Current Perspective on the Location and Function of Gamma-Aminobutyric Acid (GABA) and its Metabolic Partners in the Kidney

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Abstract: Gamma-aminobutyric acid (GABA) is an inhibitory neurotransmitter located in the mammalian central nervous system, which binds to $GABA_A$ and $GABA_B$ receptors to mediate its neurological effects. In addition to its role in the CNS, an increasing number of publications have suggested that GABA might also play a role in the regulation of renal function. All three enzymes associated with GABA metabolism; glutamic acid decarboxylase, GABA α-oxoglutarate transaminase (GABA-T) and succinic semialdehyde dehydrogenase (SSADH) have been localised to the kidney providing the necessary machinery for localised GABA synthesis and metabolism. Moreover GABA receptors have been localised to both tubular and vascular structures in the kidney, and GABA is excreted in urine (~3 μM) in humans. Despite the collective evidence describing the presence of a GABA system in the kidney, the precise function of such a system requires further clarification. Here we provide an overview of the current renal GABA literature and provide novel data that indicates GABA can act at contractile pericyte cells located along vasa recta capillaries in the renal medulla to potentially regulate medullary blood flow.

Keywords: Gamma-aminobutyric acid, Pericytes, Kidney, Renoprotective, $GABA_A$, $GABA_B$.

INTRODUCTION

Gamma-aminobutyric acid (GABA) is an established inhibitory neurotransmitter, most commonly associated with having a functional role in the mammalian central nervous system (CNS). This endogenous amino acid is expressed in the vertebrate CNS [1], peripheral nervous system [2], and in several non-neural tissues [3]. In the CNS, GABA acts at $GABA_A$ receptors ($GABA_AR$) to exert fast inhibitory action and at $GABA_B$ receptors ($GABA_AR$) to mediate slower inhibitory transmission. $GABA_ARs$ are ligand-gated chloride channels, which belong to a superfamily of heteropentameric ligand-gated ion channel receptors [4]. $GABA_AR$ subunits are encoded by 19 different genes, which are grouped into eight subclasses determined by their sequence homology (α1–6, β1–3, γ1–3, δ, ε, θ, π, ρ1–3) [5]. $GABA_BRs$ are metabotropic receptors belonging to the G protein-coupled receptor superfamily (GPCRs) [6]. $GABA_BRs$ principally consist of $GABA_{B1}$ and $GABA_{B2}$ subunits [7] and an auxiliary K^+ channel tetramerisation domain (KCTD) subunit [8, 9]. The $GABA_{B1}$ subunit has two isoforms, $GABA_{B1a}$ and $GABA_{B1b}$, which combined with $GABA_{B2}$ to form functional $GABA_BRs$.

GABA is synthesised *in vivo* by the metabolic GABA shunt pathway, which acts to both synthesise and conserve GABA in a closed-loop system [10]. GABA synthesis occurs following the decarboxylation of glutamic acid by glutamate decarboxylase (GAD) [1], whilst conservation of GABA is achieved by GABA-T-mediated transamination of GABA to succinic semialdehyde (SSA), which is subsequently utilised to regenerate glutamic acid [10].

Whilst much of our knowledge regarding the function of GABA has historically originated from studies performed in the CNS, GABA and its metabolic enzymes have since been detected in numerous peripheral tissues, including the liver [11], spleen [12], oviduct [13, 14], testis [15], pancreas [16, 17], adrenal gland [18], and the kidney [19-21]. There are now a significant number of studies emerging in the literature, which report the presence of GABA and its receptors in renal tissue, with this in mind it is perhaps surprising that this well studied amino acid has not attracted more attention from renal physiologists. This review will summarise what is currently known about the expression and function of GABA, metabolic enzymes, and its receptors in the mammalian kidney and will seek to identify a potential role for a GABA system in the regulation of renal function.

THE RENAL GABA SYSTEM

Expression of GABA in the Kidney

The rat is a commonly used model for the study of renal function and multiple studies now report a wide

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distribution of GABA along the rat nephron [22-27]. Immunohistochemistry studies performed on rat kidney tissue report GABA immunoreactive structures in epithelial cells of the thin and thick ascending limbs of the loop of Henle, the connecting tubules, principal cells of the collecting duct [22, 28], the distal tubules [28] and the juxtamedullary cortex [23]. Such studies describe the densest expression of GABA to be in the inner stripe of the outer medulla [22], which is in contrast to earlier chromatography studies (performed on multiple mammalian species) which identify the renal cortex as having the greatest abundance of GABA [24]. As such there may be species-specific expression patterns for GABA. Interestingly, of the studies in which GABA expression in human renal tissue has been investigated, the highest concentration of GABA was also detected in the renal cortex, when investigating 'healthy humans' [24-26].

