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Inhibition of γ-aminobutyric acid uptake: anatomy, physiology and effects against epileptic seizures

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Abstract

The transport of γ-aminobutyric (GABA) limits the overspill from the synaptic cleft and serves to maintain a constant extracellular level of GABA. Two transporters, GABA transporter-1 (GAT-1) and GAT-3, are the most likely candidates for regulating GABA transport in the brain. Drugs acting either selectively or nonselectively at GATs exert distinct anticonvulsant effects, presumably because of distinct regions of action. Here I shall give a brief review of the localization and physiology of GATs and describe effects of selective and nonselective inhibitors thereof in different animal models of epilepsy.

Keywords: GABA transport; Tiagabine; GAT-1 (GABA transporter-1); GAT-2 (GABA transporter-2); GAT-3 (GABA transporter-3); Epilepsy; Tonic conductance; Extrasynaptic

1. Introduction

The early characterization of uptake of the neurotransmitter γ-amino butyric acid (GABA) in brain tissue suggested the existence of at least two pharmacologically distinct routes for its transport (Iversen and Kelly, 1975; Sidhu and Wood, 1986; Wood and Sidhu, 1987). Next, the purification, sequence analysis (Radian et al., 1986) and cloning (Guastella et al., 1990) of GABA transporter-1 (GAT-1) then proved to be the first member of a large family of Na^+/Cl^- coupled neurotransmitter transporters (Miller et al., 1997). As opposed to transport mechanisms for norepinephrine, dopamine and 5-hydroxy tryptamin, each for which a single transporter is known, GATs comprise a group of four different transporters (Table 1), a diversity shared with glycine transporters for which four subtypes are known. Since the initial discovery that nipecotic acid and guvacine are substrate inhibitors of GABA transport (Krogsgaard-Larsen and Johnston, 1975) with effects against pentylenetetrazol- and electroshock-induced convulsions (Croucher et al., 1983), the inhibitors tiagabine, NNC 05-0711, CI-966 and SKF 100330-A were developed and shown to be selective for GAT-1 (Yunger et al., 1984; Ali et al., 1985; Braestrup et al., 1990; Ebert and Krnjevic, 1990; Borden et al., 1994b). Based on good efficacy in animal models of epilepsy and clinical trials, tiagabine were approved for treatment of mesial temporal lobe epilepsy in humans. Compounds with limited selectivity for other GABA transporters have also been developed (Dhar et al., 1994; Borden et al., 1994a; Thomsen et al., 1997; Dalby et al., 1997). These compounds were identified based on effect against a region-dependent tiagabine-insensitive component of GABA transport in the central nervous system (CNS), and they have furthermore been shown to possess a, compared to tiagabine, distinct anticonvulsant profile (Dalby and Nielsen, 1997; Dalby et al.,1997). In addition, a variety of drugs with no apparent affinity for GATs have been shown to affect GABA transport through mechanisms presumably related to the machinery presenting transport proteins to the plasma membrane.

Our understanding on the role of GATs has greatly expanded from being a ubiquitous machinery to re-utilize released transmitter, to proteins whose distinct expression pattern in the CNS are key players in limiting the synaptic overspill of GABA and activation of GABA receptors at extrasynaptic loci. The distinct location in- and outside synapses of $GABA_A$ receptors are dictated by their subunit composition. For example, receptors containing the α_1-

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Table 1
Nomenclature of GABA transporters over species

	Plasma membrane localized				Vesicular localized
Mouse	mGAT1[a,b]	mGAT2[a,b]	MGAT3[c]	mGAT4[c]	VIAAT[d]
Mouse (suggested)	mGAT-1	mBGT-1	MGAT-2	mGAT-3	
Rat	rGAT-1[e,f]	[BGT-1][g]	rGAT-2[h]	rGAT-3[f,h]	VGAT[i]
Canine	NC	cBGT-1[j]	NC	NC	
Human	hGAT-1[k]	hBGT-1[l]	NC	hGAT-3[m]	
Torpedo	tGAT-1[n]	tBGT-1[o]	NC	NC	

Abbreviations: GAT, GABA transporter; BGT, betaine/GABA transporter; VGAT, vesicular GABA transporter; VIAAT, vesicular inhibitory amino acid transporter; NC, not cloned.

[a] Lopez-Corcuera et al., 1992.
[b] Liu et al., 1993.
[c] Liu et al., 1992.
[d] Sagne et al., 1997.
[e] Guastella et al., 1990.
[f] Clark et al., 1992.
[g] Burnham et al., 1996/Genbank entry.
[h] Borden et al., 1992.
[i] McIntire et al., 1997.
[j] Yamauchi et al., 1992.
[k] Nelson et al., 1990.
[l] Borden et al., 1994a.
[m] Borden et al., 1994a.
[n] Swanson et al., 1994.
[o] Guimbal and Kilimann, 1994.

subunit is located in most GABAergic synapses (Nusser et al., 1996), $\alpha_4-\alpha_5$ are located mostly outside synapses (Nusser and Somogyi, 1997), whereas the α_6- and δ-subunits are localized exclusively outside synapses (Nusser et al., 1998). The activation of synaptic and extrasynaptic $GABA_A$ receptors, recently reviewed by Mody (2001), has been coined *phasic* and *tonic* inhibitions, respectively. It is possible to separate phasic and tonic inhibitions either by SR95531 (gabazine), low dose picrotoxin (Newland and Cull-Candy, 1992; Stell and Mody, 2002; Semyanov et al., 2003) or by inhibition of GABA uptake by low dose NNC 05-0711 (Nusser and Mody, 2002). Given the latter result, it is conceivable that the effect of GABA uptake inhibitors is determined by the presence of subtype GABA transporter in neurons or glia and, in particular, the different cell-type's expression of extrasynaptic GABA receptors.

