor/G-protein coupling and the actions of serotonergic drugs [**5,13,15**]. In addition, it provided the means to elucidate the surprising behaviour of spiperone that I had observed during my Ph.D. studentship [**2**] (see above). In fact, spiperone markedly inhibited the basal binding of [^{35}S]GTPγS to membranes prepared from CHO-5-HT$_{1A}$ cells, confirming its inverse agonist propertites and the capacity of 5-HT$_{1A}$ receptors to elicit constitutively activation of G-proteins *in vitro* [**8**]. This was later extended to native rat hippocampal 5-HT$_{1A}$ receptors, which were shown to constitutively activate a specific G-protein subtype (Gαo) using a G-protein targeting procedure (see next section) [**69**]. In contrast to spiperone, WAY1000635 did not exhibit any positive or negative efficacy but blocked the actions of both agonists and inverse agonists, consistent with "neutral antagonist" antagonist properties [**8**]. This was an important finding because other compounds which were claimed as antagonists at 5-HT$_{1A}$ receptors, such as NAN190, BMY7378, SDZ216,525 and WAY100135, had subsequently been found to display partial agonist properties when tested in systems which exhibit high degrees of receptor reserve [79,80]. These studies therefore established that stimulation of [^{35}S]GTPγS binding could usefully differentiate between agonists, antagonists and inverse agonists [**4,5,8**]. Nevertheless, many

questions remained, notably concerning the effect of changes in receptor expression level on functional responses. This is important in view of the fact that different brain regions can exhibit different levels of 5-HT_{1A} receptors with distinct physiological influence. Thus, while some laboratories had studied cell lines with different receptor expression levels [81], the relationship of receptor to G-protein expression levels had not been quantitatively evaluated. I therefore investigated this issue by determining in CHO-5-HT_{1A} cell membranes the expression level of recombinant 5-HT_{1A} receptors and also of their coupled G-protein(s) by [^{35}S]GTPγS saturation binding, providing measures of R:G ratios [**10**]. I found that cells grown in adherent culture in the presence of cationic liposomes displayed much greater receptor expression levels than cells grown in suspension culture conditions. However, no significant difference in G-protein expression was observed, resulting in a three-fold increase in receptor:G-protein ratio. Under these conditions, I observed almost a doubling of the relative efficacy of a partial agonist, eltoprazine (from 53% to 93%), without a change in its potency. In contrast, the full agonist, 5-HT, exhibited a two-fold decrease in its EC_{50} value. Notably, in addition to these changes, the increase in 5-HT_{1A} receptor:G-protein ratio roughly doubled the negative efficacy of spiperone. Two conclusions may be drawn from these observations. First, it might be expected that a higher receptor expression level might increase basal G-protein activation. However, in this study, the three-fold increase in receptor number did not affect basal [^{35}S]-GTPγS binding. This suggested that basal G-protein activation was already at its maximum (i.e. the number of G-proteins was limiting). Second, it appeared that spiperone stabilised G-protein-coupled receptors in a conformation which is not capable of G-protein activation. Thus a higher density of spiperone-occupied receptors, which 'traps' G-proteins in an inactive state, reduces the pool of G-proteins available for activation by non-spiperone-occupied receptors. This hypothesis is consistent with the 'cubic' or 'general' ternary complex model [2,82], in which inactive receptors can exist not only in an uncoupled state but also in a state which is coupled to G-protein (see Fig. 5.1).

Determination of activation at G-protein Gα subunits by antibody capture

Almost all the studies described above employed membrane preparations expressing mixed populations of G-proteins, and the resultant measures of [^{35}S]GTPγS binding were therefore composed of the cumulated responses of all the G-protein subtypes activated or inhibited by the receptor [83]. Total [^{35}S]GTPγS binding measures, which lack resolution at the level of specific G-protein subtypes, may, therefore, conceal complex interactions which may arise from differential coupling of receptors to specific G-protein subtypes as well as ligand-dependent activation of specific transduction pathways (biased agonism, see Chapter 3) [**54**].

Efforts had been made to characterise the pharmacological profiles of ligands at 5-HT_{1A} receptors by examining coupling to specific G-protein subtypes. Thus, specific G-proteins had been co-expressed or reconstituted with 5-HT_{1A} receptors in bacterial, insect and mammalian cells [84-87]. In addition, 5-HT_{1A} receptor-G-protein fusion constructs had been designed to constrain the coupling of the receptor to specific G-protein subtypes in HEK293 cells [68,88,89]. While these strategies yielded useful information, they were not adapted to detect regulatory mechanisms that depend on availability of

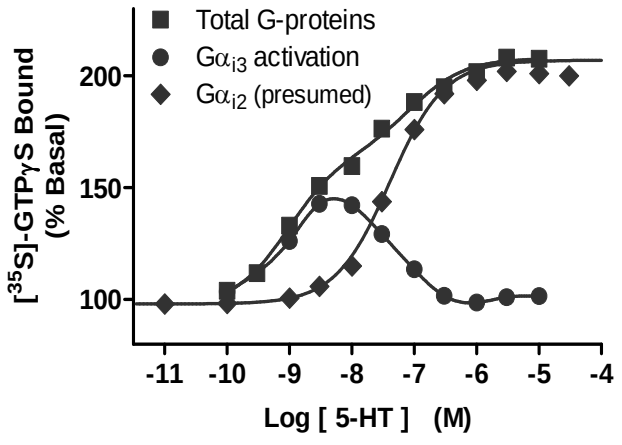
multiple G-proteins. In addition, such techniques require the use of recombinant systems expressing mutant receptor constructs and are unsuitable for investigation of receptor coupling in native tissues.

At the end of the 1990s, a novel approach for determining G-protein subtype activation was reported at muscarinic receptors [90], using a rapid methodology which lends itself to the investigation of numerous ligands and experimental conditions. The experimental principle is based on rapid immunoprecipitation and [^{35}S]GTPγS binding, whereby activated G-proteins (ie. [^{35}S]GTPγS-bound) are captured by specific antibodies [91]. These are detected using scintillant-impregnated polymer beads coated with a secondary antibody. While no data were reported concerning the action of inverse agonists, it was demonstrated that this technique allows targeting of differing G-protein families ($G\alpha_{q/11}$ and $G\alpha_{i1,2,3}$) in membranes prepared from both recombinant CHO cells and native rat striatum [90].

$G\alpha_{i3}$ activation at 5-HT_{1A} receptors: protean agonism and inverse agonism.

In my laboratory, we used the novel antibody-capture / SPA procedure to investigate the activation of G-proteins by 5-HT_{1A} receptors [**46**]. This study, revealed complex interactions in receptor-G-protein coupling, which were later confirmed in another study using siRNA knock-down technique [**100**]. Thus, 5-HT and other high-efficacy agonists, such as 5-CT, S14506 and (+)8-OH-DPAT, yielded *bell-shaped* [^{35}S]GTPγS binding isotherms at $G\alpha_{i3}$ subunits, with peaks at nanomolar concentrations (Fig. 2.3). Hence the direction of the response to agonists (stimulation or inhibition) was dependent on the ligand concentration range examined and suggested that a conformational change occurs by which 5-HT_{1A} receptors 'switched' signalling to other G-protein subtypes. This hypothesis was supported by the fact that, in classical [^{35}S]GTPγS binding assays which do not distinguish G-protein subtypes, 5-HT-induced stimulation was found to be biphasic [**46**], consistent with sequential activation of at least two G-protein subtypes. The identity of the second G-protein was probably $G\alpha_{i2}$, in view of its known high level of expression in CHO cells, and its known interaction with 5-HT_{1A} receptors [**54**] [92,93].

Figure 2.3: [^{35}S]GTPγS binding at G-protein subtypes. Simulated G-protein activation curves are shown for total G-proteins in CHO cells and for Gαi3 and Gαi2.



These unexpected data differed markedly from those obtained in previous studies. Indeed, observations of bell-shaped drug concentration-response isotherms in *in vitro* models had classically been attributed to the presence of cross-reacting receptors [94] or promiscuous coupling to two or more signal transduction systems (for example to both stimulation and inhibition of adenylyl cyclase activity [95,96]. In contrast, in the case of $G\alpha_{i3}$ activation by 5-HT_{1A} receptors,

no interfering receptors were present in the recombinant system, as demonstrated by the absence of response in membranes from non-transfected cells. Secondly, unlike 5-HT, the partial agonist, (-)pindolol, displayed *sigmoidal* stimulation isotherms for $G\alpha_{i3}$ activation, suggesting that it is not capable of inducing the conformational switch observed with higher efficacy agonists. Thus, when acting at 5-HT_{1A} receptors, (-)pindolol maintains 5-HT_{1A} receptor signalling to $G\alpha_{i3}$ over a wide concentration range. We had previously reported that pindolol behaves as a partial agonist in classical [^{35}S]GTPγS binding assays [**15**], but, for $G\alpha_{i3}$ activation, its maximal stimulation was comparable to that of 5-HT. (-)Pindolol may therefore display higher efficacy at $G\alpha_{i3}$ compared with other G-protein subtypes. Thirdly, ligand actions on $G\alpha_{i3}$ activation were highly susceptible to modulation by sodium ions. Indeed, when the sodium concentration was lowered (increasing basal $G\alpha_{i3}$ activation), the actions of (-)pindolol were reversed from activation of $G\alpha_{i3}$ to inhibition, Conversely, WAY100635, which exhibits sodium-dependent inverse agonist properties in HeLa cells [(67)], stimulated $G\alpha_{i3}$ activation in CHO cell membranes (in the presence of sodium), indicating that it possesses mild agonist properties under these conditions. Sodium ions also profoundly influenced $G\alpha_{i3}$ activation by 5-HT: *only stimulatory* actions were observed at high sodium concentration, whereas *only inhibitory* 'pseudo-inverse agonist' actions were seen at low sodium concentration. Thus, for this G-protein subtype, the actions of the endogenous agonist, 5-HT, can be altered, not just in magnitude, but also in direction. More generally, ligands which display variable direction of response, depending on the level of constitutive activity (or 'tone'), are known as 'protean agonists' [(97)] and the results discussed above for 5-HT_{1A} receptors suggests that $G\alpha_{i3}$ subunit activation may be particularly sensitive to such ligands (a later study identified similar responses for $G\alpha_{i3}$ coupling to 5-HT_{1B} receptors; Chapter 5 [**53**]). Fourthly, the inverse agonists, spiperone and methiothepin, sigmoidally inhibited [^{35}S]GTPγS binding. Their isotherms were not mirror images of those of 5-HT: no stimulatory phase was observed, producing U-shaped curves. Further, spiperone and methiothepin did not exhibit protean behaviour, that is, even when sodium concentration was modified, they invariably exhibited inhibition of basal activity. These data suggest that spiperone and methiothepin selectively stabilise/induce receptor conformation(s) which are distinct from those recognised by agonists or partial agonists. Further, these observations indicate that, to distinguish true inverse agonism from protean agonism, assays must be carried out under a variety of conditions (e.g. different sodium ion concentrations) favouring or suppressing constitutive activity. It is interesting that spiperone exhibited greater negative efficacy than methiothepin in CHO cell membranes [**9,10,19**] but opposite effects were observed in HeLa cells [(67)]. Further, in HeLa cells, the negative efficacy of spiperone, but not methiothepin, was significantly increased by removal of sodium from the incubation buffer [(67,98)], providing additional support for differential influence of these ligands on receptor-G-protein coupling.

2.4 Conclusions and perspectives.

Taken together, the experiments that I conducted demonstrated extensive complexity on the interaction of 5-HT_{1A} receptors with coupled G-protein(s). In particular, the bell-shaped concentration-repsonse curves observed for $G\alpha_{i3}$ activation were unprecedented and surprising [**46**].

Those data led me to speculate that the differences in magnitude and direction of G-protein activation may have been due to coupling of 5-HT_{1A} receptors to distinct G-protein populations, raising the prospect of identifying ligands which selectively signal via specific G-proteins subtypes (see discussion of "biased agonism", Chapter 3). It is worth noting that the complex actions of ligands on 5-HT_{1A} receptor-mediated $G\alpha_{i3}$ activation may be paralleled by similar effects at other GPCRs. In a preliminary study, Browning et al. [99] reported bell-shaped [^{35}S]GTPγS binding isotherms at adenosine A_1 receptors expressed in CHO cells. The precise G-protein subtypes activated were not identified, but the presence of bell-shaped isotherms was dependent on high receptor expression levels and, as in the case of 5-HT_{1A} receptors, the use of high efficacy agonists, whereas an inverse agonist exhibited sigmoidal inhibition isotherms [100]. These data suggest that changes in receptor conformation producing sequential activation of different G-protein populations may occur for a variety of GPCRs.

More broadly, my work contributed to the discovery of promiscuous coupling of receptors to multiple G-protein subtypes and "ligand-directed trafficking of receptor signalling", which later became known as "functional selectivity" or "biased agonism" (see next chapter). These advances highlighted the need to refine descriptions of drug efficacy for G-protein activation. In this context, antibody-capture / SPA methodology provides a useful means of elucidating diverse receptor interactions with specific G-protein subtypes. Nevertheless, it should be noted that a limitation of the antibody capture / SPA methodology is its financial cost: the antibodies and the SPA beads don't come cheap! To date, most of the published studies using this technique come from industrial laboratories [91] for which, presumably, the cost of consumables is less limiting than for many academic research labs. Thus, in the case of these scientifically innovative but financially onerous experiments, I was privileged to be able to make the most of opportunities offered by a pharmaceutical industry research environment.

3. Serotonin 5-HT$_{1A}$ receptor subpopulations and biased agonism

3.1 Key points

- The author demonstrated that the *in vitro* signaling profile of the endogenous agonist, 5-HT, differs from that of selective 5-HT$_{1A}$ receptor agonists (8-OH-DPAT, F13714 and F15599). Comparison of several intracellular responses indicated that each agonist exhibited its own distinctive "signalling fingerprint" [**61**,**88**].

- F15599 was found to preferentially activate post-synaptic 5-HT$_{1A}$ receptors at low doses that do not activate pre-synaptic 5-HT$_{1A}$ receptors in raphe nuclei [**88,94**]. This preferential post-synaptic activity is not observed with other 5-HT$_{1A}$ agonists [**102**]. Preferential cortical labelling by F15599 was also shown in micro-PET experiments in rat using [^{18}F]F15599 [**93**] but not the pre-synaptic agonist, [^{18}F]F13714 [**103**]. However, micro-PET experiments in cat generate discrepant data [**93,103**]

- In rat, F15599 exhibited potent anti-depressant-like activity in the forced swim test, inhibited stress-induced ultrasonic vocalization, and attenuateed phencyclidine-induced cognitive impairments in reversal learning and in a hole-board test [**95,97**].

- Preferential activation of post-synaptic 5-HT$_{1A}$ receptor by F15599 was found to elicit less serotonin syndrome and less disruption of attentional performance or impairment of working memory than elicited by activation of pre-synaptic receptors with F13714. F15599 was shown to be free of dirsuption of pre-pulse inhibition of startle response [**95,97**].

- The 5-HT$_{1A}$ receptor ligand (and beta-blocker), pindolol, was shown to exhibit partial agonist activity *in vitro*. This activity likely underlies its capacity to accelerate onset of therapeutic efficacy of antidepressants by interaction with pre-synaptic 5-HT$_{1A}$ receptors [**15**,**36,41,46**].

3.2 Background to the author's work

Differential functions of pre- and post-synaptic 5-HT$_{1A}$ receptors

Aboundant electrophysiological, pharmacological and neurochemical data provide compelling evidence that 5-HT$_{1A}$ receptors expressed in different brain regions possess distinct signalling and physiological properties and those region-specific effects are therapeutically-relevant. For example, at a neurochemical level, activation of pre-synaptic 5-HT$_{1A}$ receptors expressed on serotonergic neurons elicits inhibition of serotonin release in terminal regions such as hippocampus and cortex. In contrast, activation of post-synaptic cortical 5-HT$_{1A}$ hetero-receptors expressed on

glutamatergic pyramidal cells and/or GABAergic (Gamma-aminobutyric acid) interneurons, elicits increased dopamine release in cortex [**60**] [101,102].

In rodent behavioral tests, anxiolytic activity is mediated by activation of pre-synaptic 5-HT_{1A} receptors [32,38], whereas anti-depressant-like activity is mediated by activation of post-synaptic receptors [38]. Accordingly, mice that were genetically manipulated to increase raphe 5-HT_{1A} autoreceptor expression exhibited depressive-like behavior and were resistant to antidepressant treatment [103]. These observations are consistent with clinical observations in depressed patients treated with serotonin reuptake inhibitors: desensitization of pre-synaptic 5-HT_{1A} receptors is necessary before antidepressant efficacy is achieved [1,104].

In cognition tests, 8-OH-DPAT facilitated rat passive avoidance at low doses, whereas higher doses impaired performance [105,106]. This is likely due to differential effects at pre- and post-synaptic 5-HT_{1A} receptors, respectively [36]. Indeed, micro-injection of the 5-HT_{1A} receptor weak partial agonist, S15535, into the hippocampus, reversed the memory deficit elicited by systemic injection of 8-OH-DPAT in a spatial discrimination task [107], indicating that activation of post-synaptic receptors in this brain region was detrimental to mnesic performance. Finally, activation of pre-synaptic 5-HT_{1A} receptors may facilitate addiction-related behaviors, whereas activation of post-synaptic 5-HT_{1A} receptors inhibits them [108].

The diverse effects of pre- and post-synaptic 5-HT_{1A} receptor activation are not due to the presence of receptor subtypes. Indeed, only a single 5-HT_{1A} receptor gene has been identified in human and rat: it is intron-less and without splice variants [54,109] so the variety of responses described above are attributable to regional differences in other factors, such as G-protein subtypes [110], Regulators of G-protein Signaling (RGS) [111] or transcriptional regulation. Indeed, the expression of 5-HT_{1A} receptors is differentially regulated by a single nucleotide polymorphism (SNP) in the promoter region of the 5-HT_{1A} receptor gene (C-1019G substitution) [112,113]. This SNP impairs repression of the 5-HT_{1A} promoter by the NUDR/DEAF-1 transcription factors in raphe cells, consistent with over-expression of pre-synaptic 5-HT_{1A} receptors [43,114]. Thus, C-1019G polymorphism is associated with higher levels of remission failure and suicidal behavior in depressed patients, consistent with impaired antidepressant efficacy due to excessive feed-back inhibition by pre-synaptic 5-HT_{1A} receptors. Further, schizophrenia patients expressing the C-1019G polymorphism exhibit impaired negative symptoms and cognitive deficit response to antipsychotics [115-118]. This observation is likely related to the fact that the C-1019G polymorphism also causes hypofunction of 5-HT_{1A} receptors in cortex [112,119,120] thus resulting in an overall imbalance of pre- *versus* post-synaptic receptor function.

The above considerations indicate that 5-HT_{1A} receptors elicit differential, and sometimes opposing, responses in different brain regions. At a molecular level, receptor inactivation studies using N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) demonstrated the existence of 5-HT_{1A} receptor reserve in the raphe for inhibition of serotonin synthesis [59]. In contrast, receptor reserve was not observed in hippocampus for inhibition of adenylyl cyclase or for control of hypothermia [61,121].

The agonist radiotracer, [^{3}H]8-OH-DPAT showed five-fold higher affinity in hippocampus than in raphe membranes [122], suggesting that the receptor-G-protein coupling state of the receptor differs between the two brain regions. Further, whereas 5-HT$_{1A}$ receptors are coupled to inhibition of adenylyl cyclase in hippocampus, they are not coupled to this response in raphe homogenates [123]. In contrast, 5-HT$_{1A}$ receptor-mediated inhibition of inositol phosphate synthesis by 8-OH-DPAT and flesinoxan was observed in Raphe but not in hippocampus [122].

An immuno-precipitation study found that, in raphe, 5-HT$_{1A}$ receptors preferentially couple to Gαi3 subtypes whereas they couple preferentially to Gαo in hippocampus and to a combination of G-proteins in cortex and hypothalamus [110]. 5-HT$_{1A}$ receptor agonists also increased Extracellular Signal-regulated Kinase (ERK1/2) phosphorylation in the cortex, presumably by direct activation of post-synaptic 5-HT$_{1A}$ receptors. In contrast, 5-HT$_{1A}$ receptor agonists inhibited ERK1/2 phosphorylation in hippocampus, likely via an inhibition of serotonin release due to activation of pre-synaptic 5-HT$_{1A}$ receptors [124-126].

In my laboratory, we used functional ([^{35}S]GTPγS) autoradiography experiments to show that regional variations exist in native brain activation of G-proteins by 5-HT$_{1A}$ receptors [**24,36,52**]. Thus, in a comparison of the stimulatory influence of 5-HT in four different brain regions (dentate gyrus, septum, substantia nigra and cingulate cortex), 5-HT-dependent [^{35}S]GTPγS binding was greatest in the dentate gyrus, with maximal stimulation of 2.25-fold above basal values, whereas in the other brain regions stimulation was lower (1.39- to 1.56-fold) [**52**]. However, marked differences were also observed in the potency of 5-HT: low in the dentate gyrus and substantia nigra (pEC$_{50}$ values ~5) but higher in the septum and cingulate cortex (pEC$_{50}$~6.5). Some of these differences may be attributable to the presence of other receptor subtypes (e.g. 5-HT$_{1B}$) but it is likely that 5-HT$_{1A}$ receptors provide the predominant response in structures such as dentate gyrus and lateral septum. These observations are therefore consistent with brain region-dependent differences in receptor-G-protein coupling.

Biased agonism: differential activation of 5-HT$_{1A}$ receptor signaling

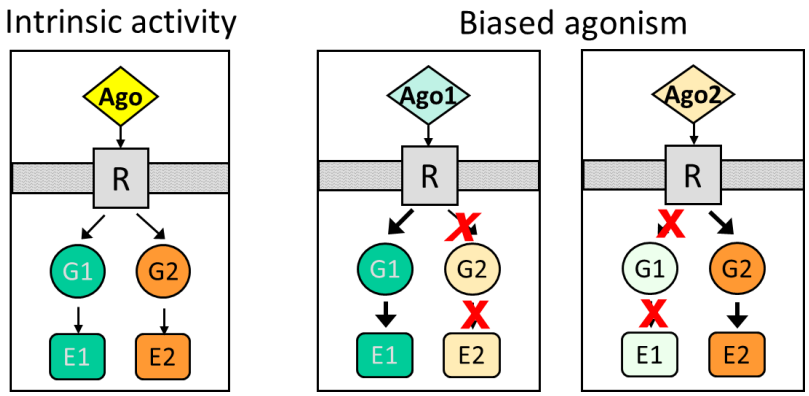
Increasing attention has recently been given to the idea of "biased agonism" (also known as "functional selectivity" or "agonist-directed signaling") [127-130]. According to this concept, agonists may preferentially direct receptor signaling to one response whilst not affecting, or even blocking, another response (Fig. 3.1). If the different signaling responses mediate distinct functional effects (e.g. therapeutic *versus* side-effects), then biased agonism offers a strategy to identify more effective and better-tolerated drugs. Examples of "biased agonism" have been reported at 5-HT2 receptors *in vitro* and *in vivo* and may underlie pro-psychotic effects of some CNS agents [**84**] [131,132].

At 5-HT$_{1A}$ receptors, a study examining G-protein subtype activation in a cloned CHO cell line [92], found that different agonists displayed a varying balance of activation of Gαi2 and Gαi3, determined using a photoreactive GTP analogue (4-azidoanilido-[α-^{32}P]GTP). Thus, whereas rauwolscine displayed similar EC$_{50}$ values for activation of the two G-protein subtypes, ipsapirone showed a nearly four-fold lower EC$_{50}$ for Gαi3 activation. 5-HT and 8-OH-DPAT had intermediate EC$_{50}$ ratios [92]. These data indicated that 5-HT$_{1A}$ receptor agonists can be distinguished by their relative capacity to activate different G-protein subtypes.

A study carried out under my direction found that G-protein activation elicited by 5-HT via 5-HT_{1A} receptors in a CHO cell line was partly blocked by pre-incubation with anti-Gαi3 antibodies, indicating that other G-protein subtypes also couple to 5-HT_{1A} receptors in this cell line [**46**], as discussed in Chapter 2. However, in the case of the partial agonist, pindolol, pre-incubation with anti-Gαi3 antibodies almost completely suppressed G-protein activation [46]. This suggested that pindolol preferentially elicits 5-HT_{1A} receptor coupling to Gαi3 and not to other G-protein subtypes, a mechanism that may underlie some of pindolol's signalling properties [**14,36,41**] and its preferential interaction with 5-HT_{1A} receptors in the raphe, as observed in positron-emission tomography (PET) studies [(133,134)]. Drug differences were also seen in rat raphe transduction: buspirone elicited 5-HT_{1A} receptor coupling to Gαi2, Gαi3 and Gαo and inhibition of adenylyl cyclase [(135)]. In contrast, (+)8-OH-DPAT only elicited coupling to Gαi3 and did not elicit the other responses.

Similar considerations apply to physiological tissues. Indeed, in a coimmunoprecipitation study, rat brain 5-HT_{1A} receptors in raphe nuclei coupled preferentially to Gαi3 subunits, whereas 5-HT_{1A} receptors in hippocampus coupled preferentially to Gαo [(110)]. In addition, although the density of 5-HT_{1A} receptors in these brain regions is similar, agonist-induced [^{35}S]GTPγS labelling was markedly lower in pre-synaptic than in post-synaptic regions in some studies [(136)]. It may be speculated that differential coupling to intracellular G-proteins may explain some of these contrasting responses of pre- and post-synaptic 5-HT_{1A} receptors.

Figure 3.1: Intrinsic activity vs Biased Agonism
The classical agonist (Ago) elicits activation of all signalling responses that are available to the receptor (R). In contrast, the biased agonists (Ago1 and Ago2) preferentially activate one response or another.
G1 & G2 = G-protein subtypes; E1 & E2 = Effectors.



Whilst the observations described above are somewhat disparate, they provided a strong rationale for supposing that some 5-HT_{1A} receptor ligands may act as "biased agonists" with distinct influence on receptor signaling in different brain regions. Hence, the identification of novel ligands that preferentially target brain regions of interest appeared pharmacologically feasible and led me to carry out detailed studies of this issue using novel, selective and efficacious 5-HT1A agonists.

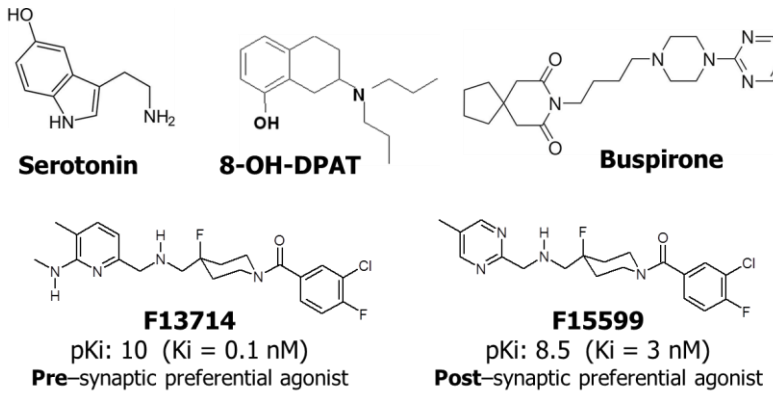
3.3 Contribution of author's work

The concept of biased agonism progressively attracted my interest over the course of the studies described in Chapter 2. For example, in ligand binding experiments the napthylpiperazine derivative, S14506, and the antipsychotic, clozapine, contrasted with other agonists by exhibiting

much smaller decreases in binding affinity when receptor-G-protein dissociation was induced by GTP [**19,35,39**]. These data suggested that S14506 and clozapine preferentially bind to receptors which are coupled to a tightly-associated G-protein subtype whereas other agonists preferentially bind to receptors coupled to other G-protein subtypes. Further, whereas ERK phosphorylation elicited by most agonists correlated with their efficacy in G-protein activation experiments, clozapine only modestly stimulated ERK phosphorylation [**35**], suggesting that it does not principally signal via this intracellular mechanism.

In later electrophysiological experiments on *Xenopus* oocytes transiently expressing 5-HT_{1A} receptors, the agonists, 5-HT, L694247 and F13714, stimulated G-protein-activated Inwardly-Rectifying K+ (GIRK) currents with similar efficacy [**61**]. In contrast, L694247 was more efficacious than 5-HT for stimulation of a G-protein-independent smooth inward current (I_{smooth}) whereas F13714 acted as an antagonist for this response, as did the selective 5-HT_{1A} receptor antagonist, WAY100635 [**61**]. These results further supported the concept that different signalling responses could be individually targeted with appropriate pharmacological tools and encouraged me to search for such tools in the course of my work. When I arrived at the Pierre Fabre research centre, I found that an ongoing serotonin drug discovery program had built a chemical library of hundreds of selective 5-HT_{1A} agonists. These provided ample material to explore and compare *in vitro* signalling properties. It became apparent that wide-ranging differences could be identified in the agonist properties of these compounds and, in particular, of F15599, a drug that can be considered as a prototypical 5-HT_{1A} biased agonist with preferential post-synaptic cortical 5-HT_{1A} activity. The rest of this chapter is devoted to describing the studies I directed on this compound, in comparison with other serotonergic ligands.

Figure 3.2: Chemical structures of 5-HT_{1A} receptor agonists.
F15599 and F13714 are chemically-related but distinct from 5-HT, 8-OH-DPAT or buspirone.



Distinct pharmacological targeting of pre- and post-synaptic 5-HT_{1A} receptors.

F15599 is a potent, selective and high efficacy agonist at 5-HT_{1A} receptors [**88**]. Chemically-related compounds include befiradol (F13640) and F13714 [137-139], but not 8-OH-DPAT or buspirone (Fig. 3.2) so F15599 marks a break with older azapirone partial agonists. Detailed studies comparing F15599 and F13714 showed that they differ markedly in their *in vitro* signaling profiles and in their *in vivo* properties at sub-populations of 5-HT_{1A} receptors. Thus, although F15599, F13714, 8-OH-DPAT and 5-HT all behaved as efficacious agonists in tests of G-protein activation, adenylyl cyclase

inhibition, ERK1/2 phosphorylation and receptor internalization, the order of potency for stimulation of these responses was specific to each agonist (Table 3.1). F15599 showed marked potency for ERK1/2 phosphorylation (EC_{50} ~15 nM) but lower potency for other responses (EC_{50} 100 to 350 nM), whereas 5-HT preferentially elicited adenylyl cyclase inhibition [**88**]. Each agonist exhibited its own "signaling fingerprint", possibly because of agonist-directed coupling of 5-HT_{1A} receptors to different G-protein subtypes. Indeed, 5-HT activated both Gαi and Gαo over a similar concentration range, whereas F15599 activated Gαi more potently and more efficaciously than Gαo, and F13714 and 8-OH-DPAT exhibited intermediate profiles [**88**]. Given that 5-HT_{1A} receptors couple to different G-protein subtypes depending on brain area [(110)], this suggests that biased agonists can, *de facto*, preferentially target certain brain regions and functional responses.

Table 3.1: Order of potency of agonists for 5-HT_{1A} receptor signalling

pERK: ERK1/2 phosphorylation; G-protein: total G-protein activation; Internal.: receptor internalization; cAMP: inhibition of adenylyl cyclase. Each agonist displayed a distinct order of potency for these responses. Adapted from [**88**].

Agonist	1st response		2nd response		3rd response		4th response
F15599	**pERK**	**>>**	**G-protein**	**>**	**Internal.**	**>**	**cAMP**
F13714	**pERK**	**≥**	**Internal.**	**=**	**G-protein**	**≥**	**cAMP**
(+)8-OH-DPAT	**pERK**	**>>**	**cAMP**	**>**	**Internal.**	**≥**	**G-protein**
5-HT	**cAMP**	**≥**	**G-protein**	**>**	**pERK**	**>**	**Internal.**

However, caution is desirable when extrapolating from *in vitro* effects to *in vivo* functional responses because cross-talk may render receptor-level biased agonism redundant in more integrated systems [(1,129)]. In the case of F15599, I directed a series of studies [**88,93,94,95,97**] that demonstrated that its distinctive "signaling fingerprint" translates to a distinctive preferential activation of post-synaptic (mainly cortical) 5-HT_{1A} receptors, with less influence on pre-synaptic 5-HT_{1A} receptors. In contrast, F13714 exhibits an opposite preference, with more pronounced activation of 5-HT_{1A} autoreceptors and less potent activity at cortical receptors. The findings supporting this assertion are summarized below.

Firstly, in rat electrophysiological tests, F15599 stimulated frontal cortex pyramidal cell electrical activity at low doses (MED 0.2 µg/kg i.v.), whereas much higher doses were necessary to inhibit raphe neuron firing (MED 8.2 µg/kg i.v.) [**94**]. Both of these effects were antagonized by WAY100635. The electrophysiological profile of F15599 is not shared by other 5-HT_{1A} agonists, such as 8-OH-DPAT, befiradol or repinotan [**102**][(140-142)].

Secondly, in microdialysis studies, F15599 stimulated dopamine release in rat frontal cortex at low doses (ED_{50} 0.03 mg/kg i.p.). This effect, associated with beneficial properties on mood and

cognitive parameters, was 5-HT_{1A}-specific, being reversed by WAY100635 [**94**][(140,143)]. In contrast, F15599 inhibited hippocampal serotonin release at doses that were about an order of magnitude higher (ED_{50} 0.24 mg/kg i.p.) [**94**] a profile of activity not shared by other 5-HT_{1A} agonists, such as F13714 or befiradol [**94,97,102**]. In addition, when F15599 was chronically administered by mini-pump, no desensitization of pre-synaptic 5-HT_{1A} receptors was observed except at very high doses (20 mg/kg/day for 14 days) whereas a low dose of F13714 was sufficient to rapidly achieve this effect (2.5 mg/kg/day for 3 days) [**67**][(144)].

Thirdly, a preferential post-synaptic action of F15599 is supported by *ex vivo* studies of expression of the immediate early gene, c-Fos. The latter provides a marker of neuronal activation state and, in the case of F15599, was markedly stimulated in frontal cortex but very little or not at all in median or dorsal raphe [**88**]. In contrast, F13714 showed an opposite profile, strongly stimulating c-Fos expression in dorsal raphe, to an extent that exceeded that in frontal cortex [(145)].

Fourthly, in *ex vivo* studies, F15599 stimulated extra-cellular signal regulated kinase (ERK1/2) phosphorylation in rat frontal cortex (a response controlled by post-synaptic 5-HT_{1A} receptors) and inhibited it in hippocampus (a response controlled by pre-synaptic receptors) at similar doses [**88**]. In contrast, F13714 and befiradol were markedly more potent for stimulation of ERK1/2 phosphorylation in hippocampus, reflecting a preferential pre-synaptic action [(125)]. These data suggest that pronounced ERK1/2 phosphorylation may underlie the preferential cortical activity of F15599, an interpretation that is consistent with the compound's potent activation of ERK1/2 phosphorylation in cellular tests *in vitro*, as discussed above [**88**].

Interestingly, deficits in ERK phosphorylation are associated with depressive states and are reversed by chronic SSRI administration [(146-148)], suggesting that the potent ERK phosphorylation-inducing activity of F15599 could underlie a favourable antidepressant profile (see below).

Effects of post-synaptic 5-HT_{1A} receptor activation in models of mood and cognition.

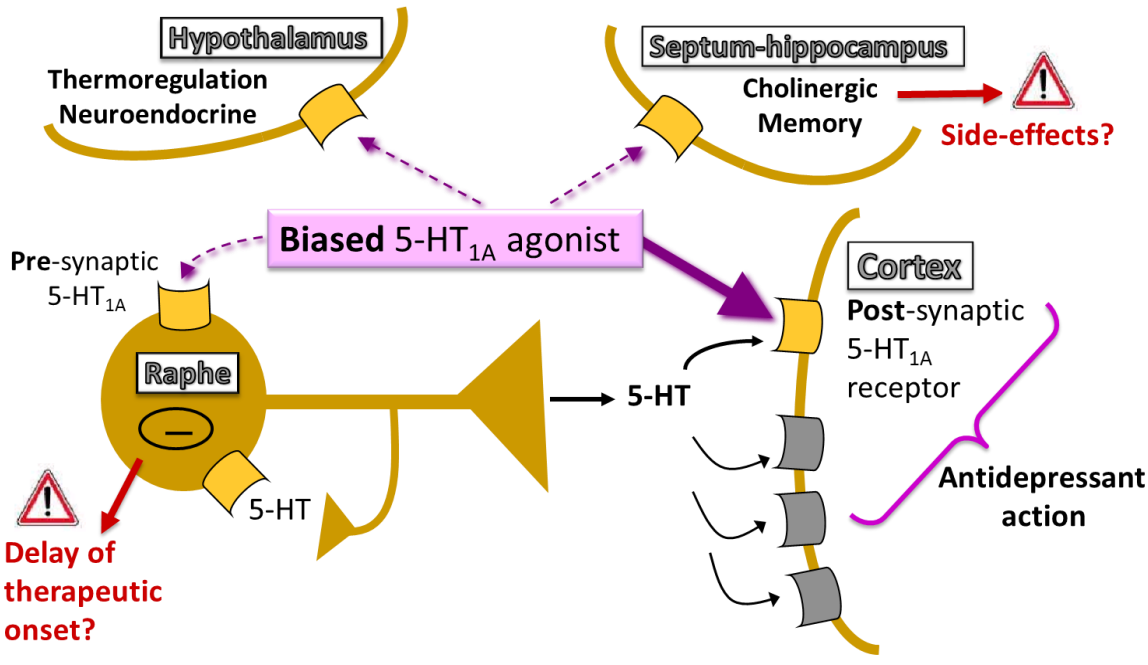
The biased agonism of F15599 at post-synaptic cortical 5-HT_{1A} receptors translates to a superior behavioral profile in models of mood and cognition. Thus, F15599 potently and completely reversed immobility in the rat forced swim test (FST), a classical model of antidepressant-like activity. It is worth underlining the fact that current antidepressants only exhibit partial activity in this test and require repeated administration to induce marked reduction in immobility behaviour. In contrast, F15599 (and F13714) achieved maximal efficacy (i.e. normalization of behaviour) upon acute treatment, consistent with a rapid and extensive antidepressant-like profile of action.

In a separate series of experiements, F15599 inhibited shock-induced ultrasonic vocalization (USV) in rat, a measure of anti-stress / anxiolytic activity [**97**] [(149)]. Notably, the potency of F15599 in both these tests (FST and USV) is as great as that of its congener, F13714, despite the fact that the latter has over 30-fold higher affinity in *in vitro* binding experiments (0.01 nM for F13714 *versus* 3.4 nM for F15599) [**88**]. The marked *in vivo* potency of F15599 suggests that preferential activation of cortical 5-HT_{1A} receptors accentuated beneficial effects on mood parameters.

On the basis of clinical data showing that adjunctive treatment with 5-HT_{1A} partial agonists improves the cognitive state of schizophrenics [143], F15599 was tested in rodent models of cognitive impairment induced by the non-competitive N-methyl-D-aspartic acid (NMDA) receptor antagonist, phencyclidine (PCP). Indeed, NMDA receptor hypo-function, particularly in cortical regions, is considered to underlie aspects of negative symptomatology and cognitive deficits in schizophrenia [150-152]. F15599 significantly attenuated PCP-induced deficits of cognitive flexibility in an operant conditioning reversal learning test in rat [**95**]. In comparison, F13714 failed to reverse the PCP-induced deficit and, in fact, tended to accentuate it [**95**]. This observation is likely related to F13714's preferential pre-synaptic 5-HT_{1A} agonism, potently inhibiting serotonin release [**67**]. Indeed, reversal learning is known to require functional serotonergic transmission in frontal cortex [153,154] as well as functional D_2 receptors [155] suggesting that F15599 is able to re-establish normal functioning in this brain region by its preferential activity at cortical 5-HT_{1A} receptors at doses that elicit dopamine release therein without suppressing serotonergic neurotransmission [**94**]. In another test of PCP-induced cognitive deficits, F15599 improved performance of rats in a hole-board test, significantly improving working and reference memory scores [**95**], possibly by opposing the release of glutamate elicited in frontal cortex by NMDA receptor blockade [156,157]. In contrast, F13714 disrupted performance when tested alone and tended to further accentuate PCP-induced deficits [**95**]. Finally, in an extensive study of the role of 5-HT_{1A} agonism on PCP-disrupted novel object recognition in rat, F15599 markedly improved the discrimination index, as did another efficacious agonist, tandospirone, whereas the partial agonist, buspirone, did not [158].

In animal tests related to side-effects, F15599 exhibited a superior profile compared to

Figure 3.3: Concept of a post-synaptic-preferential cortical 5-HT_{1A} receptors biased agonist.
Such an agonist would be expected to exhibit antidepressant properties and be beneficial against cognitive deficits with only modest impact on thermoregulation or cholinergic function. Notably, avoiding activation of 5-HT_{1A} autoreceptors should reduce the delay of therapeutic onset of antidepressant efficacy.



F13714. Thus, F15599 exhibited little propensity to elicit forepaw treading or flat body posture [**97**], which are elements of serotonin behavioral syndrome commonly observed with 5-HT_{1A} receptor agonists [159,160]. F15599 only elicited these responses at doses that were 30- to 60-fold higher than those that suppress immobility in the forced swim test [**97**]. Further, at "antidepressant" doses, F15599 did not impair working memory in the delayed non-matching to position (DNMTP) test, did not interfere with attentional performance in the 5-choice serial reaction time test (5CSRTT) and did not disrupt pre-pulse inhibition of startle response [**95**]. In contrast, F13714 potently elicited these side-effects at doses similar to those active in the forced swim test. The superior profile of F15599 could be related to preferential occupancy of subpopulations of 5-HT_{1A} receptors. Indeed, using [^{3}H]WAY100635 as a radiotracer, F15599 occupied mouse cortical and hippocampal 5-HT_{1A} receptors *in vivo* nearly as potently as F13714 [**97**], despite the fact that the latter has greater affinity *in vitro* (Table 3) [**88**]. Further, in a positron emission tomography (PET) imaging study, [^{18}F]F15599 preferentially labeled rat cortical, rather than hippocampal, 5-HT_{1A} receptors, even though the latter brain region expresses higher levels of receptors [**93**]. In cat, [^{18}F]F15599 preferentially labeled cingulate cortex 5-HT_{1A} receptors, and labeling was not observed in hippocampus. This unique regional distribution of labeling differs sharply from that observed with antagonist radioligands such as [^{18}F]MPPF [161] or [O-methyl-^{11}C]WAY-100635 [162] which label all 5-HT_{1A} receptors in different brain regions. These observations support the notion that [^{18}F]F15599 preferentially interacts with specific subpopulations of 5-HT_{1A} receptors in brain, possibly depending on their coupling to specific G-protein subtypes. Two additional comments should be made: firstly, [^{18}F]F15599 also labeled mid-brain raphe 5-HT_{1A} receptors in cat [**93**] as did [^{18}F]F13714 [**103**]. Thus, in this species, the distinct pre- / post-synaptic activation of 5-HT1A receptors observed in rat was not reflected in the distribution of receptor labelling. It will therefore be interesting to compare these radiotracers in higher species: ongoing autoradiography experiments in post-mortem human brain (Uni. of Lyon, France) and PET experiments in non-human primate (New Haven, MA) will provide clarification of this issue. Secondly, F15599 may distinguish different populations of 5-HT_{1A} receptors within cortical tissue – an assertion based on microinjection studies in the frontal cortex where agonists were locally administered: whereas F13714 and 8-OH-DPAT showed conventional monophasic dose-response relationships for inhibition of immobility in the forced swim test, F15599 yielded a biphasic dose-response curve [163]. F15599 may, possibly, distinguish cortical 5-HT_{1A} receptors expressed on pyramidal cells from those expressed on GABAergic interneurons [102,164].

3.4 Conclusions and perspectives.

5-HT_{1A} receptors are attractive targets for pharmacotherapy, in view of their localization as autoreceptors controlling serotonergic transmission and as post-synaptic receptors in brain regions controlling mood, movement, cognition and pain. However, current drugs acting as 5-HT_{1A} agonists may be sub-optimal in their profile of activity, because they indiscriminately activate 5-HT_{1A} receptors in those brain regions that mediate side-effects as well as in those that are responsible for therapeutic actions. Some attempts to circumvent this difficulty have been made with recent drugs. For example, SB-649915-B is a serotonin reuptake inhibitor (SRI) and 5-HT_{1A} antagonist [165,166] aiming

to avoid feed-back inhibition of serotonin release by blocking the activation of 5-HT_{1A} autoreceptors [104,167]. However, whilst antidepressant efficacy may be enhanced by 5-HT_{1A} autoreceptor antagonism, it ils likely to be hindered by antagonism of post-synaptic 5-HT_{1A} receptors [38,168]. Vilazodone, LuAA21004 and VN2222 exemplify another approach: they act as SRIs whilst retaining partial agonist activity at 5-HT_{1A} receptors [142,169,170]. Indeed, pindolol, which has been extensively tested in depressed patients in combination with SRIs, is a partial agonist at 5-HT_{1A} receptors [**41**][104]. However, a partial agonist strategy risks only partially achieving its objectives: is the agonism sufficiently modest to ensure blockade of 5-HT_{1A} autoreceptors? Is the agonism of sufficient magnitude to avoid blocking post-synaptic 5-HT_{1A} receptors?

The studies conducted in my laboratory demonstrated that subpopulations of cortical 5-HT_{1A} receptors may be pharmacologically targeted by biased (or "functionally selective") agonists, such as F15599, that possess specific intracellular "signaling fingerprints", possibly via preferential Gαi G-protein subtype activation and/or potent ERK1/2 activation. These observations were the first to demonstrate the feasibility of preferential targeting of cortical 5-HT_{1A} receptors and open the path to (hopefully) accelerated onset of therapeutic efficacy in depression and attenuated cognitive impairments in schizophrenia. In addition, preferential targeting of cortical 5-HT_{1A} receptors may increase the therapeutic margin with respect to side-effects that arise from the activation of other 5-HT_{1A} receptors sub-populations. Taken together, the studies I directed provided evidence that the activity of 5-HT_{1A} receptor agonists at distinct pre- and post-synaptic sub-populations of 5-HT_{1A} receptors should be considered when selecting drugs that influence serotonergic neurotransmission.

4. Serotonin 5-HT_{1A} receptor activation and psychotic disorders

4.1 Key points

- The influence of 5-HT_{1A} receptor activation in schizophrenia and in the profile of action of antipsychotic drugs constituted a major part of the author's research: over a third of the present thesis' publications are related to this field.

- The prototypical atypical antipsychotic, clozapine, was discovered to possess partial agonist activity at 5-HT_{1A} receptors [**4**]. This activity likely underlies some aspects of its "atypical" therapeutic profile and was further characterized in subsequent studies [**19,39,52,56,58,63,80**].

- Novel antipsychotic drug candidates, notably F15063, were discovered and characterized. These drugs, which combine accentuated 5-HT_{1A} receptor agonist properties with D_2 antagonism or inverse agonism [**70**], exhibited superior profiles of activity in experimental models of positive symptoms, negative symptoms and cognitive deficits of schizophrenia [**14**,**52,71,72,73,74,80,83,86,92**].

- Extensive comparative studies were carried out on reference antipsychotics possessing dual 5-HT_{1A}/D_2 properties. These studies showed that, in general, drugs targeting these receptors exhibited a broader profile of therapeutic-like activity in models of schizophrenia than older atypical or first generation antipsychotics [**58,60,62,66,76,77,81**]

- Antipsychotic drugs possessing dual 5-HT_{1A} agonism and D_2 antagonism / partial agonism were shown to display strongly reduced EPS liability [**59,65,72,76,90**] and more benign neuroendocrine impact [**64,83**].

4.2 Background to the author's work

Since the serendipitous discovery of the first antipsychotic agent, chlorpromazine [(24)], the clinical treatment of schizophrenia has been based on compounds which are capable of blocking D_2 receptors, a property which currently remains fundamental in the clinical management of psychosis [(171-173)] although other mechanisms, including activation of metabotropic glutamate (mGluR2/3) receptors [(174,175)], activation of muscarinic M1/M4 receptors [(176,177)] and indirect activation of NMDA receptors by inhibition of glycine transporters [(178-180)] may offer alternative avenues to achieve antipsychotic efficacy independently of direct dopaminergic antagonism.

'Typical' antipsychotics such as chlorpromazine and haloperidol are effective in controlling the positive symptoms of schizophrenia. However they are essentially ineffective against negative

symptoms and exhibit a marked propensity to induce 'Parkinson's-like' neuromuscular disturbances (akathisia, dystonia and tardive dyskinesia) known collectively as the extrapyramidal syndrome (EPS). 'Atypical' antipsychotic agents, such as clozapine, risperidone, olanzapine, ziprasidone, sertindole and aripiprazole, interact at multiple serotonergic and adrenergic receptors in addition to D_2-like receptors, and were originally known as 'atypical' due to their lowered EPS liability and improved capacity to alleviate (but not abolish) negative and cognitive symptoms [181-183]. Their fundamental pharmacological activity is posited to emerge from the combined interaction at D_2 and serotonergic receptors. Indeed, antagonism of 5-HT_{2A} receptors is a feature of clozapine, risperidone, olanzapine, sertindole and ziprasidone [184-187] and the 5-$HT_{2A/2C}$ antagonist, ritanserin, potentiated the clinical efficacy of risperidone on negative symptoms [188]. Nevertheless, a substantial proportion of schizophrenic patients fail to respond adequately to existing medications and continue to exhibit impairments in social functioning and cognitive performance, as well as persistent and/or recurrent psychotic episodes. In addition, both conventional and atypical antipsychotic agents may be associated with cardiac arrhythmias [189-191], and some antipsychotics, such as clozapine and olanzapine, induce marked weight gain and metabolic dysfunction linked to diabetes [192,193]. These considerations highlight the need for antipsychotic agents that display both wider therapeutic activities along with an improved security profile.

