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ORIGINAL ARTICLE

Modulation of GABA-A receptors of astrocytes and STC-1 cells by taurine structural analogs

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Abstract Taurine activates and modulates GABA receptors in vivo as well as those expressed in heterologous systems. This study aimed to determine whether the structural analogs of taurine: homotaurine and hypotaurine, have the ability to activate GABA-A receptors that include GABAρ subunits. The expression of GABA-A receptors containing GABAρ has been reported in the STC-1 cells and astrocytes. In both cell types, taurine, homo-, and hypotaurine gated with low efficiency a picrotoxin-sensitive GABA-A receptor. The known bimodal modulatory effect of taurine on GABAρ receptors was not observed; however, differences between the activation and deactivation rates were detected when they were perfused together with GABA. In silico docking simulations suggested that taurine, hypo-, and homotaurine do not form a cation–π interaction such as that generated by GABA in the agonist-binding site of GABAρ. This observation complements the electrophysiological data suggesting that taurine and its analogs act as partial agonists of GABA-A receptors. All the observations above suggest that the structural analogs of taurine are partial agonists of GABA-A receptors that occupy the agonist-binding site, but their structures do not allow the proper interaction with the receptor to fully gate its Cl^- channel.

Keywords Astrocytes · Electrophysiology · GABAρ · Homotaurine · Hypotaurine

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Abbreviations

DMEM	Dulbecco's modified eagle's medium
FBS	Fetal bovine serum
GABA	γ-amminobutyric acid
GLIC	*Gloebacter* ion channel
EGTA	Ethylene glycol tetraacetic acid
GluCl	Glutamate-gated ion channel
HEPES	2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid
ICM	Internal coordinate mechanics
RsT	Rise time constant
STC-1	Secretin tumor cell line
TPMPA	(1,2,5,6-Tetrahydropyridin-4-yl)methylphosphinic acid

Introduction

Taurine is one of the most abundant amino acids in the brain and spinal cord, leukocytes, heart and muscle cells, the retina, and indeed almost every tissue throughout the body; furthermore, taurine is known to have preventive effects against stroke and cardiovascular diseases in experimental models as well as in humans (Yamori et al. 2010; Ripps and Shen 2012). This amino acid lacks the carboxyl group and contains a sulfonate group instead; it does not form part of proteins. Taurine exerts multiple functions in the central nervous system including roles in cell volume regulation and neurotransmission. In neurons and astrocytes, taurine activates with low efficiency ionotropic GABA and glycine receptors (Pasantes-Morales et al. 1990, Moran et al. 1997; ElIdrissi and Trenkner, 2004; Le-Corronc et al. 2011). Electrophysiological and competitive binding assays have indicated that taurine is a weak agonist of the GABA-A receptor complex, interacting with

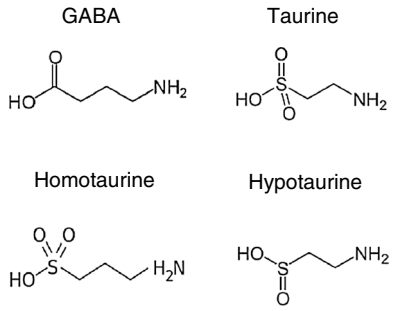

Fig. 1 Structure of agonists of the GABA-A receptor used in this study

the muscimol-displaceable, bicuculline-sensitive GABA-binding sites thereby allosterically modulating the binding of benzodiazepines (Bureau and Olsen 1991; Frosini et al. 2003).

GABA-A receptor-mediated inhibitory effects of exogenously added taurine have been reported in several brain regions such as the cerebellum, hippocampus, striatum, and cerebral cortex (Chan-Palay et al. 1982; Mori et al. 2002; Wu and Xu 2003). Experiments with recombinant GABA receptors have revealed the ability of taurine to interact with receptors composed of different combinations of subunits (Horikoshi et al. 1998; Hadley and Amin 2007). In cloned GABAρ1 subunits, taurine induces a bimodal effect, by modulating the GABA currents negatively when it is present at high concentrations but positively at low concentrations (Ochoa-de la Paz et al. 2008). This paradoxical effect could be explained by the transition of the taurine molecule from the *cis* to the *trans* conformation upon concentration changes of the molecule in solution. GABAρ1 receptors are expressed mainly in retina, although many other areas of the central nervous system express this GABA-A subunit to a lesser extent (Martínez-Delgado et al. 2008). GABAρ1, as well as GABAρ2 and GABAρ3, forms homo-oligomeric receptors when expressed in heterologous systems; upon activation by the agonist the receptor generates Cl^- currents and exhibits virtually no desensitization. Thus, to explore if the dual effect of taurine on GABAρ1 extends to other sulfonated amino acids, in this work we tested two structural analogues of taurine: hypotaurine and homotaurine (Fig. 1).