Here I shall provide a short review on data pertaining to the physiology of GABA transporters in in vitro systems and in vivo effects of drugs acting either selectively or nonselectively at GATs in the brain. I apologize at the outset to those whose excellent contributions have not been adequately referenced due to limitations in space or through an oversight on my behalf.

2. Nomenclature of GABA transporters

The cloning of the plasma membrane localized GABA transporters in the early 1990s brought about an unfortunate discrepancy in the nomenclature of species-homologue transporters, due to mouse GAT2 being the homologue of other species betaine-GABA transporter-1 (BGT-1) (i.e. mouse GAT2 = rat BGT-1 = human BGT-1, Table 1). This difference in nomenclature is confusing. I shall therefore for the remainder of the manuscript adopt the nomenclature suggested by Miller et al. (1997) and make the substitution mGAT2 = mBGT-1, which then necessitate the according changes mGAT3 = mGAT-2 and mGAT4 = mGAT-3 (Table 1). Furthermore, a prefix (m, r, h) is added where needed and hyphens are included in transporter abbreviation.

The vesicular storage of GABA is mediated by a structurally different transporter (family) than the plasma membrane localized. This family of transporters utilize both the proton electrochemical gradient ($\Delta\mu_H^+$) and the vesicular electrical membrane potential ($\Delta\psi$) for transport of GABA into the vesicular lumen (Sagne et al., 1997; McIntire et al., 1997). Although vesicular GABA transport is an important key-player in GABAergic neurotransmission (Overstreet and Westbrook, 2001; Jin et al., 2003; Deken et al., 2003), it will not be dealt with in any greater detail here.

3. Pharmacology of GABA transport

GABA transport systems have been extensively characterized in vivo, in brain slices, culture systems and synaptosomes since its original discovery by Iversen and Neal (1968). It soon became evident that at least two high-affinity systems must exist based on the complimentary effects of *cis*-3-aminocyclohexanecarboxylic acid (ACHC) and β-alanine (Fig. 1), acting as substrate inhibitors for the two systems (Levi and Raiteri, 1973; Levi et al., 1974; Iversen and Kelly, 1975; Sidhu and Wood, 1986; Radian et al.,1986; Kanner and Bendahan, 1990). However, β-alanine is also a substrate for the taurine carrier and its action in nonrecombinant systems cannot exclusively be ascribed its pharmacological selectivity between GATs (Larsson et al., 1986). Nipecotic acid and guvacine, two GABA transport substrate inhibitors (Krogsgaard-Larsen and Johnston, 1975; Schousboe et al., 1991, but see Barrett-Jolley, 2001) served as lead structures for synthesizing of a series of selective, high-affinity GAT-1 inhibitors (Fig. 1: tiagabine, NNC 05-0711, CI-966, SKF 89976-A/SKF 100330-A) which, as opposed to their parent compound, readily penetrate the blood–brain barrier (Yunger et al., 1984; Ali et al., 1985; Braestrup et al., 1990; Ebert and Krnjevic, 1990; Suzdak et al., 1992; Borden et al., 1994b). These lipophilic analogs are not substrates for transport (Larsson et al., 1988; Schousboe et al., 1991), nor are they strictly competitive. The latter is based on a higher affinity for [^{3}H]tiagabine binding than for [^{3}H]GABA uptake, and the opposite for nipecotic acid (Suzdak, 1993). Furthermore, the mutation of Tyr^{140} on GAT-1 results in a transporter incapable of binding and transport of GABA but which