Serotonin 5-HT_{1A} receptor activation: a mechanism for improved antipsychotic action

In view of the limitations associated with many antipsychotic therapies, attention has been increasingly given to the concept of a 'pharmacological cocktail' approach [194-196]. This strategy targets pharmacological properties necessary for the desired therapeutic benefits (actions against positive, negative and cognitive symptoms) whilst attempting to avoid interaction(s) with sites responsible for side effects, such as antagonism of serotonin 5-HT_{2C} or histaminergic H1 receptors, associated with weight gain, or blockade of muscarinic receptors, associated with autonomic side effects and memory disturbances [197]. A promising means of achieving this is to combine, in the same molecule, agonist properties at 5-HT_{1A} receptors with antagonist (or partial agonist) properties at D_2 receptors [**75,96,101**] [47,198,199]. Indeed, multiple observations have attracted attention to 5-HT_{1A} receptors as a key target in the treatment of schizophrenia.

First, 5-HT_{1A} receptor activation plays an important role in stimulating dopamine (DA) release in frontal cortex [**6,60**] [200-203] and recent receptor knock-out (KO) and chemical knock-down experiments in mice showed that 5-HT_{1A}, but not 5-HT_{2A}, receptor activation is required for cortical DA release by antipsychotics [101]. In fact, antagonism of 5-HT_{2A} receptors in frontal cortex indirectly elicits activation of 5-HT_{1A} receptors [204] and risperidone and olanzapine, which do not directly interact with 5-HT_{1A} receptors, increase frontal cortex DA release partly via activation of 5-HT_{1A} sites [204]. Furthermore, in microdialysis experiments, synergism between 5-HT_{2A} blockade and 5-HT_{1A} receptor activation has been demonstrated: cortical DA release is increased when a 5-HT_{2A} receptor antagonist (which is inactive by itself) is co-administered with a threshold dose of a 5-HT_{1A} receptor agonist [204]. Taken together, these results suggested that activation of 5-HT_{1A} receptors is a crucial

element in the neurochemical mechanism of action of atypical antipsychotics and may alleviate a deficiency in dopaminergic neurotransmission in frontocortical regions of schizophrenic patients. Amelioration of this 'hypofrontality' had been associated with improvement in negative and cognitive symptoms of schizophrenia [205,206].

Second, numerous laboratories, including mine, have demonstrated anti-cataleptic properties of 5-HT_{1A} agonists in rodents, indicating that activation of 5-HT_{1A} receptors should reduce EPS induced by D_2 receptor blockade [**59,65**] [207-209]. The extent to which 5-HT_{1A} agonists are able to reverse neuroleptic-induced catalepsy is dependent on the level of efficacy of the ligand: relatively high efficacy activation, for example by the prototypical agonist, 8-OH-DPAT, is necessary to completely abolish neuroleptic-induced catalepsy [**59,65**] [210-212]. Interestingly, interactions between 5-HT_{1A} and 5-HT_{2A} receptors may underlie predisposition to catalepsy [213], further illustrating the close functional relationship between these two targets.

Third, 5-HT_{1A} receptor density had been shown to be modified in schizophrenics, with most studies reporting up-regulation, as determined by post-mortem investigations of receptor expression in frontal cortex and other brain regions [214-217]. 5-HT_{1A} receptor expression may increase as a compensatory mechanism subsequent to hypoactivation of this site, a deficiency that could be remedied by administration of 5-HT_{1A} receptor agonists. Further, 5-HT_{1A} receptor activation hyperpolarizes cortical pyramidal neurons [50,218,219] and may thus attenuate dysfunctional glutamatergic function in schizophrenia by opposing the increase in glutamate release induced by NMDA receptor blockade [220,221]. Such regulation of NMDA receptor function may thus help to re-establish normal cortical activity.

Fourth, the atypical antipsychotic, clozapine, which displays improved capacity to treat negative symptoms with minimal liability for EPS, exhibits partial agonist properties at 5-HT_{1A} receptors in various *in vitro* models of receptor transduction, as first demonstrated in my laboratory in 1996 [**4**] and subsequently confirmed in a multiplicity of studies [**44,63,56,80**]. Both the elevation of DA release in frontal cortex and the anti-cataleptic properties of clozapine in rats are partially mediated by 5-HT_{1A} receptors [201,222] and the anxiolytic-like activity of clozapine in an ultrasonic vocalization test in rat, is blocked by WAY100635, a selective 5-HT_{1A} receptor antagonist [223], further supporting a role of 5-HT_{1A} receptors in the mechanism of action of clozapine.

Fifth, exploratory clinical trials have shown that buspirone and tandospirone, which act as partial agonists at 5-HT_{1A} receptors [32,224], substantially ameliorate cognitive and negative symptom scores and reduce the incidence of extrapyramidal symptoms in haloperidol-treated schizophrenic patients [225-229]. The fact that beneficial effects are seen when a 5-HT_{1A} partial agonist is administered to schizophrenic patients provides compelling clinical support for the rationale of combining D_2 antagonism with 5-HT_{1A} receptor agonism in novel antipsychotics.

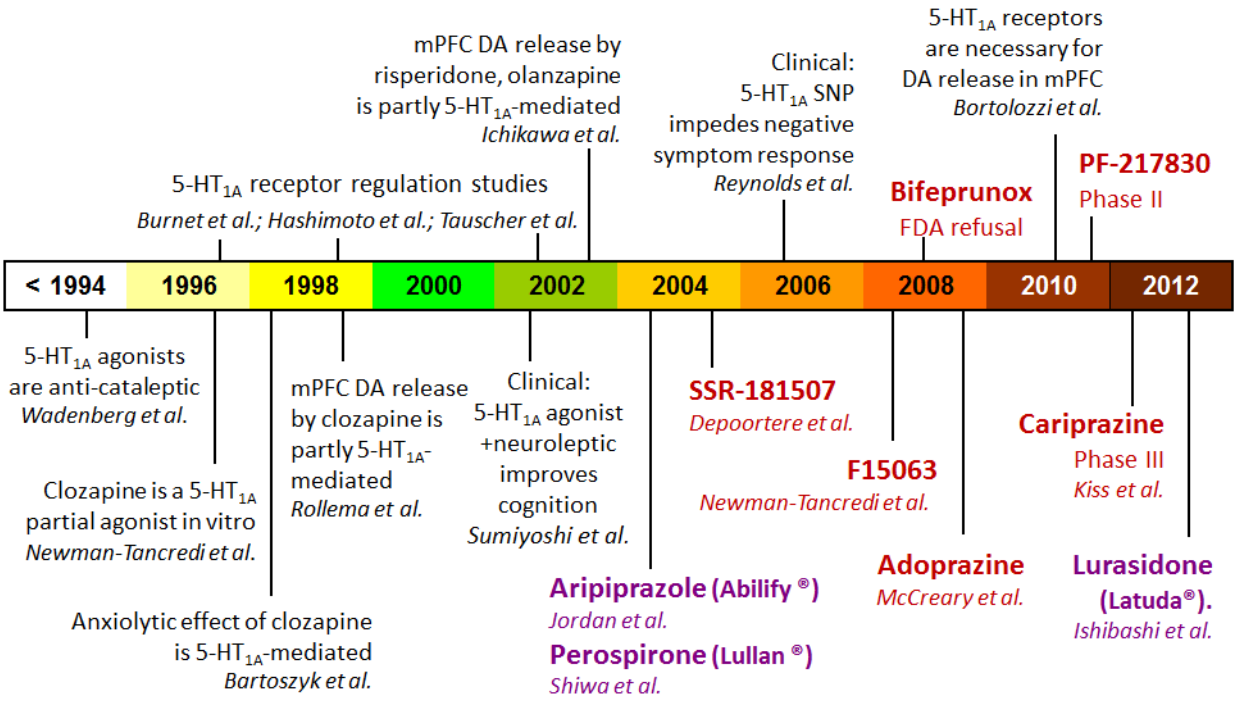
Finally, recent clinical studies have shown that schizophrenic patients expressing the C(-1019)G polymorphism of the gene coding for the 5-HT_{1A} receptor exhibit negative symptoms and cognitive deficit that are resistant to treatment with antipsychotics [115-118]. As discussed in Chapter 2, the

C(-1019)G polymorphism is located in the promoter region of the 5-HT_{1A} receptor gene and elicits hypoactivity of cortical 5-HT_{1A} receptors and is associated with mood deficits [119,120]. These findings provide powerful support for the contention that regulating 5-HT_{1A} receptor activity is an important element in successful management of schizophrenia.

4.3 Contribution of author's work

In view of the extensive evidence supporting an important role for 5-HT_{1A} receptors in the control of the negative symptoms and cognitive deficits of schizophrenia, it is not suprising that numerous pharmaceutical companies have proposed drug candidates that combine 5-HTA agonism with D_2 antagonism or partial agonism (Fig. 4.1). However, despite extensive interest in compounds of this type, few direct comparative studies have been reported, with publications often describing results on only one or two compounds [for example, see publications on SSR181507 [230], cariprazine [231] or adoprazine [232]]. This raises a considerable difficulty in evaluating the relative merits and limitations of these drugs, since experimental conditions can vary considerably between laboratories. One of the objectives of my work was to carry out systematic comparative studies under uniform conditions in order to interpret the role of different receptor targets in the effects of novel antipsychotic candidates. A key finding of these studies is that relatively modest changes in the balance of D_2 antagonism versus 5-HT_{1A} receptor activation translate to markedly divergent profiles of activity in neurochemical and behavioural tests of antipsychotic-like activity. My work led to the notion that an appropriate balance of D_2 and 5-HT_{1A} receptor properties is necessary in order to achieve optimal

Figure 4.1: Timeline of the rise of interest in 5-HT_{1A} receptors as targets for antipsychotics.
Early interest in 5-HT_{1A} receptors centered on their role in attenuating EPS but subsequent studies extended this to negative symptoms and cognitive deficits. The author's work drew attention to the 5-HT_{1A} agonist properties of clozapine and other atypical antipsychotics. Here, landmark research studies are shown in black; exploratory drugs are shown in red; commercialized antipsychotics are shown in magenta.



antipsychotic activity. The rest of this Chapter is devoted to describing the comparative studies that I directed to elucidate this issue, which is relevant to the ongoing efforts to identify and develop antipsychotics that activate 5-HT_{1A} receptors. Indeed, in addition to clozapine, ziprasidone and nemonapride, the more recent antipsychotics, aripiprazole and perospirone, also exhibit partial agonist properties at 5-HT_{1A} receptors in a variety of *in vitro* assays [**15,56,63,64,80**] [(233-236)]. Other compounds have undergone extensive clinical trials, including bifeprunox [**75**] [(237)], lurasidone [(48)] and cariprazine [(231,238)]. Further, early-stage 5-HT_{1A}/D_2 compounds such as adoprazine (SLV313; [(232)], SSR181507 [(230)] and F15063, a development candidate identified in my laboratory [**71**], have been extensively characterized at a pharmacological level and provide useful comparators for assessing the influence of combined targeting of 5-HT_{1A} and D_2 receptors.

Binding affinity of recent antipsychotics at D_2 and 5-HT_{1A} receptors *in vitro*

I carried out a ligand binding and *in vitro* functional activity comparison of a series of commercialized and potential antipsychotics [**56**], showing that they differed widely in their balance of affinity and agonist properties at 5-HT_{1A} receptors. As a follow-up, I carried out a much broader investigation of the binding profiles of 22 antipsychotics at 8 native brain and 18 cloned human receptors [(239)]. As expected, conventional antipsychotics such as haloperidol exhibited robust affinity for D_2-like receptors and little or no affinity for 5-HT_{1A} receptors. Atypical 'multi-receptor' antipsychotics such as clozapine, olanzapine, risperidone and ziprasidone, were notable for their accentuated 5-HT_{2A} and 5-HT_{2C} receptor affinity. These antipsychotics also interacted potently with diverse other receptors, including α_1 and α_2 adrenergic, muscarinic M_1 and histaminergic H1 receptors, some of which are associated with side-effects such as sedation, weight gain and metabolic disturbances. These data are consistent with previous comparative studies of antipsychotic affinity profiles [(240-242)].

In contrast, some recent compounds exhibited comparatively simple receptor profiles with decreased affinity at 5-HT_{2A} and 5-HT_{2C} receptors, and low affinity at adrenergic, M1 and H1R receptors (Fig. 4.2). This clearly marks a departure from the principle that had guided the selection of atypical antipsychotics, *i.e.,* more potent interaction at 5-HT_{2A} than at D_2 receptors and broad antagonism of monoamine receptors, as described above. Instead, recent antipsychotics exhibit affinity at 5-HT_{1A} receptors close to their affinity at D_2 receptors. Thus the "selectively non-selective" profile typified by bifeprunox, adoprazine and F15063 [**56,75,101**] [(194)], as well as that reported for cariprazine and lurasidone [(48,231)], should avoid a number of undesirable effects associated with current antipsychotics, whilst retaining desired pharmacological actions [(242-244)]. Nonetheless, differences are apparent among these compounds. For example, aripiprazole and bifeprunox have about 10-fold higher affinity at D_2 receptors than at 5-HT_{1A} [**56,75**], whereas adoprazine and SSR-181507 possess 5 to 10-fold higher affinity at 5-HT_{1A} than at D_2 receptors [**56,101**]. Thus, the balance of D_2 and 5-HT_{1A} receptor properties for these compounds varies substantially and the question of an "optimal balance" remains open, pending the availability of further clinical data drugs (Fig. 4.4).

Agonist efficacy of recent antipsychotics at D_2 and 5-HT_{1A} receptors *in vitro*

The level of agonist efficacy at 5-HT_{1A} and D_2 receptors is a key element influencing activity of the compounds in models of antipsychotic action. Some recent compounds such as aripiprazole, bifeprunox and SSR181507 exhibit different levels of partial agonism at D_2 receptors [245], as do other recently-reported compounds such as cariprazine [231] and PF-217830 [**70**] [246]. The rationale for partial agonism at D_2 receptors, although attractive (EPS reduction, "stabilization" of DA transmission, avoidance of hyper-prolactinaemia) is complex. For example, bifeprunox, which exhibits marked partial agonist activity at D_2 receptors exceeding that of aripiprazole [**70**,**75**] [247,248] did not achieve satisfactory antipsychotic efficacy in Phase III clinical trials and failed to win FDA regulatory approval. Thus it appears that D_2 receptor partial agonism should, at most, remain very modest and not exceed that of aripiprazole, which has demonstrated antipsychotic efficacy [249,250].

In our studies, a number of recent antipsychotics were compared in several tests of signal transduction at 5-HT_{1A} receptors, including G-protein activation, adenylyl cyclase inhibition, extracellular regulated kinase (ERK1/2) phosphorylation and receptor internalization [**56,63,64,80**]. In general, the drugs behaved as partial agonists in all of these tests, but distinct responses were observed in some cases. For example, in G-protein activation ([^{35}S]GTPγS binding) experiments, all the compounds exhibited agonist efficacy of about 40 to 60%, similar to that of buspirone [**56,64**]. Similarly, in the receptor internalization test, all the compounds exhibited low efficacy [**80**]. In contrast, in tests of ERK1/2 phosphorylation, clozapine exhibited modest efficacy (<30%) which was about half that of either other antipsychotics or buspirone [**63**]. F15063 was more active than the other compounds tested, with efficacy higher than that of buspirone and approaching that of 8-OH-DPAT [**71**]. The results from the adenylyl cyclase test were markedly distinct: ziprasidone,

Figure 4.2: Binding profiles of established and novel antipsychotics.
Whereas haloperidol exhibits potent and relatively selective antagonism at D_2 receptors, the atypical antipsychotics such as clozapine, olanzapine and risperidone, exhibit multi-receptor profiles. More recent drugs, such as aripiprazole and cariprazine, show preferential interactions at D_2 and 5-HT_{1A} receptors. Adapted from [**101**].

Note: for copyright reasons, this Figure is omitted from the public access version of the thesis.

adoprazine and F15063 showed high efficacy whereas bifeprunox was less active [**56**]. Taken together, these data show that recent antipsychotics exhibit various levels of partial agonism at 5-HT_{1A} receptors and this affects their balance of pharmacological properties in relation to agonism/antagonism at D_2 receptors. These data also indicate that agonist efficacy can be highly dependent on the *in vitro* efficacy system considered. Indeed, some agonists preferentially activate specific signaling pathways, a phenomenon known as "functional selectivity" or "biased agonism" [(127,251)]. Thus, aripiprazole has been reported to exhibit functional selectivity at dopamine D_2 receptors, at which it exhibited distinctive signaling properties for adenylyl cyclase inhibition, ERK1/2 phosphorylation and receptor internalization [(252,253)]. In addition, aripiprazole may also act as a biased agonist at 5-HT_{1A} receptors, *i.e.*, it preferentially elicited DA release in frontal cortex (via locally-expressed 5-HT_{1A} receptors) at low doses whereas higher doses are necessary to inhibit serotonin release (via raphe-localized 5-HT_{1A} receptors) [(254)]. These differences likely arise from distinct signal transduction properties of 5-HT_{1A} receptors in different brain regions [**99**] [(110,112)]. Thus, some differences between novel antipsychotics may arise from their potential "functional selectivity" at 5-HT_{1A} receptor subpopulations (e.g. in frontal cortex), that mediate some of the therapeutic properties of atypical antipsychotics. Preferential pharmacological targeting of post-synaptic cortical 5-HT_{1A} receptors has recently been demonstrated in my laboratory using a selective 5-HT_{1A} receptor "biased agonist", F15599, as described in Chapter 3 [**88,94,99**].

Figure 4.3: Agonist efficacy of antipsychotics at 5-HT_{1A} receptors.
Antipsychotics were tested in a variety of *in vitro* signal transduction models and in different cellular environments. Like clozapine, recent antipsychotics act as partial agonists at this receptor.
Adapted from [**56,63,71,80**].

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Activity in rodent models of positive symptoms of schizophrenia

In view of the complexity and multifactorial nature of schizophrenia, I believed strongly that no single experimental procedure in a non-human species can be considered to model the pathology. At best, different tests may model individual symptoms of the illness and an assessment of the therapeutic usefulness of putative antipsychotics may be assessed by profiling their activity in a palette of diverse types of experiments (for review see [(255)]. In the case of the positive symptoms of

schizophrenia, procedures include pharmacological reversal of stereotyped behaviors or hyper-locomotion elicited by the DA releasers, methylphenidate and amphetamine, DA agonists such as apomorphine, or non-competitive NMDA receptor antagonists such as phencyclidine, ketamine or MK-801. Inhibition of conditioned avoidance behavior and reversal of apomorphine-induced pre-pulse inhibition (PPI) deficits are also widely used. All of these procedures are sensitive to D_2 receptor antagonists and are therefore suitable for comparing drugs possessing combined D_2 and 5-HT_{1A} properties. In an extensive comparison of the activity of twelve established and novel putative antipsychotics [**65,66,72,76,77**], the compounds were active in most of the above tests, but some important differences emerged. For example, haloperidol was potently active in all of the tests over similar dose ranges. In contrast, clozapine and other atypical antipsychotics exhibited a broader variability of active doses among tests, probably reflecting their lower affinity at D_2 receptors. In particular, the total reversal ("normalization") of stereotyped behaviors induced by methylphenidate required high doses of clozapine and was not achieved with ziprasidone [**76**]. In the case of antipsychotics possessing 5-HT_{1A} and D_2 properties, aripiprazole and the other D_2 partial agonists displayed somewhat variable active dose ranges. Indeed, aripiprazole, like another D_2 receptor partial agonist, SSR181507, was active in most tests but failed to normalize methylphenidate-induced behaviors [**65**,**76**]. Thus, this test may be particularly stringent in identifying high-potency D_2 receptor antagonists, as well as differentiate "true" antagonists from partial agonists. These observations underlined the importance of potent D_2 receptor antagonism to achieve "incisive" antipsychotic-like properties. Consistent with this interpretation, the potency of the antipsychotics to inhibit apomorphine-induced climbing in mouse correlated closely with their *in vitro* affinity at D_2 receptors and with central occupancy of D_2 receptors [**68**]. In a further test of antipsychotic-like activity, haloperidol and risperidone reversed PPI deficits induced by apomorphine in rat [**66**], a model of gating deficits observed in schizophrenics [256] and consistent with previous findings [257,258]. The activity of antipsychotics in this test is related to D_2 receptor antagonism [259] but may be outweighed by excessive 5-HT_{1A} receptor agonist properties. Consequently, 5-HT_{1A} / D_2 compounds exhibited a variety of profiles, depending on their balance of properties at these sites. Aripiprazole, which acts as a partial agonist at both D_2 and 5-HT_{1A} receptors was poorly active in this test [**66,77**], although another study reported somewhat discrepant data [260]. Adoprazine and SSR181507 failed to reverse apomorphine-induced PPI deficits, due to excessive 5-HT_{1A} agonist actions, as demonstrated by the fact that activity was restored by pre-administration of a selective 5-HT_{1A} receptor antagonist [**66,77**]. In addition, adoprazine and SSR181507 elicited deficits of basal PPI, at least at some doses. This is attributable to their marked agonism at 5-HT_{1A} receptors: the deficit elicited by SSR181507 was reversed when animals were pre-treated with WAY100635 [**77**]. In a study from another laboratory, the deficit elicited by the efficacious 5-HT_{1A} receptor agonist, 8-OH-DPAT, was attenuated by administration of the partial agonist, buspirone, or D_2 receptor antagonists such as haloperidol and raclopride [261], supporting the concept of a "balance" of activity between 5-HT_{1A} receptor activation and D_2 receptor blockade. Thus, whilst F15063 exhibits substantial 5-HT_{1A} receptor agonism, it nevertheless reversed apomorphine-induced PPI deficits, likely due to its concomitant potent D_2 receptor antagonism [**72**]. It should be noted that, contrary to observations

in rats, 5-HT_{1A} receptor activation does not impair but increases PPI in mice [262], so caution is necessary when extrapolating from these data to other species.

Activity in rodent models of negative symptoms and cognitive deficits of schizophrenia

Negative symptoms of schizophrenia, including blunted affect, impoverished speech and social withdrawal, are poorly treated by many current antipsychotics. I therefore carried out a comparative microdialysis study of the effects of eleven antipsychotics on a surrogate neurochemical marker of efficacy against negative symptoms, i.e., increased DA levels in the frontal cortex of rat [**60**], a brain region that is relevant to the control of mood and cognition. Clozapine and other atypical antipsychotics, but not haloperidol, increased frontal cortex levels of DA [**60,86**]. Neither aripiprazole nor bifeprunox increased DA levels in these studies, although others have reported that aripiprazole elicited a modest increase in DA levels over a narrow dose range [263,264]. These data suggest that aripiprazole and bifeprunox may have a limited capacity to alleviate cognitive deficits or negative symptoms that arise from impaired frontocortical dopaminergic neurotransmission. Indeed, it appears that by activating inhibitory pre-synaptic VTA-located D_2 receptors controlling DA release [265], the D_2 receptor partial agonist properties of these compounds predominate in this neurochemical paradigm. In contrast, the putative 5-HT_{1A}/D_2 antipsychotics, adoprazine, SSR181507 and F15063, robustly increased DA release in frontal cortex of rat [**60,86**]. This effect was mediated by 5-HT_{1A} receptor agonism, as demonstrated by its reversal by the selective 5-HT_{1A} receptor antagonist, WAY100635.

Another neurochemical means of investigating the 5-HT_{1A} agonist properties of antipsychotics is to examine their inhibition of serotonin release, a response controlled by activation of 5-HT_{1A} autoreceptors. In a comparative microdialysis study, serotonin levels in ventral hippocampus were not significantly modified by ziprasidone or aripiprazole, indicating that their 5-HT_{1A} agonist properties are modest [**60**]. In contrast, bifeprunox, adoprazine, SSR181507 and F15063 robustly reduced serotonin levels [**60,86**], consistent with accentuated agonist efficacy at 5-HT_{1A} receptors. However, generalized reductions of serotonin release are unlikely to be beneficial in schizophrenia. Indeed, animals with impaired serotonin transmission can exhibit deficits in tests of cognitive flexibility (see [266] and discussion below) and, in mice that lack pre-synaptic serotonergic markers, atypical antipsychotics fail to oppose PCP-induced PPI deficits [267]. In addition, the antipsychotic-like activity (assessed by inhibition of MK-801-induced hyper-locomotion) of M100907 is lost in serotonin-depleted mice [268], suggesting that serotonergic neurotransmission must be maintained to achieve therapeutic efficacy with atypical antipsychotics. These considerations suggest that it may be preferable to select antipsychotics that do not substantially inhibit serotonin levels.

A behavioural model of the negative symptoms of schizophrenia is the measure of PCP-induced social interaction deficits, which resemble the social withdrawal observed in schizophrenia patients. In a comparative study of nine antipsychotics [**62**], both haloperidol and clozapine failed to show significant activity over a three-day treatment period. However, clozapine has been reported to require three weeks of treatment in order to attenuate the PCP-induced deficit [269]. In contrast, aripiprazole, SSR181507 and F15063 showed substantial activity in this model, attenuating PCP-

induced deficits within 3 days at low doses [**62,73**]. In each case, the attenuating effects of these drugs were blocked by pre-administration of a 5-HT_{1A} antagonist [**62,73**], consistent with other reports on SSR181507 [230] and aripiprazole [270]. It is interesting that adoprazine and the selective 5-HT_{1A} receptor agonist, 8-OH-DPAT, did not alleviate PCP-induced social interaction deficits, indicating that the balance of D_2 antagonist/partial agonist and 5-HT_{1A} receptor agonist properties is critical to activity in this test [**62**]. Nevertheless, in a separate study [230] the selective 5-HT_{1A} agonist, 8-OH-DPAT, was able to reverse PCP-induced social interaction deficits, possibly reflecting methodological differences and underlining the necessity for comparative studies under uniform experimental conditions.

In experiments designed to investigate the effects of antipsychotics on cognitive parameters, several recent 5-HT_{1A}/D_2 antipsychotics were compared in a reversal learning task, a model of cognitive flexibility. In this test, PCP impairs re-acquisition of an operant conditioning task, and the atypical antipsychotic clozapine tended to reduce deficits, although it required 4 to 5 days of treatment before this became apparent [**73**] [271]. In contrast, the attenuation of PCP-induced deficits with SSR181507 and F15063 was detected from the first day [**73**] [271]. Consistent with these observations, 5,7-dihydroxytryptamine lesions of pre-frontal cortex serotonergic transmission in marmosets impaired cognitive flexibility [266], an effect which is neurochemically specific, as demonstrated by the fact that depletion of frontal cortex DA did not elicit a similar effect [154]. These observations suggest that activation of cortical 5-HT_{1A} receptors may be beneficial in maintaining cognitive flexibility and

Figure 4.4: Concept of "balance"when targeting both D_2 and 5-HT_{1A} receptors
Antipsychotics that exhibit potent D_2 receptor antagonism, such as haloperidol (lower left), possess marked side-effects (indicated in red). Combining D_2 antagonism or partial agonism with 5-HT_{1A} agonism, as in the case of clozapine or aripiprazole, brings significant benefits, but a suitable balance needs to be achieved. For example, bifeprunox lacks sufficient antipsychotic efficacy due to excessive D_2 partial agonism. Buspirone is anxiolytic but lacks adequate D_2 antagonism. Figure adapted from [**96**].

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that this underlies the capacity of adoprazine and F15063 to attenuate reversal learning deficits induced by NMDA hypofunction. Indeed, these compounds have only weak affinity for other 5-HT receptors, such as 5-HT_{2A} and 5-HT_6 [**71**,**75**] [232] and exemplify the concept of "selectively non-selective" antipsychotics targeted at 5-HT_{1A} / D_2 receptors.

Taken together, the results obtained in my laboratory provided consistent evidence of the importance of the balance between 5-HT_{1A} agonist and D_2 antagonist properties in order to achieve desired neurochemical profile. Although several recent antipsychotics interact at a variety of targets other than D_2 and 5-HT_{1A}, it seems clear that agonist properties at 5-HT_{1A} receptors provide benefits in tests of negative symptoms and cognitive deficits related to schizophrenia.

Activity in models of extrapyramidal symptom liability and side effects

The classic animal model for assessing EPS liability in rodents is catalepsy, although this is essentially a model of rigidity, rather than akathisia or tardive dyskinesia [255]. Haloperidol, risperidone, olanzapine and ziprasidone all induce catalepsy to various degrees, whereas clozapine, which is a low-potency D_2 antagonist, only induces slight catalepsy at high doses [184,222]. A comparative study showed that, unlike conventional or atypical antipsychotics, D_2/5-HT_{1A} drugs were essentially free of catalepsy in rodents [**65,76**]. This was true of the D_2 receptor partial agonists aripiprazole, bifeprunox and SSR181507, but also of the potent D_2 receptor antagonists, adoprazine and F15063 [**65,76,72**]. Their freedom from catalepsy induction is remarkable, in view of the high affinity of these compounds at D_2 receptors *in vitro* and their marked *in vivo* D_2 receptor antagonism observed in a range of tests including normalization of methylphenidate-induced stereotypies [**76**] (see above). In addition, in the case of typical and atypical antipsychotics, high levels of D_2 receptor occupancy are associated with EPS induction [184,272,273] and it is therefore striking that adoprazine and F15063 are free of catalepsy induction even at doses that completely occupy D_2 receptors [**68**]. The absence of catalepsy with recent antipsychotics is at least partially due to their 5-HT_{1A} agonist properties, both in rat and mouse. Indeed, when 5-HT_{1A} receptors are blocked by prior antagonist treatment, a robust catalepsy is then unmasked [**59**] [274]. These data emphasize the role of 5-HT_{1A} activation in the low EPS liability of new generation antipsychotics [**65**]. In addition to rodent studies, we carried out a comparative study of the acute influence of thirteen antipsychotics on behavior of non-human primates [**90**]. In Cynomolgous monkeys, haloperidol produced potent dystonia-like rigidity and unusual postures at low doses. Clozapine exhibited little rigidity but elicited sedation-like behavior. In contrast, aripiprazole and other 5-HT_{1A} agonists/D_2 receptor partial agonists (bifeprunox and SSR181507) induced very little motor disturbance. Other compounds (adoprazine, F15063), which act as 5-HT_{1A} agonists and D_2 antagonists, exhibited an intermediate profile, once again suggesting that the balance of 5-HT_{1A} and D_2 receptor properties plays an important role.

It should be noted that all of the drugs interfered with cognitive function in operant conditioning tasks, when administered at higher doses. Thus, in rat, the compounds decreased accuracy of responding in a 5-choice serial reaction time task (5CSRTT), a test that requires sustained attention in order to gain food reinforcement [275]. The decrease in accuracy cannot to be due to catalepsy induction and may be due to a slowing in responding and/or sedative effect of the compounds.

Indeed, decreased performance in the 5CSRTT coincided, in most cases, with decreases in spontaneous locomotion, consistent with a general reduction in motor activity [**76**].

Antipsychotics with pronounced D_2 antagonism increase prolactin levels by blocking pituitary D_2 receptors that tonically inhibit prolactin release. I therefore undertook a comparative study of the neuroendocrine effects of antipsychotics in rats [**64**] [(276)]. Clozapine only increased prolactin levels at high doses (40 mg/kg) reflecting its low affinity at D_2 receptors, whereas haloperidol and risperidone did so at low doses, consistent with their potent antagonism of D_2 receptors. Recent antipsychotics that, like haloperidol, act as antagonists at D_2 receptors, including adoprazine and F15063, also increased plasma prolactin levels [**64,72**]. In contrast, antipsychotics possessing partial agonism at D_2 receptors, such as aripiprazole, bifeprunox and SSR181507, elicited only small increases in prolactin levels [**64**] [(276)], likely because they maintain a low level of activation of pituitary D_2 receptors.

A serious side-effect of some current antipsychotics such as clozapine and olanzapine is their detrimental impact on metabolism and body mass, with concomitant induction of diabetic disorders, commonly referred to as "metabolic syndrome" [(192,193)]. I therefore compared the effects of antipsychotics on plasma glucose levels in rats [**83**]. Upon acute administration, clozapine and olanzapine elicited substantial increases in plasma glucose levels, whereas drugs such as aripiprazole, ziprasidone and bifeprunox, which are not associated with metabolic dysfunction in humans, did not. These results are consistent with the reduced interaction by these antipsychotics with receptors associated with metabolic regulation, including 5-HT_{2C} and histamine H1 receptors [(277-279)]. However, differences were also apparent among recent 5-HT_{1A} / D_2 compounds. Thus, SSR181507 and adoprazine elicited increases in plasma glucose whereas F15063 did not [**83**]. It is possible that these differences may be related to the 5-HT_{1A} / D_2 balance of these compounds. Indeed, SSR181507 and adoprazine also elicited increases in corticosterone release, likely due to their substantial 5-HT_{1A} agonism [**83**]. In contrast, F15063 has predominant D_2 antagonist properties and did not elicit corticosterone release [**72**]. These observations emphasize, once again, the importance of considering the balance of 5-HT_{1A} / D_2 properties in the profile of novel antipsychotics.

4.4 Conclusions and perspectives.

Whilst a variety of recent antipsychotic drugs share the general property of interacting at both 5-HT_{1A} and D_2 receptors, the extensive pharmacological comparisons that I directed revealed that the "balance" of D_2 and 5-HT_{1A} properties profoundly influences their pharmacological profile [**96,101**]. Thus, insufficient 5-HT_{1A} agonism *versus* D_2 receptor antagonism would yield an antipsychotic that induces EPS and is likely to be less effective against negative and cognitive symptoms (*cf.* haloperidol). In contrast, insufficient D_2 antagonism versus 5-HT_{1A} agonism (as in the case of buspirone) would yield a compound that lacks robust antipsychotic action and may cause a serotonin syndrome, at least in rodents. Taken together, the observations indicated that antipsychotics exhibiting an appropriate combination of D_2 / 5-HT_{1A} properties may possess improved efficacy against negative symptoms and cognitive deficits of schizophrenia. Further, these drugs may be expected to have low EPS liability and little propensity to induce side-effects such as metabolic dysfunction or weight gain.

5. Serotonin 5-HT_{1B} and 5-HT_{1D} receptors

5.1 Key points

- Many of the drugs that were characterized over the course of the author's research interacted at several serotonin receptors. The signalling of 5-HT_{1B}, 5-HT_{1D}, 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors was therefore investigated as this can substantially influence CNS responses.

- 5-HT_{1B} and 5-HT_{1D} receptors were found to exhibit marked differences in constitutive activity for activation of coupled G-proteins [**25**,**31,34**]. This constitutive activity was blocked by some, but not all, antipsychotics [**37**].

- Serotonin was discovered to exhibit 'protean' agonism at 5-HT_{1B} receptors: under high sodium ion conditions, 5-HT stimulated G-protein activation but under low sodium ion conditions it inhibited basal activation [**53**].

- Using 5-HT_{1D} receptors expressed in CHO cells, I developed a technique to detect and quantify constitutive activity without using inverse agonists. The methodology relied on isotopic dilution of [^{35}S]GTPγS binding with unlabelled GTPγS [**34**].

- Despite prominent constitutive activity *in vitro*, and the availability of selective inverse agonists, tests of 5-HT_{1B} / 5-HT_{1D} receptor activation *in vivo* and *ex vivo* did not detect corresponding constitutive activation [**25**].

5.2 Background to author's work

Classical theories of G-protein-coupled receptor (GPCR) activation assume that functional responses are induced by the action of agonists at otherwise quiescent receptors [(280)]. Accordingly, the induction of an active state of the receptor, capable of stimulating signal transduction pathways, was considered to depend upon the presence of an agonist. Antagonists were thought to exert no effects themselves on the state of receptor activation, and merely interfere with the actions of agonists. However, during the 1970s and 1980s, several observations suggested that certain "antagonists" may act in a fashion opposite to agonists. One early observation was that lesions of dopaminergic pathways and dopaminergic antagonists can induce additive effects on striatal levels of acetylcholine [(281)], suggesting that some antagonists could have an effect of their own, in addition to blocking the action of endogenous dopamine. Nevertheless, it could not be formally excluded that antagonists might be blocking the actions of residual pools of dopamine. Another study reported that agonists had higher affinity for [^{3}H]dopamine-labelled dopamine receptors whereas antagonists had greater affinity for [^{3}H]haloperidol-labelled sites [(282)]. Further, at muscarinic receptors labelled with [^{3}H]quinuclidyl benzylate, antagonists had higher affinity for receptors uncoupled from G-proteins [(283)]. This led to the proposal by Costa and Herz that certain antagonists act as "inverse agonists" at δ-opioid receptors endogenously expressed by NG108-15 cells [(284)]. Indeed, whereas the agonist,

DADLE, increased GTPase activity at these δ-opioid receptors, the "antagonist," ICI174864, inhibited basal activity. Crucially, both actions were blocked by a "neutral" antagonist (cf. a simplified model of receptor coupling in Fig. 2.2). Although agonists and antagonists are classically considered to down- and up-regulate receptors, respectively, the above observations led to the suggestion that "inverse agonist" rather than "antagonist" properties may favor receptor up-regulation *in vitro* and/or *in vivo* [285]. Subsequent studies employing homogeneous populations of recombinant receptors in heterologous expression systems free of endogenous agonist underpinned the concept of "negative intrinsic efficacy" and "inverse agonism" [286,287]. Thus, many types of wild-type GPCRs have been observed to display an agonist-independent constitutive activation of intracellular transduction mechanisms inhibited by inverse agonists. In contrast, genuinely "neutral" antagonists are considered to block the actions of both agonists and inverse agonists, without themselves altering activity. Gradually, a more sophisticated model of G-protein coupling emerged (the Cubic Ternary Complex model) in which multiple states of receptor / G-protein interaction were identified, depending on the constitutive activity of the receptor and its interaction with bound ligands and/or G-protein (see Fig. 5.1). Such a model instructed my thinking about the function of serotonin receptors, which exhibit substantial constitutive activity and for which both agonists and inverse agonists can be identified (see next section).

Figure 5.1: The Cubic Ternary Complex Model of receptor-G-protein coupling.
The model represents ligand (L), receptor (R), and G protein (G) interactions. Association of L and R is represented from top to bottom, R and G interactions from front to back, and the conversion of inactive and active R states from left to right. The dynamics of G-protein activation are represented as red dashed lines. Reproduced from [2].

Note: for copyright reasons, this Figure is omitted from the public access version of the thesis.

5.3 Contribution of author's work

5-HT$_{1B}$ receptors: *in vitro* constitutive activity and R:G ratios

Serotonin 5-HT$_{1B}$ autoreceptors on serotonergic neuronal terminals modulate serotonin release both in the central nervous system [288] [**25**] and in the periphery, including the dura matter. Consequently, an understanding of mechanisms of signal transduction at 5-HT$_{1B}$ receptors is relevant to the treatment of affective and cardiovascular disorders and to the management of migraine [**13**] [289,290]. *In vitro*, 5-HT$_{1B}$ receptors couple to inhibition of adenylyl cyclase [291], a response which is abolished by pre-treatment with Bordetella pertussis toxin (PTX). This toxin ADP-ribosylates Gα subunits of the Gi/o family, indicating coupling of 5-HT$_{1B}$ receptors to this family of G proteins.

In view of the importance of 5-HT$_{1B}$ receptors, I decided to apply a similar approach to these sites as I had used for 5-HT$_{1A}$ receptors (Chapter 2). Thus, a [^{35}S]GTPγS binding study at 5-HT$_{1B}$ receptors expressed in CHO cells demonstrated robust activation of G-proteins by agonists, and also revealed that 5-HT$_{1B}$ receptors exhibit marked constitutive activity for G-protein activation [**31**]. In agreement with previous studies [292,293], several compounds were shown to act as inverse agonist at this receptor, including methiothepin and the selective 5-HT$_{1B}$ ligand, SB224,289 [**31**] as well as some antipsychotics [**37**]. In addition, an investigation was carried out of influence of receptor density on the activation of intracellular G-proteins using two cell membrane preparations (referred to as RGhigh and RGlow) that exhibited either a high or a low ratio of receptor expression to G-protein expression, determined in saturation binding experiments. Importantly, RGhigh and RGlow membranes differed in their functional responses. Firstly, the stimulation attained with 5-HT was markedly higher in RGhigh membranes than in RGlow membranes, probably reflecting a faster rate of G-protein 'cycling' due to the greater availability of 5-HT$_{1B}$ receptors per G-protein [294,295]. Secondly, the relative efficacies of the partial agonists, BMS181,101 and L775,606, were also increased in RGhigh membranes vs. RGlow membranes. The present data differ from those for 5-HT$_{1A}$ receptors expressed in CHO cells [**10**] where the potency of 5-HT, but not its efficacy, was increased by an augmentation of R:G stoichiometry: the reverse was true for 5-HT$_{1B}$ receptors. One factor potentially implicated is that the R:G ratio of RGlow membranes (~0.3) was substantially less than its counterpart in CHO-h5-HT$_{1A}$ cell membranes (1.4) [**10**]. Thus, CHO-h5-HT$_{1B}$ RGlow membranes may not have attained a 'ceiling' whereby, for example, the number of available G-proteins was limiting, as may have been the case in studies of CHO-h5-HT$_{1A}$ RGlow cell membranes [**10**] and cannabinoid CB1 receptors [296]. Thirdly, in RGlow membranes, the inhibitory effects of the inverse agonist, methiothepin, was modest, whereas in RGhigh membranes about 40 % of basal [^{35}S]-GTPγS binding was inhibited by SB224,289 and methiothepin. These data are reminiscent of observations at CHO-h5-HT$_{1A}$ cell membranes showing that an increase in R:G ratio augmented the negative efficacy of the inverse agonist, spiperone [**10**]. The most likely explanation is that inverse agonists stabilise G-protein-coupled (as well as uncoupled) receptors in inactive conformation(s) [**10**] [297]. This reduces the pool of G-proteins available for activation by non-inverse agonist-occupied receptors. The present data therefore provide evidence that receptor:G-protein stoichiometry is an important factor in detecting inverse agonism at 5-HT$_{1B}$ receptor-coupled G-proteins.

5-HT$_{1B}$ receptors: constitutive activity and "protean" agonism at G-protein subtypes

The study described above [**31**] and other studies of the influence of serotonergic ligands on G-protein activation by 5-HT$_{1B}$ receptors [77,292,293] used techniques which measured 'overall' G-protein activation, without distinguishing the precise G-protein subtype(s) involved. However, 5-HT$_{1B}$ receptors can couple to several Gα subtypes, including members of the Gαi/o family and Gα15 subunits [86,298,299]. In fact, reconstitution of 5-HT$_{1B}$ receptors expressed in Sf9 cells with different purified Gαi subunits increased the affinity of agonists, indicating coupling to these G-protein subtypes [86]. Compared with Gαi2 and GαO, the Gαi3 subtype induced the greatest increase in agonist affinity, suggesting preferential coupling of 5-HT$_{1B}$ receptors to this G-protein. In view of these observations, I decided to employ an antibody-capture technique coupled to scintillation proximity assay (SPA) detection [91] [**45,46**] to characterise Gαi3 subunit activation by recombinant human 5-HT$_{1B}$ receptors stably expressed in CHO cells [**31**]. The latter express Gαi3 subunits [92,300,301] but only low levels of Gαo and Gαi1 [92,301]. I had previously applied a similar methodology to the study of 5-HT$_{1A}$ receptor coupling to G-proteins [**46**] (Chapter 2).

Agonist-induced activation of Gαi3 subunits exhibited interesting differences from that of 'total' G-protein activation determined by classical [^{35}S]GTPγS binding filtration assays [**31,34**] [77,293]. Firstly, although BMS181,101 behaved as an efficacious agonist (Emax 85%) in conventional [^{35}S]GTPγS binding experiments, its efficacy for Gαi3 activation was accentuated, consistently exceeding that of 5-HT. Secondly, S18127 behaved as 'neutral' antagonist in [^{35}S]GTPγS binding [**34**], whereas it exhibited modest partial agonist properties for Gαi3 activation. Thirdly, the potencies of the agonists for Gαi3 activation were markedly greater than for 'total' G protein activation [**53**]. Thus, the profile of action of 5-HT$_{1B}$ receptor ligands for Gαi3 subunit activation may differ from that of other G-protein subtypes, and classical G protein activation assays may mask these differences by yielding a composite response of multiple G proteins. In contrast to the actions of agonists, it is interesting to note that the negative efficacies of inverse agonists for inhibition of Gαi3 activation corresponded closely to those reported previously by classical methods. This suggests that a major proportion of the constitutive activity in CHO-h5-HT$_{1B}$ cell membranes may be mediated by Gαi3 subunits.

We investigated the detection of constitutive activity under different conditions of sodium ion availability [**53**]. Indeed, NaCl influences receptor conformation, favouring a change from a G-protein coupled to an uncoupled state, reducing the affinity of agonists [302]. Further, NaCl reduces basal G-protein activation in membranes of cells expressing GPCRs [67,303,304]. However, few studies have examined the influence of NaCl on specific G protein subtypes [305]. In our study, NaCl concentration was inversely related to basal Gαi3 activation and the inverse agonist, methiothepin failed to inhibit [^{35}S]GTPγS binding in the presence 300mM NaCl, when constitutive activity was, it seems, totally suppressed [**53**]. These data are reminiscent of studies at 5-HT$_{1A}$ receptors, where inverse agonist properties of certain ligands could only be observed in the absence of NaCl [98]. However, a striking result concerned the influence of 5-HT on Gαi3 activation. Whereas, under standard conditions (100mM NaCl), 5-HT exhibited robust stimulation of [^{35}S]GTPγS binding to Gαi3 subunits, at low NaCl concentrations (10mM), 5-HT inhibited Gαi3 activation [**53**]. Hence, under the latter conditions,

5-HT behaved as an inverse agonist, reversing basal [^{35}S]GTPγS binding in a manner similar to that of methiothepin (Fig. 5.2). This behaviour is consistent with the concept of a protean agonist [97], by which ligands can exhibit either agonist or inverse agonist properties depending on the 'receptor tone'. When the latter is low (i.e. little constitutive G protein activation), the agonist properties of the ligand (in this case 5-HT) are observed. In contrast, when receptor tone is high (i.e. a high level of constitutive activity) the ligand stabilises the receptor in a conformation which is less able to activate G-proteins than the free, non-ligand-occupied, receptor. Indeed, it is noteworthy that, over a wide range of NaCl concentrations (10 to 100mM), saturating levels of 5-HT induced a similar degree of Gαi3 labelling, either by stimulating or inhibiting [^{35}S]GTPγS binding from basal levels.

Previous reports had described the protean agonist properties of secretin at constitutively active mutants of secretin receptor [306] and of levomedetomidine and the dexefaroxan analogue, RX831003, at α_{2A} receptors [307,308]. However, our study was the first to demonstrate that the endogenous agonist, 5-HT, can exhibit protean agonism at non-mutant 5-HT$_{1B}$ receptors. These data raise the intriguing possibility that agonist signalling at 5-HT$_{1B}$ receptors may vary markedly depending on NaCl concentration. It would be interesting to investigate whether these actions of NaCl are due to its reported allosteric regulation of a conserved aspartate residue in the intracellular end of the putative 2nd transmembrane segment of the receptor. Mutation of this residue in noradrenalin α2 receptors abolished the ability of Na+ to modulate receptor-ligand interactions [302].

Figure. 5.2: Influence of NaCl on 5-HT$_{1B}$ receptor-mediated activation of Gai3 subunits.
Adapted from [**53**].

Note: for copyright reasons, this Figure is omitted from the public access version of the thesis.

5-HT$_{1D}$ receptors: quantification of its prominent constitutive activity *in vitro*

Despite progress in defining the actions of agonists and inverse agonists at different receptor targets *in vitro*, several obstacles hinder the detection and quantification of constitutive activity. Firstly, the presence of constitutive activity has often been inferred when ligands (inverse agonists) inhibit basal activity in the absence of an endogenous ligand. However, if such inverse agonists are not available, the potential presence of constitutive activity cannot be verified. Hence, it is desirable to be able to use a procedure permitting the detection and quantification of constitutive activity independently of the use of inverse agonists. Secondly, the efficacy of agonists is expressed relative to that of a 'full'

agonist (generally the endogenous neurotransmitter). However, in the case of inverse agonists, the existence of an 'endogenous ligand' remains speculative for many GPCRs and the maximal negative efficacy that may be attained is often unclear. Indeed, there is still no consensus as to how the efficacies of inverse agonists, relative to an actual or theoretical 'full inverse agonist', should be defined.

5-HT_{1D} receptors exhibit marked constitutive activity and several inverse agonists have been identified at this site [**37**] [292,309,310]. Further, 5-HT_{1D} receptors are of potential importance as inhibitory receptors on serotonergic cell bodies in the raphe nuclei, suggesting that inverse agonist properties at 5-HT_{1D} receptors may be relevant to the treatment of affective disorders and of migraine [**25**]. In a novel approach, I investigate constitutive G-protein activation directly by analysing homologous inhibition of [^{35}S]GTPγS binding by unlabelled GTPγS. Such binding isotherms detect high affinity (HA) and low affinity (LA) binding components. Although it had been shown that HA binding may be modulated by agonists [311], the actions of inverse agonists on HA binding had not been investigated. The investigations I conducted showed that analysis of HA sites yields information concerning the presence and degree of constitutive activity at CHO-h5 HT_{1D} receptors [**34**].

