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Research Paper

Comparative study of taurine and tauropyrone: GABA receptor binding, mitochondrial processes and behaviour

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Abstract

Objectives Taurine, a sulfur-containing amino acid, has high hydrophilicity and is poorly absorbed. Tauropyrone, a taurine-containing 1,4-dihydropyridine derivative, is suggested to have greater activity than taurine owing to improved physicochemical properties that facilitate delivery of the compound to target cells. The aim of this study was to determine whether the 1,4-dihydropyridine moiety in tauropyrone improves the pharmacological efficacy of taurine *in vitro* and *in vivo*.

Methods The effects of taurine and tauropyrone, as well as of the 1,4-dihydropyridine moiety were compared in in-vitro experiments to determine the binding to GABA receptors and influence on mitochondrial processes (isolated rat liver mitochondria), and in in-vivo tests to assess the influence on behavioural effects caused by the GABA-A receptor ligands, bicuculline, diazepam and ethanol.

Key findings Unlike taurine, tauropyrone did not display binding activity for the GABA-A receptor, and only taurine (but not tauropyrone) at low doses (0.1, 1.0 and 10 mg/kg) antagonised the bicuculline-induced convulsion effect. Taurine and tauropyrone had no effect on diazepam myorelaxing action, and they both exerted a comparable 'anti-ethanol' effect (shortening of the ethanol-sleeping time). Taurine and tauropyrone did not influence processes of mitochondrial bioenergetics.

Conclusions The action of tauropyrone at the level of the GABA-A receptor differs qualitatively from that of taurine, probably because of its 1,4-dihydropyridine moiety, which may hinder access to the GABA-A receptor GABA site. Tauropyrone does not show improved pharmacological efficacy in in-vitro and in-vivo studies in comparison with taurine.

Keywords 1,4-dihydropyridine; GABA receptor ligands; mitochondrial processes; taurine; tauropyrone

Introduction

Taurine (Figure 1) is an abundant and widely distributed sulfur-containing amino acid that is involved in diverse functions including modulation of calcium fluxes, membrane stabilization, neuronal excitability, osmoregulation, antioxidation, cell proliferation and immune responses.[1–3] Taurine is found in all cellular subcompartments, even in mitochondria.[4] Moreover, studies on mitochondrial tRNA have demonstrated the existence of taurine-modified uridine residues that are suspected to be processed inside the mitochondrial matrix, indicating the importance of taurine in normal mitochondrial function.[5]

Taurine is structurally similar to the inhibitory neurotransmitter γ-aminobutyric acid (GABA), and its binding to GABA-A and GABA-B receptors has been established.[6,7] In addition, numerous studies have shown that taurine exhibits inhibitory effects on neuronal transmission not only by potentiating GABA and glycine receptor functions, but also by inhibiting glutamate receptors and calcium channel functions.[8,9]

There are numerous studies that suggest that taurine participates in cell protection, but its mechanism of action is still unclear.[10] Problems arising from the clinical use of taurine have been attributed to its hydrophilicity, zwitterionic nature and poor absorption, which has necessitated the use of extremely high doses to achieve any clinical efficacy.[11] High doses

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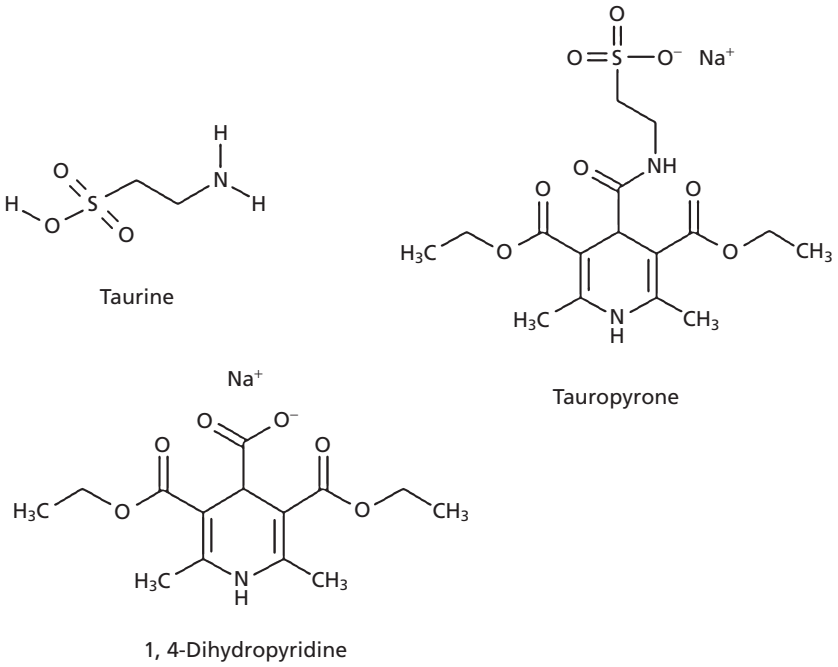

