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Interactions of taurine and structurally related analogues with the GABAergic system and taurine binding sites of rabbit brain

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1 The aim of this study was to find taurinergic compounds that do not interact with brain GABA ergic systems.

2 Washed synaptic membranes (SM) from whole rabbit brain were able to bind [^{3}H]muscimol. Saturation experiments of the binding of [^{3}H]GABA to $GABA_B$ receptors showed that SM possess two binding components; twice Triton X-100-treated SM contained 0.048 mmol endogenous taurine/kg protein and bound [^{3}H]taurine in a saturable manner ($K_d = 249.0 \pm 6.3$ nM and $B_{max} = 3.4 \pm 1.0$ pmol mg^{-1} prot).

3 Among the 19 structural analogues of taurine, 6-aminomethyl-3-methyl-4H-1,2,4-benzothiadiazine 1,1-dioxide (TAG), 2-aminoethylarsonic (AEA), 2-hydroxyethanesulfonic (ISE) and (±)*cis*-2-aminocyclohexane sulfonic acids (CAHS) displaced [^{3}H]taurine binding ($K_i = 0.13$, 0.13, 13.5 and 4.0 μM, respectively). These analogues did not interact with $GABA_A$ and $GABA_B$ receptors and did not affect taurine- and GABA-uptake systems and GABA-transaminase activity.

4 3-Aminopropanesulfonic acid (OMO), β-alanine, pyridine-3-sulfonic acid, *N*,*N*,*N*-trimethyltaurine (TMT), 2-(guanidino)ethanesulfonic acid (GES), ethanolamine-*O*-sulphate, *N*,*N*-dimethyltaurine (DMT), taurine and (±)piperidine-3-sulfonic acid (PSA) inhibited [^{3}H]muscimol binding to $GABA_A$ receptors with different affinities ($K_i = 0.013$, 7.9, 24.6, 47.5, 52.0, 91.0, 47.5, 118.1 and 166.3 μM, respectively). Taurine, 2-aminoethylphosphonic acid, DMT, TMT and OMO inhibited the binding of [^{3}H]GABA to $GABA_B$ receptors with K_i's in the μM range (0.8, 3.5, 4.4, 11.3 and 5.0, respectively). GES inhibited taurine uptake ($IC_{50} = 3.72$ μM) and PSA GABA transaminase activity ($IC_{50} = 103.0$ μM).

5 In conclusion, AEA, TAG, ISE and CAHS fulfill the criteria for taurinergic agents.
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Keywords: Taurine; GABA; taurine derivatives; GABA receptors; taurine binding site

Abbreviations: ACES, *N*-(carbamoylmethyl)-2-aminoethanesulfonic acid; AEA, 2-aminoethylarsonic acid; AEP, 2-aminoethanephosphonic acid; β-ALA, β-alanine; AMS, aminomethanesulfonic acid; ANSA, 2-aminobenzenesulfonate; CAHS, (±)*cis*-2-aminocyclohexane sulfonic acid; DMT, *N*,*N*-dimethyltaurine; EOS, ethanolamine *O*-sulfate; GES, 2-(guanidino)ethanesulfonic acid; GLY, glycine; ISE, 2-hydroxyethanesulfonic acid; MMT, *N*-methyltaurine; OMO, 3-aminopropanesulfonic acid, homotaurine; PIPES, piperazine-*N*,*N*′-bis-(2-ethanesulfonic acid); PSA, (±)piperidine-3-sulfonic acid; PYR, pyridine-3-sulfonic acid; TAG, 6-aminomethyl-3-methyl-*4*H-1,2,4-benzothiadiazine 1,1-dioxide; TAHS, (±)*trans*-2-aminocyclohexane sulfonic acid; TAU, 2-aminoethanesulfonic acid, taurine; TMT, *N*,*N*,*N*-trimethyltaurine.

Introduction

Taurine and GABA are recognized as major inhibitory amino acids distributed in large quantities in various areas of the central nervous system (CNS) (Barbeau *et al*., 1975; Yakimova, 1996). The role of GABA as an inhibitory neurotransmitter has been well established, whereas that of taurine is still under investigation. In the case of GABA, the structure and function of three types of GABA receptors, namely $GABA_A$, $GABA_B$ and $GABA_C$, have so far been identified, while the taurine receptor is not yet defined. Some very convincing evidence has outlined functional inter-relations between taurine and GABA in the brain at transporters and receptors of specific neuronal networks. From this it appears that GABA plays a major role as an inhibitory transmitter while taurine acts as a modulator of GABAergic function (Kuriyama & Hashimoto, 1998).

Since taurine binds both to $GABA_A$ and $GABA_B$ receptors (Krogsgaard-Larsen *et al*., 1980; Kontro & Oja, 1990), it has been suggested that it affects nervous functions by interacting with GABAergic systems. However, it appears that taurine possesses many actions distinct from those of GABA, such as

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growth promotion and enhancing the survival of neuronal cells (Hayes *et al*., 1975), modulation of calcium fluxes (Huxtable, 1989), transmitter release (Kamisaki *et al*., 1993) and regulation of osmolality in the mammalian brain (Hussy *et al*., 2001; Tuz *et al*., 2001). In particular, taurine is able to inhibit the depolarization-evoked release of aspartate and glutamate through a taurine-specific site(s) but not through GABA receptors (Kamisaki *et al*., 1993). Thus, it is therefore possible that taurine may exert its biologic activity by interacting with a specific taurine receptor. Wu *et al.* (1987) and Kontro & Oja (1987a,b) looked for specific synaptic receptor sites for taurine and described a sodium-independent taurine binding to synaptic membranes: this could reflect binding by postsynaptic receptor sites.

