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TRYPTOPHAN METABOLISM IN ALCOHOLISM. TRYPTOPHAN BUT NOT EXCITATORY AMINO ACID AVAILABILITY TO THE BRAIN IS INCREASED BEFORE THE APPEARANCE OF THE ALCOHOL-WITHDRAWAL SYNDROME IN MEN

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Abstract — Tryptophan (Trp) metabolism and disposition and excitatory and other amino acid concentrations were determined in alcohol-dependent subjects in relation to the alcohol-withdrawal syndrome (AWS). Parameters were examined in 12 alcohol-dependent male subjects, undergoing elective upper digestive tract tumour resection, and 12 age-, gender-, and medication-matched controls on three occasions: pre-operatively, post-operatively, and immediately before (i.e. within 24 h of) the appearance of the AWS. No significant differences were observed between controls and alcoholic subjects on the first or second of these occasions. On the third occasion, within 24 h of the appearance of the AWS, alcoholics showed a dramatic elevation (117%) in free serum Trp concentration and a consequent increase (111%) in the ratio of [free Trp]/[competing amino acids], which is an accurate predictor of Trp entry into the brain. Increases were also observed on this third occasion in concentrations of total Trp (49%), cortisol (123%), and norharman (137%). Concentrations of glutamate, glycine, aspartate, serine, and taurine did not differ significantly within or between the control and alcohol-dependent groups of subjects on any of the three occasions. The possible significance of the Trp and related metabolic changes in relation to the behavioural features of the AWS is discussed.

INTRODUCTION

The alcohol-withdrawal syndrome (AWS) occurs during the period immediately following cessation of alcohol intake after long-term and heavy consumption by alcohol-dependent subjects, and is characterized by a variety of behavioural disturbances, ranging from symptoms of mild anxiety and of sympathetic and autonomic activation to convulsions and, in some cases, the more severe state of delirium tremens (Victor and Adams, 1953; Charness *et al.*, 1989; Schuckit *et al.*, 1995). The biochemical mediators of the human AWS remain unknown, despite many investigations of the roles of γ-aminobutyric acid (GABA)ergic, cholinergic, dopaminergic, noradrenergic, and serotonergic mechanisms (for references, see Stuppaeck *et al.*, 1990). Animal studies have, however, suggested that convulsions and the other severe symptoms of hyperexcitability of the AWS may involve activation of the excitatory *N*-methyl-D-aspartate (NMDA)-type of glutamate receptors as an adaptive response to withdrawal of ethanol after its long-term consumption (for reviews, see Gonzales, 1990; Lovinger, 1995).

A number of physiologically occurring substances act as endogenous modulators of NMDA receptor function. These include the major excitatory amino acid glutamate and also aspartate and glycine, as well as a number of tryptophan (Trp) metabolites of the kynurenine pathway, such as the excitotoxic metabolites quinolinic acid and

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kynurenine, which activate these receptors, and the cytoprotective metabolite kynurenic acid, which has antagonistic properties (for review, see Stone, 1993). These Trp metabolites are products of the major Trp-oxidative route, the hepatic kynurenine–nicotinic acid pathway, the rate-limiting step of which is catalysed by the first and liver- and L-Trp-specific enzyme, Trp pyrrolase (Trp 2,3-dioxygenase, EC 1.13.11.11). These metabolites are also formed to a lesser extent in the brain from kynurenine either of peripheral origin or centrally produced from Trp by the action of the less substrate- and less tissue-specific enzyme indoleamine 2,3-dioxygenase (EC 1.13.11.17). Another major difference between these two enzymes is that, whereas liver Trp pyrrolase is inducible by glucocorticoids and activated by its substrate Trp and its co-factor haem (Badawy and Evans, 1975*b*), cytokines, particularly interferon-γ, are the principal effectors of extra-hepatic indoleamine dioxygenase (Pfefferkorn *et al.*, 1986; Taylor and Feng, 1991). We have previously shown that ethanol withdrawal enhances liver Trp pyrrolase activity (Badawy and Evans, 1973, 1975*a*) and that a dramatic activation of this enzyme associated with increased expression of its *m*RNA occurs in conjunction with the AWS (Bano *et al.*, 1996) in rats.

As a first step in studying the possible role of excitatory amino acids and neuroactive Trp metabolites in the human AWS, we have performed a preliminary study in which we compared a small group of alcohol-dependent subjects with a matched control group for possible differences in availability to the brain of the excitatory amino acids glutamate, aspartate, and glycine, and of the amino acid Trp (the precursor of the above neuroactive metabolites) and its disposition before the appearance of symptoms of the AWS. Preliminary accounts of parts of this work have appeared in abstract forms (Badawy *et al.*, 1996; Bradley *et al.*, 1998).

SUBJECTS AND METHODS

This prospective randomized study was performed on patients at the Department of Anaesthesiology and Operative Intensive Medicine of the Benjamin Franklin Medical Centre, Berlin, Germany, from whom written informed consent was obtained, and was approved by the local Ethics Committee.