Hypotaurine is a strong antioxidant, scavenging OH^- radicals with its sulfur group; this amino acid accumulates in the mammalian reproductive system where it acts as an osmolyte (Fontana et al. 2004). Homotaurine has been extensively studied because it binds Aβ-amyloid in its soluble form; thus, its use was proposed to prevent Alzheimer's disease. Unfortunately, clinical trials of this molecule have not been conclusive (Santa-María et al. 2007).

In this study, we tested the action of the taurine analogs on the cell line STC-1, cells known to express a variety of GABA-A subunits (Glassmeier et al. 1998; Jansen et al. 2000); in addition, we tested them in a primary culture of astrocytes from cerebellum, in which GABA-A, GABAρ1 and GABAρ2 are known to be expressed in vitro and in situ (Bormann and Kettenmann 1988; Martínez-Delgado et al. 2011). Finally, we generated in silico a structural model that explains the binding of homotaurine and hypotaurine to the GABA-binding site.

Materials and methods

Electrophysiology and pharmacology

STC-1 cells were maintained in Dulbecco's modified eagle's medium (DMEM) supplemented with 15 % horse serum, 2.5 % fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin and maintained at 37 °C and 5 % CO_2 (Machuca-Parra et al. 2013). For the astrocyte primary culture, one newborn CD1 mouse was decapitated for each cellular culture; the cerebellum was removed, and dissociated cells were cultured in DMEM supplemented with 10 % FBS, 2 mM glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin (Reyes-Haro et al. 2005; Martínez-Delgado et al. 2011). All experiments were conducted according to the guidelines of the National Institutes of Health Guide for Care and use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of the Universidad Nacional Autónoma de México.

Membrane currents were recorded using the whole-cell voltage-clamp technique (Hamill et al. 1981). A fragment of coverslip, on which astrocytes or STC-1 cells were cultured for 4–8 days, was placed in a recording chamber (300 μl for STC-1 cells or 500 μl for astrocytes) on the stage of an inverted microscope (Olympus IX-70, Japan). The recording chamber was continuously superfused (5 ml/min for STC-1 cells and 2 ml/min for astrocytes) with standard bathing solution containing (in mM): 150 NaCl, 2.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, and 10 glucose (pH 7.3). Recording pipettes were made with borosilicate capillaries (Sutter Instruments) and filled with a solution containing (in mM): 130 KCl, 0.5 $CaCl_2$, 5 EGTA, 2 $MgCl_2$, 10 HEPES, 3 ATP-Mg, and 0.4 GTP-Mg (pH 7.3), having a resistance of 3–5 MΩ. All experiments were carried at room temperature (20–23 °C). Currents were recorded with an Axopatch 200B amplifier (Axon Instruments, CA, USA) or EPC 10 amplifier (HEKA), filtered at 3 kHz, and acquired at 10 kHz. The data were analyzed with pClamp 8.2 (Axon Instruments, CA, USA) or TIDA software (5.19) and Microcal Origin 7 software (OriginLab, MA, USA). The data are given as mean ± standard error. Comparison of the mean values among groups was performed by the Student's *t* test, where $p < 0.05$ was taken to indicate

a statistically significant difference. The rise time (Rs) and decay time constants (τ) were calculated with Clampfit or TIDA software.

Homology modeling and docking

We used the program MODELLER v 9.10 to develop a homology model of the human GABAρ1 subunit, employing the high-resolution crystal structures of the *C. elegans* glutamate-gated chloride channel (GluCl), the pentameric ligand-gated ion channel of *Gloebacter violaceus* (GLIC), and the refined structure of the nicotinic acetylcholine receptor, downloaded from the Brookhaven Protein Data Bank (PDB) using the accession numbers 3RIF, 3EHZ, and 2BG9, respectively (Brejc et al. 2001; Bocquet et al. 2009; Hibbs and Gouaux 2011). The model of GABAρ1 was then projected fivefold on the pentameric structure of the GluCl channel to build a homopentamer of the GABAρ1 receptor. The amino acid sequence was obtained from the Uniprot Server (protein accession number: P24046). The few observed clashes were corrected using GROMOS96 (part of DeepView/Swiss-Pdb Viewer v4.0.1 software) and refined by energy minimization using Molsoft ICM v3.5. All the ligand molecules were downloaded and edited from the ChemSpider database (IDs: Taurine, 1091; Hypotaurine, 96959; Homotaurine, 1584). For Docking, we used Pocket Finder in Internal Coordinate Mechanics (ICM) for automatic detection of small molecule-binding sites defined on an interface of the cytoplasmic domain, selecting the best fitting site for each superposed molecule in the electron density, and optimized on the model (ICM score). The images were generated using the PyMOL Molecular Graphics System, Version 1.3, Schrödinger, LLC.