In the present investigation, we studied 19 taurine analogues to see if they interact with (1) $GABA_A$ and $GABA_B$ receptors, (2) uptake systems for GABA and taurine, (3) taurine binding site(s), and (4) 4-aminobutyrate transaminase (i.e. GABA-transaminase, EC 2.6.1.19) activity. In the course of this study, the K_d and B_{max} of the binding of different radioligands to $GABA_A$, $GABA_B$ and taurine binding sites or the K_m and V_{max} values relative to GABA and taurine uptake systems in the rabbit brain, with the use of different preparations obtained from whole brains, have been systematically characterized. 2-Aminoethylarsonic acid (AEA), 6-aminomethyl-3-methyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (TAG), 2-hydroxyethanesulfonic acid (ISE) and (±)*cis*-2-aminocyclohexane sulfonic acid (CAHS) fulfill the criteria of taurinergic agents by displacing [^{3}H]taurine binding without interacting with the GABAergic system. Thus, they can represent useful probes to investigate the role of taurine in the CNS.

Methods

Materials

Taurine derivatives were selected by modifying the sulfo and the amino group of taurine, by changing the carbon chain length and by considering more restricted analogues such as the cyclic derivatives. The structures and sources of the 20 compounds used in the present study are reported in Table 1.

[^{3}H]muscimol (specific activity 19.1 Ci mmol^{-1}), [^{3}H]GABA (specific activity 40.0 Ci mmol^{-1}) and [^{3}H]taurine (specific activity 24.1 Ci mmol^{-1}) were purchased from NEN™ Life Science Products, Inc. (Boston, U.S.A). All other materials were from standard local sources and of the highest grade commercially available.

Synthesis of AEA, DMT, TMT, PSA, CAHS, TAHS and GES

AEA was prepared by periodate oxidation of 2-[(2-hydroxyethyl)amino]ethylarsonic acid, itself made by treating 2-chloroethylarsonic acid with ethanolamine (Geoghegan & Dixon, 1989). (*N*,*N*-dimethyltaurine) (DMT) was prepared by methylation of taurine as described by Clarke *et al*. (1933). (*N*,*N*,*N*-trimethyltaurine) (TMT) was prepared starting from DMT, which was methylated with iodomethane in the presence of a hindered base (tributylamine) (Barnhurst, 1961). (±)Piperidine-3-sulfonic acid (PSA) was prepared by catalytic hydrogenation (nickel as a catalyst) of sodium pyridine-3-sulfonate as reported by Freifelder & Wright (1964). CAHS was prepared from 2-aminobenzenesulfonic acid by catalytic hydrogenation as reported by Egli & Eugster (1975). (±)*trans*-2-Aminocyclohexane sulfonic acid (TAHS) was prepared from cyclohexene by sulfur monochloride addition, followed by oxidation to 2-chlorosulfonic acid and substitution of chlorine as reported by Machetti *et al*. (2000). 2-(Guanidino)ethanesulfonic acid (GES) was prepared by the treatment of taurine with methyl thioisourea as reported by Fujii & Cook (1975). TAG, synthesized as described by Girard *et al*. (1982), was a generous gift of Dr G.G. Yarbrough from Merk Frosst Laboratories (Quebec, Canada). The purity of all compounds was evaluated above 95% by ^{1}H NMR or high-performance liquid chromatography (HPLC).

Receptor binding assays

$GABA_A$ receptors

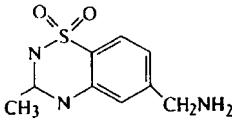
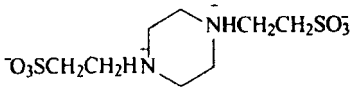
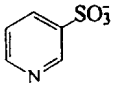
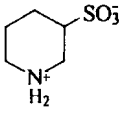
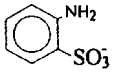
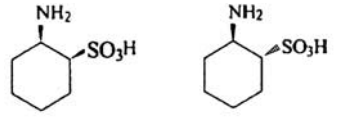
Rabbit-brain membranes were prepared and assayed for $GABA_A$ receptor binding by using the method described by Watabe *et al.* (1993) with slight modifications. The whole brain was homogenized in 10 vol. of cold 0.32 M sucrose and the homogenate was centrifuged at $2500 \times g$ for 10 min. The supernatant was then centrifuged at $48{,}000 \times g$ for 30 min and the pellet (crude synaptic membranes, CSM) was resuspended in Tris-HCl 50 mM pH 7.4 and frozen for 24 h. Thawed CSM were then resuspended in an appropriate volume of Tris-HCl containing Triton X-100 (0.05% w v^{-1}) in order to obtain a final protein concentration of 1 mg ml^{-1}. The mixture was incubated for 30 min at 37°C and then centrifuged at $48{,}000 \times g$ for 20 min. The pellet (washed synaptic membranes, WSM) was then washed three times with buffer and frozen at −20°C before use. For saturation experiments, samples containing increasing concentrations of [^{3}H]muscimol (1×10^{-9}–2.5×10^{-8} M) were added to 100 μg of WSM (final volume = 1 ml) and incubated for 30 min at 0°C. The incubation was followed by rapid filtration on Whatman GF/B glass fiber filters which were washed three times with 2 ml of cold buffer. 5×10^{-5} M GABA was used to assess nonspecific binding.