Recruitment of patients

Of 106 male patients with a malignant tumour of the upper digestive tract evaluated, 48 were diagnosed as alcohol-dependent, using DSM-IV criteria (American Psychiatric Association, 1994). Of these 48, 27 elected to receive pharmacoprophylactic therapy for alcohol withdrawal (clonidine and flunitrazepam) and were accordingly excluded. Of the remaining 21 alcohol-dependent patients, the 12 who developed the AWS after their post-operative admission to the Intensive Care Unit (ICU) were included in the study, and were matched for basic characteristics (age, body weight, and height), medical condition (cancer diagnosis), and medication with 12 males admitted to the ICU, who acted as the control group. With regard to medication, all subjects (alcohol-dependent and control) received, on the eve of their operations, flunitrazepam (0.027–0.052 μg/kg body wt *per os*); midazolam (0.19–0.25 μg/kg body wt *per os*) was given 1 h pre-operatively, and peri-operative prophylaxis was with the antibiotics mezlocillin or cefotiam and metronidazole. Anaesthesia was induced with i.v. fentanyl, propofol, and vecuronium and maintained with the same three drugs in addition to N_2O/O_2 (70%:30%). Additionally, ranitidine was given to all patients on the eve of their admission to the ICU. The alcohol-dependent subjects were also each given a 100-mg dose of vitamin B_1 daily i.v. Exclusion criteria were female gender, an age of <18 years, diagnosis of liver cirrhosis, and presence of hepatitis B or C through Child's titre screening.

Diagnostic criteria of alcohol dependence and assessment of the AWS

As stated above, alcohol dependence was diagnosed in accordance with the DSM-IV criteria of the American Psychiatric Association (1994), including a daily alcohol consumption of >60 g, and also the CAGE questionnaire (Ewing, 1984). Subjects in the control group had no previous history of alcoholism, their daily alcohol consumption did not exceed 25 g and their CAGE questionnaire score was 0. The AWS was confirmed by a symptom check-list of a diagnostic schematic instrument based on internationally accepted algorithms and used routinely by us

(Heil *et al.*, 1992), and quantified using the CIWA-Ar scale (Sullivan *et al.*, 1989). Therapy of the AWS, commenced after the final venesections (see below), was with flunitrazepam, the dose of which was titrated until the CIWA-Ar score dropped below 20. If this was not possible with the maximum dose of 16 mg, haloperidol and/or clonidine were also given depending on whether patients developed vegetative, hallucinatory or both symptoms.

Study design

All patients (control and alcohol-dependent subjects) underwent a comprehensive evaluation upon admission to the surgical ward, which included assessment of alcohol dependence and the related laboratory parameters carbohydrate-deficient transferrin (CDT), γ-glutamyltransferase (GGT), and mean corpuscular volume (MCV). Overnight-fasting venous blood samples (25 ml each) were taken between 08:00 and 09:00 from all subjects on three occasions: (1) pre-operatively, 24 h before induction of anaesthesia prior to surgery; (2) post-operatively, on the morning after the operation; (3) within 24 h before the appearance of symptoms of the AWS in alcoholic patients and at the corresponding times for each matched control. Symptoms of the AWS in alcohol-dependent patients appeared immediately upon admission to the ICU (i.e. within 24 h of surgery) in two subjects, within 2 days of this admission in two other subjects, within 3 days in four subjects, and within 4 and 5 days in two subjects each. The AWS therefore took an average of 2.8 ± 0.5 days (mean ± SEM), with a median of 3 days, to appear in this group of patients. Accordingly, to obtain the blood sample immediately preceding the appearance of the AWS, daily venesections were made up until observation of the AWS. These intermediate samples were, however, not analysed.

Laboratory investigations

Serum CDT was determined by a research procedure based on micro-anion-exchange chromatography and subsequent turbidometry as described previously (Muller *et al.*, 1993) in which a CDT level above 9 mg/l is considered pathological (Heil *et al.*, 1994), whereas GGT and MCV were determined by standard clinical laboratory procedures, in which the normal ranges for German laboratories are 6–28 U/l and 80–96 fl respectively. Serum cortisol concentration was determined by an ELISA immunoassay, whereas those of amino acids were determined by the autoanalyser procedure. Free (ultrafiltrable) and total (free + albumin-bound) serum Trp concentrations were determined fluorimetrically by a modification (Bloxam and Warren, 1974) of the method of Denckla and Dewey (1967), as described previously (Badawy and Evans, 1976). Ultrafiltration of serum was performed on fresh (unfrozen) samples within 2 h of isolation, to avoid the effect of frozen storage on Trp binding (Morgan and Badawy, 1994), using the Amicon micropartition MPS-1 assembly (Amicon GmbH, Witten, Germany) and after centrifugation of 1-ml portions of serum at room temperature at 2000 *g* for 20 min. Trp binding was expressed as the percentage free serum Trp (100 × [free serum Trp]/[total serum Trp]). Concentrations of the physiological binder of Trp, namely albumin (Doumas and Biggs, 1972), and displacers of albumin-bound Trp, namely non-esterified fatty acids (NEFA) (Mikac-Dević *et al.*, 1973) and those of glucose (Slein, 1963) and the major Trp-oxidation product kynurenine (Joseph and Risby, 1975) were determined by standard procedures. Norharman concentration was determined in plasma samples by high-performance liquid chromatography and fluorimetric detection as described previously (Spies *et al.*, 1995).