Studies focusing on renal tubular acidosis provide evidence, which suggests detectable levels of GABA and GAD in the kidney are altered, although the reports regarding the effect this has on GABA synthesis are somewhat disparate. The GABA precursors glutamine and glutamic acid, are known to produce glucose and ammonia during renal gluconeogenesis and acidosis [27, 29, 30]. Spectroscopy studies utilising cortical tubules isolated from chronically acidotic rats report an increase in the rate of gluconeogenesis [30, 31] with a decrease in the rate of glutamine, aspartate and GABA formation [31]. Similarly, chromatography studies performed in rats report that chronic acidosis simulates a significant reduction in the concentration of GABA in rat kidney cortex, whereas levels of GABA in the brain were unaffected [24]. The underlying mechanisms are however controversial, *in vivo* studies in rats describe an increase in the level of renal GABA during acidosis, specifically in response to inhibition of GABA-T, which suggest the conversion of L-glutamic acid to GABA may be increased during ammoniagenesis. Thus, the function of the renal GABA pathway may be associated with ammoniagensis during acidosis [24]. Experiments performed on rat renal cortex tissue conversely suggest that the renal GABA pathway contributes to glutamate disposal and only plays a passive role in subsequent ammoniagenesis [32]. By contrast, other studies have reported that chronic acidosis in rats causes a significant increase in GAD activity in renal homogenates which would imply an increase in synthesis of GABA [33]. Conflicting chromatography data suggests GAD and GABA-T activity in the kidney remain unaltered by acidosis [24]. The disparate findings reported may simply be due to the differential experimental techniques employed, and further research is clearly needed. Currently, data from these preliminary studies suggest the renal GABA system is directly affected by chronic acidosis, and given the acidosis-associated loss of kidney function, the resulting impact this system might have on renal function may be significant in both health and disease.

Expression of Metabolic Enzymes of GABA in the Kidney

As is true for GABA, its metabolic partners have been detected in various locations throughout the nephron in a variety of different species (Table **1**). Glutamic acid, the precursor to GABA, has been detected in proximal locations to GABA in the rat nephron, which include the epithelial cells of the thick portion of the loop of Henle in the outer medulla, the thin portion of the loop of Henle in the inner medulla, distal tubules, and principal cells of the collecting duct in both the inner and the outer medulla [20]. In addition, glutamic acid-specific immunoreactivity was also detected in areas of the nephron, in which GABA has not yet been identified, such as podocytes of the Bowman's capsule [20].

Previous studies performed in rats, have suggested that GABA can be synthesised intramurally in the kidney by multiple pathways. The majority of reports to date indicate GABA is synthesised from L-glutamic acid [24, 34, 35] *via* the intramitochondrial enzyme GAD, exclusively within tubules of the rat renal cortex [36], and that the renal GABA pathway accounts for approximately 25% of glutamic acid oxidation in the renal cortex [24]. Furthermore, one study suggests that the synthesis of GABA by decarboxylation of L-glutamic acid in the rat renal cortex, might be different from the corresponding reaction in the brain [24], due to characteristic differences between renal GAD and brain GAD [26, 35], and renal GAD having two Km values [24]. A potential explanation for the differing Km values for renal GAD being that i) there is more than one form of GAD present within the kidney or ii) GAD binds differently at differing concentrations of the substrate [24].

Notably, GABA has also been shown to be synthesised *in vivo* from putrescine, *via* an alternative biosynthetic pathway, in a range of peripheral tissues including the kidney [37, 38]. Putrescine is converted to GABA-aldehyde *via* diamine oxidase and is then converted to GABA *via* aldehyde dehydrogenase [39].