Results

Taurine and analogs act as agonists of GABA-A receptors in STC-1 cells and astrocytes

To determine whether taurine and its structural homologs activate GABA-A receptors containing GABAρ subunits we tested astrocyte primary cultures from cerebellum and STC-1 cells, because they endogenously express functional $GABA_A$ receptors that include GABAρ subunits.

GABA generated larger currents in astrocytes (GABA 50 μM, 506 ± 68 pA; $n = 9$) (Fig. 2a) than in STC-1 cells (GABA 100 μM, 150 ± 29 pA; $n = 23$, Fig. 2c), despite the fact that we doubled the concentrations of the agonist. A similar pattern for taurine (1 mM) was observed in astrocytes (193 ± 98 pA; $n = 6$) and STC-1 cells (36 ± 6 pA; $n = 6$) (Fig. 2b, d). Homotaurine evoked currents with an amplitude of 891 ± 154 pA ($n = 4$) in astrocytes and 93 ± 13 pA ($n = 6$) in STC-1 cells, while hypotaurine (1 mM vs 3 mM) evoked currents with an amplitude of 165 ± 68 pA ($n = 9$) in astrocytes and 196 ± 43 pA ($n = 6$) in STC-1 cells (Fig. 2b, d). All the currents were almost completely blocked (>85 %) by picrotoxin (100 μM), a selective antagonist of GABA-A receptors (Table 1) (Fig. 2a, c). Altogether, these results indicate that taurine, hypotaurine, and homotaurine activate GABA-A receptors in cerebellar astrocytes and STC-1 cells, with higher affinity for those expressed by astrocytes.

Modulation of $GABA_A$ receptors by taurine and its analogs

We tested the modulatory effect of taurine and its analogs on GABA-evoked currents in astrocytes and STC-1 cells (Fig. 3a, e). In astrocytes, taurine or one of the analogs was co-applied at 1 mM with 50 μM GABA; the current amplitudes were not different from the control (50 μM GABA), and the deactivation constant (τ) remained unaffected (Fig. 3b, c); however, the rise time constant (RsT) estimated for GABA (5.39 ± 0.68) increased when taurine was present in the bath (+57 %; $n = 5$; $p = 0.0204$) and decreased with homotaurine (−40 %; $n = 7$; $p = 0.034$), while hypotaurine did not affect the RsT (Fig. 3d). On the other hand, the amplitude of GABA currents (100 μM) was not modified significantly for STC-1 cells with the co-application of taurine or any of the analogs (Fig. 3f). Nevertheless, the estimated τ for GABA (4.5 ± 0.8 s) increased when taurine (35 ± 3.7 s; $n = 9$; $p < 0.001$), homotaurine (20 ± 3.5 s; $n = 8$; $p < 0.018$), or hypotaurine (11 ± 2.5 s; $n = 8$; $p < 0.001$) was co-applied (Fig. 3g). Furthermore, the estimated RsT for GABA (0.86 ± 0.18 s; $n = 23$) increased significantly when taurine (2.23 ± 0.35 s; $n = 9$; $p = 0.002$), homotaurine (2.26 ± 0.36 s; $n = 8$; $p < 0.001$), or hypotaurine (3.08 ± 0.87 s; $n = 8$; $p = 0.001$) was co-applied (Fig. 3h).

Homology modeling of the interaction of taurine and its analogs with the agonist-binding site of GABAρ1

Dose–response curves of taurine acting on the GABAρ1 receptor, as well as TPMPA blocking of the generated currents suggested that these molecules bind to the GABA-binding site of the receptor. Thus, to understand whether the structural analogs interact with the receptor, docking simulations were performed using a model based on acetylcholine receptor, GLIC and GluCl. Taurine bound to the GABA-binding site is shown in Fig. 4a. The electrostatic representation illustrates that the aromatic box of the receptor that permits receptor binding of GABA through a cation–π interaction does not seem to play this role for