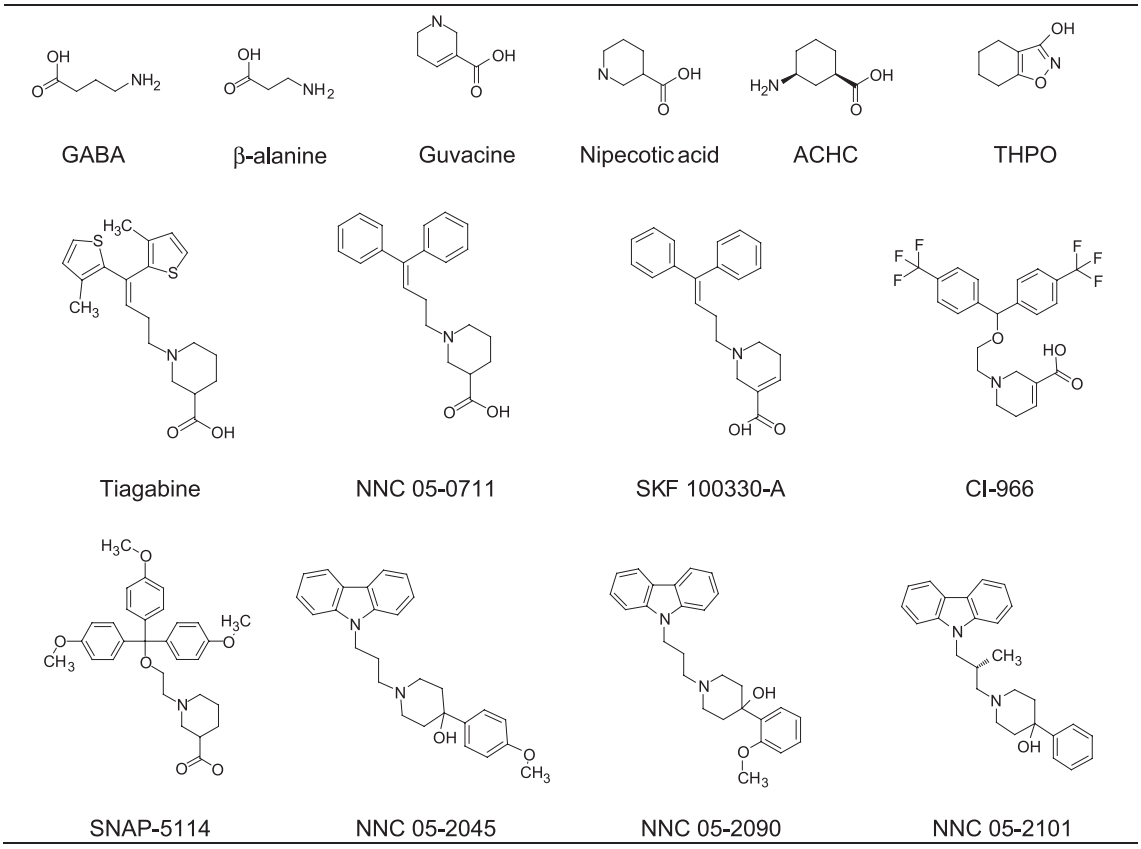

Fig. 1. Chemical structures of GABA and inhibitors of GABA uptake. ACHC: *cis*-3-aminocyclohexanecarboxylic acid; THPO: 4,5,6,7-tetrahydroisoxazolo(4,5-*c*)pyridin-3-ol; tiagabine: (*R*)-1-(4,4-bis(3-methyl-2-thienyl)-3-butenyl)-3-piperidinecarboxylic acid; SKF 100330 A: 1-(4,4-Diphenyl-but-3-enyl)-1,2,5,6-tetrahydro-pyridine-3-carboxylic acid; CI-966: 1-{2-[Bis-(4-triflouromethyl-phenyl)-methoxy]-ethyl}-1,2,5,6-tetrahydro-pyridine-3-carboxylic acid; SNAP-5114: (*S*)-(−)-1-[2-[tris-(4-methoxyphenyl)methoxy]ethyl]-3-piperidinecarboxylic acid; NNC 05-2045: 1-(3-(9*H*-carbazol-9-yl)-1-propyl)-4-(4-methoxyphenyl)-4-piperidinol; NNC 05-2090: 1-(3-(9*H*-carbazol-9-yl)-1-propyl)-4-(2-methoxyphenyl)-4-piperidinol; NNC 05-2101: (*S*)-1-((3-(carbazol-9-yl)-2-methyl)propyl)-4-phenyl-4-piperidinol.

exhibits residual binding of SKF 100330-A, suggesting that the binding site for this compound is only part overlapping that of GABA (Bismuth et al., 1997). In a functional assay for GABA transport in synaptosomes prepared from rat cerebral cortex, tiagabine and NNC 05-0711 at a dose of 1.0–10 μM inhibit ~96% of GABA uptake, but only ~67% in synaptosomes prepared from superior and inferior colliculus or thalamus (Suzdak et al., 1992; Dalby et al., 1997, Thomsen, personal communication). This is similar to a whole brain aggregate in which 15% of GABA uptake is insensitive to 10 μM NNC 05-0711, but highly sensitive to β-alanine (Borden, 1996). SNAP-5114 (Fig. 1) was the first selective inhibitor at human and mouse GAT-3 with an apparent IC_{50} of 0.8 μM at the cloned mGAT-3 (Dhar et al., 1994; Borden et al., 1994a; Thomsen et al., 1997). Five other compounds with similar IC_{50} (low μM) at mGAT-3 were identified. These compounds were generally nonselective (two- to fivefold difference in IC_{50} at mGAT-1 through mGAT-3) though NNC 05-2090 (Fig. 1) exhibits at least 10-fold selectivity towards mBGT-1 (Thomsen et al., 1997). Recently, a series of 4,5,6,7-tetrahydroisoxazolo(4,5-*c*)pyridin-3-ol (THPO) derivatives (a rigid GABA analog, Fig. 1) were shown to selectively inhibit mGAT-1 (Bolvig et al., 1999; Falch et al., 1999). These compounds may serve as structural leads for new competitive GAT-1 inhibitors and help to elucidate the differences between competitive and noncompetitive inhibition (Roepstorff and Lambert, 1994; Bernstein and Quick, 1999).