Table 1: The Location of GABA Receptor Subunits and GABA Metabolic Enzymes in the Kidney

Components	Nephron	Species	mRNA *vs.* Protein	References
$GABA_AR$	Convoluted tubules	Rat	Protein	[55]
	Collecting duct	Rat	Protein	[55]
	Thick ascending limb	Rat	Protein	[55]
$GABA_AR$ subunits				
α_1	Proximal tubules (r) (rb); whole kidney (h)	Human, rat, rabbit	Both (r) (rb); mRNA (h)	[21, 56]
α_2	Whole kidney	Mouse	Protein	[56]
α_5	Renal cortex	rat	Both	[56]
α_6	Whole kidney	Mouse	Protein	[57]
β_1	Renal cortex	rat	Both	[56]
β_2	Whole kidney (m); renal cortex (r) (rb)	Rat, mouse, rabbit	Both (r) (rb); mRNA (m)	[57, 69]
β_3	Renal cortex (r) (rb); outer medulla (r); whole kidney (h)	Human, rat, rabbit	Both (r) (rb); mRNA (h)	[21, 56, 69]
γ_1	Renal cortex	Rat	Both	[56]
γ_2	Whole kidney (m); renal cortex (r) (rb)	Rat, mouse, rabbit	mRNA (m) (r) (rb)	[56, 57]
γ_3	Whole kidney	Mouse	mRNA	[57]
π	Proximal and distal tubules (r); whole kidney (h)	Human, rat	Both (r); mRNA (h)	[21]
$GABA_BR$	Renal cortex	Rat	Both	[21, 68]
$GABA_BR$ R1 subunit	Glomeruli	Rat	Both	[21]
	Arteriole	Rat	Both	[21]
	Whole kidney	Human	mRNA	[21]
$GABA_BR$ R2 subunit	Proximal tubules	Rat	Both	[21]
	Collecting tubules	Rat	Both	[21]
	Whole kidney	Human	mRNA	[21]
GAD	Glomeruli	Rat	Both	[21, 24]
	Arteriole	Rat	Both	[21]
	Proximal tubules (r) (m)	Rat, Mouse	Protein (r) (m)	[24]
	Distal tubules	Mouse	Protein	[44]
	Whole kidney	Human	mRNA	[21]
GABAT	Proximal tubules (r) (m)	Rat, Mouse	Both (r); protein (m)	[21, 46, 48]
	Descending and ascending loops of Henle	Mouse	Protein	[48]
	Distal tubules (r) (m)	Rat, mouse	Both (r); protein (m)	[21, 46]
	Whole kidney	Human	mRNA	[21]
SSADH	Whole kidney	Human	Protein	[19]
GAT2	Cortical renal tubules	Rat	Both	[21]
	Whole kidney	Human	mRNA	[21]
BGT1	Basolateral membrane of collecting ducts	Mouse	Protein	[52]
	Thick ascending limbs of Henle	Mouse	Protein	[52]
	Outer medulla	Rat	mRNA	[51]
	Papilla	Rat	mRNA	[51]

Abbreviations: (h) refers to human, (r) refers to rat, (rb) refers to rabbit, and (m) refers to mouse.

Radioactivity studies performed in mouse kidney have shown that, following administration of [^{14}C]-putrescine, significant amounts of radioactive carbon were recovered as GABA both in mouse kidney tissue and urine [38]. Future studies are needed to determine whether the two biosynthetic pathways operate simultaneously or whether they contribute differentially in space and time to GABA synthesis in the kidney.

In addition, GAD activity has been detected in a range of peripheral tissues including the liver, oviduct, testis and the kidney [39, 40] (see Table **1**). GAD activity reported in the kidney is second highest to that reported for the brain [40]. As mentioned above, renal GAD is distinct from GAD found in the brain due to its differential dependence on the cofactor pyridoxal 5'-phosphate required for its enzymatic activity [35], sensitivity to antagonists and agonists, and sensitivity to dietary sodium. Unsurprisingly, when investigating the effect of dietary sodium on GAD activity, only renal GAD activity is reduced when rats were placed on a