The agonist, 5-HT, increased the amount of HA [^{35}S]GTPγS binding. Conversely, the inverse agonists, methiothepin and, to a lesser degree, SB224289, decreased the number of HA binding sites. Neither 5-HT nor the inverse agonists markedly influenced LA [^{35}S]GTPγS binding sites [**31,34**], suggesting that the latter are not related to 5-HT_{1D} receptor/G-protein coupling events. Further, LA (but not HA) sites were detected in membranes of control, non-transfected CHO cells, indicating that LA binding does not require the presence of h5-HT_{1D} receptors and are probably related to the spontaneous turnover of GTP/GDP by Gα subunits in CHO cell membranes. These observations indicate that HA, but not LA, [^{35}S]GTPγS binding observed under basal conditions reflects the constitutive activation of G-proteins by h5-HT_{1D} receptors [**34**]. Three components may, therefore, be distinguished in GTPγS versus [^{35}S]GTPγS binding isotherms (Fig. 5.3): (i) LA sites, which may reflect endogenous GTP/GDP exchange of G-proteins; (ii) receptor-dependent G-protein activation observed in the *absence* of receptor ligands, which provides a measure of the constitutive activity of the receptor and which can be inhibited by inverse agonists; and (iii) agonist-dependent activation of G-proteins.

Several issues arise from this model. Firstly, it appears that 5-HT_{1D} receptors display a high degree of constitutive activity with marked [^{35}S]GTPγS binding induced by the receptor itself in the absence of agonist. Correspondingly, the agonist, 5-HT, only increased [^{35}S]GTPγS binding to 129% relative to basal values (=100%) an observation in accordance with the modest stimulation induced by 5-HT that had been observed in other studies of [^{35}S]GTPγS binding at 5-HT_{1D} receptors expressed in CHO cells [77,293]. Indeed, if 5-HT_{1D} receptors predominantly exist in a state which is constitutively active, agonists may only induce a small additional stimulation of G-protein activation [312-314]. In comparison, at receptor subtypes which display a lower degree of constitutive activity, 5-HT induced 219% and 240% increases in [^{35}S]GTPγS binding in the case of 5-HT_{1B} receptors [293] [**31**] and 230% in the case of 5-HT_{1A} receptors [**19**].

Secondly, endogenous constitutive activity may be detected without the need for inverse agonists. Indeed, biphasic inhibition of basal [^{35}S]GTPγS binding by GTPγS is observed for 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors, systems in which inverse agonists have been detected [**10,31**] [(77)]. Another research group subsequently applied the same methodology to the study histamine H3 receptors reporting similar data [(315)]**.** In contrast, at D_2, D_3 and D_4 receptors, inhibition of basal [^{35}S]GTPγS binding by GTPγS only revealed LA sites and, under these conditions, we detected no inverse agonists [**12,20**]. Thus, biphasic inhibition of basal [^{35}S]GTPγS binding by GTPS may indicate the presence of constitutive G-protein activation at a variety of GPCRs and, hence, the possibility of observing inverse agonist actions.

Figure 5.3: Determination of constitutive G-protein activity
The use of isotopic dilution of [^{35}S]GTPγS binding identifies three components (i) LA sites, which may reflect endogenous GTP/GDP exchange of G-proteins; (ii) receptor-dependent G-protein activation observed in the *absence* of receptor ligands, which provides a measure of the constitutive activity of the receptor and which can be inhibited by inverse agonists; and (iii) agonist-dependent activation of G-proteins. Adapted from [**34**].

Note: for copyright reasons, this Figure is omitted from the public access version of the thesis.

Thirdly, quantification of constitutive activity enables the definition of a 'full inverse agonist': i.e. a ligand that totally abolishes constitutive activity (i.e. HA binding sites). Methiothepin and SB224,289 suppressed 77% and 33% of HA sites, respectively, compared with 56% and 22% of 'overall' (i.e. HA+LA) basal [^{35}S]GTPγS binding [**34**]. The ratio between the 'overall' percentage of inhibition and 'corrected' values (with LA sites subtracted) may vary as a function of the degree of constitutive activity, receptor expression levels and G-protein subtype activated [**10,31**]. Such factors may contribute to the wide variations in negative efficacies reported for inhibition of [^{35}S]GTPγS binding by methiothepin at 5-HT$_{1D}$ receptors: 70% [(77)], 28% [(310)] and 56% [**34**]. Expressing negative efficacy as a proportion of observed HA [^{35}S]GTPγS binding may, thus, facilitate comparisons of inverse agonist efficacy between different expression systems. In summary, the methodology that I developed showed that [^{35}S]GTPγS versus GTPγS inhibition isotherms can be employed to characterise constitutive activity at 5-HT$_{1D}$ receptors in the absence of inverse agonist ligands. It would be of interest to apply the present approach to cerebral and other native populations of GPCRs (see perspectives).

5.4 Conclusions and perspectives.

The studies described above indicate that 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors are capable of eliciting substantial constitutive activity in cellular recombinant tests. However, constitutive activation is not

easily detected in various native systems, as we found when we carried out an additional series of experiments (functional [^{35}S]GTPγS autoradiography on rat brain tissue sections; microdialysis studies of 5-HT release in frontal cortex; measures of core temperature [**25**]). In each case, 5-HT$_{1B}$ / 5-HT$_{1D}$ receptor agonists elicited the expected effects (increased [^{35}S]GTPγS binding, decreased 5-HT release and lowered core temperature) but the selective 5-HT$_{1B}$ / 5-HT$_{1D}$ receptor inverse agonist, SB224289, did not exert the opposite effects (i.e. it did not inhibit basal [^{35}S]GTPγS binding, did not increase 5-HT release or body temperature). In fact, SB224289 appeared to possess "neutral" antagonist properties in these tests, similar to another compound, S18127, which appeared "neutral" in *in vitro* measures [**25,37,53**]. In other words, when tested on native tissue systems, the inverse agonists could not be distinguished from neutral antagonists.

Several reasons may explain the absence of inverse agonist actions *in vivo*. Firstly, SB224289 may act as an inverse agonist at recombinant human 5-HT$_{1B}$/5-HT$_{1D}$ receptors but not at native rat and guinea pig receptors. However, in a comparison of recombinant guinea pig and human 5-HT$_{1B}$ receptors [(316)], SB224289 acted as inverse agonists in both cases. Secondly, agonist and inverse agonist efficacy depends on receptor and G-protein expression levels, as discussed above [**10,31**]. These may be too low at native 5-HT$_{1B}$ receptors to permit inverse agonist actions to be detected. Thirdly, the techniques may not have been sufficiently sensitive to detect modest inverse agonist actions *in vivo*. In particular, this may be the case for [^{35}S]GTPγS autoradiography, in which actions of SB224289 upon 5-HT$_{1B}$ coupled G-proteins might be diluted by other pools of G-proteins. Fourthly, for inverse agonism to be detected, the receptor must be present in a constitutively active state and, ideally, endogenous agonist(s) should not be present. However, *in vivo*, 5-HT is spontaneously released both at the dendritic and terminal level suggesting that 5-HT is available to pre- and postsynaptic receptors. Thus, spontaneous release of 5-HT may impede the detection of inverse agonist actions.

In conclusion, although extensive *in vitro* data, partly generated in my laboratory [**25,31,37,53**], supports the existence of 5-HT$_{1B}$ and 5-HT$_{1D}$ receptor constitutive activity, this still lacks *in vivo* correlates. It should be noted that constitutive activation of another serotonin receptor subtype (5-HT$_{2C}$) has been documented [(317)]. It is the author's opinion that the demonstration of *in vivo* constitutive activity at 5-HT$_{1B/1D}$ receptors will require novel technical strategies and/or specific experimental conditions. These may include, for example, the use of transgenic mice overexpressing 5-HT$_{1B}$ or 5-HT$_{1D}$ receptors in order to increase basal activation signals. In addition, it may be useful to test rodents that are 5-HT-depleted, allowing potential inverse agonist effects to be detected in the absence of concurrent agonist activation.

6. Serotonin 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors

6.1 Key points

- Agonists at 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors exhibited functional selectivity: some were more potent and efficacious for activation of Gαq G-proteins, whereas others were more potent for activation of calcium release [**84**].

- 5-HT$_{2B}$ antagonism was shown to be relevant to antipsychotic activity, inhibiting amphetamine-induced nucleus accumbens dopamine release in microdialysis experiments and opposing amphetamine-induced locomotion in rat [**98**].

- G-protein activation by 5-HT$_{2C}$ receptors was characterised using a novel methodology (antibody-capture associated with scintillation proximity assay). 5-HT$_{2C}$ receptors were found to display "promiscuous" coupling to both Gαi3 and Gαq/11 G-proteins [**40,45**].

- The novel antidepressant, agomelatine (*Valdoxan*®) was found to display antagonist properties at 5-HT$_{2C}$ and 5-HT$_{2B}$ receptors [**51**].

- At 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors, a novel rapid methodology was developed to determine *in vitro* agonism, based on depletion of [^{3}H]phosphatidylinositol by phospholipase-C, rather than on synthesis of [^{3}H]inositol triphosphate, which is a more resource-intensive procedure [**26,32,47**].

6.2 Background to the author's work

The serotonin 5-HT2 receptor family includes three receptor subtypes (5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$), which are expressed throughout the mammalian brain [318,319]. During the last three decades, much attention has been devoted to the role of 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors and they are considered as promising targets for improved treatments of neuropsychiatric disorders related to monoamine neuron dysfunctions [318,320,321]. In particular, the marked affinity and antagonist properties of atypical antipsychotics such as clozapine at 5-HT$_{2A}$ sites may permit control of psychotic symptoms in the relative absence of extrapyramidal side-effects [318,322]. Clozapine and other antipsychotics also display high affinity for closely-related 5 HT$_{2C}$ receptors [318,323,324], blockade of which exerts a tonic, inhibitory influence upon ascending dopaminergic pathways: correspondingly, by enhancing dopamine release in frontal cortex, 5-HT$_{2C}$ antagonist properties may counteract the "hypofrontality" which contributes to the deficit symptoms of schizophrenia [325,326]. 5-HT$_{2C}$ receptor antagonists have also been reported to manifest anxiolytic properties [327,328].

In contrast, relatively few studies have focused on the role of central 5-HT$_{2B}$ receptors in the control of DA neuron activity. Some early electrophysiological and microdialysis studies in rats [325] indicated

that 5-HT_{2B} did not influence dopaminergic neuronal firing or dopamine release. On the other hand, a study in mice [329], reported that central 5-HT_{2B} receptors modulate accumbal DA release induced by 3,4-methylenedioxymethamphetamine (MDMA), suggesting that they do, in fact, influence dopaminergic function.

The three 5-HT_2 receptors show high homology in their primary and secondary structure [319,330,331] and are mainly coupled via Gq/11 G-proteins to phospholipase C (PLC) [332-335]. Activation of PLC elevates cytosolic levels of inositol phosphates, and subsequently increases levels of intracellular calcium, two major parameters that have been exploited for characterization of drug efficacy at 5-HT2 receptor subtypes [336]. Furthermore, previous studies have shown that both 5-HT_{2A} and 5-HT_{2C} receptors can couple independently to PLC and phospholipase A2 (PLA2) and that the relative efficacy of the agonists at these receptors can differ depending upon whether phosphatidylinositol hydrolysis or arachidonic acid release is measured [333,337,338]. Biased agonism, or functional selectivity, has also been reported for 5-HT_{2C} receptors coupled to PLC versus ERK 1/2 phosphorylation where the rank order of relative efficacy of DOI and quipazine was reversed [339]. Although the precise nature of G-proteins involved in these transduction pathways remains to be elucidated, 5-HT_{2C} receptors are known to interact with other G-proteins than Gαq/11, notably Gαi3 and Gα13, depending also on the nature of 5-HT_{2C} receptor editing [**45**] [340-342].

6.3 Contribution of author's work

5-HT_{2A} receptors and biased agonism

Although serotonin 5-HT_{2A} receptors are thought to be a primary target of some hallucinogen compounds such as LSD and DOI[343], not all 5-HT_{2A} receptor agonists elicit hallucinations. This discrepancy may be related to the capacity of the agonists to stimulate signalling via different intracellular pathways [128], a hypothesis supported by the fact that functional selectivity was observed for a series of compounds for 5-HT_{2A} and 5-HT_{2C} receptor-mediated PLC and PLA2 activation [337,338]. In my laboratory, a series of hallucinogenic and non-hallucinogenic compounds showed differential preference for the activation of specific cellular signalling responses [**84**]. Indeed, at 5-HT_{2A} receptors, lisuride, bromocriptine, LSD and, to a lesser extent, pergolide preferentially induced activation of Gαq/11 proteins while the other agonists more potently elicited for Ca^{2+} release. Moreover, a similar tendency was also observed at 5-HT_{2B} and 5-HT_{2C}-VSV receptors [**84**]. These observations are unlikely to be accounted for by "strength of signal" dependent on receptor reserve. Indeed, lisuride was more potent than mCPP to induce Gαq/11 protein activation at 5-HT_{2A} receptor while the opposite result was observed for induction of Ca^{2+} signalling. Furthermore, both compounds behaved as partial agonists at both pathways, suggesting that a contribution of receptor reserve is unlikely (Fig. 6.1). In contrast, these results are highly suggestive of the existence of agonist-specific receptor states that preferentially engage distinct cellular pathways. Evidence for ligand-specific preferential activation of PLC, PLA2 and phospholipase D by 5-HT2 receptors have been presented repeatedly *in vitro* [128,337] and more recently *in vivo* for 5-HT_{2A} [132,344]. In NIH-3T3

cells, LSD also induced weak 5-HT_{2C} receptor coupling to Ca^{2+} response whereas efficient activation of PLC pathway was observed [345], in accordance with our results.

A concern in the interpretation of these data was the nature of the different pathways examined, i.e. activation of Gαq/11 proteins and of Ca^{2+} signalling. These pathways are closely linked to each other, such that Ca^{2+} mobilization can be considered as a downstream consequence of Gαq/11 activation and subsequent PLC stimulation. However, the pharmacological differences between these two responses observed in our experiments suggest that that Gαq/11 signalling is not the only element controlling the strength of Ca^{2+} signalling. One possible interpretation is that 5-HT_{2A} receptors couple to different G-proteins in CHO cell lines and that the propensity to activate these different G-proteins varies according to the agonist tested. Indeed, 5-HT_{2C} receptors couple to Gα13 and Gαi3, as well as to Gαq/11 [346,347][**45**], and some 5-HT_{2A} intracellular signalling involves pertussis toxin-sensitive G-proteins [338,348]. Thus, the differential signalling reported herein may be the result of the agonist-specific activation of a combination of distinct G-proteins.

Figure. 6.1: 5-HT_{2A} receptor biased agonists.
h5-HT_{2A} receptors coupled to Gq/11 activation (□) and Ca^{2+} release (■). Reproduced from [**84**].

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This interpretation is consistent with the observation that Gαq protein KO mice displayed a decreased DOI-induced head-twitch response, supporting a key role for this G-protein in hallucinogenic drug effects [349]. We likewise observed that DOI was a potent and efficacious agonist at 5-HT_{2A} receptor-coupled Gαq/11 proteins, a property which was shared also by LSD, pergolide and bromocriptine whereas lisuride was less efficacious. Pergolide and, to a lesser degree, bromocriptine have been reported to induce

hallucinations in Parkinsonian patients [350-352]. However, lisuride, which is generally considered to be non-hallucinogenic in human [343], as well as LSD, bromocriptine and pergolide exhibited a high propensity to stimulate Gαq/11 proteins in comparison with Ca^{2+} mobilization. Thus, our results tentatively suggested that differences in hallucinogenic properties between these compounds may not be simply related to a higher efficacy and potency to stimulate Gαq/11 proteins versus Ca^{2+} mobilization, but that a threshold of efficacy at 5-HT_{2A} receptor may be of importance.

5-HT_{2B} receptors and dopamine release in rat

In 2008, data was published indicating that 5-HT_{2B} receptors modulate the increase in accumbal DA release induced by 3,4-methylenedioxymethamphetamine (MDMA) [329]. This landmark study was the first to report that central 5-HT_{2B} receptors had a role in the control of CNS dopamine release. At that time, I was working on compounds that had antagonist properties at 5-HT2 receptors, including 5-HT_{2B} [**47**], so I decided to investigate the influence of 5-HT_{2B} receptors on DA release in rat using *in vivo* microdialysis. In this study [**98**], the selective 5-HT_{2B} receptor antagonists, LY266097 or RS127445 significantly decreased Nucleus Accumbens (NAc) DA outflow. Both compounds possess high (subnanomolar) affinity for 5-HT_{2B} receptors, and at least 100-fold selectivity over numerous other receptors [353,354]. Thus, given that the 5-HT_{2B} antagonists were administered at very low doses, it was concluded that their effects result from selective blockade of 5-HT_{2B} receptors. This was supported by previous microdialysis studies showing that basal DA outflow in the striatum and the NAc is unaltered by selective antagonism of 5-HT_{2A}, 5-HT3 or 5-HT4 receptors [355-357], and increased by 5-HT_{2C} antagonism [317]. Therefore the pattern of response to 5-HT_{2B} antagonists in rat was different to that observed previously for other receptors [358]. In co-treatment experiments, LY266097 significantly attenuated amphetamine-induced dopamine release in the NAc, thus showing that 5-HT_{2B} receptor blockade attenuates the effects of this psychostimulant. In addition, LY266097 was tested in behavioural experiments, at the same dose as that used in microdialysis experiments, and significantly reduced amphetamine-induced hyperlocomotion, whilst not affecting spontaneous locomotor activity. Locomotor activity is a behavioral response classically associated with increased DA function in the NAc [359]. Thus, our data suggested that reduction of amphetamine-induced hyperlocomotion by a 5-HT_{2B} receptor antagonist results from the ability of the latter to reduce accumbal DA outflow.

From a therapeutic point of view, our findings suggest that central 5-HT_{2B} receptors constitute a new pharmacological target for improved treatment of neuropsychiatric disorders resulting from DA neuron dysfunction, such as schizophrenia, depression or drug addiction. In this regard, the ability of 5-HT_{2B} receptor antagonists to achieve a selective reduction of mesoaccumbens DA hyperactivity without altering nigrostriatal DA, could avoid the side-effects induced by long term DAergic treatments which are currently available (i.e. neuroleptic-induced extrapyramidal symptoms including tardive diskynesias or levodopa-induced psychosis) [360]. Interestingly, several atypical antipsychotic drugs, such as aripiprazole and cariprazine, have been shown to possess high affinity and potent antagonist properties at 5-HT_{2B} receptors, which could contribute to their advantageous therapeutic properties [244,361]. It is also interesting to speculate whether 5-HT_{2B} receptor antagonism

plays a role in the therapeutic properties of the antidepressant, agomelatine, which was investigated in my laboratory. Agomelatine possesses melatonin agonist activity and 5-HT_{2C} antagonist properties [**51**] which are considered important for its clinical efficacy, despite being of only low potency (pKi = 6.15). However, agomelatine also acted as a 5-HT_{2B} receptor antagonist with an affinity (pKi = 6.59) which is about 3-fold greater than at 5-HT_{2C} receptors [**51**]. In the light of the findings described above, it would be interesting to determine whether such properties are relevant to agomelatine's profile of action.

5-HT_{2C} receptors and promiscuous coupling to Gαi and Gαq/11

5-HT_{2C} receptors play a major role in the etiology and treatment of affective disorders, anxious states, schizophrenia, and Parkinson's disease [334,362]. They are coupled to phospholipase C (PLC), the activity of which is classically determined by quantification of levels of inositol phosphate and/or intracellular calcium [323,333] [**26,32,47**]. In addition to PLC, 5-HT_{2C} receptors control other transduction systems including cGMP formation, adenylyl cyclase, and phospholipase A2 (PLA2) [333,363,364]. Several isoforms of 5-HT_{2C} receptors, which arise from differential extents of RNA editing, are differentially coupled to PLC as revealed by differing efficacies and potencies of agonists; furthermore, the nonedited INI isoform of 5-HT_{2C} receptors exhibits constitutive activity [317,345,365].

5-HT_{2C} receptor agonists can differentially stimulate PLC compared with PLA2 activity and cGMP production [333,366]. This suggests that agonists can induce different receptor conformations, preferentially engaging specific effector pathways. In fact, although 5-HT_{2C} receptors are classically considered to couple to Gαq, 5-HT_{2C} receptors interact with PTX-sensitive Gαi/o proteins controlling DNA synthesis and proliferation [367], adenylyl cyclase activity [363], and membrane currents in Xenopus laevis oocytes [368]. Moreover, direct coupling of 5-HT_{2C} receptors to Gαi1 and Gαo was shown by an antisense strategy [369]. Finally, in a cell line expressing high levels of 5-HT_{2C} receptors, 5-HT stimulated [^{35}S]GTPγS binding to Gαi proteins [341].

In our laboratory, we characterized the coupling of 5-HT_{2C} receptors expressed in CHO cells using [^{35}S]GTPγS binding as an index of "total" G-protein activation, i.e. a measure that is composed of the effects of all the G-protein subtypes involved [**45**]. An indication of their identity was gained by pre-treating the cells with pertussis toxin (PTX). 5-HT-mediated [^{35}S]GTPγS binding was sensitive to PTX, resulting in a decrease in [^{35}S]GTPγS binding and an increase in pEC_{50}. This demonstrates the involvement of Gαi/o proteins, but PTX did not completely abolish [^{35}S]GTPγS binding, indicating that 5-HT also stimulated PTX-insensitive G proteins, such as Gαq/11. In comparison, PTX did not affect the potency or efficacy of LSD and lisuride, suggesting that these ligands mainly activated PTX-insensitive G-proteins coupled to 5-HT_{2C} receptors. Therefore, these data suggested that some 5-HT_{2C} receptor agonists can signal preferentially through Gαq/11 and/or Gαi G-proteins. To confirm this hypothesis, we used an antibody capture assay together with a detection technique employing anti-IgG–coated scintillation proximity assay (SPA) beads, as originally described by [90,91] and applied to 5-HT_{1A} and 5-HT_{1B} receptors [**46,53,69**]. This technique permitted measurement of the stimulation of Gαq/11 and Gαi3 proteins in response to 5-HT_{2C} receptor activation by different agonists [**45**].

LSD behaved as a full agonist for Gαq/11 activation but only as a weak partial agonist at Gαi3. Previous studies in HEK293 cells had likewise shown that LSD is more efficacious at Gαq (inositol generation) than at Gαi/o ([^{35}S]GTPγS binding) [341]. However, in our study, 5-HT, RO600175 and DOI more potently activated Gαq/11 than Gαi3 [**45**], whereas differential activation was not apparent in another study [341]. One possibility that could have accounted for these divergent results was the presence of receptor reserve in the cell line we used. Indeed, coupling of GPCRs to distinct G-proteins was known to be influenced by the presence of receptor reserve [370,371], and we had previously shown that the CHO-h5-HT$_{2C}$ cell line used in these experiments displayed substantial receptor reserve for activation of PLC, using an innovative rapid experimental procedure based on measuring depletion of membrane-bound [^{3}H]phosphatidylinositols [**26,32,47**]. We therefore examined this issue by carrying out receptor inactivation experiments using the alkylating agent, EEDQ. These showed that extensive receptor reserve was present in the cell line for 5-HT$_{2C}$ receptor-mediated activation of Gαq/11 but not for Gαi3 G-proteins. Indeed, the efficacies of 5-HT, Ro600175 and DOI for Gαq/11 activation were not reduced by EEDQ pre-treatment, even though the number of 5-HT$_{2C}$ receptors was diminished 7-fold. In contrast to Gαq/11 activation, EEDQ treatment markedly diminished the efficacy of all agonists for 5-HT$_{2C}$ receptor-mediated Gαi3 activation. Thus, in the present system, potential agonist-directed trafficking by LSD and DOI must take into account the large receptor reserve. It was concluded that the markedly higher efficacy of the partial agonists, DOI and LSD, at Gαq/11 compared with Gαi3 (observed in the absence of EEDQ) was associated with the more efficient coupling of 5-HT$_{2C}$ receptors to Gαq/11 versus Gαi3.

Figure 6.2: 5-HT$_{2C}$ receptor-mediated Gq/11 and Gi3 protein activation.

Agonist concentration-response curves at Gq/11 (left-hand panels) and Gi3 (right-hand panels) from membrane preparation of CHO–h5-HT$_{2C}$ cells. [^{35}S]GTPgS binding is expressed as a percent of maximal stimulation with 5-HT (100%) under three conditions: control (■), EEDQ-treated for 60 minutes (◆) and EEDQ-treated for 90 minutes (▲). Reproduced from [**45**]**.**

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Thus, LSD, DOI and lisuride express their "functional selectivity" properties only under certain conditions of receptor reserve. This conclusion is similar to that reached for α_{2A}-adrenoceptor coupling to adenylyl cyclase via Gαi and Gαs [370] and raises the question of whether receptor reserve exists for Gαq/11 and/or Gαi3 in physiological systems. In this context, it should be noted that coupling to Gαi3 was observed herein even after extensive EEDQ treatment, reducing receptor expression to about 2 pmol/mg. In rat choroid plexus neurons, 5-HT_{2C} expression levels (for a mixture of cell types) is also in the pmol/mg range [372] suggesting that the present data in CHO cells are relevant to central populations of 5-HT_{2C} receptors.

To summarise, our data demonstrated that 5-HT_{2C} receptors (VSV isoform) couple to both Gαq/11 and Gαi3 in CHO cells and that these G-proteins subtypes are recruited in an agonist and receptor reserve-dependent manner. The differential influence of agonists upon G-protein coupling at 5-HT_{2C} receptors may be relevant to their functional profiles *in vivo*.

7. Dopamine D_2, D_3 and D_4 receptors

7.1 Key points

- Antagonism of dopamine D_2 receptors is fundamental for antipsychotic activity and, in conjunction with 5-HT_{1A} receptor activation, was the basis for a drug discovery program directed by the author and discussed in Chapter 4. However, dopamine D_3 and D_4 receptor subtypes may also contribute to the activity of antipsychotics. The author carried out studies that characterized the function of these receptors and identified novel selective ligands.

- The *in vitro* signal transduction pathways elicited by D_2 and D_3 receptors were compared and shown to differ both at the G-protein level [**20**] and on down-stream responses (ERK phosphorylation) [**23**]. These receptors signal through different G-proteins and kinase cascades. The pharmacology of D_2 receptor signalling was examined at GIRK channels [**70**].

- D_4 receptors were shown to be tightly coupled to G-proteins and to GIRK currents, but most available agonists only displayed modest efficacy [**12,82**]. Notably, some agonists showed higher efficacy for one response whereas others had an opposite profile, suggesting functional selectivity. D_4 receptors were also discovered to be "promiscuous", being activated by adrenaline and noradrenaline, as well as dopamine [**7**].

- The pharmacology of D_3 receptors was investigated using novel selective as radiotracers: [^{3}H]S14297 [**3**] and [^{3}H]S33084 [**30**]. S33084 was also characterised *in vitro* and *in vivo* [**29,33**]. Another D_3 antagonist, S33138, was characterized and later taken into clinical trials as an antipsychotic [**79**]. A highly-selective D_4 receptor antagonist, S18126, was characterized [**18**].

- The relative contributions of D_2, D_3 and D_4 receptor activation was compared in tests of penile erection in rats [**87**], agonist-induced emesis in dogs [**85**], and apomorphine-induced rotations in rats with unilateral 6-OH-DA lesions of nigro-striatal pathway [**42**].

7.2 Background to author's work

Dopamine D_2 and D_3 receptors

Dopaminergic neurotransmission is mediated by 5 receptor subtypes (D_1 to D_5) which are grouped into 2 receptor families. D_1-like receptors include the D_1 and D_5 subtypes, whereas D_2 -like receptors include the D_2, D_3 and D_4 subtypes. D_2 and D_3 receptors, in particular, display marked sequence homology and pharmacological similarity in their *in vitro* ligand binding profiles [(373,374)]. However, D_3 receptors may be distinguished from D_2 receptors by several factors. D_3 receptors are concentrated in limbic rather than striatal brain regions [(375)]. Further, they mediate stimulation, rather than inhibition, of c-fos expression in striatal neurones [(376,377)], and inhibition, rather than stimulation, of locomotor activity in rats [(378)]. In addition, whereas D_2 receptors couple efficiently to second

messenger systems, markedly inhibiting adenylyl cyclase activity, such responses proved elusive and complex for D_3 receptors [379,380]. Indeed, D_3 receptors were found to couple selectively to inhibition of adenylyl cyclase type V, but not type I or VI, and only weakly to type II [381,382]. *In vitro* studies of agonist efficacy at D_3 receptors therefore employed other measures of receptor activation, including medium acidification [383], and stimulation of mitogenesis [376,384]. However, these approaches measure responses 'downstream' of the receptor in the intracellular activation cascade and the relevance of an increase in mitogenesis for post-mitotic central nervous system neurones is unclear. In contrast, measuring receptor-mediated G-protein activation by stimulation of [^{35}S]GTPγS binding directly reflects ligand binding events at the receptor itself. Thus, I adopted this strategy to investigate the functional properties of human D_3 receptors (see below).

The extracellular signal-regulated kinases, ERK1 and ERK2, also known as mitogen-activated protein kinases (MAPK), are involved in the control of cell growth and differentiation by growth factors [385,386]. In the case of dopamine receptors, D_2 receptors expressed in C6 glioma cells had been found to stimulate ERK1/2 and thymidine incorporation via the Ras protein [387] and, when expressed in CHO cells, D_2 receptors activated ERK1/2 via PI3-kinase [388]. D_4 receptors expressed in SK-N-MC human neuroblastoma cells stimulate a pathway involving Ras activation via Shc/Grb2/Sos complex and the tyrosine kinase Src [389]. In contrast to D_2 and D_4 receptors, little comparative information was available concerning D_3 receptors, although they were known to stimulate expression of the immediate early gene, c-fos, in cultured neurones [376], and mediate stimulation of mitogenesis [376,384]. These data suggested that D_3 receptors may couple to serine/threonine kinase pathways and activate ERK1/2, but the demonstration of such coupling had not, as yet, been documented.

Dopamine D_4 receptors

Although many antipsychotics antagonise $D_{2/3}$ receptors, they also bind with high affinity to D_4 receptors [390]. In humans, D_4 receptors are upregulated in schizophrenics, independently of their antipsychotic treatment, suggesting that the changes are disorder-related [391]. Interest was stimulated by the observation that clozapine has somewhat higher affinity at D_4 receptors than for D_2 or D_3 receptors [392], suggesting that aspects of clozapine's 'atypical' profile may be attributed to actions at D_4 receptors, notably insofar as concerns cognitive function. Indeed, D_4 receptors had been shown to be preferentially localised in brain regions that are implicated in regulation of cognition, including the hippocampus and frontal cortex [393-397]. In addition, electrophysiological studies demonstrated that D_4 receptor activation influences the electrical activity of prefrontal cortex (PFC) neurons: NMDA receptor currents are decreased and NMDA receptor internalisation favoured [398]. It is interesting that D_4 receptor blockade has protective properties on cultured hippocampal neurons [399] whereas D_4 receptor activation reduces oxidative stress-induced in rat cortical neurons and hippocampus-derived cells [400,401]. Thus, an intermediate degree of partial agonist activity may provide a beneficial influence on preservation of neuronal integrity. Further observations support this hypothesis: in rodents, D_4 receptor activation is implicated in memory consolidation [402], short-term social memory [403] and increased exploratory activity [404], and reduced D4 receptor expression is associated with attentional deficits [405]. In contrast, in tests of cognition in

primates, D_4 receptor antagonists opposed the deficits in frontal cortex-dependent working memory tasks induced by stress [406] or chronic treatment with PCP [407]. The D_4 receptor antagonist, L745870, had differential effects in rats with high versus low basal memory performance, suggesting that optimal responses may be obtained with a partial agonist [408]. D_4 receptor activation is also relevant to the treatment of sexual dysfunction, a frequently-reported side-effect of antipsychotic treatment that can seriously influence treatment outcome in schizophrenia patients [409,410]. Compounds possessing D_4 agonist properties, such as ABT724 and flibanserin (a compound also possessing 5-HT_{1A} receptor agonist activity), were clinically evaluated for attenuation of sexual dysfunction [411,412].

Taken together, these observations suggested that a modest level of D_4 receptor activation may be a desirable property for novel antipsychotic agents with improved efficacy against negative and cognitive symptoms of schizophrenia. However, there was a lack of studies providing a comparative analysis of the *in vitro* efficacy at DA D_4 receptors of established and novel antipsychotics.

7.3 Contribution of author's work

D_2 and D_3 Receptor coupling to G-proteins

I compared the G-protein coupling of dopamine D_3 receptors with that of D_2 receptors expressed in a common CHO cell environment. Although both D_3 and D_2 receptors mediated stimulation of [^{35}S]GTPγS binding, a detailed investigation of the coupling of these receptor subtypes identified marked differences between D_3 and D_2 sites [**20**].

First, despite an 11-fold difference in receptor expression level (15 pmol/mg for D_3 vs only 1,4 pmol/mg for D_2 receptors), the dopamine-elicited increase in [^{35}S]GTPγS binding by D_3 receptors (1.6-fold) was less than that observed at D_2 receptors (2.5-fold). This striking difference was further underlined by the observation that partial inactivation of D_3 receptors using the alkylating agent, EEDQ, markedly reduced the stimulation of [^{35}S]GTPγS binding induced by dopamine, indicating that high receptor expression levels are necessary to observed measurable activation of G-proteins by D_3 receptors. However, when Furchgott analysis was undertaken, a hyperbolic occupancy/response plot was obtained, indicating the presence of marked D_3 receptor reserve for stimulation of [^{35}S]GTPγS binding by dopamine. Thus the limited stimulation of [^{35}S]GTPγS binding to CHO-hD_3 membranes appears to be a property of D_3 receptors themselves and not due to insufficient intrinsic efficacy of dopamine. In addition, the modest stimulation at D_3 receptors was not a consequence of augmented basal [^{35}S]GTPγS binding, because basal [^{35}S]GTPγS binding was unaffected by receptor inactivation. Further, the low fold-stimulation in CHO-hD_3 membranes was unlikely to be due to a global lack of activatable G-proteins: the amount (Bmax) of dopamine-activated G-proteins in CHO-hD_3 cell membranes was about 3-fold higher than that in CHO-hD_2 cell membranes. Taken together, these data suggested that, unlike D_2 receptors, D_3 receptors are poorly capable of inducing the conformational changes necessary for G-protein activation [413], perhaps due to a slower rate of G-protein coupling/uncoupling at D_3 receptors or, alternatively, to interaction with different G-protein subtypes at D_3 vs D_2 receptors, a possibility suggested by subsequent experiments.

Indeed, G-protein activation by dopamine at D_3 receptors was PTX-sensitive, implicating Gαi/o G-proteins similar to the known PTX sensitivity of D_2 receptors [414,415]. However, marked differences were observed between D_3 and D_2 receptors in antibody tests. Thus, dopamine-stimulated [^{35}S]GTPγS binding at hD_3 and hD_2 receptors was inhibited by an antiserum that recognizes the three Gαi subunits (Gαi1/i2/i3) and, more weakly, Gαo subunits. This antiserum inhibited dopamine-stimulated [^{35}S]GTPγS binding to CHO-hD_3 membranes more strongly than to CHO-hD_2 membranes (67 % vs 40 % inhibition). The greater effect at D_3 receptors suggested that D_3 may couple to both Gαi and Gαo proteins whereas D_2 receptors may preferentially couple to members of the Gαi protein family. This is consistent with a study which found that an attenuation of D_2 receptor-mediated inhibition of adenylyl cyclase by pretreatment with anti-Gαi1/i2 but not by anti-Gαo antibodies [416].

An interaction of D_3 receptors with a G-protein other than Gαi or Gαo is suggested by the observation that, when CHO-hD_3 cells were treated with PTX, there remained a residual capacity of dopamine to stimulate [^{35}S]GTPγS binding, consistent with the inhibition of dopamine-dependent [^{35}S]GTPγS binding at D_3 (but not hD_2) receptors by anti-Gαq/11 antibodies. Thus, these data suggest that D_3 receptors in the present CHO-hD_3 cell line may interact with $G\alpha_{q/11}$ [**20**]. Although a previous study of D_3 receptors expressed in CHO cells did not find such an effect [379], that may have been due to a 50-fold lower D_3 expression level (0.3 vs 15 pmol/mg in the cells used here).

In conclusion, this study characterized, for the first time, G-protein coupling at D_3 receptors using a functional test. The data suggested that the coupling of D_3 receptors to G-proteins is less efficacious than that at D_2 receptors. In addition, unlike D_2 receptors, D_3 receptors appeared to couple to G-proteins other than Gαi, such as Gαo and/or Gαq/11 proteins.

D_3 Receptor activation of ERK pathway

Both dopamine D_2 and D_4 receptor subtypes were known to stimulate ERK1/2 in cultured cells [388,389] but it was not known whether dopamine D_3 receptors also activate this pathway and, if so, by which intracellular signaling mechanisms. This was therefore the focus of a study undertaken in my research group [**23**]. We found that, in CHO cells stably transfected with D_3 receptors (CHO-hD_3), DA stimulated ERK1/2 phosphorylation in a time-dependent manner with a peak of activation occurring at 5 min and a rapid return to the basal level. The activation was specifically mediated by D_3 receptors, because haloperidol and the selective D_3 receptor antagonist, GR218,231, blocked DA-mediated but not FGF-induced stimulation of ERK1/2 phosphorylation. Further, the preferential D_3 agonists, (+)7-OH-DPAT and PD128,907, like DA, triggered ERK1/2 activation. It is interesting that stimulation was similar for the three agonists whereas both (+)7-OH-DPAT and PD128,907 exhibit partial agonist properties for G-protein activation in CHO-hD_3 cell membranes [**20**]. This difference suggests possible signal amplification at the level of kinase cascade following G-protein activation and ERK1/2 phosphorylation may, thus, more readily detect weak agonist actions.

PTX-treatment of CHO-hD_3 cells abolished ERK1/2 stimulation by DA, (+)7-OH-DPAT and PD128,907, suggesting the exclusive involvement of Gi and/or Go proteins, analogous to that reported for D_2

receptors [388]. As in other studies [417], inhibition of MEK (located just upstream of ERK) by the specific inhibitor, PD98059, abolished ERK1/2 activity in CHO-hD_3 cells. Further investigation revealed distinctive features in the activation of the ERK1/2 cascade by D_3 receptors versus D_2, D_4 receptors and other GPCRs. Inhibitors of tyrosine kinase activity (genistein and lavendustin A), did not reduce DA-induced ERK1/2 activation. In contrast, inhibitors of protein kinase C (PKC; Ro31-8220 and Gö6983), blocked DA-induced ERK1/2 activation. However, ERK1/2 activation was insensitive to PKC downregulation by phorbol esters, indicating the involvement of an 'atypical' PKC. Futhermore ERK1/2 activation involved phosphatidylinositol 3-kinase (PI 3-kinase) inasmuch as its inhibition by LY294002 and wortmannin reduced DA-induced ERK1/2 activation.

In conclusion, this work demonstrated that D_3 receptors activate ERK1/2 activity. Although the ERK1/2 activation pathway bore similarities to other previously-characterised GPCRs (such as dopamine D_2 receptors) a distinctive pattern of intracellular kinase cascade activation was observed. Thus, D_3 receptor-mediated ERK1/2 phosphorylation involves the activation of PI 3-kinase and 'atypical' PKC. These findings raised the possibility that ERK1/2 stimulation may be relevant to regulation of cellular events such as neuronal plasticity, morphological differentiation and synaptic transmission. Subsequent studies by other authors provided substantiation for this hypothesis [418,419].

D_4 Receptor coupling to G-proteins

In vitro profiling of antipsychotics at D_4 receptors

Although D_4 receptors had been the object of considerable interest because of their potential role in cognition and psychosis, the drugs used to investigate their function were only characterized in somewhat scattered reports using different methodologies that were not easily cross-interpreted. For example, one study estimated agonist efficacies at $D_{4.4}$ receptors (4-repeat splice variant, the most common allele in human) by comparing their affinities at different receptor states [420]. Other studies investigated agonist/antagonist activity at D_4 receptors by adenylyl cyclase determinations but only few compounds were compared in each study [421-423] and systematic comparisons of agonist efficacies and antagonist potencies were lacking. The study I conducted was the first to address this issue by evaluation of a series of 33 dopaminergic ligands, including the principal anti-Parkinson's disease drugs and antipsychotics, using competition binding and [^{35}S]GTPγS binding to membranes of CHO cells transfected with the $D_{4.4}$ receptor [**12**] [424]. High concentrations of GDP (3 μM), NaCl (100 mM) and MgCl2 (3 mM) were found to be necessary to optimize the ratio of agonist-induced over basal [^{35}S]GTPγS binding. In addition, CHO-D4.4 cells displayed a high ratio of dopamine-activated G-proteins to receptors, indicating the absence of 'spare receptors' and enabling partial agonist activity to be defined. In accordance with previous studies [421,425] the antipsychotics, clozapine and haloperidol, as well as other antipsychotics, exhibited antagonist properties, with Kb values that corresponded closely to respective Ki values. None of the antipsychotic drugs tested exhibited any intrinsic agonist activity, indicating that they acted as 'neutral' or 'silent' antagonists in this study [**12**].

Several years later, I undertook a further study comparing over 30 dopaminergic drugs by [^{35}S]GTPγS binding and by activation of G-protein-regulated inwardly-rectifying potassium (GIRK) currents in Xenopus oocytes [**82**]. As expected, the selective D_4 receptor agonists, ABT724, CP226269, PD168077 and Ro-10-5824 modestly stimulated [^{35}S]GTPγS binding, whereas the antagonists, L745870 and RBI257 did not. In contrast, antipsychotics exhibited wide variety of efficacy at D_4 receptors. However, the observed levels of agonism were highly dependent on experimental conditions. Thus, when a higher concentration of sodium (100 mM) was used in the incubation buffers, Emax values for G-protein activation were decreased and a loss of potency was seen for most agonists. This is consistent with the influence of NaCl on opposing D_4 receptor / G-protein coupling [**12**] [(426)] and raises the issue of the most physiologically-relevant conditions in which to undertake *in vitro* studies, as discussed previously for 5-HT_{1B} receptor coupling to Gαi3 subunits [**53**] (see Chapter 5). Whilst physiological extra-cellular sodium concentrations are in the 100 mM range, heterotrimeric G-proteins that are located at the intracellular face of plasma membranes where NaCl concentrations are much lower (approximately 10 mM), except under neuronal depolarisation conditions, when sodium influx transiently increases intracellular NaCl levels. Therefore, a low NaCl concentration may be more "physiological" when measuring G-protein activation. In any case, these data raised the question of the amount of receptor activation that is relevant to receptor activation under physiological conditions *in vivo*. Although the precise receptor densities and G-protein subtypes encountered by D_4 receptors in their neuronal microenvironments are still unknown, even modest *in vitro* partial agonism may translate to measurable *in vivo* effects. This is supported by the observation that apomorphine and ABT724 (Emax 47 and 36%, respectively, [**82**]) facilitated sexual activity in rodents [(427)]. Further, PD168077 (Emax 39 %) stimulated extracellular regulated kinase (ERK) phosphorylation in the paraventricular nucleus, a region associated with sexual behaviour [(428)]. Compounds such as bifeprunox, sarizotan, SLV313 and F15063 that possess partial agonist properties (Emax 13 – 32%) may therefore also be expected to influence D_4 activity under physiological conditions. Indeed, F15063 and SLV313 reversed scopolamine-induced memory deficits in a rat social recognition paradigm [**73**][(429)], an action that is blocked by pre-treatment with L745,870. Thus, D_4 receptor activation by F15063 and SLV313 appears responsible for their activity in a model of cholinergic deficit, a finding in accordance with the known pro-cholinergic action of SLV313 in neurochemical measures [(232)].

In general, the compounds that activated [^{35}S]GTPγS binding to CHO-h$D_{4.4}$ membranes also increased GIRK channel currents in the Xenopus oocytes [**82**], demonstrating that they are capable of regulating both of these transduction systems linked to D_4 receptors [(430)]. However, whereas [^{35}S]GTPγS binding experiments measure activation of G-protein Gα subunits, GIRK channel currents provide measures of Gβγ subunit activation [(431)] and some drugs exhibited preferential efficacy for one transduction system versus the other. For example, sarizotan exhibited substantial partial agonist efficacy for [^{35}S]GTPγS binding (Emax = 32%), greater than that observed with the selective agonist, CP226289 (Emax = 23%). In contrast, sarizotan was less efficacious for activation of GIRK currents (Emax <40%) than CP226289 (Emax ~70%). These data suggest that CP226289 may preferentially exert its agonist properties via GIRK channels whereas sarizotan may predominantly

influence other G-protein activation pathways. Such observations suggested that some "biased agonism" may be occurring at D_4 receptors, as has been observed at other receptors (see Chapter 3 for discussion of biased agonism at 5-HT_{1A} receptors).

In summary, the studies I conducted [**12,82**] underline the importance of comparative information using a library of reference compounds in order to 'calibrate' *in vitro* functional responses. It is interesting to note that several recent drugs, such as bifeprunox, SLV313, F15063 and aripiprazole, exhibit marked partial agonist activity. It may therefore be supposed that this will translate *in vivo* to detectable physiological activity, particularly in regards to cognitive profile (see Introduction) and/or influence on sexual dysfunction.

"Promiscuous" coupling of D_4 receptors to adrenaline and noradrenaline

Receptors are usually classified according to their endogenous neurotransmitter, with the assumption that other neurotransmitters have little, if any, capacity to activate the receptor. However, preliminary evidence had been reported that dopaminergic receptors may also exhibit high affinity for other neurotransmitters [(432-434)]. I found this to be an intriguing concept because it runs contrary to usual assumptions of receptor selectivity and signaling. When I investigated the issue at D_4 receptors, I found that, remarkably, noradrenaline and adrenaline, like dopamine, bound to D_4 receptors with high affinity [**7**]. In fact, the high-affinity components (K_H) of the biphasic competition binding curves were in the nanomolar range (3 to 12 nM; see Fig. 7.1). These K_H values are similar to the affinitites of noradrenaline and adrenaline at α1- α2- and β-adrenoceptors [(435)], suggesting that D_4 receptors are concurrently activated under physiological conditions. Nanomolar K_H values were also observed for a further D_4 receptor isoform ($D_{4.2}$) and in a different expression system (baculovirus-infected Sf9 cells), providing further support for the assertion that high affinity for noradrenaline and adrenaline is an intrinsic characteristic of D_4 receptors. In contrast, D_2 receptors exhibited low affinity for noradrenaline and adrenaline, indicating that high affinity for these neurotransmitters is not a general property of dopaminergic receptors [**7**].

Figure 7.1: Binding and G-protein activation by adrenaline and noradrenaline at D_4 receptors. The upper panel shows the binding isotherms of 5 endogenous agonists at dopamine $D_{4.4}$ receptors. The lower panel shows the effects of these agonists on G-protein activation. Adapted from [**7**].

Note: for copyright reasons, this Figure is omitted from the public access version of the thesis.

The biphasic competition isotherms of adrenaline and noradrenaline at D_4 receptors

suggested the presence of two G-protein-coupling states of the receptor. Indeed, an increase in K_H values was observed in the presence of GppNHp, consistent with a change from a high agonist affinity (G-protein coupled) to a low agonist affinity (uncoupled) conformation of the receptor (see Chapter 2 and Fig. 2.2). In G-protein activation experiments ([^{35}S]GTPγS binding), noradrenaline and adrenaline, like dopamine, acted as agonists (Fig. 7.1). It is interesting that noradrenaline and adrenaline are less potent than dopamine in stimulating [^{35}S]GTPγS binding and it is interesting to speculate whether they may promote coupling of the D_4 receptor to different G-protein populations. In any case, stimulation of [^{35}S]GTPγS binding by noradrenaline was specifically mediated by D_4 receptors, since the selective antagonist, L745,870, as well as spiperone, haloperidol and clozapine, completely antagonised it. In contrast, noradrenaline-induced [^{35}S]GTPγS binding was not inhibited by antagonists at dopamine D_2/D3, D1/D5 receptors or α1-, α2- and β-adrenoceptors [**7**].

Conclusions and perspectives.

In addition to dopamine, adrenaline and noradrenaline also potently bind D_4 receptors (but not D_2 receptors). Like dopamine, adrenaline and noradrenaline also act as agonists, stimulating D_4 receptor-mediated [^{35}S]GTPγS binding [**7**]. These observations are consistent with similar ones published almost concurrently by another laboratory [436] and with subsequent data reported by a third research group [426]. A later study extended these data by demonstrating that the "promiscuity" of D_4 receptors is also observed for activation of GIRK channels in *Xenopus* oocytes [437]. Taken together, these observations strongly suggest that D_4 receptors mediate some of the physiological actions of noradrenaline and adrenaline. For example, D_4 receptors are found in hippocampus and spinal cord [393,438], which receive noradrenergic, but little dopaminergic, innervation [439]. Similarly, mRNA encoding D_4 receptors is detected in retina, adrenal chromaffin cells, heart and kidney [440,441], tissues rich in noradrenaline and adrenaline.

8. Noradrenergic α2 receptors

8.1 Key points

- Noradrenergic α2 receptors are important targets in the control of movement disorders such as Parkinson's disease but little systematic characterisation of the *in vitro* binding profiles of anti-parkinsonian drugs was available.