Figure 1 Chemical structures of taurine, tauropyrone and the 1,4-dihydropyridine moiety.

of up to 60,[12] 126,[13] and even 200 and 600 mg/kg[14] have been used in mice, while millimolar concentrations have been used in in-vitro experiments, for example 1 mM,[15] 20 mM[16] and 85 mM.[10]

To lower the effective dose of taurine and increase its therapeutic potential, various attempts have been made to improve its liposolubility and enhance its absorption by the synthesis of analogues.[17] For instance, tauropyrone, a taurine-containing 1,4-dihydropyridine (DHP) derivative (Figure 1), has been prepared by attaching taurine to the DHP ring at position 4 via a peptide-type bond.[18] The dihydropyridine carrier system for sustained delivery of drugs to the brain is well known.[19] In this context, we suggest that the DHP moiety (Figure 1) as a carrier molecule would facilitate taurine delivery to the brain and cellular targets, including mitochondria. Hence, tauropyrone should exert more pronounced effects at lower doses than taurine.

Tauropyrone has been shown to exhibit an anti-aggregant action at lower doses than taurine,[18] a memory-improving effect in alcoholised rats,[20] a cell-death protecting activity in oxygen-glucose deprivation and glutamate-, but not in MPP^+-neurotoxicity models in cerebellar granule cells,[21,22] and a dual (pro- and antiapoptotic and inflammatory) action in azidothymidine-induced cardiotoxicity and neurotoxicity models *in vivo*.[23] Beneficial effects of tauropyrone supplementation (0.5 g/kg per day for 7 and 14 days) have been shown to prevent the progression of inflammatory processes in both a Parkinson's disease 6-OHDA model in rats and in a microglial cell line *in vitro*.[24]

The aim of the present study was to determine whether the DHP moiety in tauropyrone improves the pharmacological efficacy of taurine *in vitro* and *in vivo*. We compared the effects of tauropyrone and its constituents (taurine and DHP) on the in-vitro binding to GABA receptors (0.1 μM–1 mM) and the influence on mitochondrial respiration at concentrations of 0.1 μM–10 mM. We also studied the influence of low doses (0.1, 1.0 and 10.0 mg/kg, i.p.) of the test compounds on behavioural effects specifically altered by GABA-A receptor ligands, bicuculline (GABA site antagonist), diazepam (benzodiazepine site agonist) and ethanol (modulatory site ligand), *in vivo* in mice.

Materials and Methods

Animals

For behavioural studies (and also for GABA-A receptor binding), male ICR mice (19–22 g) from the Laboratory of Experimental Animals (Riga Stradins University, Riga, Latvia) were used. For GABA-B receptor binding studies, Wistar rats (250–350 g) from the same animal breeding facility were used. For mitochondrial studies, male Wistar rats (250–350 g) from the Central Vivarium (Faculty of Medicine, University of Coimbra, Coimbra, Portugal) were used. Animals were housed under standard conditions (21–23°C, 12-h light–dark cycle) with unlimited access to standard food and water. For each test, separate animal groups were used.

All experimental procedures were carried out in accordance with guidelines of the Directive 86/609/EEC (European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes 1986) and were approved by National Ethics Committees.

Chemicals

Tauropyrone ((2-(2,6-dimethyl-3,5-diethoxycarbonyl-1,4-dihydropyridine-4-carbox-amido)-ethansulfo acid) and DHP (1,4-dihydroisonicotinoyl derivative corresponding to the dihydropyridine part of tauropyrone or sodium

3,5-bis-ethoxycarbonyl-2,6-dimethyl-1,4-dihydropyridine-4-carboxylate) were synthesized at the Latvian Institute of Organic Synthesis (Riga, Latvia). Other chemicals were obtained from Sigma (St Louis, MO, USA).