For displacement experiments, a fixed concentration of labelled muscimol (1×10^{-8} M) was incubated, as described above, with increasing concentrations of taurine or its derivatives (1×10^{-10}–1×10^{-2} M).

$GABA_B$ receptors

Radioligand binding of [^{3}H]GABA to CSM from rabbit brain was performed as described by Hill & Bowery (1981). Briefly, a crude mitochondrial fraction (P2) enriched in synaptic membranes was prepared from the whole brain of rabbit according to the method of Gray & Whittaker (1962). The P2 fraction was collected by centrifugation at $20{,}000 \times g$ for 20 min and subjected to hypotonic shock by rehomogenization in water. The mixture was then recentrifuged for 20 min at $8000 \times g$ and the supernatant was used to gently rinse the upper layer of the pellet. The combined suspension was recentrifuged for 20 min at $20{,}000 \times g$ and washed twice by homogenization and centrifugation and then stored frozen at −18°C until use. Saturation and displacement studies were performed on thawed membranes resuspended in Tris-HCl (50 mM, pH 7.4) + $CaCl_2$ (2.5 mM) (Tris-Ca) and incubated for 45 min at 20°C before centrifugation at $7000 \times g$ for 10 min. This washing procedure was repeated three times allowing 15 min of incubation to remove endogenous GABA and other possible inhibitory

Table 1 Compounds used in the present study

		Compound (abbreviation)	*Structure*	*Origin*
Group modified		2-Aminoethanesulfonic acid, taurine (TAU)	$^{+}NH_3–CH_2–CH_2–SO_3^{-}$	Merck
		6-Aminomethyl-3-methyl-4*H*-1,2,4-benzothiadiazine-1, 1-dioxide (TAG)		Gift
$–SO_3^{-}$		2-Aminoethane phosphonic acid (AEP)	$^{+}NH_3–CH_2–CH_2–PO_3H^{-}$	Sigma
		2-Aminoethylarsonic acid (AEA)	$^{+}NH_3–CH_2–CH_2–AsO_3H^{-}$	Synth.
		β-Alanine (βALA)	$^{+}NH_3–CH_2–CH_2–COO^{-}$	Sigma
		Ethanolamine-*O*-sulphate (EOS)	$^{+}NH_3–CH_2–CH_2–O–SO_3^{-}$	Sigma
		N-methyltaurine (MMT)	$^{+}NH_2(CH_3)–CH_2–CH_2–SO_3^{-}$	Merck
		N,N-dimethyltaurine (DMT)	$^{+}NH(CH_3)_2–CH_2–CH_2–SO_3^{-}$	Synth.
$–NH_3^{+}$		*N,N,N*-trimethyltaurine (TMT)	$^{+}N(CH_3)_3–CH_2–CH_2–SO_3^{-}$	Synth
		2-(Guanidine)ethanesulfonic acid (GES)	$H_2N^{+}=C(NH)–NH–CH_2–CH_2–SO_3^{-}$	Synth
		2-Hydroxyethanesulfonic acid (ISE)	$HO–CH_2–CH_2–SO_3^{-}$	Sigma
		N-(Carbamoylmethyl)-2-aminoethane sulfonic acid (ACES)	$H_2N–CO–CH_2–NH_2^{+}–CH_2–CH_2–SO_3^{-}$	Sigma
Carbon chain length	1C	Aminomethansulfonic acid (AMS)	$^{+}NH_3–CH_2–SO_3^{-}$	Sigma
	3C	3-Aminopropane sulfonic acid, homotaurine (OMO)	$^{+}NH_3–CH_2–CH_2–CH_2–SO_3^{-}$	Sigma
Cyclic derivatives		Piperazine-*N,N'*-bis(2-ethane sulfonic acid) (PIPES)		Sigma
		Pyridine-3-sulfonic acid (PYR)		Aldrich
		(±)Piperidine-3-sulfonic acid (PSA)		Synth.
		2-Aminobenzenesulfonate (ANSA)		Aldrich
		2-Aminocyclohexane sulfonic acid	 (±) *cis isomer* (CAHS) (±) *trans isomer* (TAHS)	Synth.
Others		Glycine (GLY)	$+NH_3–CH_2–COO^{-}$	Sigma

substances. The final pellet (WSM) was resuspended in Tris-Ca for the assays. For saturation experiments, 900 μg of WSM (final volume = 0.8 ml) was incubated for 10 min at room temperature in Tris-Ca containing a fixed concentration of [^{3}H]GABA (2×10^{-8} M) + increasing concentrations of unlabelled GABA (5×10^{-8}–1×10^{-6} M) in the presence of 4×10^{-5} M isoguvacine to suppress any binding to $GABA_A$ sites. The incubation was terminated by a rapid filtration on Whatman GF/B glass fiber filters, which were washed three times with 2 ml of cold buffer. To study the displacement of radiolabelled GABA from $GABA_B$ receptors by taurine and its derivatives, a fixed concentration (2×10^{-8} M) of [^{3}H]GABA + increasing concentrations (1×10^{-8}–1×10^{-2} M) of the compounds were used. The assay was performed as described for saturation experiments.