- The author conducted the first multiparametric study of anti-Parkinson's disease agents with differing profiles of action at α2 and other monoamine receptors. This extensive study compared 14 drugs, including the principal clinically-employed pharmacotherapeutics, in ligand binding tests at 21 receptors [**48**].

- The effects of 14 antiparkinsonian agents were examined in signal transduction tests *in vitro* at 14 targets, including 6 serotonergic (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}), 4 dopaminergic (D_{2S}, D_{2L}, D_3, D_4), and 4 noradrenergic (α_1, α_{2A}, α_{2B}, α_{2C}) receptors [**49,50]**.

- The clinical anti-Parkinson's disease drug, piribedil, which was previously thought to act essentially through dopaminergic mechanisms, was shown to possess α2 receptor antagonism *in vitro* and *in vivo* [**38**]. The alpha2 properties were of a similar magnitude as the dopaminergic properties of piribedil.

- Commonly-used α2 antagonists often lack of selectivity with regards to serotonin 5-HT_{1A} receptors. This was revealed using *in vitro* binding and signal transduction studies [**17**] and in *in vivo* studies of anti-cataleptic influence [**55**].

8.2 Background to author's work

In Parkinson's disease, the progressive degeneration of nigrostriatal dopaminergic pathways is associated with diverse motor symptoms, including rigidity, tremor, bradykinesia, and postural instability [(442)]. In addition, patients exhibit sensory and cognitive/attentional deficits together with depressed mood and chronic pain [(19,443,444)]. Despite increasing interest in neuroprotective strategies, Parkinson's disease is principally treated by administration of the dopamine (DA) precursor L-dihydroxyphenylalanine (L-DOPA) [(445)]. However, L-DOPA displays variable pharmacokinetics, elicits dyskinesias and autonomic side effects, poorly improves certain motor symptoms, is largely ineffective against cognitive and mood deficits, and loses efficacy upon prolonged administration [(20)]. Abrupt transitions between "on" and "off" phases are particularly distressing to patients [(22)].

These observations encouraged efforts to identify non-dopaminergic strategies for relief of Parkinson's disease: one possibility was to target adrenergic mechanisms because parkinsonian patients show a loss of locus ceruleus-localized adrenergic neurons [(446-448)]. This depletion of noradrenaline is seen notably in the motor cortex, in limbic structures such as the nucleus

accumbens, and in the spinal cord and aggravates deficits in the control of motor behavior, mood, cognition, and attention [23,446]. α_{2A} receptors are implicated in the functional roles of adrenergic pathways and elicit activation of signaling (G-protein activation) in regions such as amygdala and lateral septum [**28**]. α_{2A} receptors are also implicated in the inhibition of frontocortical and, possibly, subcortical dopaminergic pathways as well as corticolimbic serotonergic projections [1,447]. Modulation of noradrenergic transmission by α_{2A} receptors has, therefore, well-established and broad effects on control of motor behavior, mood, and cognition [**27**][447,449-451].

In this context, α2 receptor antagonists potentiate induction of rotation by dopaminergic agonists in rats bearing unilateral lesions of the substantia nigra [447,450-452]. They also enhance the ability of dopaminergic agonists to alleviate perturbation of motor functions provoked by reserpine and haloperidol [446] (although some α_2 receptor antagonists also exhibit 5-HT_{1A} agonist properties [**17,55**]). Moreover, α2 receptor antagonists facilitate antiparkinsonian actions of L-DOPA in this model while suppressing its dyskinetic side effects [446,453]. Furthermore, in primates, α_2 receptor antagonists attenuate motor symptoms elicited by the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [454,455], whereas lesions of the locus ceruleus exacerbate pathological changes and delay recovery [450]. On a clinical level, small-scale clinical studies in parkinsonian patients had suggested a modest improvement upon administration of α2 receptor antagonists [456,457]. Moreover, coadministration of idazoxan improves L-DOPA-elicited dyskinesia in PD patients[458,459].

These observations indicate that a deficiency of adrenergic transmission may contribute to motor, cognitive, and/or emotional symptoms of Parkinson's disease, and blockade of α2 autoreceptors may be favourable for its treatment. This might be achieved by adjunctive use of α2 receptor antagonists with L-DOPA or dopaminergic agonists [23,446]. Alternatively, α2 receptor antagonist and D_2 agonist properties might be incorporated into a single molecule, such as piribedi [**38**]. The latter is an antiparkinsonian drug from Servier laboratories, where I was working at the time of the studies described in the present chapter. However, studies of the adrenergic properties of antiparkinsonian drugs had been restricted to a few ligands at poorly-characterized native sites and knowledge of the comparative agonist/antagonist profiles of antiparkinson agents was fragmentary.

8.3 Contribution of author's work

The above considerations led me to believe that (i) whereas psychopharmacologists had for many years carried out highly detailed analysis of the receptor profiles of antipsychotics (see Chapter 4), such analyses of antiparkinsonian drugs were conspicuously absent; (ii) a careful investigation of available antiparkinsonian drugs, particularly in relation to their α2 adrenergic properties, could provide a rational basis from which to interpret *in vivo* animal data and clinical observations; (iii) a comparative study of antiparkinsonian drugs should examine different chemical classes of drugs and carry out both affinity and efficacy (agonist / antagonist) measures.

The studies that I undertook evaluated the actions of 14 dopaminergic agonists (antiparkinson agents) at multiple classes of monoaminergic receptors. In addition, actions at muscarinic M1 sites and histamine H1 sites were evaluated in light of their role in the control of motor behavior, attention, mood, and cognition [460-462] and the alterations in histaminergic and cholinergic transmission that had been reported in Parkinson's disease [463,464]. The strategy adopted was as follows. First, using competition binding assays, we determined drug affinities at recombinant, stably transfected, human receptors as well as at rat α_{2D} and at native H_1 receptors. Second, to analyse the extensive database of drug profiles, data were subjected to principle components analysis (PCA) and drugs were classified by hierarchical (cluster) analysis in accordance with their overall homology [**48**]. This multivariate approach to drug comparisons had the advantage of permitting identification of patterns that are not necesserily revealed by visual inspection or drug-by-drug/receptor-by-receptor comparisons [465,466]. Third, efficacies of antiparkinson agents were determined at the majority of monoaminergic receptor subtypes incorporated into these multivariate analyses [**48,50**].

Cluster analysis of drug homology for receptor affinities.

Although multivariate techniques had been used for evaluation of biochemical abnormalities in schizophrenia [467] and characterization of drug pharmacokinetic profiles [465], this was their first utilization for characterization of drug/receptor binding. Multivariate strategies simultaneously analyse the entire database, permitting hypothesis-free exploration of overall affinity profiles. Although the experimenter still needs to select both the drugs and the variables to be measured, substantial databases such as the one that was generated in my laboratory, decrease the risk that an involuntary "bias" may distort analyses. Comparisons of the overall affinity profiles of drugs provide a framework for interpretation of their contrasting functional profiles *in vivo*. For example, comparisons of the profiles of established drugs with those of novel compounds could provide information on the latter's possible therapeutic potential. However, multivariate analyses assume that drugs behave in an identical manner at different receptor types. This is appropriate for structure-activity relationships focusing on drug affinity (pKi values) but neglects potential differences in efficacy. In other words, the multivariate analysis did not take into account the fact that some compounds possess agonist or antagonist properties. I therefore addressed this important issue in separate investigations of G-protein activation and other intracellular responses [**49,50**]. Ideally, an integrated measure would be available that reflects both affinity and efficacy, but there is not yet an accepted parameter that can be used for a multivariate analysis.

From the dendrogram of overall drug homology generated by analysis of the database, several drug clusters were observed (Fig. 8.1). The most striking separation between drugs was at the first "node", which yielded two major subdivisions. The first major group comprised quinpirole, quinelorane, ropinirole, pramipexole, piribedil, and talipexole, whereas the second comprised lisuride, terguride, roxindole, bromocriptine, cabergoline, pergolide, TL99, and apomorphine. These drugs displayed low affinities for multiple classes of 5-HT receptor, and low affinities for D_5 compared with D_{2S}/D_{2L} receptors. Drugs in this first cluster also showed low affinity for D_1 and H_1 receptors and negligible affinity for α_1 and α_2 receptors, although this feature was also seen in

certain drugs in the second major cluster. Within the first group, similarity was apparent between quinpirole and, and between ropinirole and pramipexole, which comprised two closely related subclusters. The other two agents, piribedil and talipexole, could be distinguished by their more pronounced affinities at noradrenergic α_{2A}, α_{2C} and α_1 receptors. Within the second major subdivision, lisuride and terguride revealed high affinities at all sites, notably at α_1, α_2, 5-HT_{2A} and 5-HT_{2C} receptors and all subtypes of dopamine receptor. Roxindole and bromocriptine constituted a closely related subcluster showing a similar overall pattern of affinities, notably sharing high affinity for α_1 receptors. An additional pair of ligands displaying similar receptor binding profiles was formed by cabergoline and pergolide, with the former showing higher affinities at most sites. Both revealed low affinities for H_1 receptors and α_1 and α_2 receptors. The final couple of closely-related drugs was TL99 and apomorphine, which showed less pronounced affinities at most sites than other drugs.

Figure 8.1: Cluster analysis of receptor affinity of anti-Parkinson's disease drugs.

The analysis is based on 294 values of binding affinity (pKi) at 21 receptors. The distance between two individual drugs is inversely proportional to their pharmacological homology. In broad terms, three different drug classes were identified: those with selective affinity for dopamine D_2-like receptgors (shown in magenta), those with combined α2 and D_2 properties (black) and those with mixed D_2, serotonergic and adrenergic properties (blue). Figure adapted from [**48**].

Note: for copyright reasons, this Figure is omitted from the public access version of the thesis.

Noradrenergic α2 receptors.

When the antiparkinsonian compounds were compared in functional tests of receptor activation, striking differences in drug efficacies were seen at α2 receptor subtypes [**49**]. In analogy to piribedil [**38**], lisuride, bromocriptine, and apomorphine displayed antagonist properties, observations amplifying functional studies of isolated organs and hippocampal NA release in rats [468,469]. In line with their high affinities for α2 receptors [**48**], roxindole and two further ergot-related ligands, terguride and cabergoline, also manifested potent α2 receptor antagonist properties [**22**]. In contrast, in line with previous in vivo studies at α2 receptors in rodents [470,471], TL99 displayed agonist, and talipexole partial agonist, properties at α2A, α2B and α2C receptors. In vivo studies in rodents had revealed agonist actions of pergolide at central α2 receptors [472] and particularly

pronounced agonist properties at α2B receptors were observed here [**49**]. These contrasting actions of antiparkinson agents are of considerable significance in light of evidence that blockade of α2 receptors improves motor performance, cognitive function, and perhaps mood in Parkinson's disease [23,446]. In contrast, α2 receptor agonists interfere with antiparkinsonian actions [451,473,474]. Indeed, talipexole-induced stereotypy in rats (which reflects agonist properties at striatal D_2 receptors) is only apparent upon prevention of its α2 receptor agonist properties by coadministration of idazoxan [470] and it only elicits rotation in unilateral SNPC-lesioned rats upon cotreatment with α2 receptor antagonists [475]. The overall picture that emerges from these studies is that currently-used antiparkinsonian drugs display a widely-diverging palette of mechanisms of action which, in some cases appears to be far from optimised. One drug that displays a distinctive and somewhat more attractive pharmacological profile is piribedil, as discussed below.

Table 8.1: Agonist efficacies of antiparkinsonian drugs.
Information is based on data from cloned human receptors [**38**,**49**,**50**].

	Dopaminergic			**Adrenergic**		**Serotonin 5-HT$_1$**			**Serotonin 5-HT$_2$**		
Ligand	**D_2**	**D_3**	**D_4**	**α_{1A}**	**α_{2A}**	**1A**	**1B**	**1D**	**2A**	**2B**	**2C**
Quinpirole	++++	++	++	0	0	+	0	0	0	0	0
Quinelorane	++++	++	++	0	±	0	0	0	0	0	0
Ropinirole	+++	++	++	0	0	+	0	0	0	0	0
Pramipexole	++++	++	+	0	++	+	0	0	0	0	0
Piribedil	++	++	0	–	–	0	0	0	0	0	0
Talipexole	++	++	+	0	++	0	0	0	0	0	0
Lisuride	+	+	+	–	–	+++	++	++	++	–	++
Terguride	+	+	–	–	–	++	+	++	++	–	–
Roxindole	±	+	+	–	–	++	+	±	–	–	–
Bromocriptine	+++	++	±	–	–	++	++	++	++	–	++
Cabergoline	+++	++	+	–	–	+++	+++	++	+++	+++	+++
Pergolide	+++	++	+	0	+	++	+++	++	+++	+++	++
TL 99	++	+++	++	0	+++	0	0	0	0	0	0
Apomorphine	+++	++	+	0	±	+	**0**	+	–	–	–

Piribedil as a dual dopamine D_2 and α2 adrenergic anti-parkinsonian agent.

The studies I carried out indicated that, although piribedil is not a potent agent, its affinity at α_{2A} and α_{2C} receptors was comparable to its affinity at D_2 receptors [**38**]. This suggests that at therapeutically relevant doses activating D_2 receptors, piribedil also occupies α_{2A} and α_{2C} sites, actions that likely contribute to its functional profile. Although certain other antiparkinsonian agents also interact with α2 receptors [**48,49**], piribedil behaves essentially as an antagonist, whereas some compounds, such as talipexole, are efficacious agonists [**49**][470,476], a property that is lilely to limit their clinical efficacy. In contrast, selecting compounds that exhibit α2 receptor antagonism in addition to D_2 receptor agonism may represent a promising strategy for improved management of Parkinson's disease. Even if post-synaptic α2 receptors are simultaneously antagonized, favorable actions should be mediated

via colocalized, post-synaptic α1 and β adrenergic receptors [452][**38,49**]. Correspondingly, in contrast to talipexole, piribedil was found to reinforce corticolimbic adrenergic and cholinergic transmission, actions contributing to its favorable influence upon cognitive function and mood [477-481]. Moreover, apart from mild affinity at 5-HT_{1A} sites, piribedil was devoid of activity at multiple serotonergic receptors [**48,50**]. In contrast, other agents, such as bromocriptine, pergolide, and cabergoline, acted as potent agonists at 5-HT_{2A} and 5-HT_{2C} receptors, which likely underlies their propensity to elicit psychotic side-effects in Parkinsonian patients [**48,50**].

8.1 Conclusions and perspectives.

The comprehensive analysis that I conducted of the binding profiles of antiparkinson agents revealed marked and unexpected heterogeneity [**48**]. The latter extended beyond binding affinities to remarkable diversity in agonist / antagonist actions identified in accompanying efficacy studies [**49,50**]. In particular, this work demonstrated that some antiparkinsonian drugs exhibit marked affinity at α1 and α2 adrenergic receptors. Whereas talipexole behaves as an agonist at α2 receptors [470], piribedil showed antagonist properties [**38**].

It is interesting that, in the decade since these studies were published, new research on the use of α2 receptor antagonists in treatment of Parkinson's disease has been limited (a couple of dozen references found in PubMed in response to the keywords "alpha2" and "parkinsons"). Nevertheless, a selective α2 antagonist, fipamezole, was found to decrease L-DOPA-induced dyskinesias without interfering with antiparkinsonian properties [482] and recent publications suggest that interest in noradrenergic strategies for pharmacotherapy of Parkinson's disease may be increasing [23,446]. In particular, piribedil constitutes a possible "prototype" anti-PD drug that differs structurally from imidazolines (such as idazoxan), alkaloids (such as yohimbine) and other prototypical antagonists, but shares their antagonist properties at α2 receptors [**38**].

In conclusion, the study of anti-PD drugs carried out in my laboratory and the characterization of piribedil's α2 receptor antagonism contributed to advancing concepts that provide a basis for novel drug discovery programs for the treatment of Parkinson's disease.

Part B: Abstracts of publications included in this Thesis

Reference no. 1

Biochem J. 1992 Aug 1;285 (Pt 3):933-8.

High-level stable expression of recombinant 5-HT_{1A} 5-hydroxytryptamine receptors in Chinese hamster ovary cells.

Newman-Tancredi A, Wootton R, Strange PG.
Biological Laboratory, The University, Canterbury, Kent, U.K.

The human 5-hydroxytryptamine 5-HT_{1A} receptor gene was transfected into Chinese hamster ovary cells. A series of recombinant monoclonal cell lines expressing the receptor were isolated and the properties of one cell line that expressed receptors at a high level (2.8 pmol/mg) were studied in detail. In ligand binding assays with the selective 5-HT_{1A} receptor agonist 2-(NN-di[^{3}H]propylamino)-8-hydroxy-1,2,3,4-tetrahydronaphthalene ([^{3}H]8-OH-DPAT) only a single class of saturable high-affinity binding sites was detected, with a pharmacological profile in competition experiments essentially identical to that of the 5-HT_{1A} receptor of bovine hippocampus. [^{3}H]8-OH-DPAT binding to the recombinant cell membranes was inhibited by GTP, showing that the receptors in the transfected cells couple to G-proteins. A series of 5-hydroxytryptamine agonists inhibited forskolin-stimulated adenylate cyclase activity in the cells and, despite the high level of receptor expression, their apparent efficacies were similar to those observed for inhibition of adenylate cyclase in brain. This recombinant cell line provides a complete model system for studying the 5-HT_{1A} receptor and its transmembrane signalling system. The recombinant cells can also be grown in suspension culture for long periods but, whereas 5-HT_{1A} receptor numbers and receptor regulation by guanine nucleotides are maintained in suspension-grown cells, the inhibition of adenylate cyclase by the 5-HT_{1A} receptor is gradually lost.

Reference no. 2

Biochem Pharmacol. 1993 Mar 9;45(5):1003-9.

Characterization of recombinant human serotonin 5-HT_{1A} receptors expressed in Chinese hamster ovary cells. [^{3}H]spiperone discriminates between the G-protein-coupled and -uncoupled forms.

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Biological Laboratory, The University, Canterbury, Kent, U.K.

5-HT_{1A} serotonin (5-hydroxytryptamine) receptors have been characterized by ligand binding in a recombinant Chinese Hamster Ovary cell line expressing the human receptor gene. The agonist ligand [^{3}H]2-(N,N-dipropylamino)-8-hydroxy-1,2,3,4-tetrahydronaphthalene ([^{3}H]8-OH-DPAT) and the antagonist [^{3}H]spiperone were used. For both radioligands the binding sites labelled have the properties of 5-HT_{1A} receptors and most antagonists show roughly equal affinities for the receptors labelled by either [^{3}H]8-OH-DPAT or [^{3}H]spiperone. Agonists, however, show higher affinities for the sites labelled by [^{3}H]8-OH-DPAT and the antagonist spiperone conversely shows a higher affinity for the sites labelled by [^{3}H]spiperone. Whereas [^{3}H]8-OH-DPAT binding is inhibited by guanosine triphosphate (GTP) the binding of [^{3}H]spiperone is increased by GTP. A model is proposed for the results whereby [^{3}H]8-OH-DPAT labels a form of the receptor coupled to a G-protein and [^{3}H]spiperone labels a form of the receptor uncoupled from G-proteins (or possibly coupled to a different G-protein).

Reference no. 3

Neuropharmacology. 1995 Dec;34(12):1693-6.

[^{3}H](+)S 14297: a novel, selective radioligand at cloned human dopamine D_3 receptors.

Newman-Tancredi A, Audinot V, Jacques V, Peglion JL, Millan MJ.
Institut de Recherches Servier, Department of Psychopharmacology, Croissy-sur-Seine, France.

The selective dopamine D_3 receptor antagonist [^{3}H](+)S 14297 ((+)-[7-(N,N-dipropylamino)-5,6,7,8-tetrahydro-naphtho(2,3b)dihydro,2,3- furane]), labelled to high specific activity (145 Ci/mmol), bound to cloned human dopamine D_3 receptors but displayed negligible binding to cloned human D_2 receptors. [^{3}H](+)S 14297 exhibited rapid association and dissociation, high affinity saturable binding (Kd = 7.0 nM) and a competition binding profile highly correlated with that of [125I]iodosulpride (r=0.98).

Reference no. 4

Neuropharmacology. 1996 Jan;35(1):119-21.

Clozapine is a partial agonist at cloned, human serotonin 5-HT_{1A} receptors.

Newman-Tancredi A, Chaput C, Verriele L, Millan MJ.
Institut de Recherches Servier, Department of Psychopharmacology, Croissy-sur-Seine, France.

Clozapine exhibited 10-fold higher affinity than haloperidol for human 5-HT_{1A} receptors expressed in Chinese Hamster Ovary cells (CHO-h5-HT_{1A}) (Kis = 160 and 1910 nM respectively). Whereas haloperidol did not alter the basal binding of [^{35}S]GTP gama S to CHO-h5HT_{1A} membranes, clozapine stimulated it with an EC_{50} of 2320 nM and an efficacy of 49% (compared to 5-HT). The stimulation was antagonized by the selective 5-HT_{1A} receptor antagonist, WAY100635 (1 nM).

Reference no. 5

Eur J Pharmacol. 1996 Jun 20;307(1):107-11.

S 15535 and WAY100635 antagonise 5-HT-stimulated [^{35}S]GTPgammaS binding at cloned human 5-HT$_{1A}$ receptors.

Newman-Tancredi A, Chaput C, Verrièle L, Millan MJ.
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In Chinese hamster ovary (CHO) cells expressing cloned human 5-HT$_{1A}$ receptors, S15535 (4-(benzodioxan-5-yl)1-(indan-2-yl)piperazine) exhibited high affinity (Ki = 0.79 nM), similar to that of 5-HT (0.61 nM), (+/-)-8-hydroxy-3-(di-n-propylamino)tetralin ((+/-)-8-OH-DPAT; 0.58 nM) and N-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-N- (2-pyridinyl)cyclo-hexanecarboxamide (WAY100635; 0.56 nM). In these cells, 5-HT stimulated [^{35}S]GTPγS binding 3-fold (EC$_{50}$ = 15 nM) whereas (+/-)-8-OH-DPAT exhibited 73% efficacy relative to 5-HT (EC$_{50}$ = 6.0 nM). WAY100635 completely blocked 5-HT- and (+/-)-8-OH-DPAT-stimulated [^{35}S]GTPγS binding. Likewise, S15535 antagonised 5-HT-stimulated [^{35}S]GTP gamma S binding, reducing it to 30.1% of control values. S 15535 (100 nM) also shifted the 5-HT and (+/-)-8-OH-DPAT stimulation curves to the right, to EC$_{50}$ values of 870 and 313 nM, respectively. However, unlike WAY100635, which by itself did not stimulate [^{35}S]GTPγS binding, S15535 alone increased it by 34.7% relative to 5-HT (EC$_{50}$ = 5.8 nM). In conclusion, S15535 antagonises the stimulation of 5-HT$_{1A}$ receptors by 5-HT, whilst itself exerting weak partial agonist activity at these sites.

Reference no. 6

J Pharmacol Exp Ther. 1997 Mar;280(3):1241-9.

Interactions of (+)- and (-)-8- and 7-hydroxy-2-(di-n-propylamino)tetralin at human (h)D3, hD_2 and h serotonin$_{1A}$ receptors and their modulation of the activity of serotoninergic and dopaminergic neurones in rats.

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The aminotetralins, 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) and 7-OH-DPAT behave as preferential agonists at serotonin 5-HT_{1A} and dopamine D_3 and D_2 receptors, respectively. In our study, we evaluated the influence of their (+)- and (-) isomers on the electrical activity of serotoninergic neurones of the dorsal raphe nucleus (DRN), which bear 5-HT_{1A} autoreceptors, and of dopaminergic neurones of the ventral tegmental area (VTA), which possess inhibitory D_3 and D_2 receptors. These actions were compared to their *in vitro* interactions with cloned, human (h)5-HT_{1A}, hD_3 and hD_2 receptors. In binding studies, racemic 8-OH-DPAT showed 100-fold selectivity for h5-HT_{1A} vs. hD_2 and hD_3 receptors and there was little difference between its (+)- and (-)-isomers either in terms of their potency at 5-HT_{1A} receptors or of their selectivity at 5-HT_{1A} vs hD_2/hD_3 sites. Nevertheless, the (+)-isomer was markedly more efficacious than its (-)-counterpart in stimulating the binding of guanosine 5'-O-(3-[^{35}S]thiotriphosphate) ([^{35}S]-GTPgammaS) at h5-HT_{1A} receptors, a measure of coupling to G-proteins; 90 vs. 57% maximal stimulation respectively, relative to 5-HT = 100%. Also the (+)-isomer was ca. 3-fold more potent than the (-)-isomer in inhibiting the firing rate of DRN neurones. These actions were abolished by the 5-HT_{1A} antagonist, (-)-tertatolol, but unaffected by the hD_2/hD_3 antagonist, haloperidol. Whereas (+)-8-OH-DPAT stimulated VTA neurone firing with a bell-shaped dose response curve, the (-)-isomer only inhibited VTA firing. The (+)-isomer-induced stimulation was blocked by (-)-tertatolol but not haloperidol, whereas the (-)-isomer-induced inhibition was abolished by haloperidol and unaffected by (-)-tertatolol. In contrast to 8-OH-DPAT, the (+)- and (-)isomers of 7-OH-DPAT showed marked stereoselectivity inasmuch as the latter bound with 20-fold less potency than the former at hD_3 and, at higher concentrations, hD_2 receptors. Correspondingly, (+)-7-OH-DPAT was 20-fold more potent than (-)-7-OH-DPAT in reducing VTA firing. Concerning 5-HT_{1A} receptors, the (+)-isomer showed 20-fold lower affinity than at hD_3 receptors and, accordingly, it inhibited DRN firing at 20-fold higher doses than for inhibition of VTA firing. (-)-7-OH-DPAT showed even less (5-fold) selectivity for hD_3 vs. 5-HT_{1A} sites and for inhibition of VTA vs. DRN firing. The inhibition of VTA and DRN neurone firing by (+)-7-OH-DPAT was abolished by haloperidol and (-)-tertatolol, respectively. Finally, in line with this inhibition of DRN firing, both (+)- and (-)-7-OH-DPAT showed substantial efficacy ([^{35}S]-GTPgammaS binding, 76 and 53%, respectively) at h5-HT_{1A} receptors. In conclusion, for these substituted aminotetralins, stereospecificity is a more marked feature of interactions at hD_3 (and hD_2) than at h5-HT_{1A} receptors and is more pronounced for 7- as compared to 8-OH-DPAT. Neither (+)- nor (-)-7-OH-DPAT show substantial selectivity for hD_3 vs. 5-HT_{1A} receptors and their inhibition of the firing of VTA as compared to DRN neurones is mediated by hD_3/hD_2 and 5-HT_{1A} receptors, respectively. Finally, VTA neurones are stimulated by (+)-8-OH-DPAT via 5-HT_{1A} receptors and inhibited by (-)-8-OH-DPAT via hD_3 and/or hD_2 receptors.

Reference no. 7

Eur J Pharmacol. 1997 Jan 29;319(2-3):379-83.

Noradrenaline and adrenaline are high affinity agonists at dopamine D_4 receptors.

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The activity of monoamine neurotransmitters was examined at dopamine D_4 receptors. In competition binding with [^{3}H]spiperone, noradrenaline and adrenaline exhibited a high affinity binding component (K_H = 12.1 nM and 5.0 nM, respectively), similar to that of dopamine (K_H = 2.6 nM), whereas serotonin (5-hydroxytryptamine, 5-HT) and histamine had low affinity (Ki > 1000 nM). Noradrenaline and adrenaline acted as agonists at dopamine D_4 receptors, stimulating receptor-mediated [^{35}S]guanylyl-gamma-thiotriphosphate ([^{35}S]GTPgammaS) binding (EC_{50} = 7.8 and 5.8 microM, respectively, versus 0.1 microM for dopamine). The dopamine D_4 receptor-selective ligand, 3-(4-[4-chlorophenyl]piperazin-1-yl)methyl-1H-pyrrolo[2,3b]-pyridi ne (L745,870) and the dopaminergic antagonists, spiperone, haloperidol and clozapine, inhibited noradrenaline-stimulated [^{35}S]GTPgammaS binding whereas alpha1-, alpha2- and beta-adrenoceptor antagonists did not. These results indicate that dopamine D_4 receptors are activated by noradrenaline and adrenaline, although at 50-100-fold higher concentrations than dopamine.

Reference no. 8

Br J Pharmacol. 1997 Mar;120(5):737-9.

Inhibition of the constitutive activity of human 5-HT_{1A} receptors by the inverse agonist, spiperone but not the neutral antagonist, WAY100635.

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At recombinant human 5-hydroxytryptamine (5-HT) 5-HT_{1A} receptors expressed in Chinese hamster ovary cells (CHO-5-HT_{1A}), 5-carboxamidotryptamine (5-CT), acted as a full agonist (relative to 5-HT = 100%) for stimulation of receptor-mediated [^{35}S]GTPgammaS (guanylyl-5'-[gamma-thio]-tryphosphate) binding. In contrast, spiperone inhibited basal [^{35}S]GTPgammaS binding by 30.2% (IC_{50} = 55.5 nM) in CHO-5-HT_{1A} membranes but not in control untransfected membranes. The antagonist, N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl)-cyclohexane-carboxamide (WAY100635), blocked both 5-CT-induced stimulation and spiperone-induced inhibition of [^{35}S]GTPgammaS binding without itself modifying [^{35}S]GTPgammaS binding. It is concluded that, in this heterologous expression system, 5-HT_{1A} receptors display 'constitutive' activation of G-proteins and that spiperone displays inverse agonist activity whereas WAY100635 acts as a 'neutral' antagonist at this site.

Reference no. 9

J Pharmacol Exp Ther. 1997 Jul;282(1):132-47.

S 15535, a novel benzodioxopiperazine ligand of serotonin (5-HT)$_{1A}$ receptors: I. Interaction with cloned human (h)5-HT$_{1A}$, dopamine hD$_2$/hD$_3$ and h alpha2A-adrenergic receptors in relation to modulation of cortical monoamine release and activity in models of potential antidepressant activity.

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The novel, potential anxiolytic, S 15535 (4-(benzodioxan-5-yl)1-(indan-2-yl)piperazine), is an agonist and antagonist (weak partial agonist) at pre- and postsynaptic serotonin 5-HT$_{1A}$ receptors, respectively. Herein, we characterized its influence on dialysate levels of 5-HT, dopamine (DA) and NAD simultaneously determined in single samples of the frontal cortex (FCX) of freely moving rats, and compared its activity in several other models of potential antidepressant (AD) properties with those of the 5-HT reuptake inhibitor (SSRI), fluoxetine. S 15535 displayed high affinity at cloned human (h) 5-HT$_{1A}$ receptors (Ki = 0.7 nM) and >250-fold lower affinity at cloned hD$_2$ (400 nM), hD$_3$ (248 nM) and h alpha2A-adrenergic (AR) (190 nM) receptors. S 15535 (0.08-5.0 mg/kg s.c.) markedly and dose-dependently suppressed dialysate levels of 5-HT in the FCX, nucleus accumbens and striatum of freely moving rats, whereas fluoxetine (10.0 mg/kg s.c.) elevated levels of 5-HT in each structure. In contrast to 5-HT, dialysate levels of DA and NAD in the FCX were dose-dependently increased by S 15535, and this effect was mimicked by fluoxetine. The influence of S 15535 and fluoxetine on FCX levels of DA was regionally specific inasmuch as dialysate levels of DA in the accumbens and striatum were not modified. The selective 5-HT$_{1A}$ antagonist, WAY100635 (N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl) cyclohexanecarboxamide (0.16) transiently elicited a slight increase in cortical levels of 5-HT, an action opposite to that of S 15535. Further, in the presence of WAY100635 (0.16), the influence of S 15535 (0.63) on cortical levels of 5-HT, DA and NAD was markedly attenuated. Upon chronic administration of S 15535 or fluoxetine (10.0 mg/kg s.c. daily for 14 days, in each case), there was no significant alteration in the density of beta-AR receptors in the FCX. However, in contrast to fluoxetine, S 15535 elicited a significant (25%) decrease in the density (Bmax) of 5-HT$_{2A}$ receptors labeled by [^{3}H]ketanserin in the cortex; there was no alteration in Kd. In a learned helplessness paradigm in rats, S 15535 (0.63-40.0 mg/kg p.o.) markedly reduced escape deficits on each of three consecutive days of testing. Fluoxetine (2.0-8.0 mg/kg i.p.) was also active in each session, but presented a biphasic dose-response curve. Finally, under the conditions used, neither S 15535 (0.63-10.0) nor fluoxetine (0.63-10.0) decreased immobility time in the forced swim test. In conclusion, S 15535 is a selective ligand of cloned, h5-HT$_{1A}$ receptors. Its agonist actions at 5-HT$_{1A}$ autoreceptors underlie its ability to decrease extracellular levels of 5-HT in the FCX, and likely contribute to the increase in extracellular levels of DA and NAD evoked by S 15535 in this structure. Further, S 15535 is active in several other, although not all, models of potential AD activity. Thus, although S 15535 is under development as an anxiolytic agent, a further characterization of its putative AD actions would be of interest.

Reference no. 10

Neuropharmacology. 1997 Apr-May;36(4-5):451-9.

Agonist and inverse agonist efficacy at human recombinant serotonin 5-HT_{1A} receptors as a function of receptor:G-protein stoichiometry.

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Membrane preparations were made from Chinese Hamster Ovary (CHO) cells expressing 1.6 and 4.2 pmol/mg of recombinant human 5-HT_{1A} receptors, as determined by saturation binding with the selective antagonist, [^{3}H]S 15535 ([^{3}H]4-(benzodioxan-5-yl)]-(indan-2-yl)piperazine). There was no change in the number of G-proteins activated by the full agonist, serotonin (5-HT; approximately 1.1 pmol/mg in each preparation, measured by [^{35}S]GTPgammaS saturation binding), therefore increasing the receptor:G-protein ratio from approximately 1.4:1 (RGlow) to approximately 4:1 (RGhigh). Agonist efficacy was measured by stimulation of [^{35}S]GTPgammaS binding. The serotonergic agonist, eltoprazine, behaved as a partial agonist (Emax = 52.7%) at RGlow membranes but virtually as a full agonist (Emax = 93.2%) at RGhigh membranes, relative to 5-HT (= 100%). The latter exhibited a two-fold shift to the left in its concentration-response curve in RGhigh compared to RGlow membranes ($P < 0.01$). WAY100635 (N-2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl-N-(2-pyridinyl) -cyclo-hexane-carboxamide), did not alter [^{35}S]GTPgammaS binding from basal levels in either membrane preparation. In contrast, spiperone displayed inverse agonist activity, decreasing [^{35}S]GTPgammaS binding from basal levels by 17% in RGlow membranes but by 28% in RGhigh membranes. These data indicate that an increased receptor:G-protein ratio (i) augments the potency of full agonists, (ii) increases the efficacy of partial agonists and (iii) increases the negative efficacy of inverse agonists at recombinant human 5-HT_{1A} receptors. Furthermore, these data suggest that spiperone induces, or stabilises, a G-protein-coupled, but inactive conformation of the receptor.

Reference no. 11

Eur J Pharmacol. 1997 May 30;327(2-3):247-56.

Binding profile of the novel 5-$HT_{1B}/_{1D}$ receptor antagonist, [^{3}H]GR125,743, in guinea-pig brain: a comparison with [^{3}H]5-carboxamidotryptamine.

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Native brain 5-HT_{1B}/1D) receptors were studied using the novel antagonist, [^{3}H]GR125,743 (N-[4-methoxy-3-(4-methylpiperazin-1-yl)phenyl]-3-methyl-4-(4-pyri dyl)benzamide). In guinea-pig striatal membranes, [^{3}H]GR125,743 displayed rapid association (t1/2 = 4.5 min), high (90%) specific binding and high affinity (Kd = 0.29 nM), although Bmax values (fmol/mg protein) varied according to brain region-striatum: 199; frontal cortex: 89; hippocampus: 79; cerebellum: 26. In frontal cortex, the B(max) determined with [^{3}H]5-CT ([^{3}H]carboxamidotryptamine) was significantly higher (178; P<0.05), suggesting that it also labels other binding sites. In striatal membranes, guanylylimidodiphosphate (GppNHp) inhibited [^{3}H]5-CT but not [^{3}H]GR125,743 binding, suggesting that the latter has antagonist properties. Nevertheless, in competition binding experiments, the pKi values obtained with [^{3}H]GR125,743 and [^{3}H]5-CT for 20 serotonergic ligands, including L694,247 (2-[5-[3-(4-methylsulphonylamino)benzyl-1,2,4-oxadiazol-5-yl]-1H-indole-3-yl]ethylamine), GR46,611 (3-[3-(2-dimethylamino-ethyl)-1H-indol-6-yl]-N-(4-methoxybenzyl)acrylami de), sumatriptan and alniditan, were highly correlated (r = 0.99). Ketanserin and ritanserin showed low affinity for [^{3}H]GR125,743 binding to guinea-pig striatal sites (Ki = 12600 and 369 nM), suggesting that 5-HT_{1B} (rather than 5-HT_{1D}) receptors are predominantly labelled in this tissue. The present data indicate that [^{3}H]GR125,743 is a useful tool for studying native 5-$HT_{1B}/_{1D}$ receptors.

Reference no. 12

J Pharmacol Exp Ther. 1997 Jul;282(1):181-91.

[^{35}S]Guanosine-5'-O-(3-thio)triphosphate binding as a measure of efficacy at human recombinant dopamine $D_{4.4}$ receptors: actions of antiparkinsonian and antipsychotic agents.

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Recombinant human dopamine $D_{4.4}$ receptor-mediated G protein activation was characterized in membranes of transfected mammalian (Chinese hamster ovary) cells by the use of [^{35}S]guanosine-5'-O-(3-thio)triphosphate ([^{35}S]GTPgammaS) binding. An initial series of experiments defined the conditions (3 microM GDP, 100 mM NaCl, 3 mM MgCl2) under which optimal stimulation (2.2-fold increase in specific [^{35}S]GTPgammaS binding) was achieved with the endogenous agonist dopamine. The number of dopamine-activated G-proteins in Chinese hamster ovary-$D_{4.4}$ membranes was determined through [^{35}S]GTPgammaS isotopic dilution saturation binding, yielding a Bmax value of 2.29 pmol/mg. This compared with a $D_{4.4}$ receptor Bmax value of 1.40 pmol/mg determined by [^{3}H]spiperone saturation binding, indicating that 1 or 2 G-proteins were activated per $D_{4.4}$ receptor and that there were few or no "spare receptors" in this cell line. Under these conditions, the efficacy for stimulation of [^{35}S]GTPgammaS binding at $D_{4.4}$ receptors of 12 dopaminergic agonists was determined. Several antiparkinsonian drugs, including ropinirole, quinerolane and lisuride, exhibited agonist activity at $D_{4.4}$ receptors (Emax = 74.3%, 72.4% and 32.2%, respectively, compared with dopamine = 100%). The EC_{50} values for agonist stimulation of [^{35}S]GTPgammaS binding correlated well with the inhibition constants derived from competition binding with [^{3}H]spiperone (r = +.99). However, other antiparkinsonian drugs (bromocriptine, L-DOPA and terguride) showed low affinity and/or were devoid of agonist activity at $D_{4.4}$ receptors. The potency at $D_{4.4}$ receptors of the novel, selective $D_{4.4}$ receptor antagonist L 745,870 was determined, indicating that it has high affinity (Ki = 1.99 nM) without detectable agonist activity. Furthermore, L745,870 completely inhibited dopamine-stimulated [^{35}S]GTPgammaS binding with a Kb value of 1.07 nM. The action of an additional 20 chemically diverse dopaminergic ligands, including clozapine, ziprasidone, sertindole, olanzapine and several other "atypical" antipsychotics, in advanced development was investigated. Each of these ligands shifted the dopamine stimulation curve to the right in a parallel manner consistent with competitive antagonism at this site and yielding Kb values (32.6, 22.4, 17.2 and 26.5 nM, respectively) that agreed closely with their Ki values (38.0, 14.9, 18.5 and 26.1 nM). In contrast, raclopride and seroquel exhibited low affinity at $D_{4.4}$ receptors (Ki > 1000 nM). Other compounds that showed antagonist activity at $D_{4.4}$ receptors included the 5-hydroxytryptamine$_{2A}$ receptor antagonist fananserin (RP62203), the sigma ligand BMY14,802 and the D_3 receptor antagonist GR103,691. In conclusion, dopamine $D_{4.4}$ receptor activity is unlikely to be an important factor in the clinical effectiveness of antiparkinsonian drugs, although low agonist efficacy at $D_{4.4}$ receptors might be associated with a lesser incidence of side effects. Furthermore, antagonist activity at $D_{4.4}$ receptors is a common property of many typical and atypical antipsychotic agents.

Reference no. 13

Naunyn Schmiedebergs Arch Pharmacol. 1997 Jun;355(6):682-8.

Agonist activity of antimigraine drugs at recombinant human 5-HT_{1A} receptors: potential implications for prophylactic and acute therapy.

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The actions of several serotonergic ligands in use or under development for the treatment of migraine headaches were examined at recombinant human 5-HT_{1A} receptors stably expressed in Chinese Hamster Ovary cells. Affinities (Kis) at this site were determined in competition binding experiments with [^{3}H]8-OH-DPAT ([^{3}H](+/-)8-hydroxy-N,N-dipropylaminotetralin), whilst agonist efficacy was measured by stimulation of [^{35}S]GTPgammaS (guanylyl-5'-[gamma[^{35}S]thio]-triphosphate) binding. Of the prophylactic antimigraine drugs tested, methysergide and lisuride behaved as efficacious agonists (Emax > or = 90% relative to 5-HT) whereas pitozifen and (-)propranolol acted as a partial agonist (60%) and an antagonist, respectively. This suggests that there is no correlation between agonism at 5-HT_{1A} receptors and prophylactic antimigraine action. In contrast, serotonin, dihydroergotamine, sumatriptan, naratriptan and alniditan, which are effective in acute interruption of migraine attacks, each displayed high efficacy (Emax = 100, 100, 92.6, 79.3, 79.1% respectively) and marked affinity (Ki = 18.7, 0.6, 127, 26.4 and 3.0 nM respectively) at 5-HT_{1A} receptors. EC_{50} values for agonist stimulation of [^{35}S]GTPgammaS binding correlated with respective Ki values at 5-HT_{1A} receptors (r = 0.93) and the stimulation of [^{35}S]GTPgammaS binding by these compounds was antagonised by the selective 5-HT_{1A} antagonist WAY100635 (N-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-N-(2-pyridinyl) cyclo-hexanecarboxamide; 100 nM). These data suggest that agonism at 5-HT_{1A} receptors may be involved in some actions of drugs used in acute antimigraine therapy. In comparison with the above compounds, novel ligands targeted at 5-HT_{1B}/$_{1D}$ receptors, such as GR125,743 (N-[4-methoxy-3-(4-methyl-piperazin-1-yl)phenyl] -3-methyl-4-(4-pyridyl)benzamide) and GR127,935 (N-[4-methoxy-3-(4-methylpiperazin-1-yl)-phenyl]-2'-methyl-4'-(5-m ethyl-1, 2,4-oxadiazol-3-yl)-biphenyl-4-carboxamide), only weakly activated [^{35}S]GTPgammaS binding (32.4 and 32.1% efficacy) and displayed moderate affinity at 5-HT_{1A} receptors (Kis 53.1 and 49.8 nM) suggesting that they constitute useful tools to differentiate 5-HT_{1A} and 5-HT_{1B}/$_{1D}$ receptor-mediated actions. In conclusion, the present data indicates that several antimigraine agents exhibit marked 5-HT_{1A} receptor activity and that although this is unlikely to be important for prophylactic action it may be relevant to the ancilliary properties of drugs used for acute migraine treatment.

Reference no. 14

J Pharmacol Exp Ther. 1998 Sep;286(3):1341-55.

S 16924 ((R)-2-[1-[2-(2,3-dihydro-benzo[1,4] dioxin-5-Yloxy)-ethyl]-pyrrolidin-3yl]-1-(4-fluoro-phenyl)-ethanone), a novel, potential antipsychotic with marked serotonin (5-HT)$_{1A}$ agonist properties: I. Receptorial and neurochemical profile in comparison with clozapine and haloperidol.

Millan MJ, Gobert A, **Newman-Tancredi A**, Audinot V, Lejeune F, Rivet JM, Cussac D, Nicolas JP, Muller O, Lavielle G.
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S 16924 showed a pattern of interaction at multiple (>20) native, rodent and cloned, human (h) monoaminergic receptors similar to that of clozapine and different to that of haloperidol. Notably, like clozapine, the affinity of S 16924 for hD_2 and hD_3 receptors was modest, and it showed 5-fold higher affinity for hD_4 receptors. At each of these sites, using a [^{35}S]GTPgammaS binding procedure, S 16924, clozapine and haloperidol behaved as antagonists. In distinction to haloperidol, S 16924 shared the marked affinity of clozapine for h5-HT_{2A} and h5-HT_{2C} receptors. However, an important difference to clozapine (and haloperidol) was the high affinity of S 16924 for h5-HT_{1A} receptors. At these sites, using a [^{35}S]GTPgammaS binding model, both S 16924 and clozapine behaved as partial agonists, whereas haloperidol was inactive. *In vivo*, the agonist properties of S 16924 at 5-HT_{1A} autoreceptors were revealed by its ability to potently inhibit the firing of raphe-localized serotoninergic neurones, an action reversed by the selective 5-HT_{1A} receptor antagonist, WAY100635. In contrast, clozapine and haloperidol only weakly inhibited raphe firing, and their actions were resistant to WAY100635. Similarly, S 16924 more potently inhibited striatal turnover of 5-HT than either clozapine or haloperidol. Reflecting its modest affinity for D_2 (and D_3) autoreceptors, S 16924 only weakly blocked the inhibitory influence of the dopaminergic agonist, apomorphine, upon the firing rate of ventrotegmental area-localized dopaminergic neurones. Further, S 16924 only weakly increased striatal, mesolimbic and mesocortical turnover of dopamine (DA). Clozapine was, similarly, weakly active in these models, whereas haloperidol, in line with its higher affinity at D_2 (and D_3) receptors, was potently active. In the frontal cortex (FCX) of freely moving rats, S 16924 dose-dependently reduced dialysate levels of 5-HT, whereas those of DA and NAD were dose-dependently increased in the same samples. In contrast, although S 16924 also suppressed 5-HT levels in the striatum and nucleus accumbens, DA levels therein were unaffected. Clozapine mimicked this selective increase in DA levels in the FCX as compared to striatum and accumbens. In contrast, haloperidol modestly increased DA levels in the FCX, striatum and accumbens to the same extent. In distinction to S 16924, clozapine and haloperidol exerted little influence upon 5-HT levels. Finally, the influence of S 16924 upon FCX levels of 5-HT, DA (and NAD) was attenuated by WAY100635. In conclusion, S 16924 possesses a profile of interaction at multiple monoaminergic receptors comparable to that of clozapine and distinct to that of haloperidol. In addition, S 16924 is a potent, partial agonist at 5-HT_{1A} receptors. Correspondingly, acute administration of S 16924 decreases cerebral serotoninergic transmission and selectively reinforces frontocortical as compared to subcortical dopaminergic transmission. In line with these actions, S 16924 shows a distinctive profile of activity in functional (behavioral) models of potential antipsychotic activity (companion paper).

Reference no. 15

Neuropsychopharmacology. 1998 May;18(5):395-8.

Agonist and antagonist actions of (-)pindolol at recombinant, human serotonin$_{1A}$ (5-HT$_{1A}$) receptors.

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It has been proposed that the arylalkylamine, (-)pindolol, potentiates the therapeutic action of antidepressant drugs in humans by blockade of 5-HT$_{1A}$ autoreceptors. Its interactions at human 5-HT$_{1A}$ receptors have not, however, been directly characterized. Herein, we demonstrate that (-)pindolol exhibits nanomolar affinity at human 5-HT$_{1A}$ receptors expressed in Chinese Hamster Ovary cells (CHO-h5-HT$_{1A}$; Ki = 6.4 nmol/L). In a functional test of receptor-mediated G-protein activation (stimulation of [^{35}S]GTPgammaS binding) (-)pindolol displays an efficacy of 20.3% relative to the endogenous agonist, 5-HT (= 100%). (-)Pindolol also antagonizes 5-HT (100 nmol/L)-stimulated [^{35}S]GTPgammaS binding, reducing it to 19.8% of control binding. These data indicate that (-)pindolol acts as a (weak) partial agonist at CHO-h5-HT$_{1A}$ receptors and that it blocks the action of 5-HT at these sites.

Reference no. 16

Naunyn Schmiedebergs Arch Pharmacol. 1998 Mar;357(3):205-17.

Labelling of recombinant human and native rat serotonin 5-HT_{1A} receptors by a novel, selective radioligand, [^{3}H]S 15535: definition of its binding profile using agonists, antagonists and inverse agonists.