low sodium diet [23]. Northern blot hybridisation experiments have shown that isolated cDNAs from rat and mouse brain libraries, encoding GAD65 and GAD67, do not hybridise to the RNA isolated from rat kidney [40, 41]. Functional studies in which GAD activity was assessed by measuring GABA radioactivity, report renal GAD is stabilised by ATP and NaCl (and in combination), whilst ATP inhibited brain GAD [42]. Conversely, radiometric assays performed on tissue from both rats and mice suggest that both kidney and brain derived GAD respond similarly to high concentrations of the inhibitor aminooxyacetic acid [32, 43]. Regarding the specific location of active GAD, studies utilising rat renal cortex homogenates indicate GAD activity is three times greater within proximal tubules relative to the glomeruli [23]. Conflicting immunohistochemistry studies show GAD65 and GAD67 staining predominately in the glomeruli and arterioles in rats [21] and yet mRNA for GAD was not detected in glomeruli isolated from mice [44]. GAD65 and GAD67 mRNA was however detected in the proximal and distal tubules in mice but not detected anywhere in the medulla [44]. In summary, data regarding the distribution and activity of GAD throughout the nephron, yielded from various studies, is somewhat disparate and evidence further speaks to the possibility of species differences in the importance of the GABA system in the kidney. In a disease context, GAD has been reported to be a major target for auto-antibodies generated in insulin-dependent diabetes mellitus [45] and as such the GAD expressed as an auto-antibody in renal tubules may contribute to diabetic tubulointerstitial disease. However, presently this is inconclusive and requires additional evidence for clarification [44].

In the CNS, GABA-T acts to catabolise GABA to succinate, in fitting with this established mechanism, quantification studies using rat kidney cortex homogenates have shown that GABA can be similarly catabolised by GABA-T to succinate [24]. Unlike renal GAD, renal GABA-T exhibits similar functional characteristics to neural GABA-T. Antibodies raised against mouse brain GABA-T have been shown to cross-react with renal GABA-T in the apical membrane of epithelial cells in the proximal and distal convoluted tubules [46]. Additionally studies performed on human kidney tissue, indicate neural and renal GABA-T both exhibit the necessity for the pyridoxal phosphate cofactor, respond similarly to specific inhibitors, have similar molecular weights, isoelectric pH, and substrate affinities [47]. Immunohistochemical studies have shown that GABA-T appears to be predominantly localised in the renal cortex of both rats [21, 32] and mice [46, 48] and more specifically is expressed in cortical proximal and distal tubules [46] and in medullary descending and ascending loops of Henle in mice [48]. Interestingly, others report its substrate GABA, is expressed predominately in the rat renal medulla [22]. The lack of correlation in expression of GABA and GABA-T might reflect the presence of differential enzymes and/or the presence of a unique GABA uptake mechanism in the medulla [22]. Its possible that, higher levels of GABA in the renal medulla, relative to the renal cortex, may coincide with a decreased catabolic rate of GABA in the medulla, although, this fails to equate with the dense cortical expression of $GABA_A$Rs in both rats and mice (discussed below).

In addition to the metabolic enzymes in the GABA shunt pathway, an active GABA uptake system also exists in the kidney, as it does in the CNS. Immunohistochemistry studies have detected the GABA transporter GAT2 on the basolateral side of cortical renal tubules in rats [21]. Functional studies performed on brush-border vesicles, derived from the proximal tubule luminal membrane, have shown active transport of GABA into the brush-border vesicles occurs in a sodium-dependent manner [49]. Authors of this study propose that high-affinity renal GABA transport might represent the need to maintain high intracellular levels of GABA in the intracellular space of rat renal cortical slices, or perhaps more importantly the need to minimise extracellular levels of GABA, which was also found in brush-border membrane vesicles [50]. The betaine/GABA transporter (BGT1), which has a higher affinity for GABA than betaine, has also been found in the renal medulla [51, 52] although studies to date have focused on its role in transporting the osmolyte betaine rather than establishing whether it contributes to the renal GABA system.

Lastly it has been reported that the concentration of GABA in the urine is higher than that measured in the blood [12, 53], which begs the question as to whether GABA is released by the kidney to exert an extramural effect? HPLC-fluorometric studies showed that the Na^+/K^+-ATPase inhibitor ouabain stimulates a significant increase in the efflux of endogenous GABA from slices of rat renal cortex and medulla [54]. These researchers propose that the depolarising stimulus-evoked release of GABA from kidney tissue provides preliminary evidence favouring an extramural function for GABA [54].