Taurine binding sites

CSM, prepared as described for $GABA_A$ binding assay, were frozen, thawed after 3 days, incubated for 30 min at 37°C in Tris-HCl buffer (50 mM, pH 7.1) containing Triton X-100 (0.05%, v/v^{-1}), centrifuged for 10 min at 48,000 × *g*, washed with distilled water and frozen again. This treatment was repeated not earlier than 5 days later when the binding and inhibition assays were carried out (Kontro & Oja, 1987a). In the binding experiments, 400 μg of WSM was incubated for 10 min at 4°C in 400 μl final volume of 50 mM Tris-HCl buffer (pH 7.1) with increasing concentrations of [^{3}H]taurine (1×10^{-9}–2×10^{-8} M) or with a fixed amount of [^{3}H]taurine (2×10^{-8} M) + increasing concentrations of unlabelled taurine (1×10^{-7}–2×10^{-5} M). The incubation was terminated by filtration on Whatman GF/B glass fiber

filters, which were washed three times with 2 ml of cold buffer. When the effects of taurine derivatives on [^{3}H]taurine binding were tested, 6×10^{-8} M labelled taurine + increasing amounts (1×10^{-9}–1×10^{-3} M) of the compounds were used.

GABA and taurine uptake by crude synaptosomes

Uptake of [^{3}H]GABA by crude synaptosomal preparation from rabbit whole brain was assayed by the method described by Suzdak *et al.* (1992) with some modifications. The crude synaptosomes were prepared by homogenizing the brain in 20 vol of ice-cold 0.32 M sucrose. The homogenate was then centrifuged for 20 min (17,000 × *g* at 4°C) and the resulting pellet resuspended in 20 vol of Krebs buffer pH 7.1. 300 μg-aliquots of synaptosomal suspension (final volume 0.8 ml) were incubated for 10 min at 30°C with [^{3}H]GABA alone (1×10^{-9}–1×10^{-8}) or with [^{3}H]GABA (1×10^{-8} M) + increasing concentrations of unlabelled GABA (5×10^{-8}–1×10^{-4} M) to determine K_m and V_{max}. Synaptosomes were then recovered by rapid filtration through Whatman GF/B glass fiber filters under vacuum and the filters were washed three times with 2 ml cold buffer. To study the inhibition by taurine and its derivatives on carrier-mediated [^{3}H]GABA uptake, 1×10^{-8} M [^{3}H]GABA + increasing concentrations of the compounds (1×10^{-8}–1×10^{-3} M) were used. Noncarrier-mediated uptake was determined in the presence of nipecotic acid (5×10^{-4} M) and was subtracted from total binding to give carrier-mediated [^{3}H]GABA uptake.

To study the uptake of labelled taurine by rabbit whole-brain crude synaptosomes, the method of Hruska *et al.* (1978) was used. Briefly, the brain was homogenized in 9 vol of 0.32 M sucrose and then centrifuged at 1000 × *g* for 10 min. The supernatant was centrifuged again at 17,500 × *g* for 20 min. The pellet was resuspended in the original volume of sucrose. Samples of the tissue suspension (crude synaptosomal fraction) were used in subsequent experiments within 6 h. To determine K_m and V_{max} of [^{3}H]taurine uptake, 300 μg of crude synaptosomes was resuspended in Krebs' phosphate-buffered medium (final volume = 1 ml) containing a constant amount of [^{3}H]taurine (2×10^{-8} M) and increasing concentrations of non-labelled taurine (2×10^{-8}–1×10^{-2} M). The tubes were incubated for 10 min at 4°C. The reaction was stopped by rapid filtration on Whatman GF/B glass fiber filters, which were washed three times with 2 ml of cold buffer. The inhibition of labelled taurine uptake by taurine derivatives was studied by using a fixed amount of [^{3}H]taurine (2×10^{-8} M) + increasing concentrations of the derivatives (1×10^{-9}–1×10^{-2} M). Noncarrier-mediated uptake was determined in the presence of GES (1×10^{-3} M) and was subtracted from total binding to give carrier-mediated [^{3}H]taurine uptake.

GABA-transaminase activity

Rabbit brain was homogenized in 2 vol of cold distilled water as described by Qume & Fowler (1997) and stored at −20°C before use. The inhibition by either taurine or its derivatives of GABA-transaminase activity was studied by using the fluorimetric method of Salvador & Albers (1959).

Determination of taurine and GABA levels in WSM

WSM (3.2 mg) were solubilized with Triton X-100 (0.1%v.v^{-1}) and the resulting solution was analysed for taurine and GABA concentrations by reversed-phase HPLC with *o*-phthalaldehyde precolumn derivatization (Bianchi *et al.*, 1999).

Data analysis

All the experiments were performed in triplicate or quadruplicate. Saturable binding constants relative to the binding of [^{3}H]muscimol to $GABA_A$ receptors were determined by computer-assisted nonlinear regression analysis (GraphPad Prism 3.02, GraphPad Software Inc., San Diego, CA, USA), assuming binding to either a single site or a population of binding sites or two noninteracting populations and by choosing the best fit. When a mixture of hot/cold radioligand was used (i.e. for $GABA_B$ binding and [^{3}H]taurine binding assays and for GABA and taurine uptake), the analysis of data was performed by using LIGAND Program (Munson & Rodbard, 1980; Unnerstall, 1990).