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The novel benzodioxopiperazine, 5-HT_{1A} receptor weak partial agonist, S 15535 (4-(benzodioxan-5-yl)1-(indan-2-yl)piperazine) bound with high affinity and selectivity to membranes of Chinese Hamster Ovary cells stably expressing the human (h) 5-HT_{1A} receptor (Ki = 0.6 nM versus [^{3}H]8-hydroxy-dipropylamino-tetralin, [^{3}H]8-OH-DPAT): its affinity at h5-HT_{1A} receptors was more than 70-fold higher than its affinity at > 50 other binding sites. S 15535 was tritiated to high specific activity (50 Ci/mmol) and its binding profile characterised. At 22 degrees C, [^{3}H]S 15535 associated and dissociated from h5-HT_{1A} receptors with half-times of 2.9 and 5.0 min, respectively, yielding a Kd estimate of 3.6 nM. In saturation binding experiments, [^{3}H]S 15535 displayed a Bmax value for h5-HT_{1A} receptors (1630 fmol/mg), higher than that obtained with the agonist [^{3}H]8-OH-DPAT (1023 pmol/mg). Guanylyl imidodiphosphate (GppNHp, 100 microM) reduced the binding of [^{3}H]S 15535 by only 25% compared with 79% for [^{3}H]8-OH-DPAT at h5-HT_{1A} receptors. [^{3}H]S 15535 also showed high affinity, saturable binding to rat hippocampal membranes (Bmax = 820 fmol/mg versus 647 fmol/mg for [^{3}H]8-OH-DPAT). For both h5-HT_{1A} and rat 5-HT_{1A} receptors, the Ki values for competition binding of 15 serotonergic ligands with [^{3}H]S 15535 was highly correlated with that of [^{3}H]8-OH-DPAT. However, important differences were also observed. The agonist, 5-hydroxytryptamine (5-HT), displayed biphasic competition curves with [^{3}H]S 15535 but not with [^{3}H]8-OH-DPAT at h5-HT_{1A} receptors. Similarly, the 'antagonists', spiperone, methiothepin and (+)butaclamol, showed biphasic competition isotherms versus [^{3}H]S 15535 but not [^{3}H]8-OH-DPAT. When [^{3}H]S 15535 competition binding experiments were carried out in the presence of GppNHp (100 microM) the 5-HT and 8-OH-DPAT competition curves shifted to the right, whereas the spiperone and methiothepin competition curves shifted to the left. In contrast, in the presence of GppNHp, the competition isotherms for N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl)cyclo-h exanecarboxamide (WAY100635) were not altered. Taken together, these data show that (i) [^{3}H]S 15535 is a highly selective 5-HT_{1A} receptor ligand which labels both G-protein-coupled and uncoupled 5-HT_{1A} receptors, (ii) antagonists, such as WAY100635, which yield monophasic isotherms in competition with both [^{3}H]agonists and [^{3}H]antagonists, are not sensitive to the G-protein coupling state of the receptor, but (iii) spiperone and methiothepin behaved as inverse agonists, their competition isotherms with [^{3}H]S 15535 being modulated in an opposite manner to those of agonists.

Reference no. 17

Naunyn Schmiedebergs Arch Pharmacol. 1998 Aug;358(2):197-206.

Actions of alpha2 adrenoceptor ligands at alpha$_{2A}$ and 5-HT$_{1A}$ receptors: the antagonist, atipamezole, and the agonist, dexmedetomidine, are highly selective for alpha$_{2A}$ adrenoceptors.

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This study examined the activity of chemically diverse alpha2 adrenoceptor ligands at recombinant human (h) and native rat (r) alpha$_{2A}$ adrenoceptors compared with 5-HT$_{1A}$ receptors. First, in competition binding experiments at h alpha$_{2A}$ and h5-HT$_{1A}$ receptors expressed in CHO cells, several compounds, including the antagonists 1-(2-pyrimidinyl)piperazine (1-PP), (+/-)-idazoxan, benalfocin (SKF 86466), yohimbine and RX 821,002, displayed preference for h alpha$_{2A}$ versus h5-HT$_{1A}$ receptors of only 1.4-, 3.6-, 4-, 10- and 11-fold, respectively (based on differences in pKi values). Clonidine, brimonidine (UK 14304), the benzopyrrolidine fluparoxan and the guanidines guanfacine and guanabenz exhibited intermediate selectivity (22- to 31-fold) for h alpha$_{2A}$ receptors. Only the antagonist atipamezole and the agonist dexmedetomidine (DMT) displayed high preference for alpha2 adrenoceptors (1290- and 91-fold, respectively). Second, the compounds were tested for their ability to induce h5-HT$_{1A}$ receptor-mediated G-protein activation, as indicated by the stimulation of [^{35}S]GTPgammaS binding. All except atipamezole and RX 821,002 exhibited agonist activity, with potencies which correlated with their affinity for h5-HT$_{1A}$ receptors. Relative efficacies (Emax values) were 25-35% for guanabenz, guanfacine, WB 4101 and benalfocin, 50-65% for 1-PP, (+/-)-idazoxan and clonidine, and over 70% for fluparoxan, oxymetazoline and yohimbine (relative to 5-HT = 100%). Yohimbine-induced [^{35}S]GTPgammaS binding was inhibited by the selective 5-HT$_{1A}$ receptor antagonist WAY100635. In contrast, RX 821,002 was the only ligand which exhibited antagonist activity at h5-HT$_{1A}$ receptors, inhibiting 5-HT-stimulated [^{35}S]GTPgammaS binding. Atipamezole, which exhibited negligeable affinity for 5-HT$_{1A}$ receptors, was inactive. Third, the affinities for r alpha$_{2A}$ differed considerably from the affinities for h alpha$_{2A}$ receptors whereas the affinities for r5-HT$_{1A}$ differed much less from the affinities for h5-HT$_{1A}$ receptors. This affected markedly the affinity ratios of certain compounds. For example, (+/-)-idazoxan was only 3.6-fold selective for h alpha$_{2A}$ versus h5-HT$_{1A}$ but 51-fold selective for r alpha$_{2A}$ versus r5-HT$_{1A}$ receptors. Conversely, yohimbine was tenfold selective for h alpha$_{2A}$ versus h5-HT$_{1A}$ adrenoceptors but 4.2-fold selective for r alpha$_{2A}$ versus r5-HT$_{1A}$ receptors. Nevertheless, both atipamezole and DMT were highly selective for both rat and human alpha$_{2A}$ versus rat or human 5-HT$_{1A}$ receptors. In conclusion, these data indicate that: (1) the agonist DMT and the antagonist atipamezole are the ligands of choice to distinguish alpha2-mediated from 5-HT$_{1A}$-mediated actions, whilst several of the other compounds show only low or modest selectivity for alpha$_{2A}$ over 5-HT$_{1A}$ receptors; (2) caution should be exercised in experimental and clinical interpretation of the actions of traditionally employed alpha2 ligands, such as clonidine, yohimbine and (+/-)-idazoxan, which exhibit marked agonist activity at 5-HT$_{1A}$ receptors.

Reference no. 18

J Pharmacol Exp Ther. 1998 Oct;287(1):167-86.

S 18126 ([2-[4-(2,3-dihydrobenzo[1,4]dioxin-6-yl)piperazin-1-yl methyl]indan-2-yl]), a potent, selective and competitive antagonist at dopamine D_4 receptors: an *in vitro* and *in vivo* comparison with L 745,870 (3-(4-[4-chlorophenyl]piperazin-1-yl)methyl-1H-pyrrolo[2, 3b]pyridine) and raclopride.

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The novel benzoindane S 18126 possessed > 100-fold higher affinity at cloned, human (h) D_4 (Ki = 2.4 nM) vs. hD_2 (738 nM), hD_3 (2840 nM), hD1 (> 3000 nM) and hD5 (> 3000 nM) receptors and about 50 other sites, except sigma1 receptors (1.6 nM). L 745,870 similarly showed selectivity for hD_4 (2.5 nM) vs. hD_2 (905 nM) and hD_3 (> 3000 nM) receptors. In contrast, raclopride displayed low affinity at hD_4 (> 3000 nM) vs. hD_2 (1.1 nM) and hD_3 receptors (1.4 nM). Stimulation of [^{35}S]-GTPgammaS binding at hD_4 receptors by dopamine (DA) was blocked by S 18126 and L 745,870 with Kb values of 2.2 and 1.0 nM, respectively, whereas raclopride (> 1000 nM) was inactive. In contrast, raclopride inhibited stimulation of [^{35}S]-GTPgammaS binding at hD_2 sites by DA with a Kb of 1.4 nM, whereas S 18126 (> 1000 nM) and L 745,870 (> 1000 nM) were inactive. As concerns presynaptic dopaminergic receptors, raclopride (0.01-0.05 mg/kg s.c.) markedly enhanced DA synthesis in mesocortical, mesolimbic and nigrostriatal dopaminergic pathways. In contrast, even high doses (2. 5-40.0 mg/kg s.c.) of S 18126 and L 745,870 were only weakly active. Similarly, raclopride (0.016 mg/kg i.v.) abolished inhibition of the firing rate of ventrotegmental dopaminergic neurons by apomorphine, whereas even high doses (0.5 mg/kg i.v.) of S 18126 and L 745,870 were only weakly active. As regards postsynaptic dopaminergic receptors, raclopride potently (0.01-0.3 mg/kg s.c.) reduced rotation elicited by quinpirole in rats with unilateral lesions of the substantia nigra, antagonized induction of hypothermia by PD 128, 907, blocked amphetamine-induced hyperlocomotion and was effective in six further models of potential antipsychotic activity. In contrast, S 18126 and L 745,870 were only weakly active in these models (5.0-> 40.0 mg/kg s.c.). In six models of extrapyramidal and motor symptoms, such as induction of catalepsy, raclopride was likewise potently active (0.01-2.0 mg/kg s.c.) whereas S 18126 and L 745,870 were only weakly active (10.0-80.0 mg/kg s.c.). In freely moving rats, raclopride (0.16 mg/kg s.c.) increased levels of DA by + 55% in dialysates of the frontal cortex. However, it also increased levels of DA in the accumbens and striatum by 70% and 75%, respectively. In contrast to raclopride, at a dose of 0.16 mg/kg s.c. , neither S 18126 nor L 745,870 modified frontal cortex levels of DA. However, at a high dose (40.0 mg/kg s.c.), S 18126 increased dialysate levels of DA (+ 85%) and noradrenaline (+ 100%), but not serotonin (+ 10%), in frontal cortex without affecting DA levels in accumbens (+ 10%) and striatum (+ 10%). In conclusion, S 18126 and L 745,870 behave as potent and selective antagonists of cloned, hD_4 vs. other dopaminergic receptor types *in vitro*. However, their *in vivo* effects at high doses probably reflect residual antagonist actions at D_2 (or D_3) receptors. Selective blockade of D_4 receptors was thus associated neither with a modification of dopaminergic transmission nor with antipsychotic (antiproductive) or extrapyramidal properties. The functional effects of selective D_4 receptor blockade remain to be established.

Reference no. 19

Eur J Pharmacol. 1998 Aug 21;355(2-3):245-56.

Agonist and antagonist actions of antipsychotic agents at 5-HT_{1A} receptors: a [^{35}S]GTPgammaS binding study.

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Recombinant human (h) 5-HT_{1A} receptor-mediated G-protein activation was characterised in membranes of transfected Chinese hamster ovary (CHO) cells by use of guanosine-5'-O-(3-[^{35}S]thio)-triphosphate ([^{35}S]GTPgammaS binding). The potency and efficacy of 21 5-HT receptor agonists and antagonists was determined. The agonists, 5-CT (carboxamidotryptamine) and flesinoxan displayed high affinity (subnanomolar Ki values) and high efficacy (Emax > 90%, relative to 5-HT = 100%). In contrast, ipsapirone, zalospirone and buspirone displayed partial agonist activity. EC_{50}s for agonist stimulation of [^{35}S]GTPgammaS binding correlated well with Ki values from competition binding (r=+0.99). Among the compounds tested for antagonist activity, methiothepin and (+)butaclamol exhibited 'inverse agonist' behaviour, inhibiting basal [^{35}S]GTPgammaS binding. The actions of 17 antipsychotic agents were investigated. Clozapine and several putatively 'atypical' antipsychotic agents, including ziprasidone, quetiapine and tiospirone, exhibited partial agonist activity and marked affinity at h5-HT_{1A} receptors, similar to their affinity at hD_2 dopamine receptors. In contrast, risperidone and sertindole displayed low affinity at h5-HT_{1A} receptors and behaved as 'neutral' antagonists, inhibiting 5-HT-stimulated [^{35}S]GTPgammaS binding. Likewise the 'typical' neuroleptics, haloperidol, pimozide, raclopride and chlorpromazine exhibited relatively low affinity and 'neutral' antagonist activity at h5-HT_{1A} receptors with Ki values which correlated with their respective Kb values. The present data show that (i) [^{35}S]GTPgammaS binding is an effective method to evaluate the efficacy and potency of agonists and antagonists at recombinant human 5-HT_{1A} receptors. (ii) Like clozapine, several putatively 'atypical' antipsychotic drugs display balanced serotonin h5-HT_{1A}/dopamine hD_2 receptor affinity and partial agonist activity at h5-HT_{1A} receptors. (iii) Several 'typical' and some putatively 'atypical' antipsychotic agents displayed antagonist properties at h5-HT_{1A} sites with generally much lower affinity than at hD_2 dopamine receptors. It is suggested that agonist activity at 5-HT_{1A} receptors may be of utility for certain antipsychotic agents.

Reference no. 20

Mol Pharmacol. 1999 Mar;55(3):564-74.

G protein activation by human dopamine D_3 receptors in high-expressing Chinese hamster ovary cells: A guanosine-5'-O-(3-[^{35}S]thio)-triphosphate binding and antibody study.

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Despite extensive study, the G-protein coupling of dopamine D_3 receptors is poorly understood. In this study, we used guanosine-5'-O-(3-[^{35}S]thio)-triphosphate ([^{35}S]GTPgammaS) binding to investigate the activation of G-proteins coupled to human (h) D_3 receptors stably expressed in Chinese hamster ovary (CHO) cells. Although the receptor expression level was high (15 pmol/mg), dopamine only stimulated G protein activation by 1.6-fold. This was despite the presence of marked receptor reserve for dopamine, as revealed by Furchgott analysis after irreversible hD_3 receptor inactivation with the alkylating agent, EEDQ (N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline). Thus, half-maximal stimulation of [^{35}S]-GTPgammaS binding required only 11.8% receptor occupation of hD_3 sites. In contrast, although the hD_2(short) receptor expression level in another CHO cell line was 11-fold lower, stimulation by dopamine was higher (2.5-fold). G-protein activation was increased at hD_3 and, less potently, at hD_2 receptors by the preferential D_3 agonists, PD 128,907 [(+)-(4aR,10bR)-3,4,4a, 10b-tetrahydro-4-propyl-2H,5H- [1]benzopyrano[4,3-b]-1, 4-oxazin-9-ol] and (+)-7-OH-DPAT (7-hydroxy-2-(di-n-propylamino)tetralin). Furthermore, the selective D_3 antagonists, S 14297 ((+)-[7-(N, N-dipropylamino)-5,6,7, 8-tetrahydro-naphtho(2,3b)dihydro-2,3-furane]) and GR 218,231 (2(R, S)-(dipropylamino)-6-(4-methoxyphenylsulfonylmethyl)-1,2,3,4-tetrahydronaphtalene), blocked dopamine-stimulated [^{35}S]GTPgammaS binding more potently at hD_3 than at hD_2 sites. Antibodies against Galphai/alphao reduced dopamine-induced G-protein activation at both CHO-hD_3 and -hD_2 membranes, whereas GalphaS antibodies had no effect at either site. In contrast, incubation with anti-Galphaq/alpha11 antibodies, which did not affect dopamine-induced G-protein activation at hD_2 receptors, attenuated hD_3-induced G protein activation. These data suggest that hD_3 receptors may couple to Galphaq/alpha11 and would be consistent with the observation that pertussis toxin pretreatment, which inactivates only Gi/o proteins, only submaximally (80%) blocked dopamine-stimulated [^{35}S]GTPgammaS binding in CHO-hD_3 cells. Taken together, the present data indicate that 1) hD_3 receptors functionally couple to G protein activation in CHO cells, 2) hD_3 receptors activate G-proteins less effectively than hD_2 receptors, and 3) hD_3 receptors may couple to different G-protein subtypes than hD_2 receptors, including nonpertussis sensitive Gq/11 proteins.

Reference no. 21

Eur J Neurosci. 1999 Dec;11(12):4419-32.

Contrasting mechanisms of action and sensitivity to antipsychotics of phencyclidine versus amphetamine: importance of nucleus accumbens 5-HT_{2A} sites for PCP-induced locomotion in the rat.

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In the present study, the comparative mechanisms of action of phencyclidine (PCP) and amphetamine were addressed employing the parameter of locomotion in rats. PCP-induced locomotion (PLOC) was potently blocked by the selective serotonin 5-HT_{2A} vs. D_2 antagonists, SR46349, MDL100,907, ritanserin and fananserin, which barely affected amphetamine-induced locomotion (ALOC). In contrast, the selective D_2 vs. 5-HT_{2A} antagonists, eticlopride, raclopride and amisulpride, preferentially inhibited ALOC vs. PLOC. The potency of these drugs and 12 multireceptorial antipsychotics in inhibiting PLOC vs. ALOC correlated significantly with affinities at 5-HT_{2A} vs. D_2 receptors, respectively. Amphetamine and PCP both dose dependently increased dialysate levels of dopamine (DA) and 5-HT in the nucleus accumbens, striatum and frontal cortex (FCX) of freely moving rats, but PCP was proportionally more effective than amphetamine in elevating levels of 5-HT vs. DA in the accumbens. Further, whereas microinjection of PCP into the accumbens elicited locomotion, its introduction into the striatum or FCX was ineffective. The action of intra-accumbens PCP, but not intra-accumbens amphetamine, was abolished by SR46349 and clozapine. Parachloroamphetamine, which depleted accumbens pools of 5-HT but not DA, likewise abolished PLOC without affecting ALOC. In contrast, intra-accumbens 6-hydroxydopamine (6-OHDA), which depleted DA but not 5-HT, abolished ALOC but only partially attenuated PLOC. In conclusion, PLOC involves (indirect) activation of accumbens-localized 5-HT_{2A} receptors by 5-HT. PLOC is, correspondingly, more potently blocked than ALOC by antipsychotics displaying marked affinity at 5-HT_{2A} receptors.

Reference no. 22

Naunyn Schmiedebergs Arch Pharmacol. 1999 Jun;359(6):447-53.

Actions of roxindole at recombinant human dopamine D_2, D_3 and D_4 and serotonin 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors.

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Roxindole is a potential antidepressant agent. The present study determined its affinity and agonist efficacy at recombinant human (h) dopamine hD_2, hD_3 and hD_4 and serotonin (5-HT) h5-HT_{1A}, h5-HT_{1B} and h5-HT_{1D} receptors. Roxindole exhibited high affinity at hD_3 as well as at hD_2 (short isoform) and hD_4 (4-repeat isoform) receptors (pKi values 8.93, 8.55 and 8.23, respectively). Further, it displayed high affinity at hS-HT_{1A} receptors (pKi = 9.42) but modest affinity at 5-HT_{1B} and 5-HT_{1D} receptors (pKi values 6.00 and 7.05, respectively). In [^{35}S]GTPgammaS binding experiments, roxindole was >20-fold more potent in stimulating [^{35}S]GTPgammaS binding at hD_3 than at hD_2 or hD_4 receptors (pEC_{50} = 9.23 vs. 7.88 and 7.69). However, whereas roxindole exhibited partial agonist activity at hD_3 and hD_4 sites (Emax = 30.0% and 35.1%, respectively, relative to dopamine = 100%), it only weakly activated hD_2 receptors (Emax = 10.5%). Roxindole potently blocked dopamine-stimulated [^{35}S]GTPgammaS binding at hD_2 receptors (pK_B = 9.05). In comparison, the dopamine receptor agonist, (-)quinpirole, acted as a partial agonist at hD_3 and hD_4 sites (Emax = 67.4% and 66.3%, respectively) but surpassed the efficacy of dopamine at hD_2 receptors (Emax = 132%). At h5-HT_{1A} receptors, roxindole behaved as a high affinity (pKi = 9.42) partial agonist (Emax = 59.6%, relative to 5-HT = 100%), whereas (-)quinpirole had negligible activity. The selective 5-HT_{1A} antagonist, WAY100635, blocked roxindole (100 nM)-stimulated [^{35}S]GTPgammaS binding at h5-HT_{1A} receptors in a concentration-dependent manner (pK_B = 9.28). Roxindole only weakly stimulated [^{35}S]GTPgammaS binding at 5-HT_{1B} and 5-HT_{1D} receptors (Emax = 27.1% and 13.7%). The present data suggest that roxindole activates mainly D_3 vs. D_2 or D_4 receptors and 5-HT_{1A} vs. 5-HT_{1B} or 5-HT_{1D} receptors. Activation of D_3 and/or 5-HT_{1A} receptors may thus contribute to its potential antidepressant properties.

Reference no. 23

Mol Pharmacol. 1999 Nov;56(5):1025-30.

Human dopamine D_3 receptors mediate mitogen-activated protein kinase activation via a phosphatidylinositol 3-kinase and an atypical protein kinase C-dependent mechanism.

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The mitogen-activated protein kinase (MAPK) cascade is stimulated by both receptor tyrosine kinases and G protein-coupled receptors. We show that recombinant human dopamine D_3 receptors expressed in Chinese hamster ovary cells transiently activate MAPK via pertussis toxin-sensitive Gi and/or Go proteins. The involvement of D_3 receptors was confirmed by use of the D_3 agonists PD128,907 and (+)-7-hydroxy-2-dipropylaminotetralin, which mimicked the response to dopamine (DA). Furthermore, haloperidol and the selective D_3 receptor antagonists S 14297 and GR 218,231 attenuated DA-induced MAPK activation; however, when tested alone, S 14297 weakly stimulated MAPK activity, suggesting partial agonist activity. The transduction mechanisms by which hD_3 receptors activate MAPK were explored with specific kinase inhibitors. Genistein and lavendustin A, inhibitors of tyrosine kinase activity, did not reduce DA-induced MAPK activation. In contrast, PD 98059, an inhibitor of MAPK kinase, and Ro 31-8220 and Gö 6983, inhibitors of protein kinase C (PKC), blocked DA-induced MAPK activation. However, MAPK activation was insensitive to PKC down-regulation by phorbol esters, indicating the involvement of an "atypical" PKC. Furthermore, MAPK activation involved phosphatidylinositol 3-kinase inasmuch as its inhibition by LY 294002 and wortmannin reduced DA-induced MAPK activation. In conclusion, this study demonstrates that stimulation of hD_3 receptors activates MAPK. This action is mediated via an atypical isoform of PKC, possibly involving cross-talk with products of phosphatidylinositol 3-kinase activation.

Reference no. 24

Eur J Pharmacol. 1999 Nov 19;384(2-3):111-21.

The $5HT_{1A}$ receptor ligand, S15535, antagonises G-protein activation: a [^{35}S]GTPgammaS and [^{3}H]S15535 autoradiography study.

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4-(Benzodioxan-5-yl)1-(indan-2-yl)piperazine (S15535) is a highly selective ligand at 5-HT_{1A} receptors. The present study compared its autoradiographic labelling of rat brain sections with its functional actions, visualised by guanylyl-5'-[gamma-thio]-triphosphate ([^{35}S]GTPgammaS) autoradiography, which affords a measure of G-protein activation. [^{3}H]S15535 binding was highest in hippocampus, frontal cortex, entorhinal cortex, lateral septum, interpeduncular nucleus and dorsal raphe, consistent with specific labelling of 5-HT_{1A} receptors. In functional studies, S15535 (10 microM) did not markedly stimulate G-protein activation in any brain region, but abolished the activation induced by the selective 5-HT_{1A} agonist, (+)-8-hydroxy-dipropyl-aminotetralin ((+)-8-OH-DPAT, 1 microM), in structures enriched in [^{3}H]S15535 labelling. S15535 did not block 5-HT-stimulated activation in caudate nucleus or substantia nigra, regions where (+)-8-OH-DPAT was ineffective and [^{3}H]S15535 binding was absent. Interestingly, S15535 attenuated (+)-8-OH-DPAT and 5-HT-stimulated G-protein activation in dorsal raphe, a region in which S15535 is known to exhibit agonist properties *in vivo* [Lejeune, F., Millan, M.J., 1998. Induction of burst firing in ventral tegmental area dopaminergic neurons by activation of serotonin 5-HT_{1A} receptors: WAY100635-reversible actions of the highly selective ligands, flesinoxan and S15535. Synapse 30, 172-180.]. The present data show that (i) [^{3}H]S15535 labels pre- and post-synaptic populations of 5-HT_{1A} sites in rat brain sections, (ii) S15535 exhibits antagonist properties at post-synaptic 5-HT_{1A} receptors in corticolimbic regions, and (iii) S15535 also attenuates agonist-stimulated G-protein activation at raphe-localised 5-HT_{1A} receptors.

Reference no. 25

Neuropsychopharmacology. 1999 Aug;21(2 Suppl):61S-67S.

Inverse agonists and serotonergic transmission: from recombinant, human serotonin 5-HT_{1B} receptors to G-protein coupling and function in corticolimbic structures *in vivo*.

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The concept of inverse agonism, whereby "antagonists" exert actions opposite to those of agonists at constitutively active receptors, has been documented both at receptor-modulated ion channels as well as at G-protein-coupled receptors (GPCR) in recombinant expression systems. However, it remains unclear whether physiologically or therapeutically relevant inverse agonists actions at GPCRs occur in the CNS *in vivo*. The present overview discusses our recent observations concerning 5-HT_{1B} receptors, and focuses on the relationship between actions at heterologous Chinese hamster ovary (CHO) expression systems compared with native CNS populations of receptors. To this end, we have exploited several novel and selective ligands, notably the inverse agonist and neutral antagonist at 5-HT_{1B} receptors, SB224,289 and S18127, respectively. Like 5-HT itself, the agonist, GR46611, markedly increases the binding of [35S]GTPgamma S binding to h5-HT_{1B} receptors expressed in CHO cells, while the "antagonist", GR127,935, modestly stimulates binding suggesting partial agonist properties. However, SB224,289 markedly suppresses binding at these sites. S18127, which does not alter [^{35}S]GTPgammaS binding alone, abolishes the actions of both GR46611 and SB224,289. Nevertheless, in quantitative autoradiographical studies, S18127 and SB224,289 cannot be distinguished as concerns modulation of [35S]GTPgamma S binding at substantia nigra and caudate nucleus-localized 5-HT_{1B} receptors, inasmuch as they each block the action of the 5-HT_{1B} agonist, CP93129, yet fail to modify binding alone. Further, S18217 and SB224,289, as well as GR127,935, all abolish the inhibitory influence of GR46611 upon dialysis levels of 5-HT in the frontal cortex of freely moving rats without themselves modifying release. Moreover, they all block the hypothermic actions of GR46611 without themselves modifying core temperature. Thus, differences in intrinsic activity of S18127, SB224,289 and GR127,935 seen at cloned, h5-HT_{1B} receptors cannot be detected *in vivo*. Most notably, no evidence for opposite actions of the inverse agonist, SB224,289, as compared to 5-HT_{1B} agonists is apparent. These data suggest that *in vitro* observations of inverse agonist actions cannot necessarily be extrapolated to intact systems *in vivo*.

Reference no. 26

Naunyn Schmiedebergs Arch Pharmacol. 2000 Feb;361(2):221-3.

An innovative method for rapid characterisation of phospholipase C activity: SB242,084 competitively antagonises 5-HT_{2C} receptor-mediated [^{3}H]phosphatidylinositol depletion.

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6-chloro-5-methyl-1-[6-(2-methylpyridin-3-yloxy) pyridin-3-ylcarbamoyl] indoline (SB242,084) is a novel, selective 5-HT_{2C} receptor antagonist, but its actions at these sites have been little characterised at the cellular level. We employed a rapid and innovative approach to investigate its functional activity at phospholipase C (PLC)-coupled human 5-HT_{2C} receptors expressed in CHO cells. PLC activity was determined as a decrease in the [^{3}H]phosphatidylinositol ([^{3}H]PI) content of cell membranes. Serotonin (5-HT) stimulated [^{3}H]PI depletion (pEC_{50}=8.74), and SB242,084, like mesulergine, completely reversed this action of 5-HT (pK_B=9.25 and 9.01, respectively). Further, in Schild analysis, SB242,084 behaved as a high affinity competitive antagonist, inducing a parallel, rightward displacement of the 5-HT stimulation isotherm without loss of maximum efficacy. The pA2 of 9.50 was similar to its binding affinity (pKi=9.38). SB242,084 also displayed antagonist properties when PLC activity was examined by conventional determination of [^{3}H]inositol phosphate generation. Employing this parameter, the potency of SB242,084 (pK_B=9.21) and that of mesulergine (pK_B=9.06) closely resembled those determined by [^{3}H]PI depletion. In conclusion, determination of [^{3}H]PI depletion constitutes a useful and novel technique to characterise agonist and antagonist properties of ligands at PLC-coupled receptors.

Reference no. 27

J Pharmacol Exp Ther. 2000 Jan;292(1):38-53.

S18327 (1-[2-[4-(6-fluoro-1, 2-benzisoxazol-3-yl)piperid-1-yl]ethyl]3-phenyl imidazolin-2-one), a novel, potential antipsychotic displaying marked antagonist properties at alpha(1)- and alpha(2)-adrenergic receptors: I. Receptorial, neurochemical, and electrophysiological profile.

Millan MJ, Gobert A, **Newman-Tancredi A**, Lejeune F, Cussac D, Rivet JM, Audinot V, Adhumeau A, Brocco M, Nicolas JP, Boutin JA, Despaux N, Peglion JL.
Psychopharmacology Department, Institut de Recherches Servier, Croissy-sur-Seine (Paris), France.

S18327 displayed modest affinity for human (h)D2 and hD_3 receptors and high affinity for hD_4 receptors. At each, S18327 antagonized stimulation of [^{35}S]guanosine-5'-O-(3-thio)triphosphate binding by dopamine (DA). It also blocked activation of mitogen-activated protein kinase at hD_3 receptors. The affinity of S18327 at hD_1 and hD_5 sites was modest. S18327 showed pronounced affinity for human serotonin h5-HT_{2A} receptors and human alpha1A-adrenergic receptors (hARs), at which it antagonized increases in intracellular Ca^{2+} concentration levels elicited by 5-HT and norepinephrine (NE), respectively. S18327 presented significant affinity for halpha2A-ARs and antagonized NE-induced [^{35}S]guanosine-5'-O-(3-thio)triphosphate binding both at these sites and at alpha2-ARs in rat amygdala. Reflecting blockade of alpha2-autoreceptors, S18327 enhanced firing of adrenergic neurons in locus ceruleus, accelerated hippocampal synthesis of NE, and increased dialysate levels of NE in hippocampus, accumbens, and frontal cortex. S18327 abolished inhibition of ventrotegmental area-localized dopaminergic neurons by apomorphine. However, S18327 alone did not affect their activity and only modestly enhanced cerebral turnover of DA and dialysate levels of DA in striatum and accumbens. In contrast, S18327 markedly increased dialysate levels of DA in frontal cortex, an action abolished by the selective alpha2-AR agonist, S18616. Finally, S18327 reduced synthesis and dialysate levels of 5-HT in striatum and suppressed firing of dorsal raphe-localized serotonergic neurons, an action attenuated by the alpha1-AR agonist cirazoline. In conclusion, S18327 possesses marked antagonist activity at alpha1-ARs and D_4 and 5-HT_{2A} receptors and less potent antagonist activity at alpha2-ARs and D_1 and D_2 receptors. Antagonism by S18327 of alpha2-ARs enhances adrenergic transmission and reinforces frontocortical dopaminergic transmission, whereas blockade of alpha1-ARs inhibits dorsal raphe-derived serotonergic pathways. As further described in the accompanying paper, this profile of activity may contribute to the potential antipsychotic properties of S18327.

Reference no. 28

Neuropharmacology. 2000 Apr 3;39(6):1111-3.

[^{35}S]-GTPgammaS autoradiography reveals alpha(2) adrenoceptor-mediated G-protein activation in amygdala and lateral septum.

Newman-Tancredi A, Chaput C, Touzard M, Millan MJ.
Psychopharmacology Department, Institut de Recherches Servier, Croissy-sur-Seine (Paris), France.

Alpha2-adrenoceptor-mediated G-protein activation was examined by [^{35}S]-GTPgammaS autoradiography. In alpha2-adrenoceptor-rich regions (amygdala, lateral septum), noradrenaline stimulated [^{35}S]-GTPgammaS binding. These actions were abolished by the selective alpha2 antagonist, atipamezole. Conversely, in caudate nucleus, which expresses few alpha2 receptors, noradrenaline-induced stimulation was not inhibited by atipamezole, suggesting that it is not mediated by alpha2-adrenoceptors.

Reference no. 29

Eur J Pharmacol. 2000 Apr 7;394(1):47-50.

The novel antagonist, S33084, and GR218,231 interact selectively with cloned and native, rat dopamine D_3 receptors as compared with native, rat dopamine D_2 receptors.

Cussac D, **Newman-Tancredi A**, Sezgin L, Millan MJ.
Psychopharmacology Department, Institut de Recherches Servier, Croissy-sur-Seine (Paris), France.

The novel benzopyranopyrrole, S33084 ((3aR,9bS)-N[4-(8-cyano-1,3a,4, 9b-tetrahydro-^{3}H-benzopyrano[3,4-c]pyrrole-2-yl)-butyl] (4-phenyl)benzamide)), and the aminotetralin derivative, GR218,231 (2(R,S)-(di-n-propylamino)-6-(4-methoxyphenylsulfonylmethyl)-1,2,3 , 4-tetrahydro naphthalene), displayed high affinity at cloned, rat dopamine D_3 receptors (pK(i)s of 8.72 and 8.67, respectively), as well as dopamine D_3 receptors in rat olfactory tubercle (8.62 and 8.94, respectively). In contrast, they showed low affinities at striatal dopamine D_2 receptors (6.82 and 6.64, respectively). Unlike S33084 and GR218,231, the arylpiperazine, L741,626 (4-(4-chlorophenyl)-1-(1H-indol-3-ylmethyl)piperidin-4-ol), showed lower affinity for cloned (6.46) and native (6.92) dopamine D_3 receptors than for striatal dopamine D_2 receptors (7.52). S33084, GR218,231 and L741,626 should prove useful tools for exploration of the functional roles of dopamine D_3 vs. dopamine D_2 receptors.

Reference no. 30

Naunyn Schmiedebergs Arch Pharmacol. 2000 May;361(5):569-72.

[^{3}H]S33084: a novel, selective and potent radioligand at cloned, human dopamine D_3 receptors.

Cussac D, **Newman-Tancredi A**, Sezgin L, Millan MJ.
Psychopharmacology Department, Institut de Recherches Servier, Croissy-sur-Seine Paris, France.

The novel, selective dopamine D_3 receptor antagonist, S33084 [(3aR,9bS)-N[4-(8-cyano- 1,3a,4,9b-tetrahydro-^{3}H-benzopyrano[3,4-c]pyrrole-2-yl)-butyl] (4-phenyl)benzamide], was tritium-labelled to 59 Ci/mmol specific activity. Determination of association and dissociation rate constants at recombinant, human (h) D_3 receptors stably expressed in Chinese hamster ovary (CHO) cells yielded a Kd value (0.16 nM) comparable to that observed in saturation binding experiments (0.17 nM). The competition binding profile of [^{3}H]S33084 with diverse D_3 receptor agonists and antagonists correlated highly (0.99) with that of [^{3}H]spiperone. In conclusion, [^{3}H]S33084 is a highly potent and selective radioligand at dopamine D_3 receptors, which should be of considerable use for their characterisation.

Reference no. 31

Mol Pharmacol. 2000 Nov;58(5):1042-9.

Inverse agonism and constitutive activity as functional correlates of serotonin h5-HT$_{1B}$ receptor/G-protein stoichiometry.

Newman-Tancredi A, Audinot V, Moreira C, Verrièle L, Millan MJ.
Psychopharmacology Department, Institut de Recherches Servier, Croissy-sur-Seine (Paris), France.

This study evaluated the influence of receptor/G-protein (R:G) stoichiometry on constitutive activity and the efficacy of agonists, partial agonists, and inverse agonists at human (h) 5-hydroxytryphamine1B (5-HT$_{1B}$) receptors. Two Chinese hamster ovary cell lines were used; they expressed 8.5 versus 0.4 pmol h5-HT$_{1B}$ receptors/mg (determined by [^{3}H]GR125,743 saturation analysis) and 3.0 versus 1.5 pmol receptor-activated G-proteins/mg [determined by guanosine-5'-O-(3-[^{35}S]thio)-triphosphate ([^{35}S]GTPgammaS) isotopic dilution], respectively. Thus, they displayed R:G ratios of approximately 3.0 (RGhigh) and approximately 0.3 (RGlow), respectively. In competition-binding experiments, the agonists, 5-HT and sumatriptan, displayed fewer high-affinity (HA)-binding sites and the partial agonists, BMS181, 101 and L775,606, displayed decreased affinity in RGhigh versus RGlow membranes. In contrast, the inverse agonists, SB224,289 and, to a lesser extent, methiothepin, showed increased affinity. In G-protein activation experiments, both basal and 5-HT-activated [^{35}S]GTPgammaS binding were higher in RGhigh than in RGlow membranes. Constitutive activity (determined by inhibition of basal [^{35}S]GTPgammaS binding with GTPgammaS in the absence of receptor ligands) was more pronounced in RGhigh versus RGlow membranes, as revealed by the >5-fold greater proportion of HA sites. Correspondingly, the negative efficacy of inverse agonists was strikingly augmented, inasmuch as they suppressed approximately two-thirds of HA [^{35}S]GTPgammaS binding in RGhigh membranes, but only approximately one-third in RGlow membranes. Furthermore, the efficacy of partial agonists was greater at RGhigh versus RGlow membranes, as estimated by their ability to enhance [^{35}S]GTPgammaS binding. In conclusion, an increase in R:G ratios at h5-HT$_{1B}$ receptors was associated with an increase in relative efficacy of partial agonists and, most notably, an increase in both constitutive G-protein activation and negative efficacy of inverse agonists.

Reference no. 32

Naunyn Schmiedebergs Arch Pharmacol. 2000 May;361(5):549-54.

Antagonist properties of the novel antipsychotic, S16924, at cloned, human serotonin 5-HT_{2C} receptors: a parallel phosphatidylinositol and calcium accumulation comparison with clozapine and haloperidol.

Cussac D, **Newman-Tancredi A**, Nicolas JP, Boutin JA, Millan MJ.
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The novel benzopyranopyrrolidine and potential antipsychotic, S16924 ((+)-2-[[1-[2-(2,3-dihydrobenzo[1,4] dioxin-5-yloxy)-ethyl]-pyrrolidin-3yl]]-1-(4-fluoro-ph enyl)-ethanone), displays marked affinity for serotonin (5-HT)1A, 5-HT_{2A} and dopamine D_2 receptors. Herein, we show that it also possesses high affinity for the cloned, INI isoform of h5-HT_{2C} receptors (pKi=8.28) stably expressed in CHO cells. Similarly, clozapine (8.04) was a potent ligand, whereas haloperidol (<6.0) showed low affinity. As demonstrated by fura2-detection, S16924 concentration-dependently abolished (pKb=7.93) the 5HT-induced elevation in intracellular levels of Ca^{2+} ($[Ca^{2+}]i$) in a CHO cell line stably expressing the INI isoform of 5-HT_{2C} receptors. Further, as determined by depletion of membrane-bound levels of pre-labelled [^{3}H]phosphatidylinositols ([^{3}H]PI), S16924 concentration-dependently, surmountably and competitively blocked the activation of phospholipase C by 5-HT. This action was expressed with a pA2 of 7.89 according to Schild analysis. Clozapine likewise inhibited 5-HT-induced alterations in $[Ca^{2+}]i$ and [^{3}H]PI levels with pKbs of 7.43 and 7.84, respectively, whereas haloperidol was inactive (<5.0 in each case). Applied alone, S 16924, clozapine and haloperidol modified levels of neither $[Ca^{2+}]i$ nor [^{3}H]PI. In conclusion, in analogy to clozapine, and in contrast to haloperidol, S16924 behaves as a potent and competitive antagonist at h5-HT_{2C} receptors, the blockade of which may contribute to its distinctive functional profile of activity.

Reference no. 33

J Pharmacol Exp Ther. 2000 Jun;293(3):1048-62.

S33084, a novel, potent, selective, and competitive antagonist at dopamine D_3-receptors: I. Receptorial, electrophysiological and neurochemical profile compared with GR218,231 and L741,626.

Millan MJ, Gobert A, **Newman-Tancredi A**, Lejeune F, Cussac D, Rivet JM, Audinot V, Dubuffet T, Lavielle G.
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The benzopyranopyrrole S33084 displayed pronounced affinity (pKi = 9.6) for cloned human hD_3-receptors, and >100-fold lower affinity for hD_2 and all other receptors (>30) examined. S33084 concentration dependently, potently, and competitively (pA2 = 9.7) antagonized dopamine (DA)-induced [^{35}S]guanosine-5'-O-(3-thio)triphosphate (GTPgammaS) binding at hD_3-receptors. It also concentration dependently abolished stimulation by DA of hD_3-receptor-coupled mitogen-activated protein kinase. Administered alone, S33084 did not modify dialysate levels of DA in the frontal cortex, nucleus accumbens, or striatum of freely moving rats, nor the firing rate of ventrotegmental dopaminergic cell bodies. Furthermore, it had minimal effect on DA turnover in mesocortical, mesolimbic, and nigrostriatal projection regions. However, S33084 dose dependently blocked the suppressive influence of the preferential D_3-agonist PD128,907 on frontocortical release of DA. Furthermore, it likewise antagonized the inhibitory influence of PD128,907 on the electrical activity of ventrotegmental dopaminergic neurons. Although less potent than S33084, GR218,231 likewise behaved as a selective hD_3- versus hD_2-receptor antagonist and its neurochemical and electrophysiological profiles were similar. In contrast, L741,626 was a preferential antagonist at hD_2 versus hD_3 sites. *In vivo*, on administration alone, L741,626 increased frontocortical, mesolimbic, and (more potently) striatal DA release, enhanced the firing rate of dopaminergic perikarya, and accelerated cerebral DA synthesis. It also blocked the actions of PD128,907. In conclusion, S33084 is a novel, potent, selective, and competitive antagonist at hD_3-receptors. Although GR218,231 behaves similarly, L741,626 is a preferential D_2-receptor antagonist. DA D_2- but not D_3-(auto) receptors tonically inhibit ascending dopaminergic pathways, although the latter may contribute to phasic suppression of DA release in frontal cortex.

Reference no. 34

Neuropharmacology. 2001;40(1):57-64.

Constitutive activity at serotonin 5-HT_{1D} receptors: detection by homologous GTPgammaS versus [^{35}S]-GTPgammaS binding isotherms.

Audinot V, **Newman-Tancredi A**, Millan MJ.
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Although many G-protein-coupled receptors (GPCRs) may display constitutive activity, their detection has, to date, depended on the use of inverse agonists. The present study exploited a novel procedure to investigate constitutive activity at recombinant human (h) serotonin (5-HT) 5-HT_{1D} receptors stably expressed in Chinese hamster ovary (CHO) cells. 5-HT modestly stimulated guanosine-5'-O-(3-[^{35}S]thio)-triphosphate ([^{35}S]-GTPgammaS) binding to CHO-h5-HT_{1D} membranes whereas methiothepin and the 5-HT_{1B}/1D-selective ligand, SB224,289, exerted robust inhibition of basal [^{35}S]-GTPgammaS binding (inverse agonism). These actions were specific inasmuch as they were reversed by the novel, selective 5-HT_{1B}/$_{1D}$ ligand, S18127. Constitutive activity was investigated by homologous inhibition of [^{35}S]-GTPgammaS binding to CHO-h5-HT_{1D} membranes with unlabelled GTPgammaS. Under 'basal' conditions (absence of receptor ligand), biphasic isotherms were observed. Most (80%) [^{35}S]-GTPgammaS binding sites were in the high affinity (HA) versus low affinity (LA) component of the isotherms. HA binding was augmented by 5-HT (to 155%; relative to basal values=100%), but decreased by methiothepin (to 23%) and by SB224,289 (to 67%). In contrast, LA binding was not altered. Further, membranes of untransfected CHO cells exhibited only LA binding sites, indicating that the latter are not related to h5-HT_{1D} receptor-G-protein coupling. Thus, at 5-HT_{1D} receptors expressed in this CHO cell line, HA binding detected in homologous inhibition experiments (GTPgammaS versus [^{35}S]-GTPgammaS) under basal conditions provides a measure of constitutive G-protein activation. Thus, it is suggested that for h5-HT_{1D} receptors and, possibly, other GPCRs, inverse agonists will be detectable by [^{35}S]-GTPgammaS binding if a HA component is present under basal conditions.

Reference no. 35

Br J Pharmacol. 2001 Jan;132(2):518-24.

Differential modulation by GTPgammaS of agonist and inverse agonist binding to h5-HT_{1A} receptors revealed by [^{3}H]WAY100635.

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1. The interaction of serotonergic ligands at human (h) 5-HT_{1A} receptors expressed in Chinese hamster ovary cells was examined with the selective 'neutral' 5-HT_{1A} antagonist [^{3}H]WAY100635. Its binding was saturable (K_D=0.056 nM) with a Bmax (3.65 pmol/mg) significantly higher than that of two other selective 5-HT_{1A} radioligands: the partial agonist, [^{3}H]S15535 (2.77 pmol/mg) and the agonist, [^{3}H]8-OH-DPAT (2.02 pmol/mg).

2. The influence of GTPgammaS (100 microM) on the binding affinity of 15 serotonergic agonists, partial agonists, antagonists and inverse agonists was investigated in competition binding experiments with [^{3}H]WAY100635.

3. Agonists, including 5-HT, 8-OH-DPAT and buspirone, displayed biphasic isotherms which shifted to the right in the presence of GTPgammaS. In contrast, isotherms of the inverse agonists, methiothepin, (+)butaclamol and spiperone, were shifted to the left in the presence of GTPgammaS. Unlabelled WAY100635 was the only ligand that was unaffected by GTPgammaS, consistent with 'neutral' antagonist properties.

4. The magnitude of affinity changes induced by GTPgammaS for 13 ligands was highly correlated (r=0.98) with their efficacy (positive and negative) previously determined by [^{35}S]GTPgammaS binding.

5. In contrast, the napthylpiperazine derivative and high efficacy agonist, S14506, displayed only a modest GTPgammaS shift, in accordance with previous indications of 'atypical' binding properties of this ligand. A further full agonist, S14671, which is chemically closely-related to S14506, also displayed a minimal GTPgammaS shift, underpinning this observation.

6. In conclusion, [^{3}H]WAY100635 constitutes a useful neutral antagonist radioligand for the characterization of drug actions at h5-HT_{1A} receptors. GTPgammaS-induced affinity changes of agonist and inverse agonist competition isotherms generally correlate well with ligand efficacy, with the notable exception of two chemically-similar agents, S14506 and S14671, which are efficacious agonists, yet relatively insensitive to h5-HT_{1A} receptor/G-protein coupling changes.

Reference no. 36

Naunyn Schmiedebergs Arch Pharmacol. 2001 Apr;363(4):391-8.

Pindolol antagonises G-protein activation at both pre- and postsynaptic serotonin 5-HT_{1A} receptors: a GTPgammaS autoradiography study.

Newman-Tancredi A, Chaput C, Touzard M, Millan MJ.
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The arylalkylamine, pindolol, may potentiate the clinical actions of antidepressant agents. Although it is thought to act via blockade of 5-HT_{1A} autoreceptors, its efficacy at these sites remains controversial. Herein, we evaluated the actions of pindolol at 5-HT_{1A} autoreceptors and specific populations of postsynaptic 5-HT_{1A} receptors employing [^{35}S]GTPgammaS autoradiography, a measure of receptor-mediated G-protein activation. Both 8-OH-DPAT (1 microM) and 5-HT (10 microM) elicited a pronounced increase in [^{35}S]GTPyS binding in the dorsal raphe nucleus, which contains serotonergic cell bodies bearing 5-HT_{1A} autoreceptors. Pindolol abolished their actions. In the dentate gyrus, lateral septum and entorhinal cortex, structures enriched in postsynaptic 5-HT_{1A} receptors, 8-OH-DPAT (1 microM) and 5-HT (10 microM) also elicited a marked increase in [^{35}S]GTPgammaS binding which was likewise blocked by pindolol. The antagonism of 5-HT-induced [^{35}S]GTPgammaS labelling in the dentate gyrus was shown to be concentration-dependent, yielding a pIC_{50} of 5.82. Pindolol did not, itself, affect [^{35}S]GTPgammaS binding in any brain region examined. In conclusion, these data suggest that, as characterised by [^{35}S]GTPgammaS autoradiography, and compared with 5-HT and 8-OH-DPAT, pindolol possesses low efficacy at both pre- and postsynaptic 5-HT_{1A} receptors.

Reference no. 37

Neuropsychopharmacology. 2001 Sep;25(3):410-22.

Inverse agonist properties of antipsychotic agents at cloned, human (h) serotonin 5-HT_{1B} and h5-HT_{1D} receptors.