GABA binding experiments

All assays were performed in duplicate.

Brain membrane preparation and GABA-A receptor binding experiments

Membranes were prepared as described previously.[25] The membranes were incubated with 10 nM [^{3}H]GABA and the various dilutions (over a concentration range of 0.1 μM–1 mM) of taurine, tauropyrone or DHP in 50 mM TRIS/HCl + KCl buffer for 20 min at 4°C for the determination of specific binding. Non-specific binding was detected by using 10 mM GABA. After incubation, the suspensions were rapidly filtered and washed through Whatman GF/B filters. Filters were subjected to liquid scintillation counting using OptiPhase scintillation cocktail. The amount of bound radioactivity was counted for 1 min with a 1450 Microbeta Trilux (Wallac, Finland) liquid scintillation and luminescence counter.

Brain membrane preparation and GABA-B receptor binding experiments

A modified procedure for the preparation of GABA-B receptor was performed using rat brain membranes according to Bischoff *et al*.;[26] the use of hot ligand [^{3}H]CGP54626 has been described by Asay and Boyd.[27] In receptor binding assays, rat brain membranes (0.16 mg of protein per assay) were incubated with the GABA-B receptor-selective radioligand [^{3}H]CGP54626 (approx. 2 nM) and competing drugs, in 200 μl of KH buffer (pH 7.5) for 1.5 h at room temperature.[27] Non-specific binding for each assay was determined in the presence of 10 mM baclofen. The termination of binding assays was performed by filtering and washing on Whatman GF/C filters. Radioactivity was counted in the same way as in the GABA-A binding experiment.

Mitochondrial processes

Rat liver mitochondria were isolated by differential centrifugation according to conventional methods,[28] and the protein content was determined by the biuret method,[29] using bovine serum albumin as a standard.

Mitochondrial bioenergetics

Mitochondrial bioenergetics was evaluated by measuring the mitochondrial transmembrane potential using a TPP^+-selective electrode as previously described.[30]

Calcium-induced mitochondrial permeability transition

Mitochondrial permeability transition (MPT) was evaluated by measuring extramitochondrial $Ca^{[2+]}$ movement using the fluorescent calcium-sensitive probe Calcium Green 5-N.[31] Fluorescence was continuously recorded in a water-jacketed cuvette holder at 30°C, using a Perkin-Elmer LS-50B fluorescence spectrometer with excitation and emission wavelengths of 506 and 531 nm, respectively. Assays were performed in the presence of $Ca^{[2+]}$ at a concentration of 80 nmol/mg protein to evaluate the ability of the compounds (10 μmol/mg protein) to increase the susceptibility of mitochondria to MPT induction, and in the presence of $Ca^{[2+]}$ at a concentration of 120 nmol/mg protein to assess MPT protection by the studied compounds (at 1 μmol/mg protein). These concentrations were found to be appropriate and were tested in our previous studies.[32] Ciclosporine A was used as a specific inhibitor of MPT.[33]

Mitochondrial oxidative stress

Mitochondrial oxidative stress was evaluated by measuring hydrogen peroxide (H_2O_2) generation. It was measured fluorimetrically using a modification of the method previously described.[34] The fluorescence of supernatants was measured at an excitation wavelength of 312 nm and an emission wavelength of 420 nm. The peroxide generation was calculated using a standard curve of H_2O_2.

Behavioural tests in mice

All the tested drugs were dissolved in 0.9% saline. There were 8–10 animals in each group for each test.

Bicuculline-induced seizure test

Seizure threshold was determined according to the method described elsewhere.[35] The determination of seizure threshold was made by intravenous infusion (Syringe Infusion Pump, model SP100iZ; World Precision Instruments, Sarasota, FL, USA) of bicuculline (dissolved in 0.1 N HCl and diluted with isotonic saline to a final concentration of 0.1 mg/ml, pH 7) at a constant rate of 0.5 ml/min and by recording the bicuculline dose that caused the first myoclonic jerk of the head and neck. Seizure threshold was calculated as bicuculline dose per mg/kg bodyweight in the presence and absence of the test compound. Taurine, tauropyrone and DHP were administered intraperitoneally 60 min before bicuculline infusion.