When studying the ability of taurine derivatives to inhibit the binding of [^{3}H]muscimol, [^{3}H]GABA or [^{3}H]taurine to $GABA_A$, $GABA_B$ and taurine receptors, respectively, the half-maximal concentration (i.e. the IC_{50} value) for inhibition was obtained by plotting specific binding (percentage of control) vs the inhibitor concentration (M) and fitted with a nonlinear (sigmoidal) analysis (GraphPad Prism 3.02, GraphPad Software Inc., San Diego, CA, U.S.A). The K_i was calculated according to the method of Cheng & Prusoff (1973).

Results

K_d *and* B_{max} *of the binding of different radioligands to* $GABA_A$ *and* $GABA_B$ *receptors and taurine binding sites*

Whole rabbit-brain WSM were able to bind [^{3}H]muscimol with affinity constants (K_d) of 6.5 ± 0.4 nM and binding capacities (B_{max}) of 2.4 ± 0.5 pmol mg^{-1} prot., that is quite close to those reported for cow, pig, rat and mouse brain (Figure 1a). Saturation experiments of the binding of [^{3}H]GABA to $GABA_B$ receptors showed that rabbit-brain synaptic membranes possess two binding components with K_d values of 11.0 ± 0.8 nM (K_{d1}) and 1.6 ± 0.1 μM (K_{d2}), respectively, and B_{max} values of 0.2 ± 0.02 and 12.0 ± 0.8 pmol mg^{-1} prot., respectively, (Figure 1b). Moreover, twice Triton X-100-treated SM were shown to bind in a saturable manner [^{3}H]taurine with a K_d of 249.0 ± 6.3 nM and a B_{max} of 3.4 ± 1.0 pmol mg^{-1} prot. (Figure 1c). In this preparation, taurine amounted to 0.048 ± 0.026 mmol kg^{-1} prot. ($n = 3$), while GABA was not detectable.

K_m *and* V_{max} *values of GABA and taurine uptake systems*

The kinetics of [^{3}H]GABA uptake by crude synaptosomes of the rabbit whole brain indicated both a high- and a low-affinity uptake system, with relative K_m values differing by one order of magnitude ($K_{m1} = 1.6 \pm 0.2$ and $K_{m2} = 18.9 \pm 1.5$ μM), while corresponding V_{max} values were quite similar ($V_{max1} = 100.6 \pm 10.0$ and $V_{max2} = 452.3 \pm 13.5$ pmol mg^{-1} prot. min^{-1}). By contrast, [^{3}H]taurine uptake exhibited a single high affinity system ($K_m = 15.5 \pm 3.1$ μM, $V_{max} = 146.0 \pm 18.9$ pmol mg^{-1} prot. min^{-1}).