Localisation of $GABA_A$ Receptor Subunits in the Kidney

Numerous studies now provide evidence for GABA receptor expression throughout the rat nephron. Radioreceptor binding studies and autoradiography studies performed in rats have identified binding sites for the $GABA_AR$ agonist muscimol in the convoluted tubules of the renal cortex, the collecting duct and the thick ascending limb of the loop of Henle [55]. These researchers confirmed that the binding sites were indeed conventional $GABA_A$ receptors by displacing (^3H)-muscimol with muscimol, GABA, isoguvacine ($GABA_A$ receptor agonist) and bicuculline ($GABA_A$ receptor antagonist). Accumulating evidence shows that specific $GABA_AR$ subunits are present within the kidney in a range of species (see Table **1**). Immunoprecipitation studies and ^{36}Cl-uptake studies have identified a novel functional $GABA_AR$ within the proximal tubular cells of the rat kidney, co-assembled as $\alpha_5\beta_1\Upsilon_1$ [56]. More recent studies having similarly detected α_1 and β_3 subunits in the renal cortex, have additionally detected π subunit mRNA and protein in Wistar-Kyoto rats [21]. Interestingly, $GABA_A$ π subunit mRNA levels are significantly reduced in spontaneously hypertensive rats (SHR) compared to wild-type rats on a normal salt diet, there was no significant differences reported on a high salt diet. Immunohistochemical observations indicate that the α_1, β_3 and π subunits were mainly localised apically in cortical tubules, whereas immunoblot experiments reveal a potential novel combination of these $GABA_A$ receptor subunits in the kidney [21]. Interestingly, opposing evidence exists for the specific renal expression of the α_5, β_1, β_2, β_3, γ_1 and γ_2 subunits of $GABA_A$ receptors [21, 56], this may again be due to the different PCR techniques or the different rat strains used in these two studies.

Studies in mice have detected mRNA expression of $GABA_AR$ α_2, α_6, β_2, γ_2, γ_3 subunits in the renal system. Western blot experiments reveal a greater expression of $GABA_AR$ protein in the mouse medulla than mouse cortex [57]. Currently, it is unclear as to whether the amount of GABA in the mouse medulla compliments the expression of $GABA_AR$ protein. Collectively, data suggest that different forms of $GABA_ARs$, are located along the rat nephron in different species, which likely consist of different subunit combinations, thereby exerting different pharmacological characteristics. To fully appreciate the potential signalling functions of the renal GABA system, future studies must first establish the different subunit compositions of $GABA_ARs$ located throughout the kidney.

It is well established that $GABA_ARs$ are modulated by benzodiazepines [58-61] and it has been suggested that peripheral benzodiazepine receptors (PBR), unrelated to $GABA_ARs$, also activated by benzodiazepines, are present in the kidney. Studies propose variations in benzodiazepine binding to PBRs in the kidney following acute angiotensin II infusion [62], in SHR models [63-65], and after angiotensin II-induced hypertension [66]. Moreover, more recent studies report selective PBR agonists are protective against ischemic renal injury in rats [67]. Interestingly the potential for renal GABA and $GABA_ARs$, or their potential renoprotective role is not investigated in this study and yet given the accumulating evidence for a functional GABA system in the kidney it certainly seems feasible that $GABA_ARs$ might be involved in the reported PBR agonist-evoked protection against ischemic renal injury.

Unlike $GABA_ARs$, very little is known about $GABA_BRs$ in the kidney. Autoradiographic studies have detected binding sites for the $GABA_BR$ agonist baclofen, indicating a potential presence of $GABA_BRs$ in the kidney. These binding sites were exclusively detected in the rat renal cortex and absent from the medulla [68]. Specifically, $GABA_BR$ R1 and R2 subunits have been detected at both the mRNA and protein level in the rat renal cortex [21], the R1 subunit being detected in the glomeruli, arterioles and renal tubules, and R2 subunit detected in the proximal-like tubules and collecting duct-like tubules [21]. Contrary to detection of $GABA_AR$ subunits in the proximal tubules [55, 56, 69], the detection of $GABA_BRs$ within the renal cortex and the dense expression of GAD in the proximal tubules in rats [23], electron microscopy studies failed to detect GABA immunoreactive structures in both the renal corpuscles and proximal convoluted tubules in rats [22]. Future studies are required to reconcile expression of both types of GABA receptors with GABA and its metabolic enzymes in rat kidneys.

The Function of GABA in the Kidney

In light of the evidence for the presence of GABA, its metabolic enzymes and both $GABA_A$ and $GABA_B$ receptors in the kidney of a range of mammalian species, including humans [21, 56] it seems plausible that there is a physiologically relevant role, or roles, for the renal GABA system.