Audinot V, **Newman-Tancredi A**, Cussac D, Millan MJ.
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The actions of diverse antipsychotics at cloned h5-HT_{1B} and h5-HT_{1D} receptors were examined employing [^{3}H]GR125,743 and [^{35}S]-GTPgammaS for determination of affinities and efficacies, respectively. Compared with hD_2 receptors, haloperidol, chlorpromazine and olanzapine showed markedly (>100-fold) lower affinity for h5-HT_{1D} and h5-HT_{1B} receptors at which they expressed inverse agonist properties. Clozapine, risperidone and ocaperidone likewise behaved as inverse agonists at h5-HT_{1B} and h5-HT_{1D} receptors but their affinities were only approximately 10-fold lower than at hD_2 receptors. Moreover, ziprasidone, S16924 and ORG5222 interacted at h5-HT_{1B} and h5-HT_{1D} receptors with affinities similar to hD_2 sites. While S16924 and ORG5222 were inverse agonists at h5-HT_{1B} and h5-HT_{1D} sites, ziprasidone was an inverse agonist at h5-HT_{1D} receptors yet a partial agonist at h5-HT_{1B} receptors. These actions of antipsychotics were abolished by the selective, neutral antagonist, S18127. In conclusion, with the exception of ziprasidone, all antipsychotics were inverse agonists at h5-HT_{1B} and h5-HT_{1D} receptors, although they differed markedly in their potency at these sites as compared to hD_2 receptors.

Reference no. 38

J Pharmacol Exp Ther. 2001 Jun;297(3):876-87.

Antiparkinsonian agent piribedil displays antagonist properties at native, rat, and cloned, human alpha2-adrenoceptors: cellular and functional characterization.

Millan MJ, Cussac D, Milligan G, Carr C, Audinot V, Gobert A, Lejeune F, Rivet JM, Brocco M, Duqueyroix D, Nicolas JP, Boutin JA, **Newman-Tancredi A**.
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Compared with cloned, human (h)D_2 receptors (pKi = 6.9), the antiparkinsonian agent piribedil showed comparable affinity for halpha2A- (7.1) and halpha2C- (7.2) adrenoceptors (ARs), whereas its affinity for halpha2B-ARs was less marked (6.5). At halpha2A- and halpha2C-ARs, piribedil antagonized induction of [^{35}S]guanosine-5'-O-(3-thio)triphosphate (GTPgammaS) binding by norepinephrine (NE) with pKb values of 6.5 and 6.9, respectively. Furthermore, Schild analysis of the actions of piribedil at halpha2A-ARs indicated competitive antagonism, yielding a pA_2 of 6.5. At a porcine alpha2A-AR-Gi1alpha-Cys351C (wild-type) fusion protein, piribedil competitively abolished (pA_2 = 6.5) GTPase activity induced by epinephrine. However, at a alpha2A-AR-Gi1alpha-Cys351I (mutant) fusion protein of amplified sensitivity, although still acting as a competitive antagonist (pA_2 = 6.2) of epinephrine, piribedil itself manifested weak partial agonist properties. Similarly, piribedil weakly induced mitogen-activated protein kinase phosphorylation via wild-type halpha2A-ARs, although attenuating its phosphorylation by NE. As demonstrated by functional [^{35}S]GTPgammaS autoradiography in rats, piribedil antagonized activation by NE of alpha2-ARs in cortex, amygdala, and septum. Antagonist properties were also expressed in a dose-dependent enhancement of the firing rate of adrenergic neurons in locus ceruleus (0.125-4.0 mg/kg i.v.). Furthermore, piribedil (2.5-4.0 mg/kg s.c.) accelerated hippocampal NE synthesis, elevated dialysis levels of NE in hippocampus and frontal cortex, and blocked hypnotic-sedative properties of the alpha2-AR agonist xylazine. Finally, piribedil showed only modest affinity for rat alpha1-ARs (5.9) and weakly antagonized NE-induced activation of phospholipase C via halpha1A-ARs (pKb = 5.6). In conclusion, piribedil displays essentially antagonist properties at cloned, human and cerebral, rat alpha2-ARs. Blockade of alpha2-ARs may, thus, contribute to its clinical antiparkinsonian profile.

Reference no. 39

Eur J Pharmacol. 2001 Oct 5;428(2):177-84.

Efficacy of antipsychotic agents at human 5-HT_{1A} receptors determined by [^{3}H]WAY100635 binding affinity ratios: relationship to efficacy for G-protein activation.

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5-HT_{1A} receptors are implicated in the aetiology of schizophrenia. Herein, the influence of 15 antipsychotics on the binding of the selective 'neutral' antagonist, [^{3}H]WAY100635 ([^{3}H]N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl)-cyclo-hexanecarboxamide), was examined at human 5-HT_{1A} receptors expressed in Chinese Hamster Ovary cells. In competition binding experiments, 5-HT displayed biphasic isotherms which were shifted to the right in the presence of the G-protein uncoupling agent, GTPgammaS (100 microM). In analogy, the isotherms of ziprasidone, quetiapine and S16924 (((R-2-[1-[2-(2,3-dihydro-benzo[1,4]dioxin-5-yloxy)-ethyl]-pyrrolidin-3yl]-1-(4-fluoro-phenyl)-ethanone), were displaced to the right by GTPgammaS, consistent with agonist actions. Binding of several other antipsychotics, such as ocaperidone, olanzapine and risperidone, was little influenced by GTPgammaS. Isotherms of the neuroleptics, haloperidol, chlorpromazine and thioridazine were shifted to the left in the presence of GTPgammaS, suggesting inverse agonist properties. For most ligands, the magnitude of affinity changes induced by GTPgammaS (alteration in pKi values) correlated well with their previously determined efficacies in [^{35}S]GTPgammaS binding studies [Eur. J. Pharmacol. 355 (1998) 245]. In contrast, the affinity of the 'atypical' antipsychotic agent, clozapine, which is a known partial agonist at 5-HT_{1A} receptors, was less influenced by GTPgammaS. When the ratio of high-/low-affinity values was plotted against efficacy, hyperbolic isotherms were obtained, consistent with a modified ternary complex model which assumes that receptors can adopt active conformations in the absence of agonist. In conclusion, modulation of [^{3}H]WAY100635 binding by GTPgammaS differentiated agonist vs. inverse agonist properties of antipsychotics at 5-HT_{1A} receptors. These may contribute to differing profiles of antipsychotic activity.

Reference no. 40

Psychopharmacology (Berl). 2001 Jun;156(1):58-62.

The "selective" dopamine D1 receptor antagonist, SCH23390, is a potent and high efficacy agonist at cloned human serotonin$_{2C}$ receptors.

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RATIONALE: The benzazepine and "selective" dopamine D1 receptor antagonist, SCH23390 [(R)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-benzazepine-7-ol], shows significant affinity at native serotonin (5-HT)$_{2C}$ receptors.

OBJECTIVES: We examined its functional actions at cloned human (h)5-HT$_{2C}$ receptors (VSV isoform) stably expressed in CHO cells.

METHODS: Since 5-HT$_{2C}$ receptors are positively coupled to phospholipase C (PLC), their activation was determined by depletion of membrane-bound pools of pre-labelled [^{3}H]phosphotidylinositol ([^{3}H]PI).

RESULTS: SCH23390 showed high affinity (Ki, 9.3 nM) at h5-HT$_{2C}$ sites and depleted [^{3}H]PI with an EC$_{50}$ of 2.6 nM. Its efficacy was equivalent to that of 5-HT. [^{3}H]PI depletion elicited by SCH23390 was concentration-dependently abolished by the selective 5-HT$_{2C}$ antagonist, SB242,084, with a KB of 0.55 nM. Further, in the presence of a fixed concentration of SB242,084 (10 nM), the concentration-response curve for SCH23390 was shifted to the right without loss of maximal effect, yielding a KB of 0.57 nM.

CONCLUSIONS: SCH23390 is a potent and high efficacy agonist at h5-HT$_{2C}$ receptors. Activation of 5-HT$_{2C}$ receptors by SCH23390 may contribute to its functional properties both in animals and in humans.

Reference no. 41

Eur J Pharmacol. 2001 Jul 13;424(1):13-7.

Agonist properties of pindolol at h5-HT_{1A} receptors coupled to mitogen-activated protein kinase.

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At h5-HT_{1A} receptors, stably transfected into Chinese Hamster Ovary Cells (CHO-h5-HT_{1A}), the selective 5-HT_{1A} receptor agonist, (+)8-hydroxy-dipropyl-amino-tetralin, ((+)8-OH-DPAT), transiently activated mitogen-activated protein kinase (MAPK) with a pEC_{50} of 8.5. The arylalkylamine, (-)-pindolol, also behaved as an agonist with a maximal effect of 57% relative to (+)8-OH-DPAT (100%), and with a pEC_{50} of 7.2. The selective 5-HT(1A) receptor antagonist, N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl)cyclo-hexane carboxamide (WAY100635), blocked (+)8-OH-DPAT- and (-)-pindolol-induced MAPK activation with pK(B)s of 9.7 and 9.9, respectively, whereas the selective 5-HT(1B) receptor antagonist, 1'-Methyl-5-[2'-methyl-4'-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-ylcarbonyl]-2,3,6,7-tetrahydro-5H-spiro[furo[2,3-f]indole-3,4'-piperidine] (SB224,289) was inactive. Pertussis toxin blocked the actions of (+)8-OH-DPAT and (-)-pindolol demonstrating implication of G(i)/G(o) proteins. Thus, stimulation of MAPK provides an intracellular marker and signal for expression of the agonist actions of (-)-pindolol at h5-HT_{1A} receptors.

Reference no. 42

Brain Res. 2001 Nov 30;920(1-2):41-54.

Dopamine D_2 receptor-mediated G-protein activation in rat striatum: functional autoradiography and influence of unilateral 6-hydroxydopamine lesions of the substantia nigra.

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Unilateral 6-hydroxydopamine (6-OHDA) lesions of substantia nigra pars compacta (SNPC) neurons in rats induce behavioural hypersensitivity to dopaminergic agonists. However, the role of specific dopamine receptors is unclear, and potential alterations in their transduction mechanisms remain to be evaluated. The present study addressed these issues employing the dopaminergic agonist, quinelorane, which efficaciously stimulated G-protein activation (as assessed by [^{35}S]GTPgammaS binding) at cloned hD_2 (and hD_3) receptors. At rat striatal membranes, dopamine stimulated [^{35}S]GTPgammaS binding by 1.9-fold over basal, but its actions were only partially reversed by the selective D_2/D3 receptor antagonist, raclopride, indicating the involvement of other receptor subtypes. In contrast, quinelorane-induced stimulation (48% of the effect of dopamine) was abolished by raclopride, and by the D_2 receptor antagonist, L741,626. Further, novel antagonists selective for D_3 and D_4 receptors, S33084 and S18126, respectively, blocked the actions of quinelorane at concentrations corresponding to their affinities for D_2 receptors. Quinelorane potently induced contralateral rotation in unilaterally 6-OHDA-lesioned rats, an effect abolished by raclopride and L741,626, but not by D_3 and D_4 receptor-selective doses of S33084 and S18126, respectively. In functional ([^{35}S]GTPgammaS) autoradiography experiments, quinelorane stimulated G-protein activation in caudate putamen and, to a lesser extent, in nucleus accumbens and cingulate cortex of naive rats. In unilaterally SNPC-lesioned rats, quinelorane-induced G-protein activation in the caudate putamen on the non-lesioned side was similar to that seen in naive animals (approximately 50% stimulation), but significantly greater on the lesioned side (approximately 80%). This increase was both pharmacologically and regionally specific since it was reversed by raclopride, and was not observed in nucleus accumbens or cingulate cortex. In conclusion, the present data indicate that, in rat striatum, the actions of quinelorane are mediated primarily by D_2 receptors, and suggest that behavioural hypersensitivity to this agonist, induced by unilateral SNPC lesions, is associated with an increase in D_2, but not D_3 or D_4, receptor-mediated G-protein activation.

Reference no. 43

Pharmacol Biochem Behav. 2002 Apr;71(4):589-98.

Specific labelling of serotonin 5-HT_{1B} receptors in rat frontal cortex with the novel, phenylpiperazine derivative, [^{3}H]GR125,743. A pharmacological characterization.

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Although several tritiated agonists have been used for radiolabelling serotonin (5-hydroxytryptamine, 5-HT_{1B} receptors in rats, data with a selective, radiolabelled antagonist have not been presented. Inasmuch as [^{3}H]GR125,743 specifically labels cloned, human and native guinea pig 5-HT_{1B} receptors and has been employed for characterization of cerebral 5-HT_{1B} receptor in the latter species [Eur. J. Pharmacol. 327 (1997) 247.], the present study evaluated its utility for characterization of native, cerebral 5-HT_{1B} sites in the rat. In homogenates of frontal cortex, [^{3}H]GR125,743 (0.8 nM) showed rapid association (t1/2=3.4 min), >90% specific binding and high affinity (Kd=0.6 nM) for a homogeneous population of receptors with a density (Bmax) of 160 fmol/mg protein. In competition binding studies, affinities were determined for 15 chemically diverse 5-HT_{1B} agonists, including 2-[5-[3-(4-methylsulphonylamino)benzyl-1,2,4-oxadiazol-5-yl]-1H-indole-3-yl]ethylamine (L694,247; pK(i), 10.4), 5-carboxamidotryptamine (5-CT; 9.7), 3-[3-(2-dimethylamino-ethyl)-1H-indol-6-yl]-N-(4-methoxybenzyl)acrylamide (GR46,611; 9.6), 5-methoxy-3-(1,2,5,6-tetrahydro-4-pyridinyl)-1H-indole (RU24,969; 9.5), dihydroergotamine (DHE; 8.6), 5-H-pyrrolo[3,2-b]pyridin-5-one,1,4-dihydro-3-(1,2,3,6-tetrahydro-4-pyridinyl (CP93,129; 8.4), anpirtoline (7.9), sumatriptan (7.4), 1-[2-(3-fluorophenyl)ethyl]-4-[3-[5-(1,2,4-triazol-4-yl)-1H-indol-3-yl]propyl]piperazine (L775,606; 6.4) and (minus sign)-1(S)-[2-[4-(4-methoxyphenyl)piperazin-1-yl]ethyl]-N-methyl-3,4-dihydro-1H-2-benzopyran-6-carboxamide (PNU109,291; <5.0). Similarly, affinities were established for 13 chemically diverse antagonists, including N-[4-methoxy-3-(4-methylpiperazin-1-yl)phenyl]-3-methyl-4-(4-pyridyl)benzamide (GR125,743; pK(i), 9.1), (-)cyanopindolol (9.0), (-)-tertatolol (8.2), N-(4-methoxy-3-(4-methylpiperazin-1-yl)phenyl]-2'-methyl-4'-(5-methyl-1,2,4-oxadiozol-3-yl)biphenyl-4-carboxamide (GR127,935; 8.2), N-[3-(1,4-benzodioxan-5-yl)piperidin-4-yl]N-(indan-2yl)amine (S18127; 7.9), metergoline (7.8), (-)-pindolol (7.6), 1'-methyl-5-[2'-methyl-4'-(5-methyl-1,2,4-oxadiazol-3-yl)-biphenyl-4-ylcarbonyl]-2,3,6,7-tetrahydro-5H-spiro[furo[2,3-f]indole-3,4'-piperidine] (SB224,289; 7.5) and ketanserin (<5.0). These rank orders of affinity correspond to the binding profile of 5-HT_{1B}) rather than 5-HT_{1D} receptors. The low affinities of L775,066 and PNU109,291 versus L694,247 should be noted, as well as the low affinity of ketanserin as compared to SB224,289. Finally, in line with species differences, the affinities of several ligands including CP93,129, RU24,969, (-)-pindolol and (-)-propanolol in rat 5-HT_{1B} sites were markedly different to guinea pig 5-HT_{1B} sites labelled with [^{3}H]GR125,743. In conclusion, [^{3}H]GR125,743 is an appropriate tool for the radiolabelling of native, rat 5-HT_{1B} receptors and permitted determination of the affinities of an extensive series of ligands at these sites.

Reference no. 44

Psychopharmacology (Berl). 2002 Jul;162(2):168-77. Epub 2002 Apr 30.

Stimulation by antipsychotic agents of mitogen-activated protein kinase (MAPK) coupled to cloned, human (h)serotonin 5-HT_{1A} receptors.

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RATIONALE: There is evidence that serotonergic mechanisms contribute to the functional profiles of antipsychotic drugs, several of which display affinity for human (h)5-HT_{1A} receptors. OBJECTIVE: Here, we compared the interaction of several antipsychotic agents at h5-HT_{1A} receptors employing mitogen-activated protein kinase (MAPK), an intracellular marker.

METHODS: The influence of antipsychotics on MAPK phosphorylation was quantified in Chinese hamster ovary (CHO) cells stably transfected with h5-HT_{1A} receptors by use of a highly selective antibody.

RESULTS: The novel antipsychotic agent, S16924, concentration-dependently (pEC_{50}, 8.10) stimulated the phosphorylation of MAPK. Its maximal effect (96%) was similar to that of the prototypical 5-HT_{1A} agonist, (+)8-OH-DPAT (pEC_{50}, 8.54) (defined as 100%). The selective 5-HT_{1A} receptor antagonist WAY100635, which was inactive alone, abolished stimulation of MAPK by S16924 with a pKb of 9.66. This stimulatory influence of S16924 on MAPK was potently mimicked by the benzoisoxazole, antipsychotic ziprasidone (pEC_{50}, 7.25; 93%). The atypical antipsychotic clozapine also activated MAPK, albeit with lower potency and efficacy (pEC_{50}, 5.43 and 43%). These actions of ziprasidone and clozapine were also blocked by WAY100635. Evaluated at a single, high concentration, several other antipsychotics stimulated MAPK phosphorylation with variable efficacy: quetiapine (75%), ocaperidone (74%), tiospirone (57%), olanzapine (54%) and risperidone (21%). In all cases, their actions were abolished by WAY100635. In contrast, haloperidol, thioridazine and sertindole did not stimulate MAPK.

CONCLUSIONS: Antipsychotics display contrasting efficacies in modulating MAPK phosphorylation at h5-HT_{1A} receptors, ranging from high (e.g. S16924 and ziprasidone), via intermediate (e.g. clozapine) to low (e.g. haloperidol). Differential modulation of 5-HT_{1A} receptor-coupled MAPK may contribute to their contrasting functional profiles.

Reference no. 45

Mol Pharmacol. 2002 Sep;62(3):578-89.

Differential activation of Gq/11 and Gi3 proteins at 5-hydroxytryptamine$_{2C}$ receptors revealed by antibody capture assays: influence of receptor reserve and relationship to agonist-directed trafficking.

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As determined by a guanosine 5'-O-(3-[^{35}S]thio)triphosphate ([^{35}S]GTPgammaS) binding assay, which does not distinguish G protein subtypes, 5-hydroxytryptamine (5-HT) and 2(S)- 1-(6-chloro-5-fluoro-1H-indol-1-yl)-2-propanamine fumarate (Ro600175) behaved as full agonists at human 5-HT$_{2C}$ (h5-HT$_{2C}$) receptors (VSV isoform) stably expressed in Chinese hamster ovary (CHO) cells, whereas 1-2,5-dimethoxy-4-iodophenyl-2-aminopropane (DOI), d-lysergic acid diethylamide (LSD), and lisuride exhibited partial agonist properties. After treatment with pertussis toxin to uncouple 5-HT$_{2C}$ receptors from Gi/Go but not Gq/11, DOI and LSD were as efficacious as 5-HT and Ro600175 in stimulating [^{35}S]GTPgammaS binding, whereas lisuride still exhibited low efficacy (40%). Correspondingly, in a scintillation proximity assay employing specific antibodies against Gq/11, 5-HT, Ro600175, DOI, and LSD behaved as high-efficacy agonists, whereas lisuride showed efficacy of 36%. In contrast, when employing a specific antibody recognizing Gi3, DOI and LSD were less efficacious (80 and 30%, respectively) than 5-HT and Ro600175, and lisuride was inactive. Agonist actions were specifically mediated by h5-HT$_{2C}$ receptors inasmuch as the selective 5-HT$_{2C}$ antagonist SB242,084 blocked [^{35}S]GTPgammaS binding at both Gq/11 and Gi3. Agonist potency for stimulation of Gi3 was ~6- to 8-fold less than for Gq/11, indicating that the latter was preferentially engaged by h5-HT$_{2C}$ receptors. Inactivation of h5-HT$_{2C}$ receptors with the alkylating agent N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline did not modify the efficacy of 5-HT, Ro600175, and DOI at Gq/11, whereas their efficacies were substantially reduced at Gi3, indicating a greater receptor reserve for the former. Finally, the preferential activation of Gq/11 versus Gi3 by DOI, LSD, and lisuride was diminished in the presence of lower receptor number. In conclusion, h5-HT$_{2C}$ receptors couple to both Gq/11 and Gi3 in CHO cells, and efficacy for G protein subtype activation is both ligand- and receptor reserve-dependent.

Reference no. 46

Mol Pharmacol. 2002 Sep;62(3):590-601.

Antibody capture assay reveals bell-shaped concentration-response isotherms for h5-HT_{1A} receptor-mediated Galpha(i3) activation: conformational selection by high-efficacy agonists, and relationship to trafficking of receptor signaling.

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Although serotonin 5-HT_{1A} receptors couple to several Gi/o G-protein subtypes, little is known concerning their differential activation patterns. In this study, in membranes of Chinese hamster ovary cells expressing h5-hydroxytryptamine$_{1A}$ receptors (CHO-h5-HT_{1A}), isotherms of 5-HT-stimulated guanosine-5'-O-(3-[^{35}S]thio)-triphosphate ([^{35}S]GTPgammaS) binding were biphasic, suggesting coupling to multiple G-protein subtypes. The high potency component was abolished by preincubation with an antibody recognizing Galpha(i3) subunits and was resistant to induction of [^{35}S]GTPgammaS dissociation by unlabeled GTPgammaS, thus yielding a bell-shaped concentration-response isotherm. To directly investigate Galpha(i3) activation, we adopted an antibody-capture/scintillation proximity assay. 5-HT and other high-efficacy agonists yielded bell-shaped [^{35}S]GTPgammaS binding isotherms, with peaks at nanomolar concentrations. As drug concentrations increased, Galpha(i3) stimulation progressively returned to basal values. In contrast, the partial agonists (-)-pindolol and 4-(benzodioxan-5-yl)1-(indan-2-yl)piperazine (S15535) displayed sigmoidal stimulation isotherms, whereas spiperone and other inverse agonists sigmoidally inhibited [^{35}S]GTPgammaS binding. Agonist-induced stimulation and inverse agonist-induced inhibition of Galpha(i3) activation were i) abolished by pretreatment of CHO-h5-HT_{1A} cells with pertussis toxin; ii) reversed by the selective 5-HT_{1A} antagonist (N-[2-[4-(2-methoxy-phenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl)-cyclohexane-carboxamide) fumarate (WAY100635), and iii) absent in nontransfected CHO cell membranes. 5-HT isotherms could be modified by altering sodium concentration; only stimulatory actions were observed at 300mM NaCl, whereas only inhibitory actions were seen at 10 mM NaCl. Furthermore, bell-shaped isotherms were not detected at short incubation times, suggesting time-dependent changes in receptor/Galpha(i3) coupling. Taken together, these data show that low but not high concentrations of high-efficacy 5-HT_{1A} agonists direct receptor signaling to Galpha(i3). In contrast, partial agonists favor h5-HT_{1A} receptor signaling to Galpha(i3) over a wide concentration range, whereas inverse agonists inhibit constitutive Galpha(i3) activation.

Reference no. 47

Naunyn Schmiedebergs Arch Pharmacol. 2002 Mar;365(3):242-52. Epub 2002 Jan 16.

Characterization of phospholipase C activity at h5-HT_{2C} compared with h5-HT_{2B} receptors: influence of novel ligands upon membrane-bound levels of [^{3}H]phosphatidylinositols.

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Employing a novel, rapid and sensitive method for evaluation of phospholipase C (PLC) activity, the present study characterized the actions of diverse agonists and antagonists at human (h)5-HT_{2C} receptors expressed in Chinese Hamster Ovary (CHO) cells. In addition, affinities and efficacies at these sites were compared with those obtained at h5-HT_{2B} receptors.5-HT elicited a robust and rapid reduction in levels of the pre-labelled, membrane-bound substrate of PLC, [^{3}H]phosphatidylinositols ([^{3}H]PI). The time-course of [^{3}H]PI depletion paralleled that of [^{3}H]inositol phosphate ([^{3}H]IP) accumulation, as determined by conventional anion exchange chromatography. Inactivation of h5-HT_{2C} receptors with the alkylating agent, N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), revealed a large receptor reserve, with half-maximal PLC activation induced by a concentration of 5-HT occupying only 5% of sites. In analogy to 5-HT (Emax=100%), DOI, MK212 and mCPP, as well as the novel ligands, Ro600332, Ro600175 and BW723C86, showed "full" efficacy at h5-HT_{2C} sites. Their efficacies were similar at h5-HT_{2B} sites, with the exception of mCPP and MK212, which acted as partial agonists. Further, lisuride and Ro600869 behaved as partial agonists and antagonists at h5-HT_{2C} and h5-HT_{2B} receptors, respectively. As concerns functional selectivity (potency for induction of [^{3}H]PI depletion), only Ro600175 preferentially activated h5-HT_{2B} sites. In contrast, Ro600332 preferentially activated h5-HT_{2C} receptors. Amongst antagonists, RS102221 and SB242084 displayed a marked preference for h5-HT_{2C} sites, whereas LY266097, S33526 and SB204741 behaved as selective antagonists at h5-HT_{2B} receptors. At both h5-HT_{2C} and h5-HT_{2B} receptors, antagonist potency (pKb) and binding affinity (pKi) were highly correlated. In conclusion, this rapid and innovative method for determination of PLC activity permitted characterization of an extensive range of novel ligands at h5-HT_{2C} receptors. Although several antagonists clearly differentiated h5-HT_{2C} from h5-HT_{2B} receptors under these conditions, highly selective agonists remain to be identified.

Reference no. 48

J Pharmacol Exp Ther. 2002 Nov;303(2):791-804.

Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor. I. A multivariate analysis of the binding profiles of 14 drugs at 21 native and cloned human receptor subtypes.

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Because little comparative information is available concerning receptor profiles of antiparkinson drugs, affinities of 14 agents were determined at diverse receptors implicated in the etiology and/or treatment of Parkinson's disease: human (h)D1, hD_2S, hD_2L, hD_3, hD_4, and hD_5 receptors; human 5-hydroxytryptamine 5-HT_{1A}, h5-HT_{1B}, h5-HT_{1D}, h5-HT_{2A}, h5-HT_{2B}, and h5-HT_{2C} receptors; halpha1A-, halpha1B-, halpha1D-, halpha2A-, halpha2B-, halpha2C-, rat alpha2D-, hbeta1-, and hbeta2-adrenoceptors (ARs); and native histamine1 receptors. A correlation matrix (294 pKi values) demonstrated substantial "covariance". Correspondingly, principal components analysis revealed that axis 1, which accounted for 76% variance, was associated with the majority of receptor types: drugs displaying overall high versus modest affinities migrated at opposite extremities. Axis 2 (7% of variance) differentiated drugs with high affinity for hD_4 and H1 receptors versus halpha1-AR subtypes. Five percent of variance was attributable to axis 3, which distinguished drugs with marked affinity for hbeta1- and hbeta2-ARs versus hD5 and 5-HT_{2A} receptors. Hierarchical (cluster) analysis of global homology generated a dendrogram differentiating two major groups possessing low versus high affinity, respectively, for multiple serotonergic and hD5 receptors. Within the first group, quinpirole, quinerolane, ropinirole, and pramipexole interacted principally with hD_2, hD_3, and hD_4 receptors, whereas piribedil and talipexole recognized dopaminergic receptors and halpha2-ARs. Within the second group, lisuride and terguride manifested high affinities for all sites, with roxindole/bromocriptine, cabergoline/pergolide, and 6,7-dihydroxy-N,N-dimethyl-2-ammotetralin (TL99)/apomorphine comprising three additional subclusters of closely related ligands. In conclusion, an innovative multivariate analysis revealed marked heterogeneity in binding profiles of antiparkinson agents. Actions at sites other than hD_2 receptors likely participate in their (contrasting) functional profiles.

Reference no. 49

J Pharmacol Exp Ther. 2002 Nov;303(2):805-14.

Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor. II. Agonist and antagonist properties at subtypes of dopamine D_2-like receptor and alpha1/alpha2-adrenoceptor.

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The accompanying multivariate analysis of the binding profiles of antiparkinson agents revealed contrasting patterns of affinities at diverse classes of monoaminergic receptor. Herein, we characterized efficacies at human (h)D_{2S}, hD_{2L}, hD_3, and $hD_{4.4}$ receptors and at halpha2A-, halpha2B-, halpha2C-, and halpha1A-adrenoceptors (ARs). As determined by guanosine 5'-O-(3-[^{35}S]thio)triphosphate ([^{35}S]GTPgammaS) binding, no ligand displayed "full" efficacy relative to dopamine (100%) at all "D2-like" sites. However, at hD_{2S} receptors quinpirole, pramipexole, ropinirole, quinerolane, pergolide, and cabergoline were as efficacious as dopamine (Emax 100%); TL99, talipexole, and apomorphine were highly efficacious (79-92%); piribedil, lisuride, bromocriptine, and terguride showed intermediate efficacy (40-55%); and roxindole displayed low efficacy (11%). For all drugs, efficacies were lower at hD_2L receptors, with terguride and roxindole acting as antagonists. At hD_3 receptors, efficacies ranged from 33% (roxindole) to 94% (TL99), whereas, for hD_4 receptors, highest efficacies (approximately 70%) were seen for quinerolane, quinpirole, and TL99, whereas piribedil and terguride behaved as antagonists and bromocriptine was inactive. Although efficacies at hD_{2S} versus hD_{2L} sites were highly correlated (r = 0.79), they correlated only modestly to hD_3/hD_4 sites (r = 0.44-0.59). In [^{35}S]GTPgammaS studies of halpha2A-ARs, TL99 (108%), pramipexole (52%), talipexole (51%), pergolide (31%), apomorphine (16%), and quinerolane (11%) were agonists and ropinirole and roxindole were inactive, whereas piribedil and other agents were antagonists. Similar findings were obtained at halpha2B- and halpha2C-ARs. Using [^{3}H]phosphatidylinositol depletion, roxindole, bromocriptine, lisuride, and terguride displayed potent antagonist properties at halpha1A-ARs. In conclusion, antiparkinson agents display diverse agonist and antagonist properties at multiple subtypes of D_2-like receptor and alpha1/alpha2-AR, actions, which likely contribute to their contrasting functional profiles.

Reference no. 50

J Pharmacol Exp Ther. 2002 Nov;303(2):815-22.

Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor. III. Agonist and antagonist properties at serotonin, 5-HT1 and 5-HT2, receptor subtypes.

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Although certain antiparkinson agents interact with serotonin (5-HT) receptors, little information is available concerning functional actions. Herein, we characterized efficacies of apomorphine, bromocriptine, cabergoline, lisuride, piribedil, pergolide, roxindole, and terguride at human (h)5-HT_{1A}, h5-HT_{1B}, and h5-HT_{1D} receptors [guanosine 5'-O-(3-[^{35}S]thio)triphosphate ([^{35}S]GTPgammaS) binding], and at h5-HT_{2A}, h5-HT_{2B}, and h5-HT_{2C} receptors (depletion of membrane-bound [^{3}H]phosphatydilinositol). All drugs stimulated h5-HT_{1A} receptors with efficacies (compared with 5-HT, 100%) ranging from modest (apomorphine, 35%) to high (cabergoline, 93%). At h5-HT_{1B} receptors, efficacies varied from mild (terguride, 37%) to marked (cabergoline, 102%) and potencies were modest (pEC_{50} values of 5.8-7.6): h5-HT_{1D} sites were activated with a similar range of efficacies and greater potency (7.1-8.5). Piribedil and apomorphine were inactive at h5-HT_{1B} and h5-HT_{1D} receptors. At h5-HT_{2A} receptors, terguride, lisuride, bromocriptine, cabergoline, and pergolide displayed potent (7.6-8.8) agonist properties (49-103%), whereas apomorphine and roxindole were antagonists and piribedil was inactive. Only pergolide (113%/8.2) and cabergoline (123%/8.6) displayed pronounced agonist properties at h5-HT_{2B} receptors. At 5-HT_{2C} receptors, lisuride, bromocriptine, pergolide, and cabergoline were efficacious (75-96%) agonists, apomorphine and terguride were antagonists, and piribedil was inactive. MDL100,907 and SB242,084, selective antagonists at 5-HT_{2A} and 5-HT_{2C} receptors, respectively, abolished these actions of pergolide, cabergoline, and bromocriptine. In conclusion, antiparkinson agents display markedly different patterns of agonist and antagonist properties at multiple 5-HT receptor subtypes. Although all show modest (agonist) activity at 5-HT_{1A} sites, their contrasting actions at 5-HT_{2A} and 5-HT_{2C} sites may be of particular significance to their functional profiles *in vivo*.

Reference no. 51

J Pharmacol Exp Ther. 2003 Sep;306(3):954-64. Epub 2003 May 15.

The novel melatonin agonist agomelatine (S20098) is an antagonist at 5-hydroxytryptamine$_{2C}$ receptors, blockade of which enhances the activity of frontocortical dopaminergic and adrenergic pathways.

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Agomelatine (S20098) displayed pKi values of 6.4 and 6.2 at native (porcine) and cloned, human (h)5-hydroxytryptamine 5-HT$_{2C}$ receptors, respectively. It also interacted with h5-HT$_{2B}$ receptors (6.6), whereas it showed low affinity at native (rat)/cloned, human 5-HT$_{2A}$ (<5.0/5.3) and 5-HT$_{1A}$ (<5.0/5.2) receptors, and negligible (<5.0) affinity for other 5-HT receptors. In antibody capture/scintillation proximity assays, agomelatine concentration dependently and competitively abolished h5-HT$_{2C}$ receptor-mediated activation of Gq/11 and Gi3 (pA$_2$ values of 6.0 and 6.1). As measured by [^{3}H]phosphatidylinositol depletion, agomelatine abolished activation of phospholipase C by h5-HT$_{2C}$ (pKB value of 6.1) and h5-HT$_{2B}$ (pK$_B$ value of 6.6) receptors. *In vivo*, it dose dependently blocked induction of penile erections by the 5-HT$_{2C}$ agonists (S)-2-(6-chloro-5-fluoroindol-1-yl)-1-methylethylamine (Ro60,0175) and 1-methyl-2-(5,8,8-trimethyl-8H-3-aza-cyclopenta[a]inden-3-yl) ethylamine (Ro60,0332). Furthermore, agomelatine dose dependently enhanced dialysis levels of dopamine in frontal cortex of freely moving rats, whereas they were unaffected in nucleus accumbens and striatum. Although the electrical activity of ventrotegmental dopaminergic neurons was unaffected agomelatine, it abolished their inhibition by Ro60,0175. Extracellular levels of noradrenaline in frontal cortex were also dose dependently enhanced by agomelatine in parallel with an acceleration in the firing rate of adrenergic cell bodies in the locus coeruleus. These increases in noradrenaline and dopamine levels were unaffected by the selective melatonin antagonist N-[2-(5-ethyl-benzo[b]thien-3-yl)ethyl] acetamide (S22153) and likely flect blockade of 5-HT$_{2C}$ receptors inhibitory to frontocortical dopaminergic and adrenergic pathways. Correspondingly, distinction to agomelatine, melatonin showed negligible activity 5-HT$_{2C}$ receptors and failed to modify the activity of adrenergic and dopaminergic pathways. In conclusion, in contrast to melatonin, agomelatine behaves as an antagonist at 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors: blockade of the latter reinforces frontocortical adrenergic and dopaminergic transmission.

Reference no. 52

Naunyn Schmiedebergs Arch Pharmacol. 2003 Sep;368(3):188-99. Epub 2003 Aug 16.

Comparison of hippocampal G protein activation by 5-HT_{1A} receptor agonists and the atypical antipsychotics clozapine and S16924.

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This study employed [^{35}S]guanosine 5'- O-(3-thiotriphosphate) ([^{35}S]GTPgammaS) binding to compare the actions of antipsychotic agents known to stimulate cloned, human 5-HT_{1A} receptors with those of reference agonists at postsynaptic 5-HT(1A) receptors. In rat hippocampal membranes, the following order of efficacy was observed (maximum efficacy, Emax, values relative to 5-HT=100): (+)8-OH-DPAT (85), flesinoxan (62), eltoprazine (60), S14506 (59), S16924 (48), buspirone (41), S15535 (22), clozapine (22), ziprasidone (21), pindolol (7), p-MPPI (0), WAY100635 (0), spiperone (0). Despite differences in species and tissue source, the efficacy and potency (pEC_{50}) of agonists (with the exception of clozapine) correlated well with those determined previously at human 5-HT_{1A} receptors expressed in Chinese hamster ovary (CHO) cells. In contrast, clozapine was more potent at hippocampal membranes. The selective antagonists p-MPPI and WAY100635 abolished stimulation of binding by (+)8-OH-DPAT, clozapine and S16924 (p-MPPI), indicating that these actions were mediated specifically by 5-HT_{1A} receptors. Clozapine and S16924 also attenuated 5-HT- and (+)8-OH-DPAT-stimulated [^{35}S]GTPgammaS binding, consistent with partial agonist properties. In [^{35}S]GTPgammaS autoradiographic studies, 5-HT-induced stimulation, mediated through 5-HT_{1A} receptors, was more potent in the septum (pEC_{50} approximately 6.5) than in the dentate gyrus of the hippocampus (pEC_{50} approximately 5) suggesting potential differences in coupling efficiency or G-protein expression. Though clozapine (30 and 100 microM) did not enhance [^{35}S]GTPgammaS labelling in any structure, S16924 (10 micro M) modestly increased [^{35}S]GTPgammaS labelling in the dentate gyrus. On the other hand, both these antipsychotic agents attenuated 5-HT (10 microM)-stimulated [^{35}S]GTPgammaS binding in the dentate gyrus and septum. In conclusion, clozapine, S16924 and ziprasidone act as partial agonists for G protein activation at postsynaptic 5-HT_{1A} receptors in the hippocampus. These data support a role of postsynaptic 5-HT_{1A} receptors in the functional profiles of certain antipsychotic agents.

Reference no. 53

Br J Pharmacol. 2003 Mar;138(6):1077-84.

h5-HT_{1B} receptor-mediated constitutive Galphai3-protein activation in stably transfected Chinese hamster ovary cells: an antibody capture assay reveals protean efficacy of 5-HT.

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1. Serotonin 5-HT_{1B} receptors couple to G-proteins of the Gi/o family. However, their activation of specific G-protein subtypes is poorly characterised. Using an innovative antibody capture/guanosine-5'-0-(3-[^{35}S]thio)-triphosphate ([^{35}S]GTPgammaS) binding strategy, we characterised Galpha(i3) subunit activation by h5-HT_{1B} receptors stably expressed in Chinese hamster ovary (CHO) cells.

2. The agonists, 5-HT, alniditan and BMS181,101, stimulated Galpha(i3), whereas methiothepin and SB224,289 behaved as inverse agonists. The selective 5-HT_{1B} receptor ligand, S18127, modestly stimulated Galpha(i3) and reversed the actions of both 5-HT and methiothepin. S18127 (1 micro M) also produced parallel, dextral shifts of the 5-HT and methiothepin isotherms.

3. Isotopic dilution experiments ([^{35}S]GTPgammaS versus GTPgammaS) revealed high-affinity [^{35}S]GTPgammaS binding to Galpha(i3) subunits in the absence of receptor ligands indicating constitutive activity. High-affinity [^{35}S]GTPgammaS binding was increased 2.8-fold by 5-HT with an increase in the affinity of GTPgammaS for Galpha(i3) subunits. In contrast, methiothepin halved the number of high-affinity binding sites and decreased their affinity.

4. h5-HT_{1B} receptor-mediated Galpha(i3) subunit activation was dependent on the concentration of NaCl. At 300 mM, 5-HT stimulated [^{35}S]GTPgammaS binding, basal Galpha(i3) activation was low and methiothepin was inactive. In contrast, at 10 mM NaCl, basal activity was enhanced and the inverse agonist activity of methiothepin was accentuated. Under these conditions, 5-HT decreased Galpha(i3) activation.

5. In conclusion, at h5-HT_{1B} receptors expressed in CHO cells: (i) inverse agonist induced inhibition of Galpha(i3), and its reversal by S18127, reveals constitutive activation of this Galpha subunit; (ii) constitutive Galpha(i3) activation can be quantified by isotopic dilution [^{35}S]GTPgammaS binding and (iii) decreasing NaCl concentrations enhances Galpha(i3) activation and leads to protean agonist properties of 5-HT: that is a switch to inhibition of Galpha(i3).

Reference no. 54

International Congress Series, 2003 August; 1249:101-117
"Inverse agonism". Proceedings of the Esteve Foundation Symposium X

Differential ligand efficacy at h5-HT_{1A} receptor-coupled G-protein subtypes: a commentary

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Activation of heterotrimeric G-protein involves the exchange by α subunits of GDP for GTP. The binding of the hydrolysis-resistant GTP analogue, guanosine-5'-O-(3-[^{35}S]thio)-triphosphate ([^{35}S]GTPγS), provides a measure of agonist, antagonist and inverse agonist actions at G-protein-coupled receptors. However, classical [^{35}S]GTPγS binding methods do not distinguish among the subtypes of G-proteins activated. This is a limitation in view of (i) promiscuity of receptor coupling to multiple families of G-proteins, and (ii) the differential influence of ligands on distinct signal transduction pathways. A recent development of [^{35}S]GTPγS binding methodology employing antibody capture and scintillation proximity assays (SPA) permits the targeting of specific Gα subtypes in both recombinant and native tissues. When applied to human serotonin$_{1A}$ receptor (h5-HT_{1A}) expressed in Chinese hamster ovary (CHO) cells, this methodology revealed surprising patterns of Gαi3 subunit activation. For example, low but not high concentrations of high-efficacy h5-HT_{1A} agonists direct receptor signalling to Gαi3. In contrast, partial agonists favour h5-HT_{1A} receptor signalling to Gαi3 over a wide concentration range. Further, alterations in buffer sodium concentration reversed these actions of agonists: stimulation of Gαi3 activation was observed at high sodium concentration, but inhibition was observed at low sodium concentration, suggestive of protean efficacy, i.e. a switch from positive to negative efficacy dependent on receptor tone. These results indicate that complex changes in both magnitude and direction of response to receptor ligands can occur for specific G-protein subtypes. Interestingly, the inverse agonist, spiperone, inhibited constitutive h5-HT_{1A} receptor-mediated Gαi3 activation under all conditions tested, consistent with the hypothesis that it selectively stabilises distinct receptor conformation(s). Further, whereas classical [^{35}S]GTPγS binding assays in CHO cell membranes failed to demonstrate negative efficacy for the neuroleptic, haloperidol, this compound exhibited robust inverse agonism for the activation of Gαi3 subunits. These data suggest that haloperidol may selectively inhibit constitutive activation of this G-protein subtype and, thus, exhibit inverse agonist-mediated trafficking of receptor signalling.

Reference no. 55

Psychopharmacology (Berl). 2005 Feb;177(4):373-80. Epub 2004 Sep 24.

Anticataleptic properties of alpha2 adrenergic antagonists in the crossed leg position and bar tests: differential mediation by 5-HT_{1A} receptor activation.

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RATIONALE: Recent studies suggest that alpha2 adrenoceptor blockade may improve the antipsychotic-like effects of neuroleptics and attenuate dopamine D_2 receptor antagonist-induced catalepsy. However, several alpha2 adrenergic antagonists also display serotonin 5-HT_{1A} receptor agonist activity, which may contribute to anticataleptic actions.

OBJECTIVES: In this study, we examined a series of alpha2 adrenergic antagonists to determine the role of activity at serotonin 5-HT_{1A} receptors in their anticataleptic effects.
METHODS: Catalepsy in rats induced by the antipsychotic haloperidol (2.5 mg/kg, SC) was measured using the cross-legged position (CLP) and bar tests. The compounds examined in this study, in decreasing rank order of alpha2 adrenergic versus 5-HT_{1A} receptor selectivity, were atipamezole, methoxy-idazoxan (RX821002), efaroxan, idazoxan, and yohimbine. Antagonism studies were conducted using the selective 5-HT_{1A} receptor antagonist N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl) cyclohexanecarboxamide dihydrochloride (WAY100635).

RESULTS: Idazoxan, efaroxan, and yohimbine significantly attenuated the cataleptic effects of haloperidol (2.5 mg/kg, SC) in the CLP test and the actions of their highest doses were significantly blocked by pre-treatment with WAY100635 (0.63 mg/kg, SC). In contrast to the other compounds, methoxy-idazoxan was ineffective in the CLP test. Atipamezole exhibited anticataleptic effects in the bar and CLP tests which were not blocked by WAY100635. Similarly, the anticataleptic effects of methoxy-idazoxan and idazoxan in the bar test were not blocked by WAY100635.

CONCLUSIONS: Serotonin 5-HT_{1A} receptors play a prominent role in anticataleptic effects of certain alpha2 adrenergic antagonists in the CLP test, whereas alpha2-adrenergic mechanisms are likely to be primarily responsible for the anticataleptic effects of these ligands in the bar test.

Reference no. 56

Int J Neuropsychopharmacol. 2005 Sep;8(3):341-56. Epub 2005 Feb 11.

Novel antipsychotics activate recombinant human and native rat serotonin 5-HT_{1A} receptors: affinity, efficacy and potential implications for treatment of schizophrenia.

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Serotonin 5-HT_{1A} receptors are promising targets in the management of schizophrenia but little information exists about affinity and efficacy of novel antipsychotics at these sites. We addressed this issue by comparing binding affinity at 5-HT_{1A} receptors with dopamine rD_2 receptors, which are important targets for antipsychotic drug action. Agonist efficacy at 5-HT_{1A} receptors was determined for G-protein activation and adenylyl cyclase activity. Whereas haloperidol, thioridazine, risperidone and olanzapine did not interact with 5-HT_{1A} receptors, other antipsychotic agents exhibited agonist properties at these sites. Emax values (% effect induced by 10 microM of 5-HT) for G-protein activation at rat brain 5-HT_{1A} receptors: sarizotan (66.5), bifeprunox (35.9), SSR181507 (25.8), nemonapride (25.7), ziprasidone (20.6), SLV313 (19), aripiprazole (15), tiospirone (8.9). These data were highly correlated with results obtained at recombinant human 5-HT_{1A} receptors in determinations of G-protein activation and inhibition of forskolin-stimulated adenylyl cyclase. In binding-affinity determinations, the antipsychotics exhibited diverse properties at r5-HT_{1A} receptors: sarizotan (pKi=8.65), SLV313 (8.64), SSR181507 (8.53), nemonapride (8.35), ziprasidone (8.30), tiospirone (8.22), aripiprazole (7.42), bifeprunox (7.19) and clozapine (6.31). The affinity ratios of the ligands at 5-HT_{1A} vs. D_2 receptors also varied widely: ziprasidone, SSR181507 and SLV313 had similar affinities whereas aripiprazole, nemonapride and bifeprunox were more potent at D_2 than 5-HT_{1A} receptors. Taken together, these data indicate that aripiprazole has low efficacy and modest affinity at 5-HT_{1A} receptors, whereas bifeprunox has low affinity but high efficacy. In contrast, SSR181507 has intermediate efficacy but high affinity, and is likely to have more prominent 5-HT_{1A} receptor agonist properties. Thus, the contribution of 5-HT_{1A} receptor activation to the pharmacological profile of action of the antipsychotics will depend on the relative 5-HT_{1A}/D_2 affinities and on 5-HT_{1A} agonist efficacy of the drugs.

Reference no. 57

J Pharmacol Exp Ther. 2005 Mar;312(3):1034-42. Epub 2004 Nov 4.

Dual, hyperalgesic, and analgesic effects of the high-efficacy 5-hydroxytryptamine $_{1A}$ (5-HT$_{1A}$) agonist F13640 [(3-chloro-4-fluoro-phenyl)-[4-fluoro-4-{[(5-methyl-pyridin-2-ylmethyl)-amino]-methyl}piperidin-1-yl]methanone, fumaric acid salt]: relationship with 5-HT$_{1A}$ receptor occupancy and kinetic parameters.

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The aim of the present study was to establish the relationship between the plasma and brain concentration-time profiles of F13640 [(3-chloro-4-fluoro-phenyl)-[4-fluoro-4-{[(5-methyl-pyridin-2-ylmethyl)-amino]-methyl}piperidin-1-yl]methanone, fumaric acid salt] after acute administration and both its hyper- and hypoanalgesic effects in rats. The maximal plasma concentration (C(max)) of F13640 after i.p. administration of 0.63 mg/kg was obtained at 15 min and decreased to half its maximal value after about 1 h. The amount of F 13640 collected by means of *in vivo* microdialysis in hippocampal dialysates could be measured reliably after 0.63 and 2.5 mg/kg, reached its maximum at about 1 h, and fell to half of its maximal value at about 3 h. 5-Hydroxytryptamine $_{1A}$ (5-HT$_{1A}$) receptor occupancy was estimated by ex vivo binding in rat brain sections. F13640 inhibited [^{3}H]8-hydroxy-2-[di-n-propylamino] tetralin binding ex vivo in rat hippocampus, entorhinal cortex, and frontal cortex (ED50, 0.34 mg/kg i.p.). Maximal inhibition was reached at approximately 30 min after 0.63 mg/kg F 13640 and fell to half of its value after about 4 to 8 h. After injection (15 min) in the paw pressure test, F 13640 (0.63 mg/kg i.p.) induced an initial hyperalgesia that was followed 4 h later by a paradoxical analgesia that lasted until 8 h. In contrast, in the formalin test, F 13640 inhibited pain behaviors until 4 h after drug administration. F 13640 also produced elements of the 5-HT syndrome that lasted up to 4 h after administration. These results demonstrate that F 13640 induces hyperalgesia and/or analgesia with a time course that parallels the occupancy of 5-HT$_{1A}$ receptors and the presence of the compound in blood and brain.