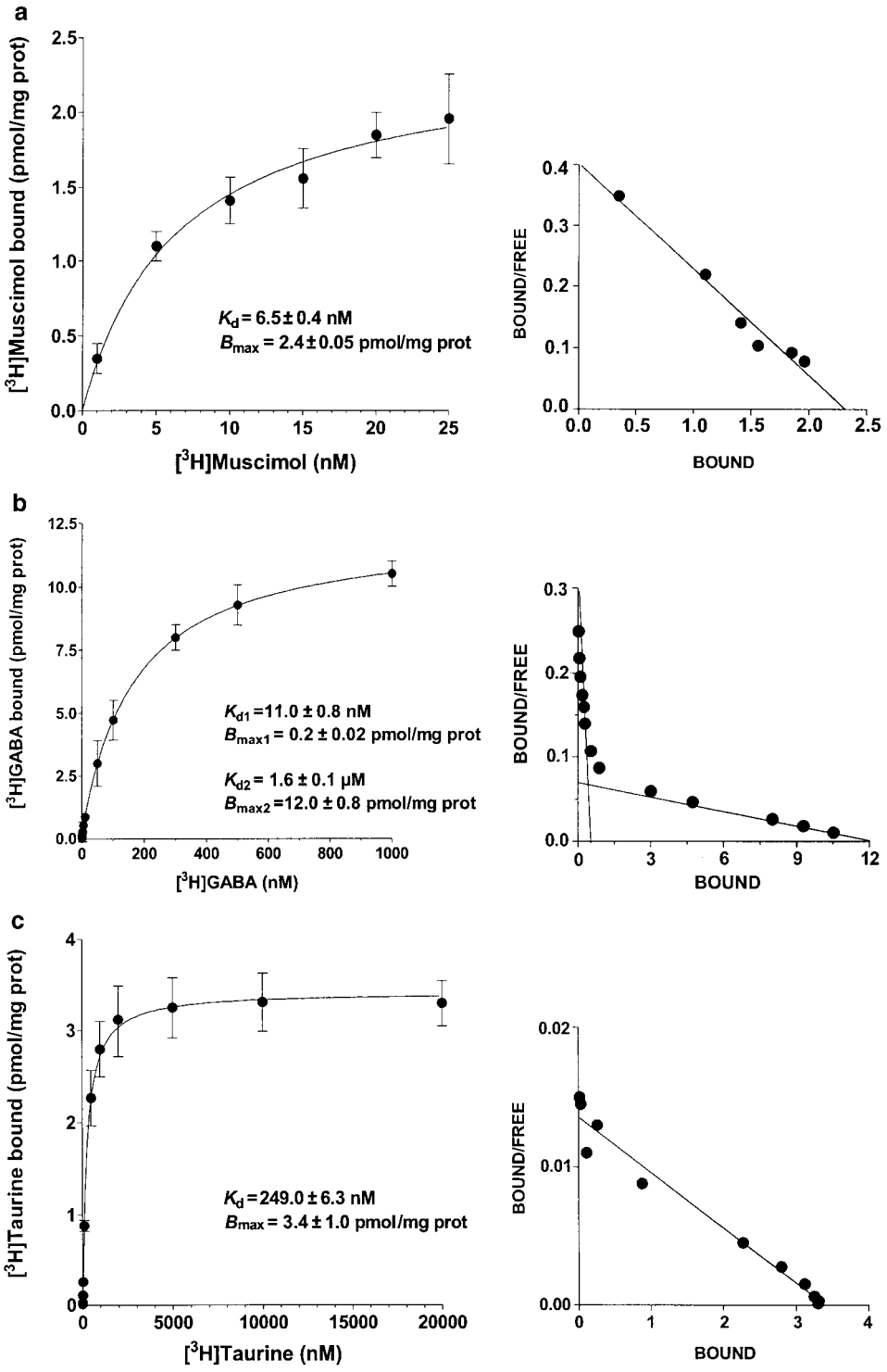


Figure 1 Binding of [^{3}H]muscimol (a), [^{3}H]GABA (b) and [^{3}H]taurine (c) to $GABA_A$, $GABA_B$ and taurine binding sites, respectively, present in different preparations of synaptic membranes of rabbit brain. Depicted saturation curves (left) and Scatchard plots (right) were obtained from a representative experiment performed in triplicate. K_d and B_{max} values are reported as mean ± s.e.m. and are obtained by at least five different experiments. For further details, see Methods section.

Displacement of specific [^{3}H]muscimol and [^{3}H]GABA binding to $GABA_A$ and $GABA_B$ receptors

Taurine and its analogues have been examined for their ability to displace [^{3}H]muscimol from $GABA_A$ receptors. As reported in Table 2, OMO was the most potent displacer with a K_i at nanomolar range, that is much lower than that of GABA. Among the other derivatives, β-ALA and PYR inhibited [^{3}H]muscimol binding with K_i values in the μM range, while TMT, GES, EOS, DMT and taurine were very weak

inhibitors. CAHS, MMT, AEP, AEA, ISE, TAG, AMS, ACES, PIPES, ANSA, GLY and TAHS did not affect [^{3}H]muscimol binding. To displace specific [^{3}H]GABA binding from $GABA_B$ receptors, taurine was, after GABA, the most potent agent, followed by DMT and β-ALA with K_i values of the same order of magnitude (μM). Also, AEP, TMT and OMO displaced the binding of [^{3}H]GABA, even though less effectively than the previous compounds. The other taurine derivatives were almost without effect.

Displacement of specific [^{3}H]taurine binding from taurine binding sites

As reported in Table 2, AEA, TAG, taurine, CAHS, GABA and ISE inhibited [^{3}H]taurine binding with corresponding K_i values ranging between 0.13 ± 0.01 (AEA) and $13.5 \pm 0.6\,\mu$M (ISE).

Inhibition of [^{3}H]taurine and [^{3}H]GABA uptake by crude synaptosomes

The effects of taurine derivatives on both taurine and GABA uptake systems were investigated. Only GES, the reported taurine uptake inhibitor in rat tissues (Huxtable 1989), was shown to inhibit [^{3}H]taurine uptake by rabbit-brain synaptosomes with an IC_{50} of $3.7 \pm 0.2\,\mu$M, while none of the other compounds affected it (data not shown). Similarly, none of the compounds tested revealed any effect on [^{3}H]GABA uptake by rabbit-brain synaptosomes. On the contrary, nipecotic acid, an inhibitor of [^{3}H]GABA uptake in many mammalian species including the rabbit, was able to inhibit with an IC_{50} of $7.8 \pm 0.1\,\mu$M.

Table 2 Comparative K_i values (μM) for displacement of specific [^{3}H]muscimol, [^{3}H]GABA and [^{3}H]taurine from $GABA_A$, $GABA_B$ receptors and taurine binding site (TAU) of rabbit brain by GABA, taurine and some taurine analogues

Compound	*$GABA_A$*	*$GABA_B$*	*TAU*
GABA	0.05 ± 0.006	0.014 ± 0.001	2.38 ± 0.2
TAU	118.1 ± 8.4	0.8 ± 0.06	0.23 ± 0.01
GES	52.0 ± 3.6	N.A.	N.T.
OMO	0.013 ± 0.001	5.0 ± 0.3	N.I.
PSA	166.3 ± 9.8	N.A.	N.T.
CAHS	N.A.	N.A.	4.0 ± 0.3
MMT	N.A.	N.A.	N.I.
AEP	N.A.	3.5 ± 0.2	N.T.
AEA	N.A.	N.A.	0.13 ± 0.01
EOS	69.1 ± 4.4	N.A.	N.T.
PYR	24.6 ± 1.7	N.A.	N.T.
ISE	N.A.	N.A.	13.5 ± 0.6
DMT	91.0 ± 13.6	1.6 ± 0.1	N.I.
TMT	47.5 ± 3.4	4.0 ± 0.3	N.I.
TAG	N.A.	N.A.	0.13 ± 0.01
AMS	N.A.	N.A.	N.T.
β-ALA	7.9 ± 0.6	1.6 ± 0.1	N.I.
ACES	N.A.	N.A.	N.I.
PIPES	N.A.	N.A.	N.I.
ANSA	N.A.	N.A.	N.T.
GLY	N.A.	N.A.	N.T.
TAHS	N.A.	N.A.	N.I.