Antihypertensive Role of GABA

There is increasing evidence that suggests GABA is able to modulate renal functions, such as urine formation, blood flow and sympathetic neurotransmitter release. Experiments performed using isolated perfused rat kidney indicate that GABA may modulate urine formation, by stimulating an increase in the fractional excretion of water, sodium and glucose, presumably by acting on $GABA_BR$ to induce vasoconstriction at the afferent arteriole [70]. In support of this, a more recent study showed that *in vivo* administration of baclofen, induced diuresis, natriuresis kaliuresis and increased glucose exaction in anaesthetised rats [71]. Alternative data collected from isolated perfused rat kidney studies, demonstrate GABA-mediated suppression of renal nerve stimulation, due to attenuation of noradrenaline release and vasoconstriction, as a result of the activation of presynaptic $GABA_BRs$ [72]. This suppressive effect of GABA on noradrenaline release has also been reported in SHR, when GABA was perfused *via* the mesenteric artery [73]. Analogous to GABA, perfusion with baclofen inhibits perivascular nerve stimulation-induced increases in perfusion pressure and noradrenaline release in the mesenteric arterial bed [73], and isolated perfused rat kidney [72].

In addition to this suppressive effect on sympathetic nerve activity, GABA has been shown to have antihypertensive properties. Interestingly, both intracerebroventricular (ICV) injections [74] and oral administration [75] of GABA reduced blood pressure in SHR [73] and to a lesser extent in normotensive rats [74]. ICV injections of GABA also caused a significant reduction in heart rate in SHR and to a lesser extent in normotensive rats, an observation that was not recapitulated when GABA was administered orally. Microinjections of baclofen, into the paraventricular nucleus of the hypothalamus caused a similar reduction in arterial blood pressure in SHR to that observed in response to GABA [75]. Also, ICV injections of muscimol, caused a decrease in mean arterial blood pressure in stroke-prone SHR [76] and in anesthetised cats [77-79]. GABA and muscimol have been shown to cause a reduction in blood pressure, heart rate and renal nerve discharge more effectively when administered by an ICV injection relative to an intravenous injection [77, 79]. This effect was reversed by intravenous administration of bicuculline [77-79]. This implies that the hypotensive effect of GABA and muscimol in anesthetised cats is mediated by CNS stimulation rather than acting on a peripheral site of action. A later study showed that pretreatment with sarthran, an angiotensin receptor antagonist, reduced the hypotensive effect of GABA and the hypertensive effect of bicuculline in SHR and normotensive rats [80] implying that the hypotensive effect of GABA is mediated *via* the angiotensin system in the CNS. The above evidence suggests that the effect of GABA on reducing blood pressure, heart rate and sympathetic nerve responses is consistent in all species studied to date. Depending on the manner in which GABA was administered, and the specific location within the body, the antihypertensive effect was mediated either through $GABA_ARs$ or $GABA_BRs$. In extension, overexpression of the $GABA_BR1$ gene in the brain, specifically the nucleus of the solitary tract, results in an increase in blood pressure and heart rate in normotensive rats [81]. This adds credibility to the notion that the GABAergic system plays an essential role in regulating blood pressure in the brain. It should be the subject of future research to determine if the antihypertensive effect of GABA, or analogues with similar properties, will have any therapeutic advantages to humans.

Renoprotective Role of GABA

In addition to the antihypertensive role of GABA in the kidney, a renoprotective role in acute renal failure in rats has been indicated. The enhancement of renal sympathetic nerve activity and noradrenaline overflow are paramount to the progression of ischemia/ reperfusion induced renal injury. Oral administration of GABA in rats, attenuates the physiological changes associated with glycerol-induced acute renal failure including, an increase in body weight, kidney weight, blood urea and creatinine [82]. More recent studies have shown that intravenous treatment with GABA or intravenous treatment with baclofen, attenuates the enhanced renal sympathetic nerve activity and the associated increase in noradrenaline overflow known to occur in ischemic acute kidney injury in rats [83]. Thus, the suppressive role of GABA on the enhanced nerve activity, seemingly *via* $GABA_BRs$, serves to suppress renal dysfunction, which may be therapeutically relevant in treating acute renal failure in humans. In support of the relevance of the renoprotective role of GABA in a clinical setting, GABA also elicits a protective role against cisplatin-induced acute kidney injury in rats [84]. Takano *et al*. (2014) proposed that since over stimulation of renal sympathetic nerve activity is associated with the renin-angiotensin-aldosterone system, activation of $GABA_BRs$ in the distal-like tubules might alter this system [21].