Reference no. 58

Brain Res. 2005 May 10;1043(1-2):32-41.

Clozapine, ziprasidone and aripiprazole but not haloperidol protect against kainic acid-induced lesion of the striatum in mice, *in vivo*: role of 5-HT$_{1A}$ receptor activation.

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Excessive activation of non-NMDA receptors, AMPA and kainate, contributes to neuronal degeneration in acute and progressive pathologies, possibly including schizophrenia. Because 5-HT$_{1A}$ receptor agonists have neuroprotective properties (e.g., against NMDA-induced neurotoxicity), we compared the effects of the antipsychotics, clozapine, ziprasidone and aripiprazole, that are partial agonists at 5-HT$_{1A}$ receptor, with those of haloperidol, which is devoid of 5-HT$_{1A}$ agonist properties, on kainic acid (KA)-induced striatal lesion volumes, in C57Bl/6N mice. The involvement of 5-HT$_{1A}$ receptors was determined by antagonist studies with WAY100635, and data were compared with those obtained using the potent and high efficacy 5-HT$_{1A}$ receptor agonist, F13714. Intra-striatal KA lesioning and measurement of lesion volumes using cresyl violet staining were carried out at 48 h after surgery. F13714, antipsychotics or vehicle were administered ip twice, 30 min before and 3 ½ h after KA injection. WAY100635 (0.63 mg/kg) or vehicle were given sc 30 min before each drug injection. Clozapine (2 x 10 mg/kg), ziprasidone (2 x 20 mg/kg) and aripiprazole (2 x 10 mg/kg) decreased lesion volume by 61%, 59% and 73%, respectively. WAY100635 antagonized the effect of ziprasidone and of aripiprazole but only slightly attenuated that of clozapine. In contrast, haloperidol (2 x 0.16 mg/kg) did not affect KA-induced lesion volume. F13714 dose-dependently decreased lesion volume. The 61% decrease of lesion volume obtained with F13714 (2 x 0.63 mg/kg) was antagonized by WAY100635. WAY100635 alone did not affect lesion volume. These results show that 5-HT$_{1A}$ receptor activation protects against KA-induced striatal lesions and indicate that some atypical antipsychotic agents with 5-HT$_{1A}$ agonist properties may protect against excitotoxic injury, *in vivo*.

Reference no. 59

Neuropharmacology. 2005 Aug;49(2):135-43. Epub 2005 Apr 1.

Novel antipsychotic agents with 5-HT_{1A} agonist properties: role of 5-HT_{1A} receptor activation in attenuation of catalepsy induction in rats.

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Compounds possessing 5-HT_{1A} agonist properties attenuate catalepsy induced by D_2 receptor blockade. Here we examined the role of 5-HT_{1A} receptor agonism in the reduced cataleptogenic potential of several novel antipsychotic agents in the crossed leg position (CLP) and the bar catalepsy tests in rats. When administered alone, ziprasidone produced marked catalepsy, whereas aripiprazole, bifeprunox, SLV313, SSR181507 and sarizotan did not. However, when 5-HT_{1A} receptors were blocked with the selective antagonist, WAY100635 (0.63 mg/kg, SC), robust cataleptogenic properties of SLV313, bifeprunox and sarizotan were unmasked and the catalepsy induced by ziprasidone was accentuated. In contrast, only modest catalepsy was induced by aripiprazole and SSR181507, even following a higher dose of WAY100635 (2.5 mg/kg). This suggests that these compounds possess other anti-cataleptic properties, such as partial agonism at dopamine D_2 receptors. The capacity to reverse neuroleptic-induced catalepsy was investigated in interaction studies with haloperidol (2.5 mg/kg, SC). Whereas ziprasidone and aripiprazole did not markedly reduce the effects of haloperidol, SLV313 and sarizotan attenuated CLP catalepsy. In contrast, SSR181507 and bifeprunox potently inhibited both CLP and bar catalepsy. Taken together, these data show that 5-HT_{1A} receptor activation reduces the cataleptogenic potential of novel antipsychotic agents but indicate marked diversity in the contribution of 5-HT_{1A} and/or other mechanisms to the profiles of the drugs.

Reference no. 60

J Pharmacol Exp Ther. 2005 Oct;315(1):265-72. Epub 2005 Jun 29.

Contrasting contribution of 5-hydroxytryptamine $_{1A}$ receptor activation to neurochemical profile of novel antipsychotics: frontocortical dopamine and hippocampal serotonin release in rat brain.

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Several novel antipsychotics, such as aripiprazole, bifeprunox, SSR181507 [(3-exo)-8-benzoyl-N-(((2S)7-chloro-2,3-dihydro-1,4-benzodioxin-1-yl)methyl)-8-azabicyclo(3.2.1)octane-3-methanamine], and SLV313 [1-(2,3-dihydro-benzo[1,4]dioxin-5-yl)-4-[5-(4-fluorophenyl)-pyridin-3-ylmethyl]-piperazine], activate serotonin 5-hydroxytryptamine (5-HT)$_{1A}$ receptors. Such activity is associated with enhanced treatment of negative symptoms and cognitive deficits, which may be mediated by modulation of cerebral dopamine and serotonin levels. We employed microdialysis coupled to high pressure liquid chromatography with electrochemical detection to examine 5-HT$_{1A}$ receptor activation in the modulation of extracellular dopamine in medial prefrontal cortex and serotonin in hippocampus of freely moving rats. The above compounds were compared with drugs that have less interaction with 5-HT$_{1A}$ receptors (clozapine, nemonapride, ziprasidone, olanzapine, risperidone, and haloperidol). Hippocampal 5-HT was decreased by bifeprunox, SSR181507, SLV313, sarizotan, and nemonapride, effects similar to those seen with the 5-HT$_{1A}$ agonist, (+)-8-hydroxy-2-(di-n-propylamino)tetralin [(+)8-OH-DPAT], consistent with activation of 5-HT$_{1A}$ autoreceptors. These decreases were reversed by the selective 5-HT$_{1A}$ antagonist, WAY100635 [N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl)cyclohexanecarboxamide]. In contrast, haloperidol, risperidone, clozapine, olanzapine, ziprasidone, and aripiprazole did not significantly modify hippocampal serotonin levels. In medial prefrontal cortex, dopamine levels were increased by SSR181507, SLV313, sarizotan, and (+)8-OH-DPAT. These effects were reversed by WAY100635, indicating mediation by 5-HT$_{1A}$ receptors. In contrast, the increases in dopamine levels induced by clozapine, risperidone, olanzapine, and ziprasidone were not blocked by WAY100635, consistent with predominant influence of other mechanisms in the actions of these drugs. Haloperidol, nemonapride, and the D_2 partial agonists, aripiprazole and bifeprunox, did not significantly alter dopamine release. Taken together, these data demonstrate the diverse contribution of 5-HT$_{1A}$ receptor activation to the profile of antipsychotics and suggest that novel drugs selectively targeting D_2 and 5-HT$_{1A}$ receptors may present distinctive therapeutic properties.

Reference no. 61

Neuropharmacology. 2005 Dec;49(7):963-76. Epub 2005 Jun 17.

Differential ion current activation by human 5-HT_{1A} receptors in Xenopus oocytes: evidence for agonist-directed trafficking of receptor signalling.

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The subject of the present study was the functional and pharmacological characterization of human 5-HT_{1A} receptor regulation of ion channels in Xenopus oocytes. Activation of the heterologously expressed human 5-HT_{1A} receptor induced two distinct currents in Xenopus oocytes, consisting of a smooth inward current (I_{smooth}) and an oscillatory calcium-activated chloride current, I(Cl_{Ca}). 5-HT_{1A} receptor coupling to both ionic responses as well as to co-expressed inward rectifier potassium (GIRK) channels was pharmacologically characterized using 5-HT_{1A} receptor agonists. The relative order of efficacy for activation of GIRK current was 5-HT ~ F 13714 ~ L694,247 ~ LY228,729 > flesinoxan ~ (+/-)8-OH-DPAT. In contrast, flesinoxan and (+/-)8-OH-DPAT typically failed to activate I(Cl_{Ca}). The other ligands behaved as full or partial agonists, exhibiting an efficacy rank order of 5-HT ~ L694,247 > F 13714 ~ LY228,729. The pharmacological profile of I_{smooth} activation was completely distinct: flesinoxan and F 13714 were inactive and rather exhibited an inhibition of this current. I(smooth) was activated by the other agonists with an efficacy order of L 694,247 > 5-HT ~ LY 228,729 > (+/-)8-OH-DPAT. Moreover, activation of I(smooth) was not affected by application of pertussis toxin or the non-hydrolyzable GDP-analogue, guanosine-5'-O-(2-thio)-diphosphate (GDPbetaS), suggesting a GTP binding protein-independent pathway. Together, these results suggest the existence of distinct and agonist-specific signalling states of this receptor.

Reference no. 62

Neuropharmacology. 2005 Dec;49(7):996-1006. Epub 2005 Jul 11.

Effects of novel antipsychotics with mixed D_2 antagonist/5-HT_{1A} agonist properties on PCP-induced social interaction deficits in the rat.

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Considerable interest has arisen in identifying antipsychotic agents with improved efficacy against negative symptoms, such as social withdrawal. In rats, a social interaction deficit can be induced by the NMDA antagonist phencyclidine (PCP). Here, we examined the effects of antipsychotics, reported to exert dual 5-HT_{1A}/D2 actions, on PCP-induced social interaction deficits. Drugs were administered daily for 3 days in combination with either vehicle or PCP (2.5mg/kg, SC) and social interaction was measured on the last day of drug treatment. Pairs of unfamiliar rats receiving the same treatment were placed in a large open field for 10 min and the number of social behaviors were scored. The results indicate that: (1) PCP significantly reduced social interaction by over 50% compared with vehicle-treated controls; (2) haloperidol (0.0025-0.16 mg/kg, SC) and clozapine (0.04-10mg/kg, IP) did not reverse PCP-induced social interaction deficits; (3) the substituted benzamide remoxipride reversed PCP-induced deficits at 0.63 and 2.5mg/kg (4) the 5-HT_{1A} agonist 8-OH-DPAT was inactive (at 0.01-0.63 mg/kg, SC); (5) among compounds reported to exert dual 5-HT_{1A}/D2 actions, SSR181507 (at 0.16 mg/kg, SC) and aripiprazole (at 0.04 and 0.16 mg/kg, IP), but not ziprasidone (0.04-2.5mg/kg, IP), SLV313 (0.0025-0.16 mg/kg, SC) or bifeprunox (0.01-0.63 mg/kg, IP), significantly reversed PCP-induced social interaction deficits; and (6) the 5-HT_{1A} receptor antagonist WAY100635 blocked the effects of SSR181507 and aripiprazole. These findings indicate that the balance of activity at 5-HT_{1A} and D_2 receptors profoundly influences the activity of antipsychotics in this model of social withdrawal, and their potential benefit on at least some of the negative symptoms of schizophrenia.

Reference no. 63

Eur J Pharmacol. 2006 Mar 18;534(1-3):63-70. Epub 2006 Feb 21.

Differential profile of antipsychotics at serotonin 5-HT_{1A} and dopamine D_2S receptors coupled to extracellular signal-regulated kinase.

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The effects of antipsychotics targeting dopamine D_2 and serotonin 5-HT_{1A} receptors were compared with conventional antipsychotics on phosphorylation of Extracellular signal-Regulated Kinase 1/2 (ERK 1/2) in CHO cell lines stably expressing either the human serotonin 5-HT_{1A} or human dopamine D_{2S} receptor. All antipsychotics except haloperidol and olanzapine exhibited agonist properties at serotonin 5-HT_{1A} receptors. Emax values (% effect of 10 microM 5-HT) were: bifeprunox (74), SSR181507 (73), SLV313 (72), aripiprazole (60), ziprasidone (56), clozapine (33). At dopamine D_{2S} receptors, partial agonist activity (% effect of 10 microM dopamine) was observed for bifeprunox (76), SSR181507 (66) and aripiprazole (59). Other antipsychotics attenuated dopamine-induced ERK phosphorylation, with pKB values of : SLV313 (8.5), haloperidol (8.1), olanzapine (7.8), ziprasidone (7.7), and clozapine (6.4). Amongst the dopamine D_2/serotonin 5-HT_{1A} receptor compounds, aripiprazole acts as a partial dopamine D_{2S} and serotonin 5-HT_{1A} receptor agonist. SSR181507 and bifeprunox possess a profile of action similar to each other, efficaciously stimulating both serotonin 5-HT_{1A} and dopamine D_{2S} receptors. In contrast, SLV313, also an efficacious serotonin 5-HT_{1A} receptor agonist, acted as a high potency dopamine D_2 receptor antagonist. Thus, antipsychotics display varying efficacies at serotonin 5-HT_{1A} and dopamine D_{2S} receptors which may play a major role in their differential functional profiles in blocking the diverse symptoms of schizophrenia.

Reference no. 64

Eur J Pharmacol. 2006 Mar 27;535(1-3):135-44. Epub 2006 Mar 22.

Partial agonist properties of the antipsychotics SSR181507, aripiprazole and bifeprunox at dopamine D_2 receptors: G protein activation and prolactin release.

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Dopamine D_2 receptor antagonists induce hyperprolactinemia depending on the extent of D_2 receptor blockade. We compared the effects of the new antipsychotic agents SSR181507 ((3-exo)-8-benzoyl-N-[[(2s)7-chloro-2,3-dihydro-1,4-benzodioxin-1-yl]methyl]-8-azabicyclo[3.2.1]octane-3-methanamine monohydrochloride), bifeprunox (DU127090: 1-(2-Oxo-benzoxazolin-7-yl)-4-(3-biphenyl)methylpiperazinemesylate) and SLV313 (1-(2,3-dihydro-benzo[1,4]dioxin-5-yl)-4-[5-(4-fluorophenyl)-pyridin-3-ylmethyl]-piperazine) with those of aripiprazole (7-{4-[4-(2,3-dichlorophenyl)-1-piperazinyl]-butyloxy)-3,4-dihydro-2(1 H)-quinolinone), clozapine and haloperidol, on functional measures of dopamine D_2 receptor activity *in vitro* and *in vivo*: [^{35}S]-GTPgammaS binding to membranes from Sf9 insect cells expressing human dopamine D_2 Long (hD_{2L}) receptors, and serum prolactin levels in the rat. All compounds antagonized apomorphine-induced G protein activation at dopamine hD_{2L} receptors. Antagonist potencies of aripiprazole, bifeprunox and SLV313 were similar to haloperidol (pK_B = 9.12), whereas SSR181507 (8.16) and clozapine (7.35) were less potent. Haloperidol, SLV313 and clozapine were silent antagonists but SSR181507, bifeprunox and aripiprazole stimulated [^{35}S]-GTPgammaS binding by 17.5%, 26.3% and 25.6%, respectively, relative to 100 microM apomorphine (Emax = 100%). pEC_{50}s were: SSR181507, 8.08; bifeprunox, 8.97; aripiprazole, 8.56. These effects were antagonized by raclopride. Following oral administration *in vivo*, the drugs increased prolactin release to different extents. SLV313 and haloperidol potently (ED_{50} 0.12 and 0.22 mg/kg p.o., respectively) stimulated prolactin release up to 86 and 83 ng/ml. Aripiprazole potently (ED_{50} 0.66 mg/kg p.o.) but partially (32 ng/ml) induced prolactin release. SSR181507 (ED_{50} 4.9 mg/kg p.o.) also partially (23 ng/ml) enhanced prolactin release. Bifeprunox only weakly increased prolactin at high doses (13 ng/ml at 40 mg/kg) and clozapine only affected prolactin at the highest dose tested (41 ng/ml at 40 mg/kg). Prolactin levels of the corresponding vehicle-treated animals were <4.3 ng/ml. These data show that (1) SSR181507, aripiprazole and bifeprunox, but not SLV313, are partial agonists at dopamine hD_{2L} receptors *in vitro*; (2) SSR181507, bifeprunox and aripiprazole exhibit reduced prolactin release *in vivo* compared with drugs that are neutral antagonists at dopamine D_2 receptors.

Reference no. 65

Neuropsychopharmacology. 2006 Sep;31(9):1869-79. Epub 2005 Oct 19.

Antipsychotic-like vs cataleptogenic actions in mice of novel antipsychotics having D_2 antagonist and 5-HT_{1A} agonist properties.

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A new generation of proven or potential antipsychotics, including aripiprazole, bifeprunox, SSR181507 and SLV313, exhibit agonist actions at serotonin 5-HT_{1A} receptors, but little comparative data are available on their pharmacological profiles. Here, we compared in mice the *in vivo* antipsychotic-like vs cataleptogenic activities of these compounds with those of drugs that exhibit little interaction at 5-HT_{1A} receptors, such as haloperidol, olanzapine and risperidone. All the drugs dose-dependently reduced apomorphine-induced climbing or sniffing and, with the exception of ziprasidone, produced complete suppression of these responses. In the bar catalepsy test, when administered alone, haloperidol, olanzapine and risperidone produced marked catalepsy, whereas, at doses up to 40 mg/kg, aripiprazole, SLV313, SSR181507, and sarizotan produced little or no catalepsy. The latter compounds, therefore, displayed a large separation between doses with 'antipsychotic-like' and those with cataleptogenic actions. When 5-HT_{1A} receptors were blocked by pretreatment with WAY100635 (2.5 mg/kg, s.c.), cataleptogenic properties of SSR181507 and sarizotan were unmasked, and the catalepsy induced by bifeprunox was enhanced. In the case of aripiprazole and SLV313, although WAY100635 produced upward shifts in their dose-response, the magnitude of catalepsy appeared to reach an asymptotic plateau, suggesting that other mechanisms may be involved in their low cataleptogenic liability. The present data confirm that 5-HT_{1A} receptor activation reduces or even completely prevents the cataleptogenic potential of novel antipsychotic agents. Further, they indicate that the balance of affinity and/or efficacy between D_2 and 5-HT_{1A} receptors profoundly influences their pharmacological activities, and will likely impact their therapeutic profiles.

Reference no. 66

Neuropsychopharmacology. 2006 Sep;31(9):1900-9. Epub 2006 Jan 18.

Actions of novel antipsychotic agents on apomorphine-induced PPI disruption: influence of combined serotonin 5-HT_{1A} receptor activation and dopamine D_2 receptor blockade.

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The dopamine D_1/D_2 agonist apomorphine (0.63 mg/kg) disrupted prepulse inhibition (PPI) of acoustic startle in rats, a model of sensorimotor gating deficits observed in schizophrenia. All current antipsychotics, which antagonize D_2 receptors, prevent this apomorphine-induced deficit. A novel class of antipsychotics possesses, in addition to D_2 antagonist property, various levels of 5-HT_{1A} agonist activity. Considering that the latter itself produces PPI deficits, it appeared necessary to assess the potential of this novel class of antipsychotics to reverse apomorphine-PPI deficits. Potent D_2 antagonists, like haloperidol (0.63-2.5 mg/kg), risperidone (0.63-10 mg/kg), and olanzapine (0.63-40 mg/kg) prevented apomorphine PPI disruption. The atypical antipsychotics, clozapine (40 mg/kg), nemonapride (0.01-2.5 mg/kg), ziprasidone (10 mg/kg), and aripiprazole (0.01 and 10 mg/kg), which all exhibit 5-HT_{1A} agonist properties, reversed PPI deficits at some doses only, whereas the anti-dyskinetic agent sarizotan (0.16-10 mg/kg), an efficacious 5-HT_{1A} agonist, did not. New generation antipsychotics with marked 5-HT_{1A} agonist properties, such as SLV313 and SSR181507 (0.0025-10 mg/kg and 0.16-10 mg/kg, respectively) did not reverse these deficits whereas bifeprunox (0.04-2.5 mg/kg) did. To reveal the contribution of 5-HT_{1A} agonist properties in the lack of effects of SLV313 and SSR181507, we pretreated rats with the 5-HT_{1A} antagonist WAY100635 (0.63 mg/kg). Under these conditions, significant reversal of PPI deficit was observed, indicating that D_2 antagonist properties of SLV313 and SSR181507 are now sufficient to overcome the disruptive effects of apomorphine. To summarize, antipsychotics possessing agonist efficacy at 5-HT_{1A} receptors exhibit diverse profiles against apomorphine-induced PPI deficits, depending on the balance between D_2 and 5-HT_{1A} activities, suggesting that they may display distinct activity on some aspects of gating deficits in schizophrenic patients.

Reference no. 67

Br J Pharmacol. 2006 Sep;149(2):170-8. Epub 2006 Aug 14.

Rapid desensitization of somatodendritic 5-HT_{1A} receptors by chronic administration of the high-efficacy 5-HT_{1A} agonist, F13714: a microdialysis study in the rat.

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BACKGROUND AND PURPOSE: Desensitization of somatodendritic 5-HT_{1A} receptors is involved in the mechanism of action of several antidepressants, but the rapidity of this effect and the amount of agonist stimulation needed are unclear. We evaluated the capacity of the high-efficacy 5-HT_{1A} agonist, F13714 (3-chloro-4-fluorophenyl-(4-fluoro-4-{[(5-methyl-6-methylamino-pyridin-2-ylmethyl)-amino]-methyl}-piperidin-1-yl-methanone) and of the partial agonist, flesinoxan, to desensitize somatodendritic 5-HT_{1A} receptors involved in the control of 5-HT release.

EXPERIMENTAL APPROACH: Intracerebral microdialysis in the hippocampus of freely moving rats was used to examine the acute and chronic effects of the two compounds (administered by osmotic pumps for 3, 7 or 14 days) on extracellular 5-HT levels, measured by HPLC with electrochemical detection.

KEY RESULTS: When given acutely, F13714, flesinoxan and the low-efficacy 5-HT_{1A} agonist, buspirone, dose-dependently decreased extracellular 5-HT concentrations (ED_{50} values: 0.04, 0.77 and 5.6 mg/kg, respectively). The selective 5-HT_{1A} antagonist WAY100635 inhibited the effects of the three compounds. F13714 (2.5 mg/kg per day for 3, 7 or 14 days and 0.63 mg/kg for 7 days) significantly attenuated the inhibition of 5-HT release induced by buspirone (10 mg/kg). In contrast, flesinoxan (10 mg/kg per day) failed to alter the response to buspirone at any of the treatment durations.

CONCLUSIONS AND IMPLICATIONS: Rat somatodendritic 5-HT_{1A} receptors controlling hippocampal 5-HT release were rapidly desensitized by chronic activation with a high-efficacy 5-HT_{1A} agonist, but not by chronic activation with a partial agonist. Thus, rapid 5-HT_{1A} autoreceptor desensitization by high-efficacy agonists may accelerate the onset of the therapeutic effects of antidepressants.

Reference no. 68

Naunyn Schmiedebergs Arch Pharmacol. 2006 Sep;373(6):441-50. Epub 2006 Sep 1.

In vivo occupancy of dopamine D_2 receptors by antipsychotic drugs and novel compounds in the mouse striatum and olfactory tubercles.

Assié MB, Dominguez H, Consul-Denjean N, **Newman-Tancredi A.**
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Interaction with dopamine D_2-like receptors plays a major role in the therapeutic effects of antipsychotic drugs. We examined *in vivo* dopamine D_2 receptor occupancy of various established and potential antipsychotics in mouse striatum and olfactory tubercles 1 h after administration of the compound, using [^{3}H]nemonapride as a ligand. All the compounds reduced *in vivo* binding of [^{3}H]nemonapride in the striatum. When administered systemically, conventional antipsychotics, D_2 antagonists, nemonapride (ID50: 0.034 mg/kg), eticlopride (0.047), haloperidol (0.11) and raclopride (0.11) potently inhibited [^{3}H]nemonapride binding. The 'atypical' antipsychotics, risperidone (0.18), ziprasidone (0.38), aripiprazole (1.6), olanzapine (0.99), and clozapine (11.1) were less potent for occupying D_2-like receptors. New compounds, displaying marked agonism at 5-HT_{1A} receptors in addition to D_2 receptor affinity, exhibited varying D_2 receptor occupancy: bifeprunox (0.25), SLV313 (0.78), SSR181507 (1.6) and sarizotan (6.7). ID_{50} values for inhibition of [^{3}H]nemonapride binding in the striatum correlated with those in the olfactory tubercles (r=0.95, P<0.0001). These values also correlated with previously-reported *in vitro* affinity of the compounds at rat D_2 receptors (r=0.85, P=0.0001) and with inhibition of apomorphine-induced climbing in mice (r=0.79 P=0.0005). In contrast, there was no significant correlation between ID50 values herein and previously-reported ED_{50} values for catalepsy in mice. These data indicate that: (1) there is no difference in D_2 receptor occupancy in limbic versus striatal regions between most classical and atypical or potential antipsychotics; and (2) high occupancy of D_2 receptors can be dissociated from catalepsy, if the drugs also activate 5-HT_{1A} receptors. Taken together, these data support the strategy of simultaneously targeting D_2 receptor blockade and 5-HT_{1A} receptor activation for new antipsychotics.

Reference no. 69

Mol Pharmacol. 2007 Mar;71(3):638-43. Epub 2006 Dec 13.

Native rat hippocampal 5-HT_{1A} receptors show constitutive activity.

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Previous studies have shown that human 5-hydroxytryptamine $(5\text{-HT})_{1A}$ receptors stably expressed in transfected cell lines show constitutive G-protein activity, as revealed by the inhibitory effect of inverse agonists, such as spiperone, on basal guanosine-5'-O-(3-[^{35}S]thio)-triphosphate ([^{35}S]GTPgammaS) binding. In the present study, we evaluated the constitutive activity of native rat 5-HT_{1A} receptors in hippocampal membranes. Using anti-Galphao-antibody capture coupled to scintillation proximity assay under low sodium (30 mM) conditions, we observed high basal [^{35}S]GTPgammaS binding to Galphao subunits (defined as 100%). Under these conditions, 5-HT and the prototypic selective 5-HT_{1A} agonist (+)8-hydroxy-2-(di-n-propylamino)tetralin [(+)-8-OH-DPAT] both stimulated [^{35}S]GTPgammaS binding to Galphao to a similar extent, raising binding to approximately 130% of basal with pEC_{50} values of 7.91 and 7.87, respectively. The 5-HT_{1A}-selective neutral antagonist [O-methyl-^{3}H]-N-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-N-(2-pyridinyl)cyclohexanecarboxamide trihydrochloride (WAY100635) could block these effects in a competitive manner with pKb values (5-HT, 9.57; (+)-8-OH-DPAT, 9.52) that are consistent with its pKi value at r5-HT_{1A} receptors (9.33). In this native receptor system, spiperone and methiothepin reduced basal [^{35}S]GTPgammaS binding to Galphao in a concentration-dependent manner to 90% of basal with pIC_{50} values of 7.37 and 7.98, respectively. The inhibition of basal [^{35}S]GTPgammaS binding induced by maximally effective concentrations of spiperone (10 microM) or methiothepin (1 microM) was antagonized by WAY100635 in a concentration-dependent manner (pK_B, 9.52 and 8.87, respectively), thus indicating that this inverse agonism was mediated by 5-HT_{1A} receptors. These data provide the first demonstration that native rat serotonin 5-HT_{1A} receptors can exhibit constitutive activity *in vitro*.

Reference no. 70

Neuropharmacology. 2007 Mar;52(4):1106-13. Epub 2007 Jan 19.

Differential agonist and inverse agonist profile of antipsychotics at D_{2L} receptors coupled to GIRK potassium channels.

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The D_2 dopaminergic receptor represents a major target of antipsychotic drugs. Using the coupling of the human D_2long (hD_{2L}) receptor to G protein-coupled inward rectifier potassium (GIRK) channels in Xenopus laevis oocytes, we examined the activity of antipsychotic agents of different classes - typical, atypical, and a "new generation" of compounds, exhibiting a preferential D_2 and 5-HT_{1A} receptor profile. When the hD_{2L} receptor was coexpressed with GIRK channels, a series of reference compounds exhibited full agonist (dopamine, and quinpirole), partial agonist (apomorphine, (-)3-PPP, and (+)-UH232) or inverse agonist (raclopride, and L741626) properties. Sarizotan exhibited only very weak partial agonist action. At higher levels of receptor cRNA injected per oocyte, both partial agonist activity and inverse agonist properties were generally more pronounced. The inverse agonist action of L741626 was reversed by interaction with sarizotan, thus confirming the constitutive activity of wild-type hD_{2L} receptors in the oocyte expression system. When antipsychotic agents were tested for their actions at the hD_{2L} receptor, typical (haloperidol) as well as atypical (nemonapride, ziprasidone, and clozapine) compounds acted as inverse agonists. In contrast, among D_2/5-HT_{1A} antipsychotics, only SLV313 and F15063 behaved as inverse agonists, whilst the other members of this group (bifeprunox, SSR181507 and the recently marketed antipsychotic, aripiprazole) exhibited partial agonist properties. Thus, the X. laevis oocyte expression system highlights markedly different activity of antipsychotics at the hD_{2L} receptor. These differential properties may translate to distinct therapeutic potential of these compounds.

Reference no. 71

Br J Pharmacol. 2007 May;151(2):237-52. Epub 2007 Mar 20.

F15063, a potential antipsychotic with D_2/D_3 antagonist, 5-HT_{1A} agonist and D_4 partial agonist properties. I. *In vitro* receptor affinity and efficacy profile.

Newman-Tancredi A, Assié MB, Martel JC, Cosi C, Slot LB, Palmier C, Rauly-Lestienne I, Colpaert F, Vacher B, Cussac D.
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BACKGROUND AND PURPOSE: Combining 5-HT_{1A} receptor activation with dopamine D_2/D3 receptor blockade should improve negative symptoms and cognitive deficits in schizophrenia. We describe the *in vitro* profile of F15063 (N-[(2,2-dimethyl-2,3-dihydro-benzofuran-7-yloxy)ethyl]-3-(cyclopent-1-enyl)-benzylamine).

EXPERIMENTAL APPROACH: F15063 was characterised in tests of binding affinity and in cellular models of signal transduction at monoamine receptors.

KEY RESULTS: Affinities (receptor and pKi values) of F15063 were: rD_2 9.38; hD_{2L} 9.44; hD_{2S} 9.25; hD_3 8.95; hD_4 8.81; h5-HT_{1A} 8.37. F15063 had little affinity (40-fold lower than D_2) at other targets. F15063 antagonised dopamine-activated G-protein activation at hD_2, rD_2 and hD_3 receptors with potency (pK_B) values 9.19, 8.29 and 8.74 in [^{35}S]GTPgammaS binding experiments) similar to haloperidol. F15063 did not exhibit any hD_2 receptor agonism, even in tests of ERK1/2 phosphorylation and G-protein activation in cells with high receptor expression. In contrast, like (+/-)8-OH-DPAT, F15063 efficaciously activated h5-HT_{1A} (Emax 70%, pEC_{50} 7.57) and r5-HT_{1A} receptors (52%, 7.95) in tests of [^{35}S]GTPgammaS binding, cAMP accumulation (90%, 7.12) and ERK1/2 phosphorylation (93%, 7.13). F15063 acted as a partial agonist for [^{35}S]GTPgammaS binding at hD_4 (29%, 8.15) and h5-HT_{1D} receptors (35%, 7.68). In [^{35}S]GTPgammaS autoradiography, F15063 activated G-proteins in hippocampus, cortex and septum (regions enriched in 5-HT_{1A} receptors), but antagonised quinelorane-induced activation of D_2/D3 receptors in striatum.

CONCLUSIONS and IMPLICATIONS: F15063 antagonised dopamine D_2/D3 receptors, a property underlying its antipsychotic-like activity, whereas activation of 5-HT_{1A} and D_4 receptors mediated its actions in models of negative symptoms and cognitive deficits of schizophrenia (see companion papers).

Reference no. 72

Br J Pharmacol. 2007 May;151(2):253-65. Epub 2007 Mar 20.

F15063, a compound with D_2/D_3 antagonist, 5-HT_{1A} agonist and D_4 partial agonist properties. II. Activity in models of positive symptoms of schizophrenia.

Depoortère R, Bardin L, Auclair AL, Kleven MS, Prinssen E, Colpaert F, Vacher B, **Newman-Tancredi A.**
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BACKGROUND AND PURPOSE: F15063 is a high affinity D_2/D_3 antagonist, D_4 partial agonist, and high efficacy 5-HT_{1A} agonist, with little affinity (40-fold lower than for D_2 receptors) at other central targets. Here, the profile of F15063 was evaluated in models of positive symptoms of schizophrenia and motor side-effects.

EXPERIMENTAL APPROACH: Rodent behavioural tests were based on reversal of hyperactivity induced by psychostimulants and on measures of induction of catalepsy and 'serotonin syndrome'.

KEY RESULTS: F15063 potently (ED_{50}s: 0.23 to 1.10 mg/kg i.p.) reversed methylphenidate-induced stereotyped behaviors, blocked d-amphetamine and ketamine hyperlocomotion, attenuated apomorphine-induced prepulse inhibition (PPI) deficits, and was active in the conditioned avoidance test. In mice, it reversed apomorphine-induced climbing (ED_{50}=0.30 mg/kg i.p.). F15063, owing to its 5-HT_{1A} agonism, did not produce (ED_{50}>40 mg/kg i.p.) catalepsy in rats and mice, a behavior predictive of occurrence of extra-pyramidal syndrome (EPS) in man. This absence of cataleptogenic activity was maintained upon sub-chronic treatment of rats for 5 days at 40 mg/kg p.o. Furthermore, F15063 did not induce the 'serotonin syndrome' in rats (flat body posture and forepaw treading: ED_{50} >32 mg/kg i.p.).

CONCLUSIONS AND IMPLICATIONS: F15063 conformed to the profile of an atypical antipsychotic, with potent actions in models of hyperdopaminergic activity but without inducing catalepsy. These data suggest that F15063 may display potent antipsychotic actions with low EPS liability. This profile is complemented by a favourable profile in rodent models of negative symptoms and cognitive deficits of schizophrenia (companion paper).

Reference no. 73

Br J Pharmacol. 2007 May;151(2):266-77. Epub 2007 Mar 20.

F15063, a compound with D_2/D_3 antagonist, 5-HT_{1A} agonist and D_4 partial agonist properties. III. Activity in models of cognition and negative symptoms.

Depoortère R, Auclair AL, Bardin L, Bruins Slot L, Kleven MS, Colpaert F, Vacher B, **Newman-Tancredi A.**
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BACKGROUND AND PURPOSE: The D_2/D3 receptor antagonist, D_4 receptor partial agonist, and high efficacy 5-HT_{1A} receptor agonist F15063 was shown to be highly efficacious and potent in rodent models of activity against positive symptoms of schizophrenia. However F15063 induced neither catalepsy nor the 'serotonin syndrome'. Here, we evaluated its profile in rat models predictive of efficacy against negative symptoms/cognitive deficits of schizophrenia.

EXPERIMENTAL APPROACH: F15063, given i.p., was assessed in models of behavioural deficits induced by interference with the NMDA/glutamatergic (phencyclidine: PCP) or cholinergic (scopolamine) systems.

KEY RESULTS: Through 5-HT_{1A} activation, F15063 partially alleviated (MED: 0.04 mg/kg) PCP-induced social interaction deficit between two adult rats, without effect by itself, underlining its potential to combat negative symptoms. At doses above 0.16 mg/kg, F15063 reduced interaction by itself. F15063 (0.16 mg/kg)) selectively re-established PCP-impaired 'cognitive flexibility' in a reversal learning task, suggesting potential against adaptability deficits. F15063 (0.04-0.63 mg/kg) also reversed scopolamine-induced amnesia in a juvenile-adult rat social recognition test, indicative of a pro-cholinergic influence. Activity in this latter test is consistent with its D_4 partial agonism, as it was blocked by the D_4 antagonist L745,870. Finally, F15063 up to 40 mg/kg did not disrupt basal prepulse inhibition of startle reflex in rats, a marker of sensorimotor gating.

CONCLUSIONS AND IMPLICATIONS: The balance of D_2/D_3, D_4 and 5-HT_{1A} receptor interactions of F15063 yields a promising profile of activity in models of cognitive deficits and negative symptoms of schizophrenia.

Reference no. 74

Naunyn Schmiedebergs Arch Pharmacol. 2007 Jun;375(4):241-50. Epub 2007 Apr 24.

F15063, a potential antipsychotic with dopamine D_2/D_3 antagonist, $5\text{-}HT_{1A}$ agonist and D_4 partial agonist properties: (IV) duration of brain D_2-like receptor occupancy and antipsychotic-like activity versus plasma concentration in mice.

Assié MB, Bardin L, Auclair A, Consul-Denjean N, Sautel F, Depoortère R, **Newman-Tancredi A.**
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F15063 (N-[(2,2-dimethyl-2,3-dihydro-benzofuran-7-yloxy)ethyl]-3-(cyclopent-1-enyl)-benzylamine fumarate salt) is a novel potential antipsychotic with dopamine D_2/D_3 blocking properties and agonist activity at $5\text{-}HT_{1A}$ and D_4 receptors. The pertinent parameter for pharmacological activity of antipsychotics appears to be central D_2-like receptor occupancy. However, its duration is not necessarily correlated with drug plasma levels, on which clinical dosing regimens are often based. Thus, we compared in mice the duration of actions of F15063 and haloperidol to (1) inhibit apomorphine-induced climbing and sniffing (behavioural measures of D_2-like receptor antagonism) and (2) occupy D_2-like receptors *in vivo* in the striatum and olfactory tubercles (inhibition of [^{3}H]nemonapride binding). Finally, we measured plasma levels of F15063. D_2-like receptor occupancy in the striatum remained elevated at 1, 4 and 8 h postadministration, with both F15063 (ID_{50}: 7.1, 3.6 and 16.5 mg/kg p.o., respectively) and the typical antipsychotic, haloperidol (ID_{50}: 1.4, 0.52 and 0.53 mg/kg p.o., respectively). This was paralleled by a protracted inhibition of apomorphine-induced climbing (ED_{50}: 0.9, 2.8 and 3.6 mg/kg p.o., and 0.21, 0.37 and 0.87 mg/kg p.o., respectively, for F15063 and haloperidol). In contrast, after administration of 10 mg/kg p.o. of F15063, its plasma levels decreased rapidly: 15.2, 2.1 and 0.6 ng/ml, 1, 4 and 8 h after administration, respectively. A similar pattern of results was observed when F15063 and haloperidol were administered i.p. and s.c., respectively. To summarise, the time-course of D_2-like receptor occupancy and inhibition of apomorphine-climbing (and sniffing) behaviours was similarly long lasting with F15063 and haloperidol. In addition, the durations of action of F15063 and haloperidol in a behavioural model of antipsychotic-like activity were closely correlated to their occupancy of central D_2-like receptors, and much longer than their presence in plasma.

Reference no. 75

Curr Opin Investig Drugs. 2007 Jul;8(7):539-54.

Neuropharmacological profile of bifeprunox: merits and limitations in comparison with other third-generation antipsychotics.

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Schizophrenia is characterized by a range of positive and negative symptoms, and cognitive deficits. While positive symptoms respond to current antipsychotic agents, negative symptoms and cognitive deficits are often resistant to pharmacopea. Thus research is now focused on developing third-generation antipsychotics that combine antagonism or partial agonism at dopamine D_2-like receptors with agonism at serotonin 5-HT_{1A} receptors. Such an association is anticipated to provide therapeutic benefits against a broader range of schizophrenia symptoms. Bifeprunox is one such third-generation antipsychotic agent which acts as a partial agonist at D_2-like receptors and is an efficacious agonist at 5-HT_{1A} receptors, with little interaction at $5HT_{2A/2C}$, muscarinic or histaminergic H1 receptors. This review summarizes the pharmacological profiles of the current antipsychotic agents and describes the rationale behind the development of third-generation antipsychotics. It also evaluates current data concerning bifeprunox in comparison with currently available antipsychotics, as well as those that are still under clinical development.

Reference no. 76

Behav Pharmacol. 2007 Mar;18(2):103-18.

Pharmacological profiles in rats of novel antipsychotics with combined dopamine D_2/serotonin 5-HT_{1A} activity: comparison with typical and atypical conventional antipsychotics.

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Combining antagonist/partial agonist activity at dopamine D_2 and agonist activity at serotonin 5-HT_{1A} receptors is one of the approaches that has recently been chosen to develop new generation antipsychotics, including bifeprunox, SSR181507 and SLV313. There have been, however, few comparative data on their pharmacological profiles. Here, we have directly compared a wide array of these novel dopamine D_2/5-HT_{1A} and conventional antipsychotics in rat models predictive of antipsychotic activity. Potency of antipsychotics to antagonize conditioned avoidance, methylphenidate-induced behaviour and D-amphetamine-induced hyperlocomotion correlated with their affinity at dopamine D_2 receptors. Potency against ketamine-induced hyperlocomotion was independent of affinity at dopamine D_2 or 5-HT_{1A} receptors. Propensity to induce catalepsy, predictive of occurrence of extrapyramidal side effects, was inversely related to affinity at 5-HT_{1A} receptors. As a result, preferential D_2/5-HT_{1A} antipsychotics displayed a large separation between doses producing 'antipsychotic-like' vs. cataleptogenic actions. These data support the contention that 5-HT_{1A} receptor activation greatly reduces or prevents the cataleptogenic potential of novel antipsychotics. They also emphasize that interactions at 5-HT_{1A} and D_2 receptors, and the nature of effects (antagonism or partial agonism) at the latter has a profound influence on pharmacological activities, and is likely to affect therapeutic profiles.

Reference no. 77

Psychopharmacology (Berl). 2007 Jul;193(1):45-54. Epub 2007 Mar 29.

Putative antipsychotics with pronounced agonism at serotonin 5-HT_{1A} and partial agonist activity at dopamine D_2 receptors disrupt basal PPI of the startle reflex in rats.

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INTRODUCTION: Prepulse inhibition (PPI) of the startle reflex has been extensively studied because it is disrupted in several psychiatric diseases, most notably schizophrenia. In rats, and to a lesser extent, in humans, PPI can be diminished by dopamine (DA) D_2/D_3 and serotonin 5-HT_{1A} receptor agonists. A novel class of potential antipsychotics (SSR181507, bifeprunox, and SLV313) possess partial agonist/antagonist properties at D_2 receptors and various levels of 5-HT_{1A} activation.

MATERIALS AND METHODS: It thus appeared warranted to assess, in Sprague-Dawley rats, the effects of these antipsychotics on basal PPI.

RESULTS: SSR181507, sarizotan, and bifeprunox decreased PPI, with a near-complete abolition at 2.5-10 mg/kg; SLV313 had a significant effect at 0.16 mg/kg only. Co-treatment with the 5-HT_{1A} receptor antagonist WAY100635 (0.63 mg/kg) showed that the 5-HT_{1A} agonist activity of SSR181507 was responsible for its effect. By contrast, antipsychotics with low affinity and/or efficacy at 5-HT_{1A} receptors, such as aripiprazole (another DA D_2/D_3 and 5-HT_{1A} ligand), and established typical and atypical antipsychotics (haloperidol, clozapine, risperidone, olanzapine, quetiapine, and ziprasidone) had no effect on basal PPI (0.01-2.5 to 2.5-40 mg/kg).

DISCUSSION: The present data demonstrate that some putative antipsychotics with pronounced 5-HT_{1A} agonist activity, coupled with partial agonist activity at DA D_2 receptors, markedly diminish PPI of the startle reflex in rats.

CONCLUSIONS: These data raise the issue of the influence of such compounds on sensorimotor gating in humans.

Reference no. 78

Eur J Pharmacol. 2007 Nov 21;574(1):15-9. Epub 2007 Jul 13.

WAY-100635 has high selectivity for serotonin 5-HT_{1A} versus dopamine D_4 receptors.

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The serotonin 5-HT_{1A} receptor antagonist WAY-100635 was recently reported to have potent agonist properties at dopamine D_4 receptors (Chemel et al., 2006, Psychopharmacology 188, 244-251.). Herein WAY-100635 (pKi at human (h) serotonin 5-HT_{1A} receptors=9.51; pKi at dopamine $hD_{4.4}$ receptors=7.42) stimulated [^{35}S]GTPgammaS incorporation in membranes of Chinese Hamster Ovary cells expressing dopamine $hD_{4.4}$ receptors with only moderate potency and modest efficacy (pEC_{50}=6.63; Emax=19% of dopamine). Moreover, in antagonism experiments, WAY-100635 had a much lower potency at dopamine $hD_{4.4}$ receptors (pKB=7.09), than at serotonin h5-HT_{1A} receptors (pK_B=9.47). These data demonstrate that WAY-100635 has high selectivity for serotonin h5-HT_{1A}versus dopamine $hD_{4.4}$ receptors.

Reference no. 79

J Pharmacol Exp Ther. 2008 Feb;324(2):587-99. Epub 2007 Nov 16.

S33138 [N-[4-[2-[(3aS,9bR)-8-cyano-1,3a,4,9b-tetrahydro[1]-benzopyrano[3,4-c]pyrrol-2(^{3}H)-yl)-ethyl]phenylacetamide], a preferential dopamine D_3 versus D_2 receptor antagonist and potential antipsychotic agent: I. Receptor-binding profile and functional actions at G-protein-coupled receptors.

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The novel, potential antipsychotic, S33138 (N-[4-[2-[(3aS,9bR)-8-cyano-1,3a,4,9b-tetrahydro[1]benzopyrano[3,4-c]pyrrol-2(^{3}H)-yl)-ethyl]phenylacetamide), displayed approximately 25-fold higher affinity at human (h) dopamine D_3 versus hD_{2L} (long isoform) and hD_{2S} (short isoform) receptors (pKi values, 8.7, 7.1, and 7.3, respectively). Conversely, haloperidol, clozapine, olanzapine, and risperidone displayed similar affinities for hD_3, hD_{2L}, and hD_{2S} sites. In guanosine-5'-O-(3-[^{35}S]thio)-triphosphate ([^{35}S]-GTPgammaS) filtration assays, S33138 showed potent, pure, and competitive antagonist properties at hD_3 receptors, displaying pKB and pA2 values of 8.9 and 8.7, respectively. Higher concentrations were required to block hD_2L and hD_2S receptors. Preferential antagonist properties of S33138 at hD_3 versus hD_2L receptors were underpinned in antibody capture/scintillation proximity assays (SPAs) of Galpha(i3) recruitment and in measures of extracellular-regulated kinase phosphorylation. In addition, in cells cotransfected with hD_3 and hD_2L receptors that assemble into heterodimers, S33138 blocked (pK_B, 8.5) the inhibitory influence of quinpirole upon forskolin-stimulated cAMP formation. S33138 had low affinity for hD_4 receptors (<5.0) but revealed weak antagonist activity at hD1 receptors (Galphas/SPA, pK_B, 6.3) and hD_5 sites (adenylyl cyclase, 6.5). Modest antagonist properties were also seen at human serotonin ($5\text{-}HT_{2A}$) receptors (Galpha(q)/SPA, pK_B, 6.8, and inositol formation, 6.9 and at 5-HT7 receptors (adenylyl cyclase, pKB, 7.1). In addition, S33138 antagonized $halpha_{2C}$ adrenoceptors ([^{35}S]GTPgammaS, 7.2; Galpha(i3)/SPA, 6.9; Galpha(o)/SPA, 7.3, and extracellular-regulated-kinase, 7.1) but not $halpha_{2A}$ or $halpha_{2B}$ adrenoceptors (<5.0). Finally, in contrast to haloperidol, clozapine, olanzapine, and risperidone, S33138 displayed negligible affinities for multiple subtypes of alpha1-adrenoceptor, muscarinic, and histamine receptor. In conclusion, S33138 possesses a distinctive receptor-binding profile and behaves, in contrast to clinically available antipsychotics, as a preferential antagonist at hD_3 versus hD_2 receptors.

Reference no. 80

Eur J Pharmacol. 2008 Feb 26;581(1-2):37-46. Epub 2007 Nov 28.

Antipsychotics differ in their ability to internalise human dopamine D_2S and human serotonin 5-HT_{1A} receptors in HEK293 cells.

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Antipsychotic drugs act preferentially via dopamine D_2 receptor blockade, but interaction with serotonin 5-HT_{1A} receptors has attracted interest as additional target for antipsychotic treatment. As receptor internalisation is considered crucial for drug action, we tested the propensity of antipsychotics to internalise human (h)D_{2S} receptors and h5-HT_{1A} receptors. Agonist-induced internalisation of hemaglutinin (HA)-tagged hD_{2S} and HA-h5-HT_{1A} receptors expressed in HEK293 cells was increased by coexpression of G-protein coupled receptor kinase 2 and beta-arrestin2. At the HA-hD_{2S} receptor, dopamine, quinpirole and bromocriptine behaved as full agonists, while S(-)-3-(3-hydroxyphenyl)-N-n-propylpiperidine [(-)-3PPP] and sarizotan were partial agonists. The typical antipsychotic, haloperidol, and the atypical compounds, olanzapine, nemonapride, ziprasidone and clozapine did not internalise HA-hD_{2S} receptors, whereas aripiprazole potently internalised these receptors (>50% relative efficacy). Among antipsychotics with combined D_2/5-HT_{1A} properties, bifeprunox and (3-exo)-8-benzoyl-N-[[(2S)7-chloro-2,3-dihydro-1,4-benzodioxin-1-yl]methyl]-8-azabicyclo-[3.2.1]octane-3-methanamine (SSR181507) partially internalised HA-hD(2S) receptors, piperazine, 1-(2,3-dihydro-1,4-benzodioxin-5-yl)-4-[[5-(4-fluorophenyl)-3-pyridinyl]methyl (SLV313) and N-[(2,2-dimethyl-2,3-dihydro-benzofuran-7-yloxy)ethyl]-3-(cyclopent-1-enyl)-benzylamine (F15063) were inactive. At the HA-h5-HT_{1A} receptor, serotonin, (+)-8-hydroxy-2-(di-n-propylamino)tetralin [(+)-8-OH-DPAT] and sarizotan were full agonists, buspirone acted as partial agonist. (-)-Pindolol showed little activity and no internalising properties were manifested for the 5-HT_{1A} receptor antagonist N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]-ethyl]-N-(2-pyridinyl)cyclohexanecarboxamide (WAY100635). Most antipsychotics induced HA-h5-HT_{1A} receptor internalisation, with an efficacy rank order: nemonapride>F15063>SSR181507>bifeprunox ~ SLV313 ~ ziprasidone>aripiprazole and potencies: SLV313>SSR181507 ~ F15063>bifeprunox ~ nemonapride ~ aripiprazole>ziprasidone. Interestingly, the internalisation induced by clozapine was only minimal, whereas aripirazole and bifeprunox were more potent for internalisation than for G-protein activation. These different profiles of antipsychotics for receptor internalisation may help to evaluate their potential therapeutic impact in the treatment of schizophrenia.