N.A. (not active) = $IC_{50} > 500\,\mu$M. N.I.: no inhibition at 1×10^{-3} M. K_i values are reported as mean $\pm$ s.e.m. of data from three or more experiments for each analogue (concentration range: 0.1 nM–1000 μM). The concentration of [^{3}H]muscimol and [^{3}H]GABA were 10 and 20 nM, respectively, while that of [^{3}H]taurine was 60 nM. For further details, see Methods section.

Effects on GABA-transaminase activity

As reported in Table 3, among the compounds studied, PSA was the most potent inhibitor of rabbit-brain GABA-transaminase activity with an IC_{50} of $103.0 \pm 3.9\,\mu$M. Vigabatrin, the GABA-transaminase inhibitor, in clinical use, is effective towards the enzymes of many species (Suzdak *et al*., 1992), including the rabbit ($IC_{50} = 287.1 \pm 17.3\,\mu$M). AEP, ANSA and AMS were weak inhibitors (IC_{50} in the mM range), while the other derivatives were inactive at 1000 μM concentration.

Discussion

In the present study, the binding characteristics of $GABA_A$ and $GABA_B$ receptors, GABA and taurine uptake and GABA-transaminase activity in different rabbit-brain preparations were investigated. Data for rat, mouse, pig and cow brain are already present in the literature. Equilibrium binding experiments on $GABA_A$ and $GABA_B$ receptors carried out in the present study have shown that the relative K_d and B_{max} found in rabbit-brain preparations are very similar to those reported for rat, mouse and pig (Krogsgaard-Larsen *et al*., 1980; Bowery *et al*., 1985; Yang & Olsen, 1987; Bureau & Olsen, 1991; Facklam & Bowery, 1993). Also findings on the uptake of [^{3}H]GABA, by rabbit whole-brain crude synaptosomes, match those reported for rat brain by some authors (Cupello *et al*., 1993), but are at variance with those obtained by Debler & Lajtha (1987), which indicated only one high-affinity system in rat brain cortex synaptosomes. Furthermore, nipecotic acid was shown here to inhibit GABA uptake with an IC_{50} value close to those already described for rat and mouse ($IC_{50} = 3.6$ and $2.79\,\mu$M, respectively) (Suzdak *et al*., 1992; Mantz *et al*., 1994).

Table 3 Comparative IC_{50} values (μM) of taurine and some of its derivatives toward GABA transaminase activity in rabbit brain crude homogenate

Compounds	*IC_{50} (μM)*	*Compounds*	*IC_{50} (μM)*
PSA	103.5 ± 3.9	EOS	N.I.
AEP	2494.5 ± 74.8	PYR	N.I.
ANSA	2023.0 ± 172.7	ISE	N.I.
AMS	3572.7 ± 588.4	DMT	N.I.
TAU	N.I.	TMT	N.I.
GES	N.I.	TAG	N.I.
OMO	N.I.	β-ALA	N.I.
PIP	N.I.	ACES	N.I.
CAHS	N.I.	PIPES	N.I.
MMT	N.I.	GLY	N.I.
AEA	N.I.	TAHS	N.I.

N.I. = no inhibition of the enzyme at 1000 μM concentration. The concentration of GABA used in the assay was 12.5 mM. IC_{50} values are reported as mean $\pm$ s.e.m. from three or more experiments for each analogue (concentration range: 1 nM–1 mM). In the same assay, IC_{50} value of vigabatrin was $287.1 \pm 17.3\,\mu$M.

Meiners *et al.* (1980) found two saturable processes for the uptake of [^{3}H]taurine by different brain preparations and cell types. These were characterized by K_m values in the μM (high affinity) and mM (low affinity) ranges, respectively. Nevertheless, we found only a single type of taurine uptake with a K_m of $15.5 \pm 3.1\,\mu$M.