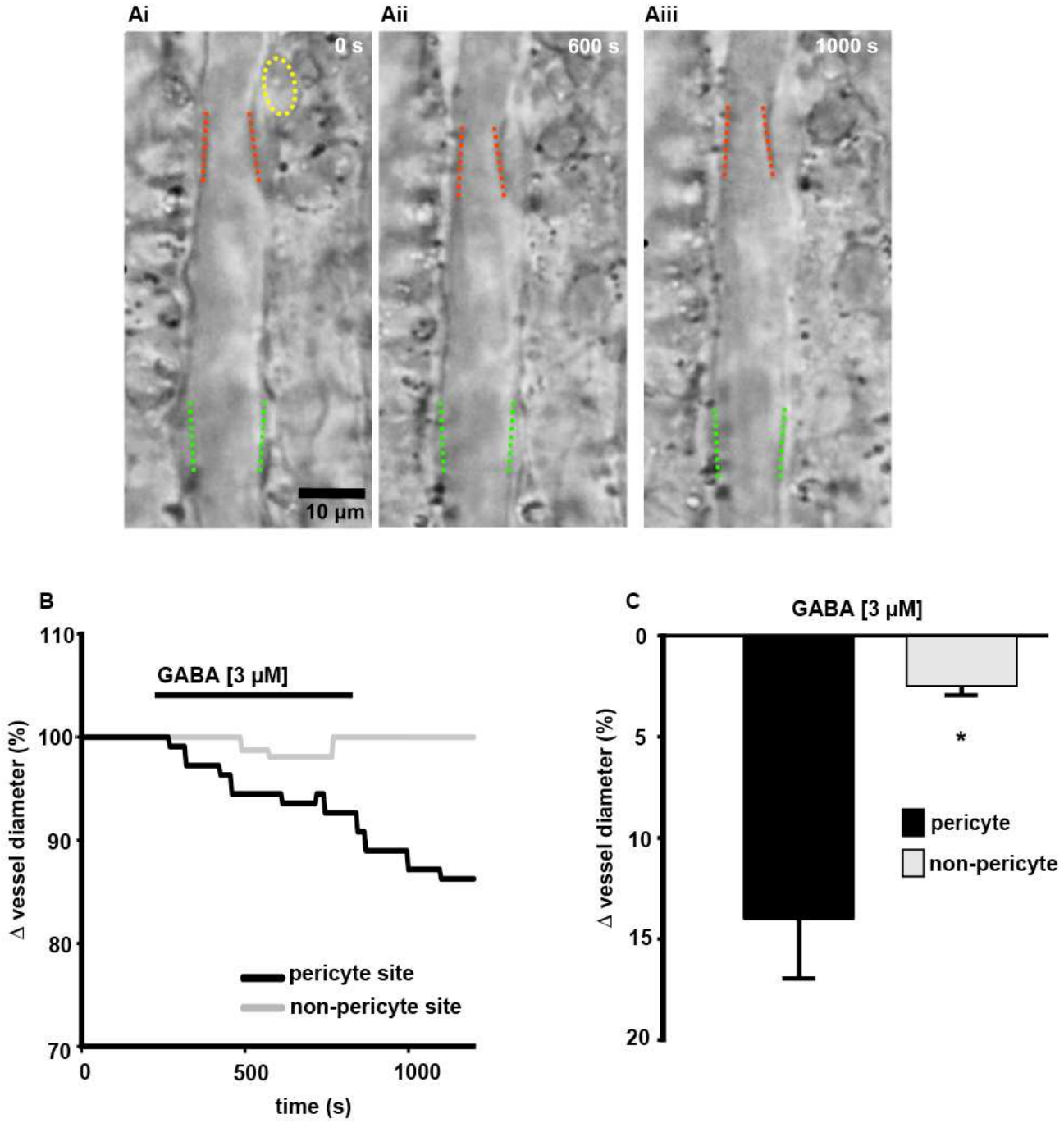

Figure 1: DIC imaging of GABA-evoked pericyte-mediated constriction of *in situ* vasa recta capillaries. (**Ai-iii**) Images of vasa recta capillaries are taken from a time series experiment in which kidney slices were exposed to GABA (3 µM). Vessel diameter was measured every 5 s throughout the course of the experiment at the pericyte site (red dashed lines) and the corresponding non-pericyte site (green dashed lines) of the same vasa recta. (**Ai-iii**) Yellow dotted circles denote the pericyte. (**Ai**) A typical field of view of vasa recta superfused with PSS under control conditions. (**Aii**) Application of GABA (3 µM) caused a reduction in vasa recta diameter at the pericyte site. (**Aiii**) Following the removal of GABA from the perfusate, a further reduction in vasa recta diameter at the pericyte site occurs. (**B**) Depicts a representative trace of percentage change in vasa recta diameter at pericyte site (black trace) and non-pericyte site (grey trace) over time in response to GABA (3 µM) exposure. (**C**) Mean data showing percentage change in vessel diameter at pericyte sites (black bar) and non-pericyte sites (grey bar) during the presence of GABA (3 µM). GABA-evoked a significantly greater vasoconstriction of vasa recta at pericyte sites relative to non-pericyte sites. Values are mean SEM, * $P<0.001$, $n = 12$.

Additional evidence favouring a protective role for GABA in the kidney has been demonstrated following oral administration of GABA in nephrectomised rats, the effect of GABA being attenuation of fibrosis and

atrophy, primarily in renal tubules. Specifically, GABA resulted in a reduction in renal functional losses as determined by measuring creatinine, serum urea nitrogen, urinary protein levels, and the increased expression of TGF- β_1 and fibronectin in renal tubules [85]. Conversely, GABA failed to exert significant protective effects in the glomeruli. Selective $GABA_AR$ and $GABA_BR$ agonists reproduced the GABA-mediated amelioration of renal functions, suggesting a role for both $GABA_ARs$ and $GABA_BRs$ in improved outcomes in nephrectomised rats. Interestingly, expressions of both $GABA_ARs$ and $GABA_BRs$ in proximal tubules were decreased in nephrorectomised rats, which was later increased following GABA administration [85]. These preliminary results imply that oral administration of GABA might offer beneficial outcomes such as reducing fibrosis and attenuating renal dysfunction, when used in conjunction with other medication, to treat renal failure.

Regulation of Blood Flow by GABA