Statistical analysis of results

Intergroup differences between numerical variables were analysed by means of the Wilcoxon rank-sum test for unrelated variables. Differences between groups for dichotomous variables were analysed by the χ^2-test. For intra-group analyses, the Friedman test was used for global differences. All statistical analyses were performed using the PC version of the Statistical Package for the Social Sciences (SPSS for Windows, Chicago, USA).

RESULTS

Patient group diagnostic characteristics

The results in Table 1 show that the control and alcohol-dependent patient groups did not differ significantly in age. By contrast, the alcoholic group had a CAGE score for alcohol-dependence

Table 1. Alcohol-dependent and control group diagnostic characteristics

Parameter	Control group	Alcohol-dependent group	Significance (*P*)*
Age (years)	60.1 ± 2.3	58.9 ± 3.2	n.s.
CIWA score	9.3 ± 1.8	32.5 ± 3.1	0.0000
CAGE score	0.0 ± 0.0	3.4 ± 0.1	0.0000
CDT (mg/l)	5.2 ± 0.8	31.7 ± 9.0	0.0001
GGT (U/l)	12.6 ± 1.7	37.4 ± 7.3	0.0002
MCV (fl)	93.6 ± 1.6	97.8 ± 2.4	0.0297

The above parameters were determined as described in the Subjects and methods section. Values are means ± SEM for each group of 12 subjects. *The significance of the differences between the control and alcohol-dependent subject groups (*P*) has been calculated using the Wilcoxon exact two-tailed test. For carbohydrate-deficient transferrin (CDT), the pathological cut-off point for the research method used is 9 mg/l, whereas the normal ranges for the routine clinical laboratory procedures used for determination of γ-glutamyl transferase (GGT) and mean corpuscular volume (MCV) in German laboratories are 6–28 U/l and 80–96 fl respectively.

diagnosis of 3.4 ± 0.1 (mean ± SEM) against a score of 0 for the control group, and a CIWA-Ar score for severity of withdrawal assessment of 32.5 ± 3.1 against a low score for controls of 9.3 ± 1.8. Alcoholics also had CDT values much higher than those of controls, which were below the pathological level of 9 mg/l. GGT and MCV values were also raised in alcoholics, but not in controls, as would be expected in a diagnosis of alcohol dependence.

Comparison of parameters of Trp metabolism and disposition in alcoholics and controls

These parameters were examined on three occasions in the two groups of patients, namely pre-operatively, post-operatively and immediately before (i.e. within 24 h of) the appearance of the AWS (or the corresponding times for matched controls). The results in Table 2 show that there were no significant differences in free serum or total serum Trp concentrations or Trp binding to albumin (expressed as the percentage free serum

Table 2. Comparison of parameters of Trp metabolism and disposition in sera of alcohol-dependent and control subjects

Parameter	First occasion (pre-operative)	Second occasion (post-operative)	Third occasion (before the AWS)
Free [Trp]			
Control	3.00 ± 0.37	2.69 ± 0.28	2.60 ± 0.29
Alcohol-dependent	3.20 ± 0.52	3.45 ± 0.44	5 65 ± 0.64*
Total [Trp]			
Control	8.83 ± 0 41	8.70 ± 0.52	8.77 ± 0.49
Alcohol-dependent	10.47 ± 0.91	10.13 ± 0.83	13.07 ± 1.65^{+}
Free Trp (%)			
Control	33.40 ± 3.20	31.93 ± 4.13	29.96 ± 3.52
Alcohol-dependent	29.93 ± 3.08	33.86 ± 2.70	46.95 ± 4.07**
[Kynurenine]			
Control	0.73 ± 0.12	0.71 ± 0 11	0.82 ± 0.11
Alcohol-dependent	0.70 ± 0.12	0.77 ± 0.13	1.05 ± 0.23
[CAA]			
Control	654 ± 61	643 ± 68	600 ± 58
Alcohol-dependent	701 ± 50	724 ± 46	717 ± 60
[Free Trp]/[CAA] ratio			
Control	0.023 ± 0.002	0.019 ± 0.002	0.018 ± 0.001
Alcohol-dependent	0.021 ± 0.002	0.025 ± 0.004	0.038 ± 0 003***
[Total Trp]/[CAA] ratio			
Control	0.074 ± 0.005	0.068 ± 0.006	0.071 ± 0.008
Alcohol-dependent	0 073 ± 0.009	0.072 ± 0.010	0.089 ± 0.011

Experimental details are as described in the Subjects and methods section. Values are expressed as ratios or in μg/ml, except for the [CAA], which is in μM, and are means ± SEM for each group of 12 subjects. The significance of the differences between the control and alcohol-dependent groups, given as exact two-tailed *P*, which were compared using the Mann–Whitney U test, is as follows: $^{+}P < 0.0145$; $*P < 0.0034$; $**P < 0\ 002$; $***P < 0.0000$. Abbreviations used: Trp, tryptophan; CAA, sum of amino acids competing with Trp for entry into the brain; AWS, alcohol-withdrawal syndrome.