Apart from direct inhibition of GABA translocation, GAT-1-mediated transport is modulated by activation of protein kinase C, causing a reduction in the number of GAT-1 transporters in the cell membrane which is presumably mediated by GAT-1 interaction with the vesicle exocytosis apparatus (reviewed by Beckman and Quick, 1998). In contrast, BDNF-mediated phosphorylation of tyrosine in GAT-1 increases the number of transporters in the cell membrane (Law et al., 2000). Finally, GABA transport is increased in hippocampal cultures after incubation with gabapentin (Whitworth and Quick, 2001) and valproate administration to control rats has been shown to decrease GAT-1 in the hippocampus (Ueda and Willmore, 2000). These latter two examples of GABA transporter modulation are probably not relevant for the pharmacology described above, but have been included here to stress the fact that

transport of GABA is indeed subject to regulation in a multitude of ways.

GAT-1, GAT-2, GAT-3 and BGT-1 have different affinities for GABA. Except for mGAT-1 and mGAT-3, the affinity for GABA is largely independent on expression system, *Xenopus* oocytes or baby hamster kidney (BHK) cells, and is in decreasing order of affinity mGAT-3 ≤ mGAT-1 = mGAT-2 ≪ mBGT-1. An apparent K_m for GABA close to 100 μM was calculated for mBGT-1, two orders of magnitude higher than that of mGAT-1 to mGAT-3 (0.8–12 μM) (Liu et al., 1993; Thomsen et al., 1997). Given that BGT-1 exhibits such a low affinity for GABA, its effectivity at regulating GABA levels in the brain are dubious. Although it could be located at synapses where GABA concentrations are high enough to be transported by BGT-1, anatomical data may argue against such a location.

4. Localization of GABA transporters

Plasma membrane localized GATs are heterogeneously localized in different brain regions and no two transporters exhibit overall identical distribution.

4.1. BGT-1 and GAT-2

BGT-1 protein has been described in mice, humans, dog and torpedo but only an mRNA transcript has been identified in rat liver (Table 1, Burnham et al., 1996/Genbank entry). In mice, the message for BGT-1 is expressed in kidneys, cerebellum, cerebral cortex, brain stem and a smaller transcript in liver (Lopez-Corcuera et al., 1992). Likewise, Northern blots of human brain RNA for BGT-1 show expression of a 3.1-kb transcript in approximate equal amounts in the amygdala, caudate nucleus, corpus callosum, hippocampus, hypothalamus, substantia nigra, subthalamic nucleus and thalamus (Borden et al., 1995). However, in the dog, cBGT-1-mRNA is not expressed in the brain or liver, whereas levels are high in the kidney (Yamauchi et al., 1992). These findings indicate that BGT-1 is differentially expressed in various tissues over species. At least in brains from human and mice, BGT-1 appears uniformly distributed but a detailed histological analysis of localization in the brain of these (or other) species has hitherto not been carried out.

In the adult mouse, GAT-2-mRNA is highly expressed in the liver and kidney but in the brain exclusively in the leptomeningeal cells constituting the pia and arachnoid meninges (Liu et al., 1993). In adult rat brain, GAT-2 immunoreactivity is seen in the arachnoidea meninges and the ependymal cells lining the ventricles (Ikegaki et al., 1994).

4.2. GAT-1 and GAT-3

GAT-1 and GAT-3 are the only two GABA transporters exclusively expressed in the brain. GAT-1 protein and its message are widely distributed in the rat brain. Intense to moderate in situ labeling of GAT-1-mRNA is observed in cells in the retina, entire limbic system, basal forebrain, the entire rostrocaudal extent of the cortex, in particular layers IV–V, and in the cerebellar cortex. Moderate labelling is observed in several nuclei of the thalamus and hypothalamus, interpeduncular nuclei, inferior colliculus, substantia nigra and striatum whereas the pons and medulla are less intense labelled (Liu et al., 1993; Swan et al., 1994; Ikegaki et al., 1994; Minelli et al., 1995; Durkin et al., 1995). These findings are consistent with light microscopy studies of GAT-1 immunoreactivity (Radian et al., 1990; Ikegaki et al., 1994; Minelli et al., 1995). In comparison, GAT-3 mRNA and protein is expressed predominantly in the thalamus, hypothalamus, a minor part of the pyriform cortex (amygdala region), inferior colliculus, pons, brainstem and deep cerebellar nuclei and at much lower levels in cerebral cortex, basal forebrain, striatum, hippocampus and cerebellar cortex (Ikegaki et al., 1994). Although in situ hybridisation studies display GAT-1- and GAT-3-mRNA in glia and GAT-1-mRNA in interneurons, ultrastructural analyses are needed to determine the localization of GATs at the level of the synapse to understand its relation to the physiology of GABA transport. In the cerebral cortex of the rat, GAT-1 is expressed in astrocyte processes and in nerve terminals forming symmetric synapses (i.e. GABAergic) with soma and dendrites of pyramidal and granule like (layer IV) cells, whereas asymmetric terminals and postsynaptic localizations (soma, dendrite) were unlabelled (Minelli et al., 1995). In contrast, GAT-3 protein in the cortex was exclusively found in astrocytic processes in the vicinity of both symmetric and asymmetric synaptic profiles (Minelli et al., 1996). Localization of GAT-1 in the hippocampus (Ribak et al., 1996b), superior colliculus (Yan and Ribak, 1998b) and cerebellum (Morara et al., 1996; Ribak et al., 1996a) is largely similar to its cortical localization. In the thalamus, however, employing the same antibody as described for studies in the cortex, GAT-1 is restricted to the distal end of astrocytic processes and is found juxta-positioned both symmetric and asymmetric synapses (De Biasi et al., 1998). A number of studies have noted the existence of subpopulations of glutamate amino decarboxylase-negative principal neurons expressing the message for GAT-1-mRNA, indicating the possibility for a postsynaptic localized GAT-1 (Snow et al., 1992; Minelli et al., 1995; Frahm et al., 2000). In the hippocampus, thalamus, superior colliculus and cerebellum, GAT-3 is exclusively localized at proximal loci in astrocytic processes, nondiscriminately surrounding both symmetric and asymmetric synapses (Morara et al., 1996; Ribak et al., 1996b; De Biasi et al., 1998; Yan and Ribak, 1998a,b). Thus, purkinje cells and thalamic GABAergic neurons appear without a high-affinity GABA uptake system.