Beyond its role as a neurotransmitter in the CNS, some studies have shown that GABA can regulate blood flow. For instance, the uptake of GABA into astrocytes has been shown to stimulate vasoconstriction of blood vessels, within the nerve layer of the olfactory bulb, which was attenuated by the inhibition of GABA uptake by an mGAT4 inhibitor, SNAP 5114, in mice [86]. Other studies have shown that GABA can serve as a vasodilator *via* its action on vascular smooth muscle cells surrounding blood vessels in different regions of the brain in dogs [87], rats [88], and rabbits [89].

Contractile pericytes are known to regulate capillary diameter in a similar way to their counterpart vascular smooth muscle cells that surround larger vessels [90-98]. In whole mount retinae, application of GABA receptor blockers caused pericyte-mediated vasoconstriction; implying endogenous GABA may act as a tonic vasodilator in the retina. Pericytes are expressed throughout all mammalian tissues and organs including the kidney and recent studies have highlighted the importance of renal pericytes in regulating medullary blood flow and the associated maintenance of the cortico-medullary gradient required for appropriate urine concentration [99]. Given that GABA is known to induce vasoconstriction in the afferent arteriole in the rat kidney [70] and may act to tonically regulate capillary diameter *via* pericytes in the retina [96], we examined whether GABA acted at vasa recta pericytes in the renal medulla to alter capillary diameter and blood flow in this region. Using a live kidney slice model [98], we have demonstrated that GABA causes pericyte-mediated vasoconstriction of vasa recta (Figure **1**). These data describe a novel role for GABA in pericyte-mediated regulation of medullary blood flow (MBF).

CONCLUSION

Although the expression of the renal GABA system has been explored for several decades, the precise function, and importance, of this system within the kidney remains obscure. More recent progress has established GABA as having both an antihypertensive and renoprotective role within the kidney, although the precise mechanism(s) remain undetermined at present. Further investigations are needed to establish the physiological relevance of these GABA-mediated effects and whether there is a therapeutic potential for these findings in humans. In light of the research discussed, it is evident that GABA can be synthesised within the kidney and it is also present within blood and urine. Future studies focusing on defining the function of the renal GABA system may help identify if it can be used as therapeutic targets for treatment of kidney diseases. As to the role of GABA as a modulator of MBF *via* its action at vasa recta pericytes, further investigations are underway in our laboratory. It is apparent that a role for GABA in non-neural tissues is emerging and more research is needed to provide greater insight in the significance of the renal GABA system.

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