It seems that the ability or affinity of taurine to displace [^{3}H]muscimol binding from $GABA_A$ receptors and [^{3}H]GABA from $GABA_B$ receptors varies according to the animal species considered. Thus taurine affinity for $GABA_A$ receptors differs significantly among cow ($IC_{50} = 2.2\,\mu$M) (Krogsgaard-Larsen *et al.*, 1981), rat ($IC_{50} = 50.0\,\mu$M) (Bureau & Oslen, 1991) and rabbit ($IC_{50} = 300.0\,\mu$M, this study). Hill & Bowery (1981) found a taurine IC_{50} value relative to its ability to displace [^{3}H]baclofen from $GABA_B$ receptors greater than 800 μM, while Kontro & Oja (1990) showed that it inhibits the binding of [^{3}H]GABA to $GABA_B$ receptors of mouse brain with an IC_{50} value of 5.12 μM. This is very close to that reported in the present study with rabbit. Among taurine derivatives, OMO was shown to interact with $GABA_A$ receptors with a very high affinity (IC_{50} and K_i values in the nM range) similarly to what was reported by Robinson *et al.* (1989) for rat ($IC_{50} = 0.049\,\mu$M) and by Krogsgaard-Larsen *et al.* (1981) for cow ($IC_{50} = 0.080\,\mu$M). Furthermore, OMO was able to displace the binding of [^{3}H]GABA from $GABA_B$ receptors with an IC_{50} value of 14.2 μM ($K_i = 5.0 \pm 0.3\,\mu$M), close to that reported by Hill & Bowery for rat (1981). Among the other compounds, only the interactions of β-ALA and PSA have been tested as to their interaction with rat brain $GABA_A$ and $GABA_B$ receptors and $GABA_A$ receptors, respectively. The resulting data (Krogsgaard-Larsen *et al.*, 1980, 1981; Hill & Bowery, 1981) are very close to those found in the present study for rabbit-brain GABA receptors.

The one taurine analogue effective in inhibiting GABA transaminase was PSA (Table 3), which is similar in effectiveness to vigabatrin, used clinically to inhibit this enzyme. Furthermore, EOS, an inhibitor of rat and mouse brain GABA-transaminase activity (Phillips & Fowler, 1982; Qume & Flower, 1997) was found to be ineffective towards the rabbit enzyme.

It has been suggested that taurine acts as a neurotransmitter in the CNS (Kontro & Oja, 1987a). This implies its interaction with a postsynaptic receptor. The demonstration of a sodium-independent binding of taurine to brain synaptic membranes has been controversial. Some authors failed to find a specific taurine binding to synaptic membranes from different brain regions of rat (Lopez-Colomè & Pasantes-Morales, 1981) and calf (Lähdesmäki *et al.*, 1977). They, however, did not use detergents for preparing membranes. Detergents, such as Triton X-100, make the receptor sites more accessible, both by removing extrajunctional plasma membranes and by diminishing nonspecific binding as Enna & Snyder (1977) showed for GABA. Moreover, detergents aid in washing out inhibiting compounds, such as endogenous taurine and GABA, and by breaking resealed membrane pouches. Kontro & Oja (1987a), in fact, could detect a saturable taurine binding in synaptic membranes isolated from mouse brain only after Triton X-100 treatment; the binding was maximal in those preparations treated twice with this detergent. Although this treatment completely removed endogenous GABA from the mouse synaptic membranes, removal of endogenous taurine was incomplete (Kontro & Oja, 1987a); we similarly found incomplete removal of endogenous taurine from the rabbit-brain synaptic membranes. In rabbit WSM, the persistence of small amounts of taurine, albeit much lower than that found in mouse WSM (i.e. 1.6 ± 0.2 mmol kg^{-1} prot.), could lead to underestimate the number of taurine binding sites. Kontro & Oja (1987a) found a sodium-independent, taurine binding with outlines of positive cooperativity, suggesting two or more taurine molecules interacting at a single binding site and an apparent K_d value of 270 nM and a B_{max} of 5.8 pmol mg^{-1} prot. By contrast, Kontro & Oja (1983) had found that, in the rat, the binding fitted a one-site curve with a K_d value of 539 nM and a B_{max} of 4.6 pmol mg^{-1} prot. Furthermore, GABA was able to displace taurine binding with an IC_{50} value of 3.0 μM (Kontro & Oja, 1987a). Other authors, however, have demonstrated the existence of a GABA-insensitive taurine binding in pig brain (Wu *et al.*, 1992) with a K_d value of 92 nM and a B_{max} of 6.0 pmol mg^{-1} prot. The membrane preparation used in the latter study, however, required a procedure involving cycles of thawing and freezing and extensive washing of the membranes with buffers, but not the use of detergents. Our procedure, based on the use of Triton X-100, is less time-consuming and gave similar results. The K_d value for [^{3}H]taurine binding found in the present study is very similar to that found by Kontro & Oja in the mouse (1987a), and both values are one order of magnitude higher than those reported for several labelled $GABA_A$ agonists for $GABA_A$ receptors (Krogsgaard-Larsen *et al.*, 1980, 1981; Yang & Olsen, 1987; Bureau & Olsen, 1991). This may be because of some unremovable endogenous binding inhibitors such as taurine itself. The same happened for the earlier GABA binding studies, wherein endogenous GABA hampered the characterization of $GABA_A$ receptors until detergents were introduced into the procedure (Napias *et al.*, 1980). In the present study, GABA was shown to displace bound [^{3}H]taurine, thus giving rise to the possibility that GABA receptors were responsible for taurine binding. However, the fact that OMO, DMT, TMT and β-ALA displaced both [^{3}H]muscimol and [^{3}H]GABA binding to $GABA_A$ and $GABA_B$ receptors, respectively, but did not affect [^{3}H]taurine binding, allows one to exclude that GABA receptors were responsible for [^{3}H]taurine binding. Finally, since CAHS, AEA, ISE and TAG did not interact either with GABAergic receptors or with the taurine uptake system but were able to displace [^{3}H]taurine binding from rabbit WSM, although with different affinities – AEA and TAG exhibiting an affinity two orders of magnitude higher than ISE and CAHS – they fulfill the criteria for taurinergic agents and represent useful probes to investigate the role of taurine in the CNS.

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