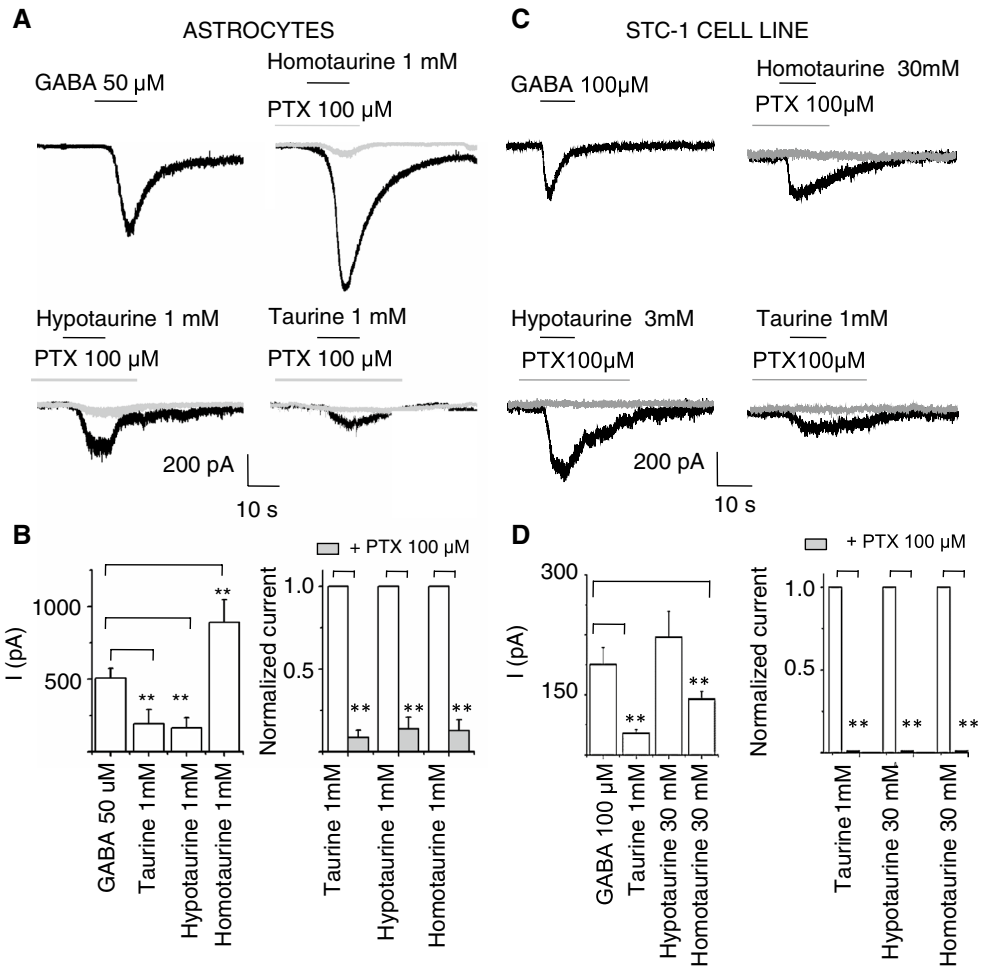

Fig. 2 Taurine and its analogs act as agonists of GABA-A receptors expressed in astrocytes and in the STC-1 cell line. **a**, **c** Representative currents elicited by GABA (50 μM for astrocytes, $n = 9$; 100 μM for STC-1 cells, $n = 6$), homotaurine (1 mM for astrocytes, $n = 4$; 30 mM for STC-1 cells, $n = 6$), hypotaurine (1 mM for astrocytes, $n = 9$; 3 mM for STC-1 cells, $n = 6$) and taurine [1 mM for astrocytes ($n = 6$) and STC-1 cells ($n = 6$)]. All the currents were antagonized by the GABA-A antagonist picrotoxin (PTX, 100 μM) in astrocytes (A, $n \geq 3$) and in the STC-1 cell line (**c**, $n = 6$ for all). **b**, **d** (*left*), Summary of the current amplitudes recorded with each molecule in astrocytes and in the STC-1 cell line. Notice that GABA-A receptors expressed in each cell type responded differently to homotaurine and hypotaurine **b**, **d** (*right*). The currents evoked by taurine and its analogs were normalized, and the antagonism by picrotoxin in astrocytes and STC-1 cells is summarized. Data are given as the mean ± S.E.M. $^{**}p < 0.01$

Table 1 Comparison between the current amplitudes generated by GABA, taurine and structural analogs in astrocytes and STC-1 cells

Amplitude (pA)					
Astrocyte			STC-1 cells		
Molecule		+Picrotoxin 100 μM	Molecule		+Picrotoxin 100 μM
GABA 50 μM	506 ± 68 ($n = 9$)	70 % ($n = 6$)	GABA 100 μM	150 ± 29 pA ($n = 23$)	100 % ($n = 6$)
Taurine 1 mM	193 ± 98 ($n = 6$)	92 % ($n = 4$)	Taurine 1 mM	36 ± 6 pA ($n = 6$)	100 % ($n = 6$)
Homotaurine 1 mM	891 ± 154 ($n = 4$)	87 % ($n = 3$)	Homotaurine 30 mM	93 ± 13 pA ($n = 6$)	100 % ($n = 6$)
Hypotaurine 1 mM	165 ± 68 ($n = 9$)	86 % ($n = 5$)	Hypotaurine 3 mM	196 ± 43 pA ($n = 6$)	100 % ($n = 6$)

Blockage effect of picrotoxin (100 M) is shown in the right column

taurine binding. Instead, we found the following residues to be fundamental: Q104, R125, Y219, and S263 (Fig. 4b). Hypotaurine fits the GABA-binding site; however, the docking analysis does not suggest a cation–π interaction but rather, the generation of two polar contacts with R125 and K238 in the aromatic box B (Fig. 4b). Finally, homotaurine docking reveals only a weak interaction, a polar contact with R125 in the aromatic box C of the receptor (Fig. 4b).