The almost exclusive expression of GAT-2 in the brain meninges of the adult individual quite certainly rules out any role this transporter could play at regulating GABA in

and outside intracerebral synapses. Examination of the subcellular localization of GABA transporters determined by their C-terminal signal-sequence in cultured hippocampal neurons and Mardin–Darby canine kidney (MDCK) cells revealed that GAT-1 was sorted to the axon, GAT-3 to the axon and cell body, whereas BGT-1 was sorted exclusively to the cell body and dendritic membrane (Ahn et al., 1996; Muth et al., 1998). A dendritic localization of BGT-1 in neurons is not consistent with it mediating a synaptic uptake mechanism for GABA unless BGT-1 is expressed at the postsynaptic loci, which remains to be elucidated. At present, the two most likely candidates for regulating ambient levels of GABA in the brain are GAT-1 and GAT-3.

5. Physiology of GABA transport

GABA transport is electrogenic with a stoichiometry for Na^+:Cl^-:GABA transport of 2:1:1 (all inwards) (Radian et al., 1986; Guastella et al., 1990; Kavanaugh et al., 1992; Clark and Amara, 1994; Cammack et al., 1994) although the stoichiometry for BGT-1 expressed in *Xenopus* oocytes may be 3:2–1:1 for Na^+:Cl^-:GABA (Matskevitch et al., 1999). Furthermore, when GAT-1 is expressed in HEK293 cells, a small leak current mediated by the transporter is present in the absence of GABA, demonstrating that the transporter in these cells can act like a channel (Cammack et al., 1994). Given the stoichiometry of GABA transport and provided a depolarized membrane potential and sufficient intracellular concentration of Na^+, Cl^- and GABA, it should be possible for it to reverse (Attwell et al., 1993; Cammack et al., 1994). This is a phenomenon of some interest as it relate to the source of ambient levels of GABA in the brain, an as yet unresolved issue (below).

The rise and decay of GABA in the synaptic cleft during synaptic activity is determined by several factors, a few of which are geometry of the synapse and extracellular space, variation in the number of vesicles released, diffusion and rate of uptake (Clements, 1996; Nusser et al., 2001). A cautious estimate of the peak concentration of GABA in the synaptic cleft is 0.5–1 mM based on patch responses to different concentrations of GABA (Maconochie et al., 1994; Jones and Westbrook, 1995). The extracellular level of GABA has been calculated to be 0.4 μM at steady state and 3.1 μM at peak by employing a model of an astrocytic GABA transporter operating at a membrane potential of −80 mV (steady state) or −20 mV (peak, glutamate depolarization) at 37 °C (a number of assumptions apply, see (Attwell et al., 1993). These extracellular levels of GABA are well within the range to exert a tonic activation of extrasynaptic α_6- or δ-subunit containing $GABA_A$ receptors (Saxena and Macdonald, 1996) as has been demonstrated by a bicuculline-mediated reduction of an outward current at negative holding potentials in hippocampal or cerebellar granule cells (Otis et al., 1991; Brickley et al., 1996; Wall and Usowicz, 1997; Nusser et al., 2001). GABA-evoked inhibitory postsynaptic currents (IPSCs) are multisynaptic in origin and cause a larger release and eventually synaptic GABA over spill as compared to single-synaptic spontaneous or mIPSCs. This overspill is reflected in the late decay phase of an evoked IPSC and is significantly increased by the application of nonsubstrate GABA uptake inhibitors (Roepstorff and Lambert, 1992, 1994; Thompson and Gahwiler, 1992; Isaacson et al., 1993; Overstreet and Westbrook, 2003). However, spontaneous IPSCs or mini-IPSCs are much less or not at all affected by application of NNC 05-0711 (Nusser and Mody, 2002; Overstreet and Westbrook, 2003) whereas the tonic inhibition is selectively increased as shown by a decrease in the holding current in dentate gyrus granule cells (Nusser and Mody, 2002). Given the regional heterogeneity of GABA transporter localization (above), it is very likely that the effect mediated by subtype-specific GABA uptake inhibitors on IPSCs, excitatory postsynaptic currents (EPSCs) or the discriminative effects on phasic and tonic inhibitions are region-, cell- and synapse-specific. For example, the slow-decaying IPSCs in hippocampus stratum oriens, initially described by Pearce (1993), are likely due to the lower expression of GAT-1 in stratum oriens interneurons than stratum radiatum and ensuing differential effect of tiagabine on evoked IPSCs in these same lamina (Engel et al., 1998). A more direct example on the effect of a tonic conductance and possible role of GABA uptake was recently provided by Semyanov et al. (2003). Whereas hippocampal granule cells (Otis et al., 1991) and interneurons in the CA1 stratum radiatum are under the influence of a GABA-mediated tonic conductance, CA1 stratum oriens interneurons and pyramidal cells are much less so (Semyanov et al., 2003). In these cells, a higher tonic conductance could be evoked by NNC 05-0711 and subsequently shown to be composed of a picrotoxin-sensitive and SR95531 (0.5 μM)-insensitive current. By selectively blocking the tonic but not the phasic conductance in stratum radiatum interneurons (1 μM picrotoxin), the IPSC frequency onto pyramidal cells was increased. Such data demonstrate that the tonic conductance on specific cell popoulations must depend on these cells expression of extrasynaptic GABA receptors, and also (in this case) that GAT inhibitors can alter the level of disinhibition in specific cellular circuits. The presence of pre- and/or post-synaptic $GABA_B$ receptors at both inhibitory and excitatory synapses (Charles et al., 2001; Kulik et al., 2002) also affects crosstalk mediated by GABA between adjacent synapses (Solis and Nicoll, 1992a,b). For example, the GABA release by synchronized interneurons during hippocampal theta rhythmic activity is sufficient to activate $GABA_B$ receptors as demonstrated by its modulation by tiagabine (decreasing frequency) in a CGP62349-dependent manner (Scanziani, 2000). Although the effects in this preparation were shown to rely on postsynaptic $GABA_B$ receptors, the presence of pre- and post-synaptic