Table 3. Comparison of parameters related to Trp metabolism and disposition in sera and plasmas of alcohol-dependent and control subjects

Parameter	First occasion (pre-operative)	Second occasion (post-operative)	Third occasion (before the AWS)
[Albumin]			
Control	37.1 ± 2.0	34.0 ± 1.4	34.7 ± 1.6
Alcohol-dependent	40.2 ± 1.5	34.8 ± 1.7	33.6 ± 1.4
[NEFA]			
Control	0.55 ± 0.07	0.44 ± 0.08	0.54 ± 0.12
Alcohol-dependent	0.52 ± 0.08	0.55 ± 0.06	0.65 ± 0.09
[Glucose]			
Control	135 ± 14	140 ± 11	138 ± 13
Alcohol-dependent	133 ± 13	147 ± 14	124 ± 5
[Cortisol]			
Control	349 ± 62	463 ± 76	421 ± 63
Alcohol-dependent	306 ± 55	685 ± 126	941 ± 225**
[Norharman]			
Control	7.85 ± 1.23	12.63 ± 2.10	11.95 ± 1.35
Alcohol-dependent	17.45 ± 3.53*	14.36 ± 4.85	28.35 ± 4.33***

The above parameters were determined in serum (except norharman, which was determined in plasma) as described in the Subjects and methods section, and are expressed as follows: albumin (g/l), NEFA (mM), glucose (mg/dl), cortisol (nM), and norharman (pg/ml). Values are means ± SEM for each group of 12 subjects. The significance of the differences between the alcohol-dependent and control subjects at the relevant occasions shown is indicated as follows: $*P < 0.0387$; $**P < 0.0029$; $***P < 0.0014$ (Mann–Whitney U exact two-tailed test). Abbreviations used: NEFA, non-esterified fatty acids; AWS, alcohol-withdrawal syndrome; Trp, tryptophan.

Trp) between the control and alcohol-dependent patient groups on the first of the above occasions. On the second occasion, both free and total serum [Trp] were respectively 28% and 16% higher in the alcohol-dependent group; however, these increases did not reach statistical significance. Trp binding (expressed as above) also remained unaltered on this second occasion. On the third occasion, shortly before the appearance of the AWS, free serum [Trp] was dramatically elevated, by 117%, with total serum [Trp] also rising by 49% in comparison with the corresponding values in the control group ($P = 0.0145$ at least). Because of the greater rise in free, compared to total, serum [Trp] on this occasion, the percentage free serum Trp was significantly increased in the alcohol-dependent group, by 57% ($P < 0.002$). The results in Table 2 also show that the serum concentration of the major Trp-oxidative product kynurenine was not significantly different between the control and alcohol-dependent subjects, though a trend towards an increase was observed in the third sample of the latter subjects. Trp availability to the brain, expressed as the [Trp]/[CAA] ratio was also assessed. As the data in Table 2 show, the [free Trp]/[CAA] ratio was not significantly different between controls and alcohol-dependent patients on the first or second occasion, although on the latter occasion there was a trend towards an increase in the alcoholic group. On the third occasion, i.e. within 24 h of the appearance of the AWS, the [free Trp]/[CAA] ratio was dramatically increased in the alcohol-dependent, to 111% above the corresponding value in the control, subjects ($P < 0.0000$). By contrast, there were no significant within- or between-group differences in the [total Trp]/[CAA] ratio, although a trend towards an increase was observed on the third occasion in the alcohol-dependent group. The above increase in the [free Trp]/[CAA] ratio on the third occasion was due to the dramatic increase in [free Trp] and not any likely decrease in the sum of [CAA], which was in fact higher in the alcohol-dependent patients than in the control subjects, though the difference was not statistically significant.

Comparison of parameters related to Trp metabolism and disposition in alcoholics and controls

The results in Table 3 show such comparisons. There were no significant between- or within-

Table 4. Comparison of concentrations of glutamate and other amino acids in sera of alcohol-dependent and control subjects

Serum concentration (μM)	First occasion (pre-operative)	Second occasion (post-operative)	Third occasion (before the AWS)
Glutamate			
Control	233 ± 47	201 ± 32	200 ± 38
Alcohol-dependent	256 ± 44	179 ± 40	229 ± 55
Glycine			
Control	290 ± 33	233 ± 17	259 ± 27
Alcohol-dependent	298 ± 29	244 ± 22	336 ± 50
Aspartate			
Control	53 ± 10	48 ± 8	52 ± 9
Alcohol-dependent	52 ± 8	48 ± 10	47 ± 9
Serine			
Control	151 ± 17	125 ± 11	129 ± 12
Alcohol-dependent	151 ± 14	130 ± 12	161 ± 22
Taurine			
Control	175 ± 23	111 ± 20	147 ± 23
Alcohol-dependent	147 ± 21	137 ± 26	125 ± 30

Concentrations of the above amino acids were determined in sera as described in the Subjects and methods section. Values are means ± SEM for each group of 12 subjects. No significant within- or between-group differences were observed at any of the above three time intervals. Abbreviation used: AWS, alcohol-withdrawal syndrome.

group differences in concentrations of the physiological binder of Trp (albumin), displacers of albumin-bound Trp (NEFA), or glucose (changes in levels of which can trigger an insulin-mediated modulation of Trp entry into the brain) at any of the three time intervals tested. By contrast, concentration of cortisol (the major glucocorticoid inducer of human liver Trp pyrrolase) was increased in the alcohol-dependent, but not the control, group by 124 and 207% on the second and third sampling occasions respectively, in comparison with the value on the first occasion ($P < 0.02$, by Student's t-test). In comparison with the control group, the alcohol-dependent patient group showed a dramatic increase in cortisol concentration on the third occasion (124%; $P < 0.0029$). Plasma norharman (β-carboline), a complex metabolite of indolylamines of interesting pharmacological properties, was also higher in the alcohol-dependent patients, compared with controls, on the first and third sampling occasions (by 122 and 137% respectively; $P = 0.0387$–0.0014, Mann–Whitney U exact two-tailed test).