Discussion

It is now well established that GABA as well as glycine receptors can be activated by paracrine release of taurine (Le-Corronc et al. 2011). In the brain and retina, taurine accumulates in glial cells, especially in astrocytes and the Bergmann glia of the cerebellum; this amino acid is released in a non-synaptic fashion independently of calcium (Zhang and Ottersen 1992; Rodríguez-Navarro et al.

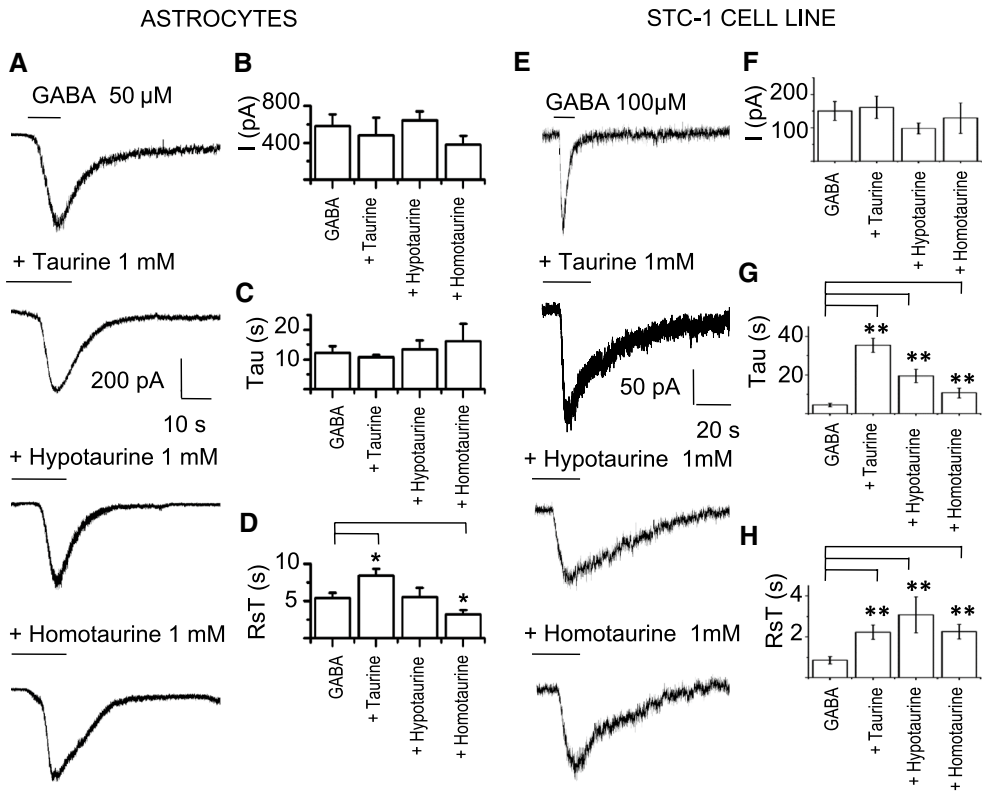

Fig. 3 Co-application of GABA with taurine and its analogs in astrocytes and STC-1 cells. **a**, e Representative currents elicited by GABA alone (50 µM for astrocytes, $n = 10$; 100 µM for STC-1, $n = 23$) or co-applied with taurine [1 mM, $n = 6$ (**a**) and $n = 9$ (**e**)], hypotaurine (1 mM; $n = 7$ (**a**) and $n = 8$ (**e**)) or homotaurine (1 mM, $n = 7$ (**a**) and $n = 8$ (**e**)), in astrocytes (**a**) or STC-1 cells (**e**), respectively. **b**, **f** Summary of current amplitudes showed no significant differences between GABA alone or co-applied with taurine, hypotaurine, or homotaurine for both astrocytes (**b**) and STC-1 cells (**f**). **c**, **g** Summary of the inactivation constant (τ) with GABA and GABA co-applied with taurine, hypotaurine, or homotaurine. No significant differences were observed in astrocytes, while in STC-1 cells all the molecules co-applied with GABA significantly increased τ. **d**, **h** Summary of the rise time (RsT) calculated for GABA alone or co-applied with taurine, hypotaurine, or homotaurine. All the molecules significantly modified the RsT for both astrocytes (**d**) and STC-1 cells (**h**) except for hypotaurine in astrocytes, where no significant change was observed. Data are given as the mean ± S.E.M. $*p < 0.05$; $**p < 0.01$

2009). GABAρ1 subunits are both positively and negatively modulated by taurine, depending on its concentration; in addition, taurine acts as a weak agonist of the receptor when expressed in *X. laevis* oocytes (Ochoa-de la Paz et al. 2008). This study aimed to understand whether taurine executes the same actions on GABAρ1 when this subunit is expressed endogenously in astrocytes and STC-1 cells and to establish whether structural analogs of taurine, hypo-, and homotaurine, activate GABA-A receptors. These cells exhibit GABA currents generated by three populations of ionotropic receptors: (1) GABA-A, made up of α, β, γ and/or δ subunits, (2) GABAρ subunits, and (3) heteromeric receptors composed of GABAρ and other GABA-A subunits. Thus, activation and modulation of taurine and structural analogs are expected to occur on all these GABA receptors.