$GABA_B$ receptors have been demonstrated in excitatory synapses of the CA3 (Lei and McBain, 2003).

The source of the ambient levels of GABA in the cerebrospinal fluid is as yet not clear. Perfusion of tetrodotoxin through a microdialysis probe has no effects on measured GABA levels in wake or anaesthetized rats (Timmerman et al., 1992; Britton et al., 1996) and it does not reduce the tonic activation in hippocampal granule and cerebellar golgi and granule cells (Otis et al., 1991; Wall and Usowicz, 1997; Rossi et al., 2003). The Ca-independent release of GABA that can be blocked by GABA uptake inhibitors in retinal cells (Schwartz, 1987), cerebral neurons (Belhage et al., 1993) and Bergman glia in the cerebellum (Barakat and Bordey, 2002) is possibly due to a reversed GABA transport (caused by high cytosolic Na^+), suggesting this mechanism as a source of extracellular GABA. However, in all cases where assessed, the net result of uptake inhibition by GAT-1 results in an increased tonic inhibition (see above), disclosing a role for these transporters as a source of GABA sufficient to induce a tonic conductance.

Very little work has been done to study the role of GAT-3 in synaptic transmission, mainly due to the lack of proper pharmacological tools. Given the macroscopically more restricted expression of GAT-3 than GAT-1 in the brain, it appears unlikely that major effects of GAT-3 inhibitors should be seen in the cortex, hippocampus or cerebellar cortex, as has been shown with synaptosome assays for the nonselective GAT-3 inhibitors in Fig. 1 (Dalby et al., 1997).

6. The role of GABA uptake in epilepsy

Much of the above is concerned about hippocampal expression of GATs and effect of, in particular, GAT-1 inhibitors. Now moving to a field where we do have limited information as to the effects of non-GAT-1 inhibitors, primarily in animal models of epilepsy and isolated seizures.

Clonic convulsions induced by electrical stimulations in typically limbic areas (kindling) and isolated generalized tonic convulsive seizures induced by electroshock are dependent on different areas in the brain for their expression. Limbic kindled seizures are focal in origin which drive a secondary generalization causing characteristically clonic convulsions with rearing (Goddard et al., 1969; Racine, 1972), a behaviour associated with forebrain structures (Browning and Nelson, 1986; Mirski and Ferrendelli, 1986). Isolated electroshock is a model of primary generalized seizures and the typical response is a tonic convulsion with full extension of hind legs whose expression are dependent on basal ganglia input to brainstem areas (Browning and Nelson, 1986; Redgrave et al., 1992a,b). GABA uptake inhibitors acting at different transporters do not display similar efficacy against these types of seizures. Specifically, GAT-1 inhibitors are effective against the kindled focal and secondary generalized seizures and clonic convulsions induced by chemoconvulsants but have no effect against tonic convulsions induced by electroshock (Nielsen et al., 1991; Swinyard et al., 1991; Dalby and Nielsen, 1997). Contrary, nonspecific GAT inhibitors are very effective against electroshock-induced convulsions but has no effect against clonic convulsions induced by chemoconvulsants, and their effect against the focal seizures in kindled rats appear to be due to ratio of GAT-1/GAT-3 affinity (Croucher et al., 1983; Nielsen et al., 1991; Swinyard et al., 1991; Suzdak et al., 1992; Morimoto et al., 1993; Dalby and Nielsen, 1997; Dalby et al., 1997). Table 2 gives ED_{50} values of four GABA uptake inhibitors against amygdala kindled seizures and electroshock in mice. There appear to be some relation between the GAT-1/GAT-3 ratio and the effect of these drugs against the focal component of kindled seizures (Table 3).