Rota-rod coordination test

The rota-rod test was performed according to the method described elsewhere[36] by use of a rota-rod apparatus (model 7600; Ugo Basile, Comerio, Italy). On the day of the experiment, the compounds were injected intraperitoneally and then after 30 min diazepam (5.0 mg/kg, i.p.) or saline (vehicle, i.p.) were administered. After 30 min (i.e. 60 min after injection of the test compound), the mice were placed on the rotating rod (15 rev/min, diameter 3 cm) and time spent on it (fall latency) for each mouse was recorded for 180 s. For the control, drugs were administered without diazepam and mice were tested after 60 min.

Ethanol-sleeping time test

The sleeping time test was carried out according to the method described previously.[37] The tested compounds were administered intraperitoneally 60 min before administration of ethanol (4.0 g/kg 20% w/v in saline, i.p.) and the test compounds. When mice became ataxic, they were placed in the supine position and were not able to turn onto all four paws three times within 30 s. Sleeping time was defined as the

time (s) from mice being placed in the supine position until they regained their righting reflex.

Statistical analysis

The data were analysed with GraphPad Prism 5 software (GraphPad Software Inc., San Diego, CA, USA). All results are expressed as mean ± SEM. Statistical significance was evaluated using one-way parametric analysis of variance followed by Dunnett's multiple comparison test to assess statistical differences between experimental groups. Kruskal-Wallis test with Dunn's multiple comparison test was performed on data obtained from the bicuculline-induced seizure test. A significance level was set at $P < 0.05$.

Results

Binding ability to GABA receptors

Taurine, tauropyrone and DHP over a concentration range from 0.1 nM to 1 mM were tested for binding to the mouse forebrain membrane GABA-A receptor *in vitro* using a radioligand binding assay. GABA-A receptor GABA site ligand [^{3}H]GABA was used as labelled ligand. As seen in Figure 2a, tauropyrone and DHP failed to displace [^{3}H]GABA from the binding site, whereas taurine slightly competed for binding to the GABA site with an affinity of 10 μM.

Drugs at the above concentrations were also tested for binding to the rat forebrain membrane GABA-B receptor using the radioligand binding assay. The GABA-B receptor ligand [^{3}H]CGP54626 was used as the labelled ligand. As seen in Figure 2b, none of the compounds (taurine, tauropyrone and DHP) bound to the GABA-B receptor.

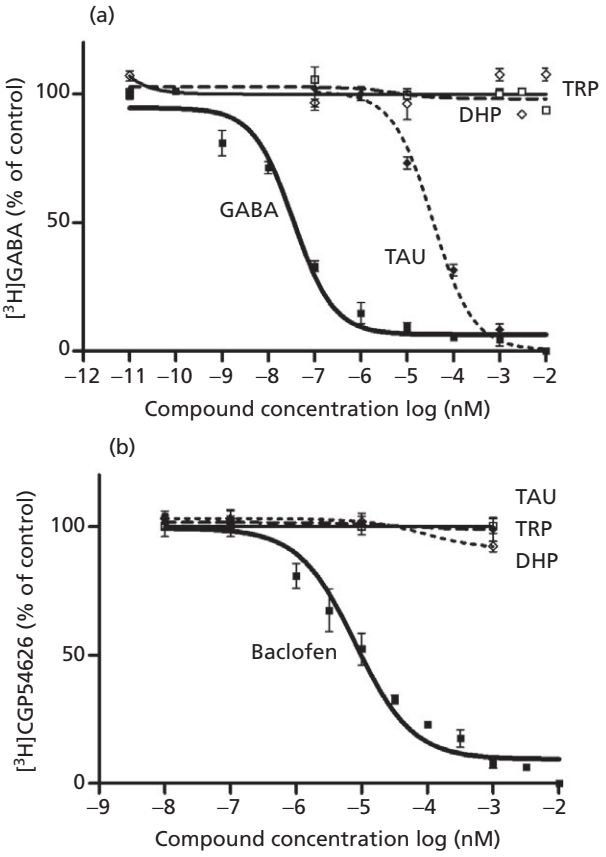

Figure 2 GABA receptor binding. Concentration–response relationship for taurine (TAU), tauropyrone (TRP) and 1,4-dihydropyridine (DHP) binding to: (a) the mouse forebrain membrane GABA-A receptor; and (b) the rat forebrain membrane GABA-B receptor. Each forebrain membrane was incubated with 10 nM [^{3}H]GABA (a) and ~2 nM [^{3}H]CGP54626 (b), and concentrations ranging from 0.1 nM to 1 mM of TAU, TRP and DHP. Non-specific binding was determined in the presence of 10 mM GABA (a) or 10 mM baclofen (b). Data are shown as mean ± SEM.