Partial agonism of the taurine analogs is consistent with the docking simulation of their interaction with the agonist-binding site of GABAρ1. In both cases, the cation–π interaction necessary for GABA-binding (Lummis et al. 2005; Padgett et al. 2007) is not predicted, and the interactions with the GABA-binding pocket are weak. It has been suggested that taurine can induce an allosteric modulation of GABA-A receptors by binding to the benzodiazepine site (Quinn and Miller 1992); the results obtained in our co-application assay are not yet conclusive and do not establish whether hypo- and homotaurine can also allosterically modulate GABAρ1; however, it is clear that both molecules interact with the agonist-binding site. In any case, GABAρ1 is not modulated by benzodiazepines (Polenzani et al. 1991; Shimada et al. 1992). On the other hand, isethionic acid, which did not induce any detectable current in oocytes that expressed GABAρ1 (not shown), did not fit in the GABA-binding site, and docking simulations (not shown) corroborated the electrophysiological findings.

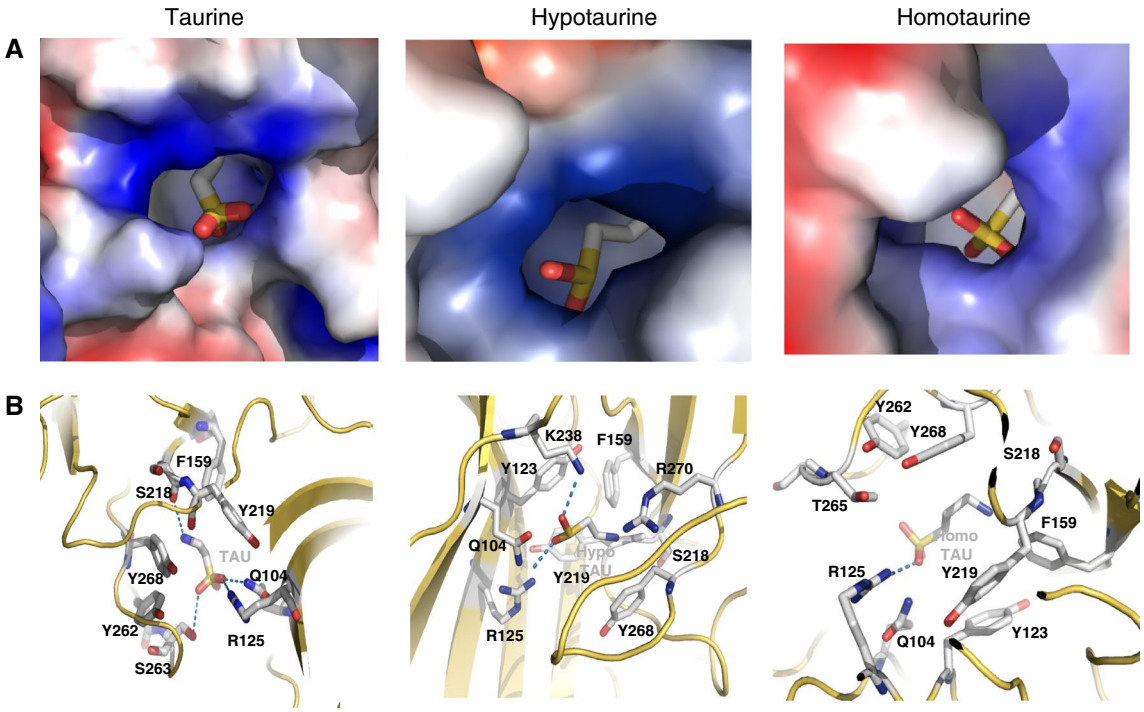

Fig. 4 Homology models of taurine and structural analogs in the GABAρ1 subunit. **a** Electrostatic representations of the ligand-binding site accommodating a molecule of agonist. Several of the known elements stabilizing the GABA-binding site are present, highlighting the aromatic box that is common in the cys-loop family, which permits the cation–π interaction but does not play the same role in binding analogs as it does with GABA itself. **b** The most significant residues stabilizing the interaction for taurine are: Q104, R125, S218, Y219, and S263. For hypotaurine the interaction seems to be somewhat less stable, involving only two residues that form polar contacts: R125, K238. Homotaurine keeps only a single polar contact with R125 besides the aromatic box B

Hypotaurine and taurine are required for the process of activating mammalian spermatozoa, and taurine is widely distributed in the male reproductive system (Guérin et al. 1995; Yang et al. 2010). Sperm cells are known to express GABA-A receptors including GABAρ1 subunits (Li et al. 2008; Kanbara et al. 2011; Puente et al. 2011); given these facts, is feasible that taurine and its analogs may function as agonists of these receptors and induce or modulate their activation. On the other hand, although interactions of homotaurine with ionotropic GABA receptors have not been previously reported, it is known that this molecule acts on a population of GABA-B receptors, giving rise to analgesia in experimental models of nociception (Serrano et al. 1998). Whether there is an involvement of ionotropic GABA receptors in this process remains to be determined.