Table 2

	Kindling ED_{50}, μmol/kg		MES ED_{50}, μmol/kg
	Focal (AD)	Generalized (behav.)	Tonic
Tiagabine	36	6	>73
NNC 05-2045	>242	>242	30
NNC 05-2090	>242	222	73
NNC 05-2101	>251	205	79
SNAP-5114	NT	NT	>198

ED_{50} values for inhibiting kindled focal seizures (behavioral grades 1 and 2) or generalized seizures (behavioral grades 3–5) and maximal electroshock (MES). All compounds tested had significant effect in reducing afterdischarge duration at two highest doses tested. NT, not tested.

Table 3

	Cerebral cortex (GAT-1 like)	Inferior colliculus (GAT-3 like)
Tiagabine	54 ± 9 nM	>100 nM
NNC 05-2045	12 ± 2 μM	1.0 ± 0.1 μM
NNC 05-2090	4.4 ± 0.8 μM	2.5 ± 0.7 μM
NNC 05-2101	13.5 ± 3 μM	1.5 ± 0.2 μM
SNAP-5114	>30 μM	0.8 ± 0.1 μM

IC_{50} values for inhibition of [3H]GABA uptake into synaptosomes prepared from rat cerebral cortex and inferior colliculus. Measurements from inferior colliculus were performed in the presence of 1.0 μM NNC 05-0711.

Given the heterogeneous expression of GAT-1 and GAT-3 in limbic (high GAT-1/low GAT-3) and brainstem areas (medium GAT-1/high GAT-3), it is likely that such different effects in vivo can be explained on the basis of a different site of action in the brain. Microdialysis experiments with tiagabine and SNAP-5114 perfused through the probe display regional heterogeneity of the effect of selective GAT inhibitors that parallel the localization of GAT-1 and GAT-3. The relatively nonspecific NNC 05-2045 has similar effect in both regions (Fig. 2). Note also that the maximal effect of tiagabine is higher in the hippocampus than the thalamus. While there could be many reasons for this result, it should be stressed that regionally co-localized GABA transporters could act like connected sinks and thus limiting the effect of selective blockade of one GABA uptake site. However, SNAP-5114,

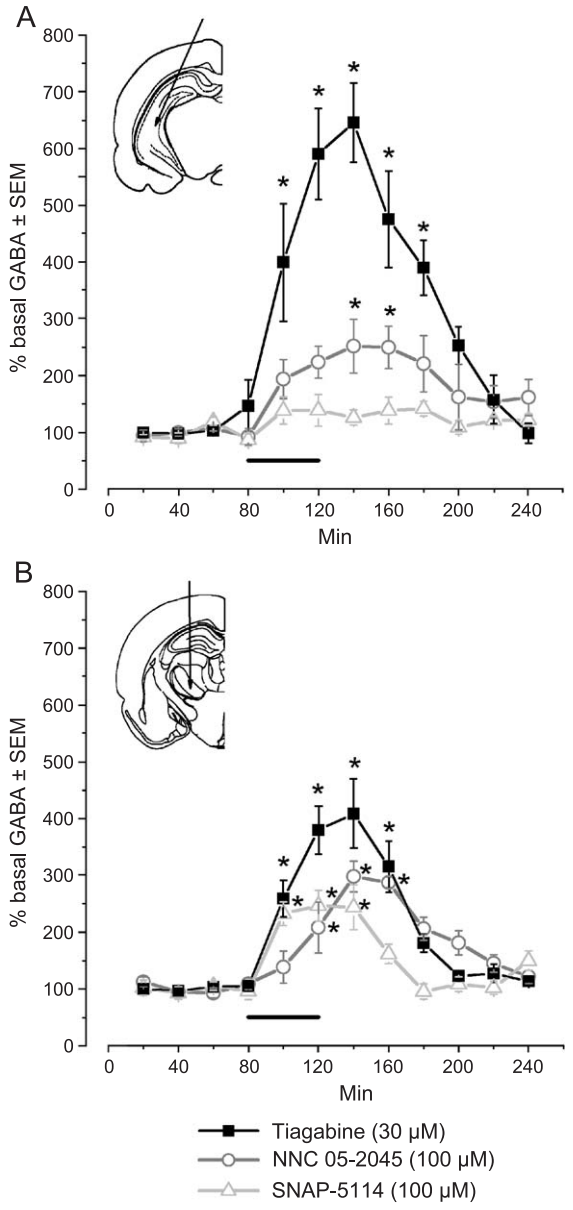

Fig. 2. Microdialysis in anesthetized rats with probe inserted in the hippocampus or thalamus (insert). Tiagabine (30 μM) and NNC 05-2045 (100 μM) significantly (ANOVA, $*P<0.05$) increase GABA levels in hippocampus and thalamus, whereas SNAP-5114 (100 μM) only has effect in the thalamus. Black bars indicate the period of drug perfusion through the microdialysis probe.

which is the only commercially available semi-selective GAT-3 inhibitor, is virtually without effect in animal models tested except for a weak effect against audiogenic seizures in DBA-2 mice. Since the compound block transport in synaptosomes has effect in microdialysis (Fig. 2), and block electroshock-induced convulsions when administered in the substantia nigra (Dalby, 2000), the poor effect this compound display in animal models when administered intraperitoneal is most likely due to a poor pharmacokinetic profile or alternatively does not pass the blood–brain barrier. It is possible that a transport inhibitor with a mixed affinity to GAT-1/GAT-3 would display effect in a broader number of animal models, but whether such a compound has an improved adverse-effect profile as compared to the selective GAT-1 inhibitor tiagabine is dubious.