Comparison of excitatory and other amino acid concentrations in alcoholics and controls

The results in Table 4 show that the serum concentrations of the excitatory amino acids glutamate, glycine, and aspartate, and also those of serine and taurine did not differ significantly between controls and alcohol-dependent subjects, nor within each group, at any of the three time intervals at which they were examined.

DISCUSSION

Current views suggest that the behavioural features of the AWS may involve hyperexcitability resulting from modulation of function of the NMDA-type of glutamate receptors. Although evidence in support of this concept has been obtained from animal studies (for reviews, see Gonzales, 1990; Lovinger, 1995), no evidence has as yet emerged to implicate NMDA receptor function modulation in the human AWS. In the present study, we have attempted to address this question in alcohol-dependent patients by examining the availability to the brain of endogenous modulators of NMDA receptor function shortly before (within 24 h of) the appearance of the AWS. No increase in the availability in the circulation of the major excitatory amino acid glutamate, the other excitatory amino acids aspartate and glycine, or the other neuroactive amino acids serine and taurine was observed (Table 4). These results therefore strongly suggest

that availability to the brain of neuroactive amino acids of peripheral origin (including the major NMDA receptor agonist glutamate) is not an important factor in the development of the human AWS. Whether such availability may be important at the time of appearance of the AWS requires a more detailed and longer time-course study. The alternative possibility, that of involvement of intracerebral changes in excitatory and/or other neuroactive amino acids in the AWS, remains to be investigated.

Another group of endogenous modulators of NMDA receptor function are the metabolites of the kynurenine pathway of Trp degradation, namely quinolinic acid, kynurenine and kynurenic acid (for review, see Stone, 1993). Excitotoxic quinolinate is a powerful agonist of NMDA receptors, whereas kynurenate exerts a cytoprotective influence by virtue of antagonism at these receptors. Kynurenine is also an NMDA receptor agonist, though not as strong as its metabolite quinolinate. The possible involvement of quinolinate as an excitotoxic mediator of the AWS has been proposed by Morgan (1991) on the basis of the following previous findings in animals: (1) increased blood–brain barrier permeability to Trp after chronic ethanol administration; (2) dramatic elevation of brain quinolinate levels after acute Trp loading, even to 200-fold in localized brain areas; (3) increased circulating levels of Trp secondary to hepatic dysfunction, which is invariably a feature of long-term and heavy alcohol consumption; (4) enhancement of liver Trp pyrrolase activity during alcohol withdrawal and its possible mediation by glucocorticoids (for references, see Morgan, 1991).

We have not investigated the quinolinate or kynurenate status of alcoholic patients in the present preliminary study, because it was considered important to establish in the first instance the status and disposition of their precursor amino acid Trp, as these are the primary determinants of synthesis of these neuroactive metabolites. As the present results show, a dramatic increase (117%) in free serum [Trp] associated with a significant increase (49%) in that of total serum Trp were observed shortly before the appearance of the AWS in alcohol-dependent men (Table 2). Circulating Trp availability to the brain is determined by three major peripheral factors: (1) at the primary level, activity of the major Trp-degrading enzyme, hepatic Trp pyrrolase, which controls the quantitatively most important of the Trp-degradative routes, the hepatic kynurenine–nicotinic acid pathway (Badawy, 1977); (2) at the secondary, but more immediate, level, extent of protein binding of Trp and hence of its modulation by the physiological binder albumin and displacers from the albumin-binding sites' NEFA (Curzon and Knott, 1974); (3) extent of competition between Trp and five other circulating amino acids (Val, Leu, Ile, Phe, and Tyr), collectively known as the competing amino acids or CAA, for the same cerebral uptake mechanism (Fernstrom and Wurtman, 1971). In human studies of Trp metabolism and disposition, the most accurate predictor of Trp entry into the brain is therefore not serum [Trp] alone, but the ratio of the latter to the sum of the Trp competitors, i.e. the [Trp]/[CAA] ratio, and, as the results in Table 2 also show, the [free serum Trp]/[CAA] ratio was also dramatically elevated (by 111%) shortly before the appearance of the AWS, purely because of the increase in [free serum Trp] and not a decrease in the [CAA]. This increase in the [free serum Trp]/[CAA] ratio is almost certain to lead to a large increase in brain [Trp]. Brain [Trp] is the single physiologically most important determinant of cerebral serotonin (5-hydroxytryptamine, 5-HT) synthesis, because the rate-limiting enzyme of the 5-HT-biosynthetic pathway, Trp hydroxylase, is unsaturated with its Trp substrate (Fernstrom and Wurtman, 1971; Carlsson and Lindqvist, 1978; Curzon, 1979). The increase in circulating Trp availability to the brain shortly before the appearance of the AWS observed in the present work is therefore almost certain to lead to an increase in brain serotonin synthesis. Although the behavioural effects of serotonin excess are well known, their possible involvement in the human acute AWS has not previously been suggested and may merit investigation. An increase in brain [Trp] shortly before the appearance of the above syndrome, as suggested by the results in Table 2 of the present work, could also lead to increased production of the excitotoxic NMDA receptor agonist quinolinic acid, possibly in large amounts in certain brain areas, as mentioned above (Morgan, 1991). The likelihood of this possibility, however, depends on whether brain indoleamine dioxygenase is active enough under these conditions to convert Trp into adequate amounts of