Reference no. 81

Behav Pharmacol. 2008 Mar;19(2):145-52.

Effects of antipsychotics and reference monoaminergic ligands on marble burying behavior in mice.

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Antipsychotics constitute efficacious augmenting agents in the treatment of anxiety disorders, including refractory obsessive-compulsive disorder. We examined the effects of 36 compounds, including typical, atypical and novel antipsychotics with dual dopamine D_2/5-hydroxytryptamine $_{1A}$ (D_2/5-HT_{1A}) actions on marble burying behavior in mice, a putative preclinical test for anxiety disorders. One hour after drug administration, male NMRI mice were placed individually in cages containing 20 marbles, and the total number of marbles buried after 30 min was counted. The selective serotonin reuptake inhibitors, citalopram (2.5-40 mg/kg), fluoxetine (2.5-10 mg/kg) and the benzodiazepine diazepam (2.5-10 mg/kg), reduced the number of buried marbles. The atypical antipsychotic, clozapine (0.16-10 mg/kg), but not its congener olanzapine, was effective in this test. Haloperidol, a typical antipsychotic, also reduced the number of buried marbles, albeit not in a dose-dependent manner. The atypical risperidone was partially active (0.16-0.63 mg/kg), as was the benzamide derivative, amisulpride, albeit at high (10-40 mg/kg) doses. Among the 'third-generation' antipsychotics possessing combined D_2/5-HT_{1A} properties, bifeprunox was active at 0.0025 mg/kg, whereas SLV313 and aripiprazole were active only at the highest doses (2.5 and 10 mg/kg, respectively). SSR181507, F15063 and the antidyskinetic agent, sarizotan, were without any effect. Among a series of receptor subtype-selective ligands, only the 5-HT_{1A} agonist, (+)-8-OH-DPAT (0.63-2.5 mg/kg) and the 5-$HT_{2A/2B/2C}$ antagonist, ritanserin (0.63-2.5 mg/kg) were active. Among novel antipsychotics with dual D_2/5-HT_{1A} properties, only bifeprunox was able to potently reduce the number of buried marbles. Inhibition of marble burying behavior may result from the interplay of several receptor systems, including 5-HT2 receptor blockade, dopamine D_2 partial agonism and serotonin 5-HT_{1A} agonism.

Reference no. 82

Int J Neuropsychopharmacol. 2008 May;11(3):293-307. Epub 2007 Sep 26.

Agonist and antagonist properties of antipsychotics at human dopamine $D_{4.4}$ receptors: G-protein activation and K+ channel modulation in transfected cells.

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Interaction at dopamine D_4 receptors may improve cognitive function, which is highly impaired in individuals with schizophrenia, but comparative studies of recent antipsychotics in cellular models of D_4 receptor activation are lacking. Here, we report the in-vitro profile of over 30 ligands at recombinant $hD_{4.4}$ receptors. In [^{35}S]GTPgammaS binding experiments using membranes of CHO-$hD_{4.4}$ cells, apomorphine, preclamol and the selective D_4 agonists, ABT724, CP226269, Ro-10-5824 and PD168077, behaved as partial agonists (Emax 20-60% vs. dopamine), whereas L745870 and RBI257, displayed antagonist properties. The 'conventional' antipsychotic, haloperidol and the 'atypicals', clozapine and risperidone, exhibited antagonist properties, while 'third generation' compounds bifeprunox, SLV313 and F15063, acted as partial agonists (10-30%). Aripiprazole and SSR181507 slightly stimulated [^{35}S]GTPgammaS binding at micromolar concentrations. In Xenopus laevis oocytes co-expressing $hD_{4.4}$ receptors with G-protein-coupled inwardly rectifying potassium (GIRK) channels, apomorphine, preclamol, ABT724, CP226269, and PD168077 stimulated GIRK currents (Emax 70-80%). The 5-HT_{1A} receptor ligands, WAY100635 and flibanserin, also exhibited partial agonist activity (30% and 15%, respectively). Haloperidol, clozapine, olanzapine and nemonapride did not stimulate GIRK currents, whereas aripiprazole, bifeprunox, SLV313 and F15063, but not SSR181507, exhibited partial agonism (Emax 20-35%). In-vitro responses depended on experimental conditions: increasing NaCl concentration (30 mm to 100 mm) reduced agonist efficacy in [^{35}S]GTPgammaS binding, whereas decreasing the amount of $hD_{4.4}$ cRNA injected into oocytes (from 2.0 to 0.5 ng/oocyte) reduced agonist efficacy of several compounds. These data indicate that, unlike conventional or 'atypical' antipsychotics, several 'third generation' agents display D_4 receptor partial agonism that may be sufficient to influence physiological D_4 receptor activity *in vivo*.

Reference no. 83

Eur J Pharmacol. 2008 Sep 11;592(1-3):160-6. Epub 2008 Jul 4.

The antipsychotics clozapine and olanzapine increase plasma glucose and corticosterone levels in rats: comparison with aripiprazole, ziprasidone, bifeprunox and F15063.

Assié MB, Carilla-Durand E, Bardin L, Maraval M, Aliaga M, Malfètes N, Barbara M, **Newman-Tancredi A.**
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Several novel antipsychotics activate serotonin 5-HT_{1A} receptors as well as antagonising dopamine $D_{2/3}$ receptors. Such a pharmacological profile is associated with a lowered liability to produce extrapyramidal side effects and enhanced efficacy in treating negative and cognitive symptoms of schizophrenia. However, 5-HT_{1A} receptor agonists increase plasma corticosterone and many antipsychotics disturb the regulation of glucose. Here, we compared the influence on plasma glucose and corticosterone of acute treatments with 'new generation' antipsychotics which target dopamine $D_{2/3}$ receptors and 5-HT_{1A} receptors, with that of atypical antipsychotics, and with haloperidol. Olanzapine and clozapine, antipsychotics that are known to produce weight gain and diabetes in humans, both at 10 mg/kg p.o., substantially increased plasma glucose (from 0.8 to 1.7 g/l) at 1 h after administration, an effect that returned to control levels after 4 h. In comparison, F15063 (40 mg/kg p.o.) was without effect at any time point. Olanzapine and clozapine dose-dependently increased plasma glucose concentrations as did SLV313 and SSR181507. Haloperidol and risperidone had modest effects whereas aripiprazole, ziprasidone and bifeprunox, antipsychotics that are not associated with metabolic dysfunction in humans, and F15063 had little or no influence on plasma glucose. The same general pattern of response was found for plasma corticosterone levels. The present data provide the first comparative study of conventional, atypical and 'new generation' antipsychotics on glucose and corticosterone levels in rats. A variety of mechanisms likely underlie the hyperglycemia and corticosterone release observed with clozapine and olanzapine, whilst the balance of dopamine $D_{2/3}$/5-HT_{1A} interaction may contribute to the less favourable impact of SLV313 and SSR181507 compared with that of bifeprunox and F15063.

Reference no. 84

Eur J Pharmacol. 2008 Oct 10;594(1-3):32-8. Epub 2008 Jul 30.

Agonist-directed trafficking of signalling at serotonin 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C}-VSV receptors mediated Gq/11 activation and calcium mobilisation in CHO cells.

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Several examples of agonist-directed trafficking of receptor signalling at 5-HT_{2A} and 5-HT_{2C} receptors have been reported that involve independent downstream transduction pathways. We now report the functional selectivity of a series of chemically diverse agonists at human (h)5-HT_{2A}, h5-HT_{2B} and h5-HT_{2C}-VSV by examining two related responses, the upstream activation of Gq/11 proteins in comparison with its associated cascade of calcium mobilisation. At the h5-HT_{2A} receptor, d-lysergic acid diethylamide (LSD) and the antiparkinsonian agents lisuride, bromocriptine and pergolide exhibit a higher potency for Gq/11 activation than calcium release in contrast with all the other tested ligands such as 5-HT, mCPP and BW723C86, that show an opposite preference of signalling pathway. Comparable observations are made at h5-HT_{2B} and h5-HT_{2C}-VSV receptors, suggesting a similar mechanism of functional selectivity for the three serotonin receptors. Interestingly, the non-hallucinogenic compound lisuride behaves as a partial agonist for both Gq/11 activation and calcium release at the three 5-HT2 receptors, in contrast with DOI, LSD, pergolide and bromocriptine, which are known to provoke hallucinations, and behave as more efficacious agonists. Hence, a functional selectivity for Gq/11 activation together with a threshold of efficacy at h5-HT_{2A} (and possibly h5-HT_{2B} and/or h5-HT_{2C}-VSV) may contribute to hallucinogenic liability. Thus, our results extend the notion of agonist-directed trafficking of receptor signalling to all the 5-HT2-receptor family and indicate that measures of Gq/11 activation versus calcium release may be useful to identify more effective therapeutic drugs with limited side effects.

Reference no. 85

Eur J Pharmacol. 2008 Nov 12;597(1-3):34-8. Epub 2008 Aug 24.

Apomorphine-induced emesis in dogs: differential sensitivity to established and novel dopamine D_2/5-HT_{1A} antipsychotic compounds.

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Small rodents (mice, rats) are the species of choice for evaluating the pharmacology of centrally acting compounds, such as antipsychotics, whereas toxicology data are routinely obtained from other species (rabbits, dogs, monkeys). Whilst there is a substantial number of "therapeutically relevant" pharmacological models for "antipsychotic-like" activity in small rodents, based on hyperdopaminergic or hypoglutamatergic/NMDA approaches, there is a remarkable paucity of such models in other species. Here, we compared the efficacy and potency of reference and new generation dopamine D_2/5-HT_{1A} putative antipsychotics, administered orally, against apomorphine-induced emesis in dogs, a model of central D_2 receptor activation that can be implemented with relative ease. Risperidone potently and fully (10 microg/kg) prevented emesis/retching induced by 0.1 mg/kg s.c. apomorphine. SLV313 and F15063 (D2 receptor antagonists/5-HT_{1A} receptor agonists) also abolished emesis/retching, albeit less potently than risperidone (minimal effective dose, MEDs: 10 and 40 microg/kg, respectively). The D_2 receptor partial agonists/5-HT_{1A} receptor agonists aripiprazole and bifeprunox, (up to 80 microg/kg) only partially attenuated emesis, as did the peripheral D_2 receptor antagonist domperidone. Under the present experimental conditions, haloperidol was only efficacious at the highest dose tested (320 microg/kg). To summarize, dogs are very sensitive to the dopaminergic blocking effects of antipsychotics in this model of D_2 receptor activation. This model can thus be advantageously used to investigate the pharmacological activity of novel D_2 receptor antagonists/partial agonists in dogs.

Reference no. 86

Eur J Pharmacol. 2009 Apr 1;607(1-3):74-83.

F15063, a potential antipsychotic with dopamine D_2/D_3 receptor antagonist, 5-HT_{1A} receptor agonist and dopamine D_4 receptor partial agonist properties: influence on neuronal firing and neurotransmitter release.

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F15063 (N-[(2,2-dimethyl-2,3-dihydro-benzofuran-7-yloxy)-ethyl]-(3-cyclopenten-1-yl-benzyl)-amine) is a potential antipsychotic with dopamine D_2/D_3 receptor antagonist, 5-HT_{1A} receptor agonist and dopamine D_4 receptor partial agonist properties. Herein, we compared its effects on rat ventral tegmental area dopamine and dorsal raphe serotonin electrical activity with those of the dopamine D_2 receptor partial agonist/5-HT_{1A} receptor agonist, SSR181507. Further, we investigated the modulation of extracellular dopamine and noradrenaline in the medial prefrontal cortex and serotonin in the hippocampus of freely moving rats by F15063 using *in vivo* microdialysis. In the ventral tegmental area, F15063 (200-700 microg/kg, i.v.) did not alter the electrical activity of dopamine neurons whereas SSR181507 (250-1000 microg/kg, i.v.) partially inhibited it, consistent with dopamine D_2 receptor partial agonism. Both compounds reduced the inhibition of firing rate induced by the full agonist apomorphine. In the dorsal raphe, both ligands suppressed firing activity, consistent with agonism at 5-HT_{1A} autoreceptors, although SSR181507 (25-75 microg/kg, i.v.) was more potent than F15063 (100-300 microg/kg, i.v.). F15063 (0.63-40 mg/kg, i.p.) dose-dependently increased dopamine levels in the prefrontal cortex and decreased hippocampal 5-HT. These effects were reversed by the selective 5-HT_{1A} receptor antagonist WAY100635 (0.16 mg/kg, s.c.), indicating that they were mediated by 5-HT_{1A} receptors (at post- and pre-synaptic levels, respectively). In the medial prefrontal cortex, noradrenaline levels were moderately but significantly increased by F15063 at 2.5 mg/kg. In conclusion, whereas SSR181507 exhibits (partial) agonism at dopamine D_2 and 5-HT_{1A} receptors, F15063 blocks dopamine D_2-like receptors whilst activating 5-HT_{1A} receptors. Such a profile distinguishes F15063 from SSR181507 and currently available antipsychotic drugs.

Reference no. 87

Behav Pharmacol. 2009 Jul;20(4):303-11.

Penile erection and yawning induced by dopamine D_2-like receptor agonists in rats: influence of strain and contribution of dopamine D_2, but not D_3 and D_4 receptors.

Depoortère R, Bardin L, Rodrigues M, Abrial E, Aliaga M, **Newman-Tancredi A.**
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Dopamine (DA) is implicated in penile erection (PE) and yawning (YA) in rats through activation of D_2-like receptors. However, the exact role of each subtype (D_2, D_3 and D_4) of this receptor family in PE/YA is still not clearly elucidated. We recorded concomitantly PE and YA after treatment with agonists with various levels of selectivity for the different subtypes of D_2-like receptors. In addition, we investigated the efficacy of antagonists with selective or preferential affinity for each of the three receptor subtypes to prevent apomorphine-induced PE and YA. Wistar rats were more sensitive than Long-Evans rats to the erectogenic activity of the nonselective DA agonist apomorphine (0.01-0.08 mg/kg), whereas Sprague-Dawley rats were insensitive. However, all the three strains were equally sensitive to apomorphine-induced YA. In Wistar rats, apomorphine (0.01-0.63 mg/kg), the D_2/D_3 agonists quinelorane and (+)7-OH-DPAT (0.000625-10 mg/kg) or PD 128,907 (0.01-10 mg/kg), but not the D_4 agonists PD-168,077, RO-10-5824 and ABT-724 (0.04-0.63 mg/kg), produced PE and YA with bell-shaped dose-response curves. Similarly, ABT-724 and CP226-269 (another D_4 agonist) failed to elicit PE and YA in Sprague-Dawley rats. Furthermore, in Wistar rats, PE and YA elicited by apomorphine (0.08 mg/kg) were not modified by selective D_3 (S33084 and SB-277011, 0.63-10 mg/kg) or D_4 (L-745,870 and RBI-257, 0.63-2.5 mg/kg) antagonists, but were prevented by the preferential D_2 blocker L-741,626 (near-full antagonism at 2.5 mg/kg). The present data do not support a major implication of either DA D_3 or D_4 receptors in the control of PE and YA in rats, but indicate a preponderant role of DA D_2 receptors.

Reference no. 88

Br J Pharmacol. 2009 Jan;156(2):338-53. Epub 2009 Jan 12.

Signal transduction and functional selectivity of F15599, a preferential post-synaptic 5-HT_{1A} receptor agonist.

Newman-Tancredi A, Martel JC, Assié MB, Buritova J, Lauressergues E, Cosi C, Heusler P, Bruins Slot L, Colpaert FC, Vacher B, Cussac D.
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BACKGROUND AND PURPOSE: Activation of post-synaptic 5-HT_{1A} receptors may provide enhanced therapy against depression. We describe the signal transduction profile of F15599, a novel 5-HT_{1A} receptor agonist.

EXPERIMENTAL APPROACH: F15599 was compared with a chemical congener, F13714, and with (+)8-OH-DPAT in models of signal transduction *in vitro* and ex vivo.

KEY RESULTS: F15599 was highly selective for 5-HT_{1A} receptors in binding experiments and in [^{35}S]-GTPgammaS autoradiography of rat brain, where F15599 increased labelling in regions expressing 5-HT_{1A} receptors. In cell lines expressing h5-HT_{1A} receptors, F15599 more potently stimulated extracellular signal-regulated kinase (ERK1/2) phosphorylation, compared with G-protein activation, internalization of h5-HT_{1A} receptors or inhibition of cAMP accumulation. F13714, (+)8-OH-DPAT and 5-HT displayed a different rank order of potency for these responses. F15599 stimulated [^{35}S]-GTPgammaS binding more potently in frontal cortex than raphe. F15599, unlike 5-HT, more potently and efficaciously stimulated G(alphai) than G(alphao) activation. In rat prefrontal cortex (a region expressing post-synaptic 5-HT_{1A} receptors), F15599 potently activated ERK1/2 phosphorylation and strongly induced c-fos mRNA expression. In contrast, in raphe regions (expressing pre-synaptic 5-HT_{1A} receptors) F15599 only weakly or did not induce c-fos mRNA expression. Finally, despite its more modest affinity *in vitro*, F15599 bound to 5-HT_{1A} receptors *in vivo* almost as potently as F13714.

CONCLUSIONS AND IMPLICATIONS: F15599 showed a distinctive activation profiles for 5-HT_{1A} receptor-mediated signalling pathways, unlike those of reference agonists and consistent with functional selectivity at 5-HT_{1A} receptors. In rat, F15599 potently activated signalling in prefrontal cortex, a feature likely to underlie its beneficial effects in models of depression and cognition.

Reference no. 89

Pharmacol Biochem Behav. 2009 Apr;92(2):363-9.

The five choice serial reaction time task: comparison between Sprague-Dawley and Long-Evans rats on acquisition of task, and sensitivity to phencyclidine.

Auclair AL, Besnard J, **Newman-Tancredi A**, Depoortère R.
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The 5-choice serial reaction time task (5-CSRTT) allows examination of multiple aspects of cognition/executive functions (attention/impulsivity/ perseveration). Most 5-CSRTT studies are performed with pigmented (i.e. Long-Evans: LE) rats; however, albino strains (i.e. Sprague-Dawley: SD) are more commonly used in behavioural pharmacology experiments. Hence, we compared 5-CSRTT performances of SD and LE rats and their sensitivity to acute phencyclidine (PCP, 1-2.5 mg/kg). SD required significantly fewer sessions(35 versus 50) than LE rats for task acquisition, especially at shortest stimulus light duration (1 s). However,once trained, under vehicle conditions, both strains performed similarly. In contrast, PCP treatment differentially affected the two strains. Thus, whilst percentage of accuracy was decreased for both strains, in SD rats number of premature responses was more markedly decreased, whereas omissions and latency time to correct responses were more notably increased. In addition, PCP monotonically diminished in SD, but augmented (1-1.5 mg/kg) in LE rats compulsive responding. To summarize, under our experimental conditions, the SD offer advantages over LE strain for speed of acquisition of 5-CSRTT. Once trained, basal performances of both strains were equivalent and stable enough for challenge with pharmacological compounds. However, PCP differentially affected the strains on several parameters considered.

Reference no. 90

Behav Brain Res. 2009 Nov 5;203(2):288-95. Epub 2009 May 21.

Differences among conventional, atypical and novel putative $D_2/5\text{-}HT_{1A}$ antipsychotics on catalepsy-associated behaviour in cynomolgus monkeys.

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Typical antipsychotics such as haloperidol exert their therapeutic effects via blockade of dopamine (DA) D_2 receptors, leading to extrapyramidal symptoms (EPS) in humans and catalepsy in rodents. In contrast, atypical antipsychotics and new generation $D_2/5\text{-}HT_{1A}$ antipsychotics have low cataleptogenic potential. However, there has been no systematic comparative study on the effects of these different classes of antipsychotics in non-human primates, a species displaying a more sophisticated repertoire of behavioural/motor activity than rats. Once weekly, six young adult female non-haloperidol-sensitised cynomolgus monkeys were treated i.m. with a test compound and videotaped to score catalepsy-associated behaviour (CAB: static postures, unusual positions and crouching). Haloperidol, risperidone, olanzapine, nemonapride and remoxipride induced, to different extents, an increase in unusual positions (a response akin to dystonia), some crouching and static postures. In contrast, clozapine, quetiapine, ziprasidone and aripiprazole produced much lower or no unusual positions; clozapine also produced marked increases in static postures and crouching. Among novel $D_2/5\text{-}HT_{1A}$ antipsychotics, SLV313 and F15063 augmented the number of unusual positions, albeit at doses 16-63 times higher than those of haloperidol for approximately the same score. SSR181507 and bifeprunox produced moderate static postures, little crouching and negligible unusual positions. These data provide the first comparative analysis in cynomolgus monkeys of EPS liability of conventional, atypical and novel $D_2/5\text{-}HT_{1A}$ antipsychotics. They indicate that the latter are less prone than haloperidol to produce CAB, and provide a basis for comparison with rodent catalepsy studies.

Reference no. 91

Br J Pharmacol. 2009 Sep;158(1):232-42. Epub 2009 Jun 5.

5-HT_{1A} receptors are involved in the effects of xaliproden on G-protein activation, neurotransmitter release and nociception.

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BACKGROUND AND PURPOSE: Xaliproden (SR57746A) is a 5-HT_{1A} receptor agonist and neurotrophic agent that reduces oxaliplatin-mediated neuropathy in clinical trials. The present study investigated its profile on *in vitro* transduction, neurochemical responses and acute nociceptive pain tests in rats.

EXPERIMENTAL APPROACH: Xaliproden was tested on models associated with 5-HT_{1A} receptor activation including G-protein activation, extracellular dopamine and 5-HT levels measured by microdialysis and formalin-induced pain. Activation of 5-HT_{1A} receptors was confirmed by antagonism with WAY100635.

KEY RESULTS: Xaliproden exhibited high affinity for rat (r) and human (h) 5-HT_{1A} receptors (pKi= 8.84 and 9.00). In [^{35}S]GTPgammaS (guanosine 5'-O-(3-[^{35}S]thio)triphosphate) assays it activated both hippocampal r5-HT_{1A}[pEC_{50}/E_{MAX} of 7.58/61% (%5-HT)] and recombinant h5-HT_{1A} receptors (glioma C6-h5-HT_{1A}: 7.39/62%; HeLa-h5-HT_{1A}: 7.24/93%). In functional [^{35}S]GTPgammaS autoradiography, xaliproden induced labelling in structures enriched with 5-HT_{1A} receptors (hippocampus, lateral septum, prefrontal and entorhinal cortices). Xaliproden inhibited *in vivo* binding of [^{3}H]WAY100635 to 5-HT_{1A} receptors in mouse frontal cortex and hippocampus (ID_{50}: 3.5 and 3.3 mg/kg, p.o. respectively). In rat, it increased extracellular dopamine levels in frontal cortex and reduced hippocampal 5-HT levels (ED_{50}: 1.2 and 0.7 mg/kg, i.p. respectively). In a rat pain model, xaliproden inhibited paw licking and elevation (ED_{50}: 1 and 3 mg/kg, i.p. respectively) following formalin injection in the paw. All effects were reversed by pretreatment with WAY100635.

CONCLUSIONS AND IMPLICATIONS: These results indicate that activation of 5-HT_{1A} receptors is the principal mechanism of action of xaliproden and provide further support for the utility of 5-HT_{1A} receptor activation as an anti-nociceptive strategy.

Reference no. 92

Eur J Pharmacol. 2009 Oct 12;620(1-3):27-35. Epub 2009 Aug 18.

F15063, a potential antipsychotic with dopamine D_2/D_3 receptor antagonist and 5-HT_{1A} receptor agonist properties: influence on immediate-early gene expression in rat prefrontal cortex and striatum.

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Brain region-specific modulation of immediate-early gene (IEG) may constitute a marker of antipsychotic drug-like activity. We investigated the effects of the putative antipsychotic drug N-[(2,2-dimethyl-2,3-dihydro-benzofuran-7-yloxy)ethyl]-3-(cyclopent-1-enyl)-benzylamine (F15063), a compound that targets both dopamine D_2 and serotonin 5-HT_{1A} receptors, in comparison with haloperidol and clozapine on rat mRNA expression of IEG i.e. the zinc-fingered transcription factors c-fos, fosB, zif268, c-jun and junB, two transcription factors of the nuclear receptor family nur77 and nor1, and the effector IEG arc. F15063 (10 mg/kg) and clozapine (10 mg/kg), but not haloperidol (0.63 mg/kg), induced c-fos and fosB mRNA expression in prefrontal cortex, a region associated with control of cognition and negative symptoms of schizophrenia. In striatum, only c-fos, fosB, junB and nur77 were induced by clozapine whereas all IEG mRNAs were increased by haloperidol and F15063 (from 2.5 mg/kg) with similar high efficacy despite a total absence of F15063-induced catalepsy. However, at 0.63 mg/kg, F15063 induced a lower degree of striatal IEG mRNA expression than haloperidol and pretreatment with the serotonin 5-HT_{1A} receptor antagonist N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl-N-(2-pyridinyl)cyclohexane carboxamide trihydrochloride (WAY100635) (0.63 mg/kg) increased the level of IEG mRNA induction by F15063. Furthermore, (+)-8-hydroxy-2-(di-n-propylamino)tetralin [(+)-8-OH-DPAT] at 0.16 mg/kg decreased haloperidol-induced striatal IEG mRNA expression although it exerted no effects on its own. These results are consistent with an activation of serotonin 5-HT_{1A} receptors by F15063, thus reducing D_2 blockade-induced striatal IEG mRNA. Furthermore, the substantial F15063-induced expression of IEGs such as c-fos in striatum is not related to cataleptogenic activity and may act more as a marker of efficacious dopamine D_2 receptor blockade.

Reference no. 93

Eur J Nucl Med Mol Imaging. 2010 Mar;37(3):594-605. Epub 2009 Sep 30.

[^{18}F]F15599, a novel 5-HT$_{1A}$ receptor agonist, as a radioligand for PET neuroimaging.

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PURPOSE: The serotonin-1A (5-HT$_{1A}$) receptor is implicated in the pathophysiology of major neuropsychiatric disorders. Thus, the functional imaging of 5-HT$_{1A}$ receptors by positron emission tomography (PET) may contribute to the understanding of its role in those pathologies and their therapeutics. These receptors exist in high- and low-affinity states and it is proposed that agonists bind preferentially to the high-affinity state of the receptor and therefore could provide a measure of the functional 5-HT$_{1A}$ receptors. Since all clinical PET 5-HT$_{1A}$ radiopharmaceuticals are antagonists, it is of great interest to develop a [^{18}F]-labelled agonist.

METHODS: F15599 (3-chloro-4-fluorophenyl-(4-fluoro-4{[(5-methyl-pyrimidin-2-ylmethyl)-amino]-methyl}-piperidin-1-yl)-methanone) is a novel ligand with high affinity and selectivity for 5-HT$_{1A}$ receptors and is currently tested as an antidepressant. In pharmacological tests in rat, it exhibits preferential agonist activity at post-synaptic 5-HT$_{1A}$ receptors in cortical brain regions. Here, its nitro-precursor was synthesised and radiolabelled via a fluoronucleophilic substitution. Radiopharmacological evaluations included *in vitro* and ex vivo autoradiography in rat brain and PET scans on rats and cats. Results were compared with simultaneous studies using [^{18}F]MPPF, a validated 5-HT$_{1A}$ antagonist radiopharmaceutical.

RESULTS: The chemical and radiochemical purities of [^{18}F]F15599 were >98%. *In vitro* [^{18}F]F15599 binding was consistent with the known 5-HT$_{1A}$ receptors distribution (hippocampus, dorsal raphe nucleus, and notably cortical areas) and addition of GppNHp inhibited [^{18}F]F15599 binding, consistent with a specific binding to G protein-coupled receptors. *In vitro* binding of [^{18}F]F15599 was blocked by WAY100635 and 8-OH-DPAT, respectively, prototypical 5-HT$_{1A}$ antagonist and agonist. The ex vivo and *in vivo* studies demonstrated that the radiotracer readily entered the rat and the cat brain and generated few brain radioactive metabolites. Remarkably, in microPET studies, [^{18}F]F15599 notably displayed a pattern of brain labelling that did not correlate with *in vitro* observations. Thus, in cat, the highest binding was observed in dorsal raphe and cingulate cortex with little binding in other cortical regions and none in hippocampus. *In vivo* binding was abolished by WAY100635, indicating specific labelling of 5-HT$_{1A}$ receptors.

CONCLUSION: [^{18}F]F15599 is a radiofluorinated agonist presenting interesting characteristics for probing *in vitro* and *in vivo* the high-affinity states of the 5-HT$_{1A}$ receptors. Its differential labelling of 5-HT$_{1A}$ receptors *in vitro* and *in vivo* may result from its reported preferential interaction with receptors coupled to specific G-protein subtypes.

Reference no. 94

Br J Pharmacol. 2010 Aug;160(8):1929-40.

Preferential *in vivo* action of F15599, a novel 5-HT_{1A} receptor agonist, at postsynaptic 5-HT_{1A} receptors.

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BACKGROUND AND PURPOSE: F15599, a novel 5-hydroxytryptamine 5-HT_{1A} receptor agonist with 1000-fold selectivity for 5-HT compared with other monoamine receptors, shows antidepressant and procognitive activity at very low doses in animal models. We examined the *in vivo* activity of F15599 at somatodendritic autoreceptors and postsynaptic 5-HT_{1A} heteroreceptors.

EXPERIMENTAL APPROACH: *In vivo* single unit and local field potential recordings and microdialysis in the rat.

KEY RESULTS: F15599 increased the discharge rate of pyramidal neurones in medial prefrontal cortex (mPFC) from 0.2 microg/kg i.v and reduced that of dorsal raphe 5-hydroxytryptaminergic neurones at doses >10-fold higher (minimal effective dose 8.2 microg/kg i.v.). Both effects were reversed by the 5-HT_{1A} antagonist (+/-)WAY100635. F15599 did not alter low frequency oscillations (approximately 1 Hz) in mPFC. In microdialysis studies, F15599 increased dopamine output in mPFC (an effect dependent on the activation of postsynaptic 5-HT_{1A} receptors) with an ED_{50} of 30 microg/kg i.p., whereas it reduced hippocampal 5-HT release (an effect dependent exclusively on 5-HT_{1A} autoreceptor activation) with an ED_{50} of 240 microg/kg) i.p. Likewise, application of F15599 by reverse dialysis in mPFC increased dopamine output in a concentration-dependent manner. All neurochemical responses to F15599 were prevented by administration of (+/-)WAY100635.

CONCLUSIONS AND IMPLICATIONS: These results indicate that systemic administration of F15599 preferentially activates postsynaptic 5-HT_{1A} receptors in PFC rather than somatodendritic 5-HT_{1A} autoreceptors. This regional selectivity distinguishes F15599 from previously developed 5-HT_{1A} receptor agonists, which preferentially activate somatodendritic 5-HT_{1A} autoreceptors, suggesting that F15599 may be particularly useful in the treatment of depression and of cognitive deficits in schizophrenia.

Reference no. 95

Eur Neuropsychopharmacol. 2010 Sep;20(9):641-54. Epub 2010 May 21.

F15599, a preferential post-synaptic 5-HT_{1A} receptor agonist: activity in models of cognition in comparison with reference 5-HT_{1A} receptor agonists.

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We assessed the activity of F15599, a selective and high efficacy 5-HT_{1A} agonist that preferentially activates post- versus pre-synaptic receptors, in rat cognition/memory models. F15599 (0.16 mg/kg i.p.) partially alleviated detrimental effects of phencyclidine on working and reference memory deficit in a hole-board model. It also attenuated phencyclidine-induced deficit of cognitive flexibility in a reversal learning task, without effects of its own. F13714 (0.04 mg/kg) a chemical congener of F15599, and 8-OH-DPAT (0.01 or 0.16), were inactive against these phencyclidine-induced deficits, and/or even worsened basal performances. F15599 (0.04-2.5) was less disruptive than F13714 (0.005-0.16) or 8-OH-DPAT (0.01-0.63), on basal performance in models of attention (5-choice serial reaction time task) and working memory (delayed non-matching to position). Finally, unlike either comparator, F15599 reduced PPI with modest potency and only partially. To conclude, F15599, in models of memory/cognition, has a more favourable profile than F13714 and 8-OH-DPAT. This suggests that preferential activation of post-synaptic 5-HT_{1A} receptors could prove useful in pathologies characterized by cognitive/memory deficiencies, such as schizophrenia and depression.

Reference no. 96

Curr Opin Investig Drugs. 2010 Jul;11(7):802-12.

The importance of 5-HT_{1A} receptor agonism in antipsychotic drug action: rationale and perspectives.

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Serotonin 5-HT_{1A} receptors are attractive targets for the development of improved antipsychotics. Indeed, extensive evidence in rodent models indicates that the activation of these receptors prevents extrapyramidal symptoms (EPS) induced by dopamine D_2 receptor blockade, favors dopaminergic neurotransmission in the frontal cortex, has a positive influence on mood, and opposes NMDA receptor antagonist-induced cognitive and social interaction deficits. Therefore, 'third-generation' antipsychotics that combine partial agonism at 5-HT_{1A} receptors with antagonism (or partial agonism) at D_2 receptors have been investigated, including aripiprazole, perospirone, lurasidone (Dainippon Sumitomo Pharma Co Ltd), cariprazine (Gedeon Richter Ltd/Forest Laboratories Inc/Mitsubishi Tanabe Pharma Corp), PF-217830 (Pfizer Inc), F-97013-GD, F-15063 and bifeprunox. Such compounds appear to provide therapeutic benefits against a broader range of symptoms of schizophrenia, including negative symptoms and cognitive deficits that are poorly controlled by established antipsychotics. Recently developed compounds are essentially free of EPS liability, and exhibit little or no interaction at sites that are potentially involved in causing side effects such as weight gain, metabolic disorders or autonomic disturbance. These compounds differ in their balance of 5-HT_{1A}/D_2 receptor affinity and agonist or antagonist properties; such differences are likely to translate into distinct therapeutic profiles. The balance of 5-HT_{1A}/D_2 receptor properties should therefore be considered when selecting compounds as antipsychotic development candidates.

Reference no. 97

Int J Neuropsychopharmacol. 2010 Nov;13(10):1285-98. Epub 2010 Jan 11.

F15599, a highly selective post-synaptic 5-HT_{1A} receptor agonist: in-vivo profile in behavioural models of antidepressant and serotonergic activity.

Assié MB, Bardin L, Auclair AL, Carilla-Durand E, Depoortère R, Koek W, Kleven MS, Colpaert F, Vacher B, **Newman-Tancredi A.**
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F15599 is a novel agonist with high selectivity and efficacy at serotonin 5-HT_{1A} receptors (5-HT_{1A}Rs). In signal transduction, electrophysiological and neurochemical tests, F15599 preferentially activates post-synaptic 5-HT_{1A}Rs in rat frontal cortex. Such a profile may translate to an improved profile of therapeutic activity for mood disorders. The in-vivo effects of F15599 were therefore compared with those of a related compound, F13714, in rat models of antidepressant activity and 5-HT_{1A}R activation: forced swimming test (FST), conditioned stress-induced ultrasonic vocalization, 5-HT syndrome, plasma corticosterone and body temperature. Acute administration of F15599 or F13714 reduced immobility in the FST at low doses; these effects were long lasting and the effects of F15599 were maintained after repeated (5 d, p.o.) administration. Both compounds decreased ultrasonic vocalization duration at low doses. In contrast, higher doses of F15599 were required to induce lower lip retraction, elements of the 5-HT behavioural syndrome, hypothermia and to increase plasma corticosterone levels. Notably, there was a greater separation of ED50 between FST and other effects for F15599 than for F13714. Thus, the in-vivo potency of F15599 in models of antidepressant/anti-stress activity is similar to that of F13714, despite the fact that the latter has an in-vitro potency two orders of magnitude greater. In contrast F15599 has a lower propensity than F13714 to induce other serotonergic signs. The distinctive pharmacological profile of F15599 suggests that preferential targeting of post-synaptic 5-HT_{1A}Rs constitutes a promising strategy for improved antidepressant therapy.

Reference no. 98

J Neurochem. 2010 Sep 1;114(5):1323-32. Epub 2010 Jun 7.

The central serotonin $_{2B}$ receptor: a new pharmacological target to modulate the mesoaccumbens dopaminergic pathway activity.

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The function of the serotonin$_{2B}$ receptor (5-HT$_{2B}$R) in the mammalian brain is poorly characterized, especially with regard to its influence on dopamine (DA) neuron activity. Here, we assessed this issue by evaluating effects of 5-HT$_{2B}$Rs ligands in the control of striatal and accumbal DA outflow, using *in vivo* microdialysis in halothane-anesthetized rats, and amphetamine-induced hyperlocomotion in vigil rats. The selective 5-HT$_{2B}$R antagonist 1-[(2-chloro-3,4-dimethoxyphenyl)methyl]-2,3,4,9-tetrahydro-6-methyl-1H-pyrido[3,4-B]indole (LY 266097; 0.16 mg/kg, i.p.) had no influence on basal accumbal and striatal DA outflow but reduced significantly accumbal DA outflow when injected at 0.63 mg/kg. A significant reduction of basal DA outflow in the nucleus accumbens was also observed after i.p. administration of 0.16 mg/kg 2-amino-4-(4-fluoronaphth-1-yl)-6-isopropylpyrimidine, another selective 5-HT$_{2B}$R antagonist. In contrast, the 5-HT$_{2B}$R agonist alpha-methyl-5-(2-thienylmethoxy)-1H-indole-3-ethanamine (3 mg/kg, s.c.) had no influence on basal DA outflow in either brain region. The increase in striatal and accumbal DA outflow induced by the 5-HT$_{2C}$R inverse agonist 5-methyl-1-(3-pyridylcarbamoyl)-1,2,3,5-tetrahydropyrrolo[2,3-f] indole (5 mg/kg, i.p.) was unaltered by LY 266097 (0.63 mg/kg) pre-treatment. Conversely, LY 266097 (0.63 mg/kg) significantly diminished the increase in DA outflow induced by haloperidol (0.01 mg/kg, s.c.) or amphetamine (0.5 mg/kg, i.p.) in the nucleus accumbens, but not in the striatum. Amphetamine-induced hyperlocomotion (1 mg/kg) was also attenuated by LY 266097 (0.63 mg/kg). These findings demonstrate that 5-HT$_{2B}$Rs exert a facilitatory control on mesoaccumbens DA pathway activity, and suggest that they may constitute a new target for improved treatment of DA-related neuropsychiatric disorders.

Reference no. 99

Neuropsychiatry (2011) 1(2), 149–164

Biased agonism at serotonin 5-HT_{1A} receptors: preferential postsynaptic activity for improved therapy of CNS disorders.

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Serotonin or 5-hydroxytryptamine 5-HT_{1A} receptors are widely expressed in the brain and have extensive influence in the control of mood, cognition, movement and pain. In order to achieve optimal therapeutic benefit from targeting these receptors, 'biased agonists' (also known as 'functionally selective agonists') are desirable in order to preferentially activate receptor subpopulations in brain regions that mediate therapeutic activity, whilst avoiding those that control other effects. For example, clinical studies indicate that antidepressant activity is favored when 5-HT_{1A} autoreceptor activation is minimized and postsynaptic 5-HT_{1A} receptor activation is reinforced. F15599 is a novel biased agonist that exhibits a distinctive signal transduction 'fingerprint' *in vitro* and preferential postsynaptic activation of cortical 5-HT_{1A} receptors *in vivo*. This profile confers on F15599 a superior activity in animal models of depression and cognition, with a wide therapeutic margin relative to side effects. The use of biased agonists at 5-HT_{1A} receptors constitutes an attractive strategy to manage CNS disorders arising from dysfunctional serotonergic neurotransmission.

Reference no. 100

Cell Signal. 2011 Jan;23(1):58-64. Epub 2010 Aug 17.

Competitive interaction of 5-HT_{1A} receptors with G-protein subtypes in CHO cells demonstrated by RNA interference.

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Following agonist action, G-protein-coupled receptors may exhibit differential coupling to G-proteins or second messenger pathways, supporting the notion of agonist-directed trafficking. To explore these mechanisms, we have designed and transfected synthetic siRNA duplexes to knockdown different Gα subunits in Chinese hamster ovary (CHO) cells expressing human (h)5-hydroxytryptamine-1A receptors (CHO-h5-HT_{1A}). siRNAs against Gαi2 and Gαi3 transfected alone or in combination caused a large decrease in the corresponding mRNA level (64-80%) and also at the protein level for Gαi3 (60-70%), whereas a non-specific siRNA showed no effect. In membranes of CHO-h5-HT_{1A}, 5-HT-stimulated guanosine-5'-O-(3-[^{35}S]thio)-triphosphate ([^{35}S]GTPγS) binding was differentially affected by transfection of siRNAs against Gαi protein, siRNAs against Gαi2 inducing a more important decrease in the efficacy of 5-HT than transfection of siRNAs against Gαi3. The high potency component was abolished after transfection of siRNAs against Gαi3 and the lower potency component was suppressed after transfection of siRNAs against Gαi2. To directly investigate Gαi3 activation we used an antibody-capture/scintillation proximity assay. (+)8-OH-DPAT yielded bell-shaped curves for Gαi3 activation, a response that was abolished after transfection of siRNAs against Gαi3 protein. Interestingly, (+)8-OH-DPAT yielded a sigmoidal response when only Gαi3 protein was expressed. These data suggest that when efficacious agonists attain a high level of occupation of h5-HT_{1A} receptors, a change occurs that induces coupling to Gαi2 protein and suppresses signalling through Gαi3 subunits.

Reference no. 101

Psychopharmacology (Berl). 2011 Aug;216(4):451-73. Epub 2011 Mar 11.

Comparative pharmacology of antipsychotics possessing combined dopamine D_2 and serotonin 5-HT_{1A} receptor properties.

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RATIONALE: There is increasing interest in antipsychotics intended to manage positive symptoms via D_2 receptor blockade and improve negative symptoms and cognitive deficits via 5-HT_{1A} activation. Such a strategy reduces side-effects such as the extrapyramidal syndrome (EPS), weight gain, and autonomic disturbance liability.

OBJECTIVE: This study aims to review pharmacological literature on compounds interacting at both 5-HT_{1A} and D_2 receptors (as well as at other receptors), including aripiprazole, perospirone, ziprasidone, bifeprunox, lurasidone and cariprazine, PF-217830, adoprazine, SSR181507, and F15063. METHODS: We examine data on *in vitro* binding and agonism and *in vivo* tests related to (1) positive symptoms (e.g., psychostimulant-induced hyperactivity or prepulse inhibition deficit), (2) negative symptoms (e.g., phencyclidine-induced social interaction deficits and cortical dopamine release), and (3) cognitive deficits (e.g., phencyclidine or scopolamine-induced memory deficits). EPS liability is assessed by measuring catalepsy and neuroendocrine impact by determining plasma prolactin, glucose, and corticosterone levels.

RESULTS: Compounds possessing "balanced" 5-HT_{1A} receptor agonism and D_2 antagonism (or weak partial agonism) and, in some cases, combined with other beneficial properties, such as 5-HT_{2A} receptor antagonism, are efficacious in a broad range of rodent pharmacological models yet have a lower propensity to elicit EPS or metabolic dysfunction.

CONCLUSIONS: Recent compounds exhibiting combined 5-HT_{1A}/D_2 properties may be effective in treating a broader range of symptoms of schizophrenia and be better tolerated than existing antipsychotics. Nevertheless, further investigations are necessary to evaluate recent compounds, notably in view of their differing levels of 5-HT_{1A} affinity and efficacy, which can markedly influence activity and side-effect profiles.

Reference no. 102

Psychopharmacology (Berl). 2012 May;221(2):261-72. Epub 2011 Dec 3.

In vivo electrophysiological and neurochemical effects of the selective 5-HT_{1A} receptor agonist, F13640, at pre- and postsynaptic 5-HT_{1A} receptors in the rat.

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RATIONALE: F13640 (befiradol) is a novel 5-HT_{1A} receptor agonist with exceptional selectivity vs. other receptors and binding sites. It shows analgesic activity in animal models and is currently developed for human use.

OBJECTIVES: Given the potential dual role of the serotonergic system in pain, through the modulation of ascending signals in spinal cord and their emotional processing by corticolimbic areas, we examined the *in vivo* activity of F13640 at somatodendritic autoreceptors and postsynaptic 5-HT_{1A} heteroreceptors in medial prefrontal cortex (mPFC).

METHODS: *In vivo* single unit recordings and intracerebral microdialysis in the rat.

RESULTS: F13640 reduced the activity of dorsal raphe serotonergic neurons at 0.2-18.2 μg/kg, i.v. (cumulative doses; ED_{50} = 0.69 μg/kg, i.v.) and increased the discharge rate of 80% of mPFC pyramidal neurons in the same dose range (ED_{50} = 0.62 μg/kg, i.v.). Both effects were reversed by the subsequent administration of the 5-HT_{1A} receptor antagonist (±)WAY100635. In microdialysis studies, F13640 (0.04-0.63 mg/kg, i.p.) dose-dependently decreased extracellular 5-HT in the hippocampus and mPFC. Likewise, F13640 (0.01-2.5 mg/kg, i.p.) dose-dependently increased extracellular DA in mPFC, an effect dependent on the activation of postsynaptic 5-HT_{1A} receptors in mPFC. Local perfusion of F13640 in mPFC (1-1,000 μM) also increased extracellular DA in a concentration-dependent manner. Both the systemic and local effects of F13640 were prevented by prior (±)WAY100635 administration.

CONCLUSIONS: These results indicate that, upon systemic administration, F13640 activates both 5-HT_{1A} autoreceptors and postsynaptic 5-HT_{1A} receptors in prefrontal cortex with a similar potency. Both activities are likely involved in the analgesic properties of the compound.

Reference no. 103

J Nuclear Medicine 2012 May; 53:969–976. Epub 2012 May 10

Radiosynthesis and preclinical evaluation of [^{18}F]F13714 as a fluorinated 5-HT$_{1A}$ receptor agonist radioligand for PET neuroimaging.

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PET brain imaging of the serotonin 1A (5-hydroxytryptamine 1A [5-HT$_{1A}$]) receptor has been widely used in clinical studies. Currently, only a few well-validated radiolabeled antagonist tracers are available for in vivo imaging of this central receptor. 5-HT$_{1A}$ receptors exist in high- and low-affinity states, depending on their coupling to G proteins. Agonists bind preferentially to receptors in the high-affinity state and thereby could provide a measure of functional 5-HT$_{1A}$ receptors. Therefore, it is of great interest to develop an 18F-labeled full agonist 5-HT$_{1A}$ receptor radiotracer. In this study, we radiolabeled the high-affinity 5-HT1A receptor agonist [^{18}F]F13714 and investigated its potential as a PET tracer.

Methods: F13714 nitro precursor was synthesized and radiolabeled via a fluoronucleophilic substitution. In vitro binding assays were performed using established protocols. Radiopharmacologic evaluations included in vitro autoradiography in rat brain and PET scans on anesthetized cats.

Results: The chemical and radiochemical purities of [^{18}F]F13714 were greater than 98%. F13714 has a high affinity (0.1 nM) and selectivity for 5-HT$_{1A}$ receptors. In vitro [^{18}F]F13714 binding in rats was consistent with the known 5-HT1A receptors distribution (hippocampus and cortical areas) and was particularly high in the dorsal raphe. In vitro binding of [^{18}F]F13714 was blocked in a dose-dependent fashion by WAY100635, the prototypical 5-HT$_{1A}$ antagonist, and by the endogenous agonist, serotonin (5-HT). Addition of Gpp(NH)p also inhibited in vitro [^{18}F]F13714 binding, consistent with a preferential binding of the compound to G-protein–coupled receptors. Ex vivo tissue measurements in rat revealed an absence of brain radioactive metabolites. In vivo studies showed that the radiotracer entered the cat brain readily and displayed a preferential labeling of 5-HT$_{1A}$ receptors located in cingulate cortex. In vivo labeling was prevented by preinjection of WAY100635.

Conclusion: [^{18}F]F13714 is a radiofluorinated agonist that presents suitable characteristics for probing the high-affinity states of the 5-HT$_{1A}$ receptors in vitro and in vivo. Thus, it is a promising tool for investigation of 5-HT$_{1A}$ agonist binding in the living human brain.

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