In conclusion, homotaurine and hypotaurine reduce the rate of deactivation of GABA responses in STC-1 cells, while in astrocytes only the rise time constant is modulated. Finally, modeling of the agonist-binding site of GABAρ1 suggests that these molecules do not form a cation–π interaction; therefore, they form only a weak interaction with the receptor.

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Conflict of interest The authors declare no conflict of interest.

References

Bocquet N, Nury H, Baaden M, Le Poupon C, Changeux JP, Delarue M, Corringer PJ (2009) X-ray structure of a pentameric ligand-gated ion channel in an apparently open conformation. Nature 457:111–114

Bormann J, Kettenmann H (1988) Patch-clamp study of γ-aminobutyric acid receptor Cl- channels in cultured astrocytes. Proc Natl Acad Sci USA 85:9336–9340

Brejc K, van Dijk WJ, Klaassen RV, Schuurmans M, van Der Oost J, Smit AB, Sixma TK (2001) Crystal structure of an ACh-binding protein reveals the ligand-binding domain of nicotinic receptors. Nature 411:269–276

Bureau MH, Olsen RW (1991) Taurine acts on a subclass of $GABA_A$ receptors in mammalian brain in vitro. Eur J Pharmacol 207:9–16

Chan-Palay V, Ito M, Tongroach P, Sakurai M, Palay S (1982) Inhibitory effects of motilin, somatostatin, [Leu]enkephalin, [Met] enkephalin, and taurine on neurons of the lateral vestibular

nucleus: interactions with gamma-aminobutyric acid. Proc Natl Acad Sci USA 79:3355–3359

ElIdrissi A, Trenkner E (2004) Taurine as a modulator of excitatory and inhibitory neurotransmission. Neurochem Res 29:189–197

Fontana M, Pecci L, Duprè S, Cavallini D (2004) Antioxidant properties of sulfinates: protective effect of hypotaurine on peroxynitrite-dependent damage. Neurochem Res 29:111–116

Frosini M, Sesti C, Dragoni S, Valoti M, Palmi M, Dixon HBF, Machetti F, Sgaragli G (2003) Interactions of taurine and structurally related analogues with the GABAergic system and taurine binding sites of rabbit brain. Br J Pharmacol 138:1163–1171

Glassmeier G, Herzig KH, Höpfner M, Lemmer K, Jansen A, Scherubl H (1998) Expression of functional GABAA receptors in cholecystokinin-secreting gut neuroendocrine murine STC-1 cells. J Physiol 510:805–814

Guérin P, Guillaud J, Ménézo Y (1995) Hypotaurine in spermatozoa and genital secretions and its production by oviduct epithelial cells in vitro. Hum Reprod 10:866–872

Hadley SH, Amin J (2007) Rat α6β2δ $GABA_A$ receptors exhibit two distinct and separable agonist affinities. J Physiol 581:1001–1018

Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ (1981) Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflugers Arch 391:85–100

Hibbs RE, Gouaux E (2011) Principles of activation and permeation in an anion-selective Cys-loop receptor. Nature 474:54–60

Horikoshi T, Asanuma A, Yanagisawa K, Anzai K, Goto S (1998) Taurine and β-alanine act on both GABA and glycine receptors in *Xenopus* oocyte injected with mouse brain messenger RNA. Brain Res 464:97–105

Jansen A, Hoepfner M, Herzig KH, Riecken EO, Scherübl H (2000) $GABA_C$ receptors in neuroendocrine gut cells: a new GABA-binding site in the gut. Pflugers Arch 441:294–300

Kanbara K, Mori Y, Kubota T, Watanabe M, Yanagawa Y, Otsuki Y (2011) Expression of the $GABA_A$ receptor/chloride channel in murine spermatogenic cells. Histol Histopathol 26:95–106

Le-Corronc H, Rigo JM, Branchereau P, Legendre P (2011) $GABA_A$ receptor and glycine receptor activation by paracrine/autocrine release of endogenous agonists: more than a simple communication pathway. Mol Neurobiol 44:28–52

Li S, Zhang Y, Liu H, Yan Y, Li Y (2008) Identification and expression of $GABA_C$ receptor in rat testis and spermatozoa. Acta Biochim Biophys Sin 40:761–767