quinolinate. Although brain indoleamine dioxygenase could not be examined directly in alcoholic patients, an indirect indicator of its activity is the level of serum (or urinary) neopterin (see Fuchs *et al.*, 1996 and references cited therein), measurement of which may therefore be necessary in studying the above possibility. As stated in the Introduction, the principal effector of indoleamine dioxygenase is interferon-γ and activity of this enzyme is induced dramatically under conditions of immune activation (see Pfefferkorn *et al.*, 1986; Taylor and Feng, 1991). Neither the immune status of alcohol-dependent humans during acute withdrawal nor the effect of withdrawal on indoleamine dioxygenase activity in animals are known, but it is tempting to speculate that, should the immune status be shown to be enhanced, the resulting induction of indoleamine dioxygenase, in conjunction with increased entry of Trp into the brain, could provide the optimum conditions for enhanced intracerebral synthesis of quinolinic acid.

The mechanisms by which the 117% increase in [free serum Trp] and the 49% increase in [total serum Trp] (Table 2) occur shortly before the appearance of the AWS are not fully understood at present. As regards the large increase in [free serum Trp], NEFA could not be held responsible, since their levels were not altered (Table 3). By contrast, serum [albumin] was decreased by 16% ($P < 0.005$ by Student's *t*-test) in the alcohol-dependent patients on the third occasion, in comparison with the value on the first occasion. We have previously shown (Badawy *et al.*, 1985) that a 16% decrease in serum [albumin] (during late pregnancy in rats) is the threshold value at which Trp binding to albumin becomes significantly decreased. It may therefore be concluded that part of the above increase in [free serum Trp] is due to decreased albumin binding (as also suggested by the associated significant increase in the percentage free serum Trp). As regards the 49% increase in [total serum Trp], inhibition of liver Trp pyrrolase activity is usually the main cause of elevation of total serum Trp, which in a typical case is also accompanied by a similar increase in [free serum Trp] (see, e.g. Badawy *et al.*, 1985). The only other likely explanation of the increase in [total serum Trp] is that of release from protein breakdown. This possibility is, however, unlikely in view of the fact that levels of other amino acids (e.g. the Trp competitors or excitatory amino acids) were not elevated (Tables 2 and 4). It may therefore be concluded that the increases in circulating [Trp] observed in the present work may be caused by decreased albumin binding and inhibition of liver Trp pyrrolase activity. The mechanism of such a possible inhibition is not understood, but one possibility is that of mediation by norharman [a known inhibitor of Trp pyrrolase activity (Eguchi *et al.*, 1984)], whose circulating concentration was also dramatically increased in alcoholics on the third occasion (Table 3), and possibly also by catecholamines (Satoh and Moroi, 1969), whose circulating levels are usually also increased during alcohol withdrawal.

If liver Trp pyrrolase activity is inhibited shortly before the appearance of the AWS, as the present results suggest, a strong possibility that it will become subsequently enhanced is indicated by the dramatic elevation of serum [cortisol] (Table 3). This hormone is the major glucocorticoid inducer of human liver Trp pyrrolase and an elevation of its circulating concentration of the magnitude observed in the present work is almost certain to herald pyrrolase induction. If such induction were to occur on subsequent days, one would expect levels of Trp to decrease and those of the major oxidative product kynurenine to rise. As the results in Table 2 show, a trend towards kynurenine elevation in the alcohol-dependent subjects was already apparent on the third occasion. A longer time-course study will therefore be required to examine these additional aspects of Trp disposition. It is tempting to speculate here, however, that the cortisol elevation observed in the present work, which is a well known feature of acute alcohol withdrawal (see, e.g. Adinoff *et al.*, 1991) may help to accelerate hepatic Trp degradation to decrease its availability to the brain for quinolinate synthesis. Another potential mechanism of inhibition of this synthesis is that involving norharman, a known inhibitor of indoleamine dioxygenase activity (Eguchi *et al.*, 1984), an increase in the circulating concentration of which was also observed in the present work (Table 3), as well as previously by us in other alcohol-dependent subjects (Spies *et al.*, 1995).

The absence of a significant change in serum kynurenine concentration observed in the present work (Table 2) deserves further comment. An increase in serum kynurenine concentration in the

presence of a rise in that of Trp usually reflects increased flux of the amino acid through its oxidative pathway without a net change in Trp pyrrolase activity (e.g. after a small Trp-loading dose), whereas activation of Trp pyrrolase leads to an increase in serum kynurenine concentration and a decrease in that of Trp. The finding (Table 2) that serum kynurenine concentration was only slightly increased on the third sampling occasion when serum [Trp] was dramatically elevated suggests that either an increase in circulating kynurenine concentration may follow shortly afterwards or that flux of Trp through its oxidative pathway is impaired at this time, because of a possible pyrrolase inhibition as stated above. A longer time-course study of Trp disposition and metabolism should establish a clearer picture of the Trp metabolic status in alcohol withdrawal.