Several reports have demonstrated that the uptake of GABA mediated by GAT-1 is compromised in the hippocampus of epileptic brains. The GABA release induced by glutamate in a Ca-free medium perfused through a microdialysis probe in the focus of epileptic patients or amygdala kindled rats is decreased compared to control subjects and control animals (During et al., 1995). The most likely explanation provided at present is the decrease in some hippocampal regions of the expression of GAT-1 in epileptic patients and in some animal models of spontaneous seizures (Mathern et al., 1999; Kang et al., 2001; Patrylo et al., 2001 but see also Andre et al., 2001). However, although the basal levels of GABA in amygdala kindled rats measured by microdialysis were increased twofold from 0.74 to 1.58 μM (Cleton et al., 2000), no difference in the effect of tiagabine in vitro could be measured. Also, when measuring the $GABA_A$ receptor Cl^-/HCO_3^- conductance in hippocampal granule and CA1 pyramidal cells at a 10 μM GABA extracellular concentration (thus measuring the effect on predominately extrasynaptic receptors), no difference between the effect of tiagabine in control or pilocarpine epileptic rats was observed (Frahm et al., 2003). Thus, although the latter experiment suggests there is no net difference in the effect of tiagabine in control and epileptic animals in its ability to secondarily activate extrasynaptic GABA receptors, the endstage epileptic condition seems to be characterized by a locally reduced level of GAT-1 and ability of the transporter to reverse. However, the pathophysiology of GABAergic transmission in the epileptic brain is extremely complex and numerous changes in different cell types occur at different stages of epilepsy. The role of each component can in several cases be viewed as being either "protective towards" or "the cause" of increased excitatory activity. With the dissection of regional differences in GABA uptake and a detailed knowledge on the localization of GABA receptors with a specific subtype composition and pharmacology, we now have a better understanding of the very different effects that GABA agonists, modulators and uptake inhibitors can have in vivo. While inhibition of uptake by tiagabine has a strong net anticonvulsant effect, the widespread increase in GABA levels by tiagabine can also have other effects, some of which most likely do not contribute to its anticonvulsant effects. For example, a local collapse of the Cl^- gradient can occur during high frequency stimulation in the CA1 and cause the HCO_3^- conductance to dominate and lead to a major dendritic GABA-induced depolarization, an effect which is strengthened by GABA uptake inhibitors (Staley et al., 1995; Jackson et al., 1999). Also, an increased tonic conductance by GABA uptake inhibition can lead to disinhibition of certain interneurons causing a diminished inhib-

itory drive to principal cells (Semyanov et al., 2003). Given that tiagabine despite good effect against human mesial temporal lobe epilepsy (Schachter, 2001) in a few cases also has proconvulsant effects (Ostrovskiy et al., 2002; Kellinghaus et al., 2002) underscores both the complexity of GABAergic neurotransmission in epilepsy and the diversity among individual patients diagnosed with mesial temporal lobe epilepsy. Not related to its anticonvulsant effects, tiagabine has also been shown to mimic the action of 4,5,6,7-tetrahydroisothiazolo-[5,4-*c*]pyridin-3-ol (THIP), an agonist at $\alpha_4\beta_3\delta$ and benzodiazepine insensitive $GABA_A$ receptors (Brown et al., 2002; Storustovu and Ebert, 2003). While THIP is a poor anticonvulsant (Holland et al., 1992), it has, like tiagabine, pronounced effects in promoting slow-wave sleep (Lancel et al., 1998; Mathias et al., 2001a; Mathias et al., 2001b), by a mechanism distinguishable from that exerted by benzodiazepines. Given the increase in tonic conductance through extrasynaptic GABA receptors mediated by NNC 05-0711 at hippocampal synapses, it is possible that an increase in tonic conductance onto extrasynaptic $\alpha_4\beta_3\delta$ $GABA_A$ receptors in other regions (sleep-related) by tiagabine underlies the similar effects of tiagabine and THIP on sleep.

Thus, GABA uptake inhibition has several effects on CNS disorders owing to a more or less global action. It is conceivable that nonselective GABA uptake inhibitors could be designed with improved or at least different efficacy against either epilepsy or sleep by variation of affinity ratio at GABA transporters as compared to selective inhibitors. However, the degrees of freedom of such a selection are determined by the regional expression of GATs and may appear low when compared to the possibilities for (specific) pharmacological action at the extrasynaptic $GABA_A$ receptors of different subunit compositions.

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The European patent EP 823905 A1 cover the non-selective GABA uptake inhibitors NNC05-2045, NNC 05-2090 and NNC 05-2101 and is currently owned by British Technology Group (BTG), based in London, England.

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