Influence on mitochondrial bioenergetics

Taurine and tauropyrone, over a concentration range of 1–10 μmol/mg protein did not affect mitochondrial transmembrane potential induced by glutamate/malate-supported respiration or the phosphorylation time (results not shown), thus indicating that these compounds did not affect rat liver mitochondrial bioenergetics.

To study drug influence on the induction of MPT we used an amount of $Ca^{[2+]}$ that by itself did not induced MPT (80 nmol/mg protein), whereas to study MPT protection we used an amount of $Ca^{[2+]}$ that did induce MPT (120 nmol/mg protein). Both types of assays were performed using succinate as respiratory substrate. As seen in Figure 3, taurine and tauropyrone did not increase the susceptibility of mitochondria to MPT (at 80 nmol/mg protein $Ca^{[2+]}$), nor did they protect mitochondria against MPT (at 120 nmol/mg protein $Ca^{[2+]}$).

Table 1 shows that taurine did not affect the generation of H_2O_2 by mitochondria under any of the experimental conditions.

Influence on bicuculline-induced seizures

The intravenous dose of bicuculline that induced the first myoclonic jerks was approximately 1.2 mg/kg. The data in Figure 4a demonstrate that taurine (at doses of 0.1, 1.0 and 10.0 mg/kg) administered intraperitoneally 60 min before bicuculline antagonised the bicuculline effect, which manifested as an requirement for an increased bicuculline dose to induce the first myoclonic jerks. Taurine was most active at 0.1 mg/kg, when the bicuculline dose needed to be doubled to induce clonic events. Intraperitoneal administration of tauropyrone at doses of 0.1–10 mg/kg did not influence the convulsive action of bicuculline. In turn, DHP administration at doses of 0.1 and 10 mg/kg (but not 1.0 mg/kg) lowered the bicuculline convulsive dose (Figure 4a). The test compounds by themselves did not show any convulsing activity during the pretreatment period of 60 min.

Influence on diazepam myorelaxing effect

The data shown in Figure 4b demonstrate that taurine, tauropyrone and DHP at the tested doses had no influence on muscle tone and coordination in the rota-rod test in mice, with the exception of taurine at a dose of 10 mg/kg, which had a tendency to prolong the diazepam fall latency (Figure 4b). The tested compounds alone did not cause any muscle relaxation (data not shown).

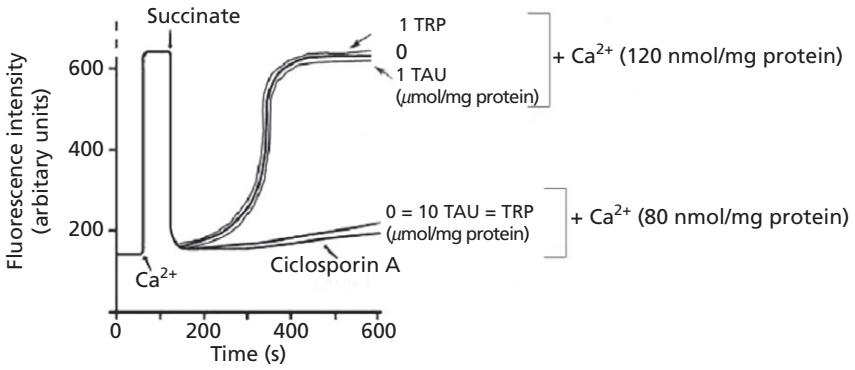

Figure 3 Mitochondrial bioenergetics. Effects of taurine (TAU) and tauropyrone (TRP) on their ability to increase the susceptibility of mitochondria to mitochondrial permeability transition (MPT) induction, and to protect mitochondria against MPT induction. The traces are typical recordings representative of three experiments obtained from different mitochondrial preparations. Ca^{2+} (80 or 120 nmol/mg protein), succinate and 1 μM ciclosporin A were added at the indicated times.