In conclusion, we have explored in the present work the availability to the brain of some endogenous modulators of NMDA receptor function in relation to the AWS in alcohol-dependent men. Our results show that, whereas availability of excitatory and other neuroactive amino acids to the brain is not increased, that of Trp is. Trp is the precursor of an important group of neuroactive metabolites known to modulate NMDA receptor function and to influence mood and behaviour, and the changes in its availability and disposition observed in the present work justify a detailed investigation of its metabolism and role in determining cerebral levels of the neuroactive metabolites quinolinate and kynurenate in relation to alcohol dependence.

REFERENCES

Adinoff B., Risher-Flowers, D., De Jong, J., Raviz, B., Bone, G. H. A., Nutt, D. J., Roehrich, L., Martin, P. R. and Linnoila, M. (1991) Disturbances of hypothalamic–pituitary–adrenal axis functioning during ethanol withdrawal in six men. *American Journal of Psychiatry* **148**, 1023–1025.

American Psychiatric Association (1994) *Diagnostic and Statistical Manual of Mental Disorders*, 4th edn, pp. 195–196. American Psychiatric Association, Washington, DC.

Badawy, A. A.-B. (1977) Minireview: The functions and regulation of tryptophan pyrrolase. *Life Sciences* **21**, 755–767.

Badawy, A. A.-B. and Evans, M. (1973) Tryptophan pyrrolase in ethanol administration and withdrawal. *Advances in Experimental Medicine and Biology* **35**, 105–123.

Badawy, A. A.-B. and Evans, M. (1975*a*) The effects of ethanol on tryptophan pyrrolase activity and their comparison with those of phenobarbitone and morphine. *Advances in Experimental Medicine and Biology* **59**, 229–251.

Badawy, A. A.-B. and Evans, M. (1975*b*) The regulation of rat liver tryptophan pyrrolase by its cofactor haem-experiments with haematin and 5-aminolaevulinate and comparison with the substrate and hormonal mechanisms. *Biochemical Journal* **150**, 511–520.

Badawy, A. A.-B. and Evans, M. (1976) Animal liver tryptophan pyrrolases — absence of apoenzyme and of hormonal induction mechanism from species sensitive to tryptophan toxicity. *Biochemical Journal* **158**, 79–88.

Badawy, A. A.-B., Davis, N. R., Morgan, C. J., Dacey, A. and Evans, M. (1985) Effects of ethanol on tryptophan metabolism and disposition in pregnant rats. In *Alcohol and the Developing Brain*, Rydberg, U., Alling, C. and Engel, J., eds, pp. 47–56. Raven Press, New York.

Badawy A. A.-B., Spies, C. D., Morgan, C. J., Bradley, D. M., Bonner, A., Ehlert, A., Blum, S. and Rommelspacher, H. (1996) Tryptophan metabolism and disposition in acute alcohol withdrawal. *Alcoholism: Clinical and Experimental Research* **20** (Suppl. to No. 2), 56A.

Bano, S., Oretti, R. G., Morgan, C. J., Badawy, A. A.-B., Buckland, P. and McGuffin, P. (1996) Effects of chronic administration and subsequent withdrawal of ethanol-containing liquid diets on rat liver tryptophan pyrrolase and tryptophan metabolism. *Alcohol and Alcoholism* **31**, 205–215.

Bloxam, D. L. and Warren, W. H. (1974) Error in the determination of tryptophan by the method of Denckla and Dewey. A revised procedure. *Analytical Biochemistry* **60**, 621–625.

Bradley, D. M., Spies, C. D. and Badawy, A. A.-B. (1998) No increase in availability of excitatory amino acids or taurine to the brain before the appearance of the alcohol withdrawal syndrome in male alcoholics. *Alcoholism: Clinical and Experimental Research* **22** (Suppl. to No. 3), 175A.

Carlsson, A. and Lindqvist, M. (1978) Dependence of 5-HT and catecholamine synthesis on concentrations of precursor amino acids in rat brain. *Naunyn-Schmiedebergs Archives of Pharmacology* **303**, 157–164.

Charness, M. E., Simon, R. P. and Greenberg, D. A. (1989) Ethanol and the nervous system. *New England Journal of Medicine* **321**, 442–454.

Curzon, G. (1979) Relationship between plasma, CSF and brain tryptophan. *Journal of Neural Transmission* **15** (Suppl.), 81–92.

Curzon, G. and Knott, P. J. (1974) Effects on plasma and brain tryptophan in the rat of drugs and hormones that influence the concentration of unesterified fatty acids in the plasma. *British Journal of Pharmacology* **50**, 197–204.

Denckla, W. D. and Dewey, H. K. (1967) The determination of tryptophan in plasma, liver and

urine. *Journal of Laboratory and Clinical Medicine* **69**, 160–169.

Doumas, B. T. and Biggs, H. T. (1972) Determination of serum albumin. *Standard Methods in Clinical Chemistry* **7**, 175–188.