Lummis SC, L Beene D, Harrison NJ, Lester HA, Dougherty DA (2005) A cation-π binding interaction with a tyrosine in the binding site of the $GABA_C$ receptor. Chem Biol 12:993–997

Machuca-Parra AI, Miledi R, Martínez-Torres A (2013) Identification of the minimal promoter for specific expression of the GABAρ1 receptor in retinal bipolar cells. J Neurochem 124:175–188

Martínez-Delgado G, Estrada-Mondragón A, Miledi R, Martínez-Torres A (2008) An update on GABAρ receptors. Curr Neuropharmacol 8:422–433

Martínez-Delgado G, Reyes-Haro D, Espino-Saldaña AE, Rosas-Arellano A, Pétriz A, Juárez-Mercado P, Miledi R, Martínez-Torres A (2011) Dynamics of GABAρ2 receptors in retinal bipolar neurons and cerebellar astrocytes. Neuroreport 22:4–9

Moran J, Morales-Mulia S, Hernandez-Cruz A, Pasantes-Morales H (1997) Regulatory volume decrease and associated osmolyte fluxes in cerebellar granule neurons are calcium independent. J Neurosci Res 47:144–154

Mori M, Gähwiler BH, Gerber U (2002) β-Alanine and taurine as endogenous agonists at glycine receptors in rat hippocampus in vitro. J Physiol 539:191–200

Ochoa-de la Paz LD, Martínez-Dávila IA, Miledi R, Martínez-Torres A (2008) Modulation of human GABAρ1 receptors by taurine. Neurosci Res 61:302–308

Padgett CL, Hanek AP, Lester HA, Dougherty DA, Lummis SC (2007) Unnatural amino acid mutagenesis of the $GABA_A$ receptor binding site residues reveals a novel cation–π interaction between GABA and γ2Tyr97. J Neurosci 27:886–892

Pasantes-Morales H, Moran J, Schousboe A (1990) Volume sensitive release of taurine from cultured astrocytes: properties and mechanism. Glia 3:427–432

Polenzani L, Woodward RM, Miledi R (1991) Expression of mammalian γ-aminobutyric acid receptors with distinct pharmacology in *Xenopus* oocytes. Proc Natl Acad Sci USA 88:4318–4322

Puente MA, Tartaglione CM, Ritta MN (2011) Bull sperm acrosome reaction induced by γ-aminobutyric acid (GABA) is mediated by GABAergic receptors type A. Anim Reprod Sci 127:31–37

Quinn MR, Miller CL (1992) Taurine allosterically modulates flunitrazepam binding to synaptic membranes. J Neurosci Res 33:136–141

Reyes-Haro D, Miledi R, García-Colunga J (2005) Potassium currents in primary cultured astrocytes from the rat corpus callosum. J Neurocytol 34:411–420

Ripps H, Shen W (2012) Taurine: a "very essential" amino acid. Mol Vis 18:2673–2686

Rodríguez-Navarro JA, Gonzalo-Gobernado R, Herranz AS, González-Vigueras JM, Solís JM (2009) High potassium induces taurine release by osmosensitive and osmoresistant mechanisms in the rat hippocampus in vivo. J Neurosci Res 87:208–217

Santa-Maria I, Hernández F, Del Rio J, Moreno FJ, Avila J (2007) Tramiprosate, a drug of potential interest for the treatment of Alzheimer's disease, promotes an abnormal aggregation of tau. Mol Neurodegener 2:17

Serrano MI, Serrano JS, Fernández A, Asadi I, Serrano-Martino MC (1998) $GABA_B$ receptors and opioid mechanisms involved in homotaurine-induced analgesia. Gen Pharmacol 30:411–415

Shimada S, Cutting G, Uhl GR (1992) γ-Aminobutyric acid A or C receptor? γ-Aminobutyric acid ρ1 receptor RNA induces bicuculline-, barbiturate-, and benzodiazepine-insensitive γ -aminobutyric acid responses in *Xenopus* oocytes. Mol Pharmacol 41:683–687

Wu ZY, Xu TL (2003) Taurine-evoked chloride current and its potentiation by intracellular Ca^{2+} in immature rat hippocampal CA1 neurons. Amino Acids 24:155–161

Yamori Y, Taguchi T, Hamada A, Kunimasa K, Mori H, Mori M (2010) Taurine in health and diseases: consistent evidence from experimental and epidemiological studies. J Biomed Sci 17(Suppl 1):S1–S6

Yang J, Wu G, Feng Y, Lv Q, Lin S, Hu J (2010) Effects of taurine on male reproduction in rats of different ages. J Biomed Sci 17(Suppl 1):S1–S9

Zhang N, Ottersen OP (1992) Differential cellular distribution of two sulphur-containing amino acids in rat cerebellum. An immunocytochemical investigation using antisera to taurine and homocysteic acid. Exp Brain Res 90:11–20