Eguchi, N., Watanabe, Y., Kawanishi, K., Hashimoto, Y. and Hayaishi, O. (1984) Inhibition of indoleamine 2,3-dioxygenase and tryptophan 2,3-dioxygenase by β-carboline and indole derivatives. *Archives of Biochemistry and Biophysics* **232**, 602–609.

Ewing, J. A. (1984) Detecting alcoholism: the CAGE questionnaire. *Journal of the American Medical Association* **252**, 1905–1907.

Fernstrom, J. D. and Wurtman, R. J. (1971) Brain serotonin content: physiological dependence on plasma tryptophan levels. *Science* **173**, 149–152.

Fuchs, D., Schrocksnadel, H., Baier-Bitterlich, G., Dapunt, O. and Wachter, H. (1996) Activated cellular immunity and decreased serum tryptophan in healthy pregnancy. *Advances in Experimental Medicine and Biology* **398**, 149–153.

Gonzales, R. A. (1990) NMDA receptors excite alcohol research. *Trends in Pharmacological Sciences* **11**, 137–139.

Heil, T., Spies, C., Hannenmann, L., Reinhart, K. and Eyrich, K. (1992) Pharmakologische Ansatze zur Prophylaxe und Therapie des postoperativen Alkoholentzugssyndroms. *Anaesthesiologie Intensivmedizin* **33**, 33–37 (in German).

Heil, T., Spies, C. D., Bullmann, C., Neumann, T., Byrich, K., Muller, C. and Rommelspacher, H. (1994) The relevance of CDT (carbohydrate-deficient transferrin) in the preoperative diagnosis of chronic alcohol abuse in intensive care patients after elective tumor resection. *Anaesthesist* **43**, 447–453 (in German).

Joseph, M. H. and Risby, D. (1975) The determination of kynurenine in plasma. *Clinica Chimica Acta* **63**, 197–204.

Lovinger, D. M. (1995) Ethanol and the NMDA receptor: implications for intoxication, tolerance, dependence and alcoholic brain damage. In *Acamprosate in Relapse Prevention in Alcoholism*, Soyka, M. ed., pp. 1–26. Springer, Berlin.

Mikac-Dević, D., Stanković, H. and Bošdković, K. (1973) A method for determination of free fatty acids in serum. *Clinica Chimica Acta* **45**, 55–59.

Morgan, P. F. (1991) Is quinolinic acid an endogenous excitotoxin in alcohol withdrawal? *Medical Hypotheses* **36**, 118–121.

Morgan, C. J. and Badawy, A. A.-B. (1994) Effects of storage on binding and stability of tryptophan in human serum. *Annals of Clinical Biochemistry* **31**, 190–192.

Muller, C., Brautigam, K., Moritz, R., Ruggeberg, J. and Kottgen, E. (1993) A simplified test procedure for measuring carbohydrate-deficient transferrin in serum. *European Journal of Clinical Chemistry and Clinical Biochemistry* **31**, A42.

Pfefferkorn, E. R., Rebhun, S. and Eckel, M. (1986) Characterization of an indoleamine 2,3-dioxygenase induced by gamma interferon in cultured human fibroblasts. *Journal of Interferon Research* **6**, 267–279.

Satoh, T. and Moroi, K. (1969) Inhibition of tryptophan pyrrolase of rats by phenols, tryptophan analogs and hydrazine derivatives. *Chemical and Pharmaceutical Bulletin (Japan)* **17**, 1560–1563.

Schuckit, M., Tipp, J. E., Reich, T., Hesselbrook, V. and Bucholz, K. K. (1995) The histories of withdrawal convulsions and delerium tremens in 1648 alcohol dependent subjects. *Addiction* **90**, 1335–1347.

Slein, M. W. (1963) D-Glucose determination with hexokinase and glucose-6-phosphate dehydrogenase. In *Methods of Enzymatic Analysis*, Bergmeyer, H.-U. ed., pp. 117–123. Academic Press, New York.

Spies, C. D., Rommelspacher, H., Schnapper, C., Muller, C., Marks, C., Berger, G. *et al.* (1995) β-Carbolines in chronic alcoholics undergoing elective tumor resection. *Alcoholism: Clinical and Experimental Research* **19**, 969–976.

Stone, T. W. (1993) Neuropharmacology of quinolinic and kynurenic acids. *Pharmacological Reviews* **45**, 309–385.

Stuppaeck, Ch. H., Barnas, Ch., Hackenberg, K., Miler, C. H. and Fleischhacker, W. W. (1990) Carbamazepine monotherapy in the treatment of alcohol withdrawal. *International Clinical Psychopharmacology* **5**, 273–278.

Sullivan, J. T., Sykora, K., Schneiderman, J. *et al.* (1989) Assessment of alcohol withdrawal: The Revised Clinical Institute Withdrawal Assessment for Alcohol Scale. *British Journal of Addiction* **84**, 1353–1357.

Taylor, M. W. and Feng, G. (1991) Review: Relationship between interferon-γ, indoleamine 2,3-dioxygenase, and tryptophan catabolism. *FASEB Journal* **5**, 2516–2522.

Victor, M. and Adams, R. D. (1953) The effect of alcohol on the nervous system. *Research Publication — Association for Research in Nervous and Mental Disease* **32**, 526–573.