Table 1 Effect of taurine on H_2O_2 generation by rat liver mitochondria

Conditions	H_2O_2 (nmol/mg protein per 15 min)					
	Taurine (μmol/mg protein)					
	0	0.0001	0.001	0.01	0.1	1
No substrate	0.28 ± 0.10	0.25 ± 0.20	0.39 ± 0.21	0.42 ± 0.22	0.41 ± 0.25	0.50 ± 0.35
Substrate	0.35 ± 0.14	0.37 ± 0.14	0.32 ± 0.06	0.31 ± 0.06	0.25 ± 0.06	0.33 ± 0.06
Substrate + rotenone	0.45 ± 0.14	0.39 ± 0.14	0.42 ± 0.06	0.39 ± 0.01	0.34 ± 0.16	0.55 ± 0.18
Substrate + antimycin A	1.25 ± 0.62*	0.94 ± 0.36	0.89 ± 0.27	1.31 ± 0.84	0.99 ± 0.36	1.49 ± 1.02
Substrate + rotenone + antimycin A	0.90 ± 0.45	0.68 ± 0.28	0.70 ± 0.24	0.68 ± 0.28	0.58 ± 0.53	0.85 ± 0.06
Substrate + rotenone + antimycin A + catalase	0.14 ± 0.01	0.12 ± 0.04	0.15 ± 0.01	0.16 ± 0.01	0.11 ± 0.05	0.32 ± 0.24

The data correspond to the mean ± SEM of three independent experiments. *$P < 0.05$, significantly different compared with mitochondria respiring in either the absence (no substrate) or presence of glutamate/malate (substrate).

Influence on ethanol-sleeping time

As seen in Figure 4c, taurine at doses of 1.0 and 10 mg/kg showed a significant effect of shortening the ethanol-induced sleeping time. A similar effect was observed after treatment with tauropyrone (Figure 4c), however a tendency to exert a more expressed effect was observed at a dose of 10 mg/kg. DHP did not influence the effect of ethanol (Figure 4c). The test compounds alone did not induce sleep in animals during the 60-min pretreatment period.

Discussion

The first aim of this study was to clarify whether tauropyrone, a taurine-containing DHP analogue, would bind to GABA receptors either with similar or improved binding activity in comparison with taurine. Taurine had been previously been shown to possess good but variable (depending on animal species) affinity to GABA-A receptor ranging from 2.2 to 300 μM.[38] In the present study, taurine bound to the brain tissue membrane GABA-A receptor site in mice (Figure 2a) with an affinity of 10 μM (or $K_i = 1.013 \times 10^{-5}$ M), which agreed with literature data.[38] In contrast, tauropyrone and DHP did not display binding activity to the GABA-A receptor (Figure 2a). The present study also showed that taurine, tauropyrone and DHP did not bind the GABA-B receptor. Some studies have reported weak taurine binding (5.12–800 μM) using [^{3}H]baclofen from GABA-B receptor.[38] To date, the functional consequences of taurine binding to GABA-B receptors have not been established.[39] The inability of tauropyrone to bind with GABA receptors, particularly to the GABA-A receptor, indicates that joining of taurine to the DHP ring has altered the compound's binding properties, probably by changing the conformation that is necessary for effective binding to the GABA receptor.

In isolated liver mitochondria, both taurine and tauropyrone acted similarly (concentrations from 0.0001 to 10 μmol/mg of protein that correspond to 0.1 μM to 10 mM) and did not interfere with mitochondrial bioenergetics. Neither compound affected ATP synthesis (data not shown), and neither increased the susceptibility of mitochondria to low concentration (80 nmol/mg protein) $Ca^{[2+]}$-induced MPT, nor protected mitochondria against high (mitochondria damaging) concentration (120 nmol/mg protein) $Ca^{[2+]}$-induced MPT opening (Figure 3). It was found that taurine also did not influence the generation of H_2O_2 by mitochondria under our experimental conditions (Table 1), indicating that taurine did not affect the propensity of mitochondria to originate and/or exacerbate oxidative stress. Unfortunately, it was not possible to assess the effect of tauropyrone in this test, since this compound interfered with the fluorescence of the probe. Our findings are in agreement with other studies demonstrating

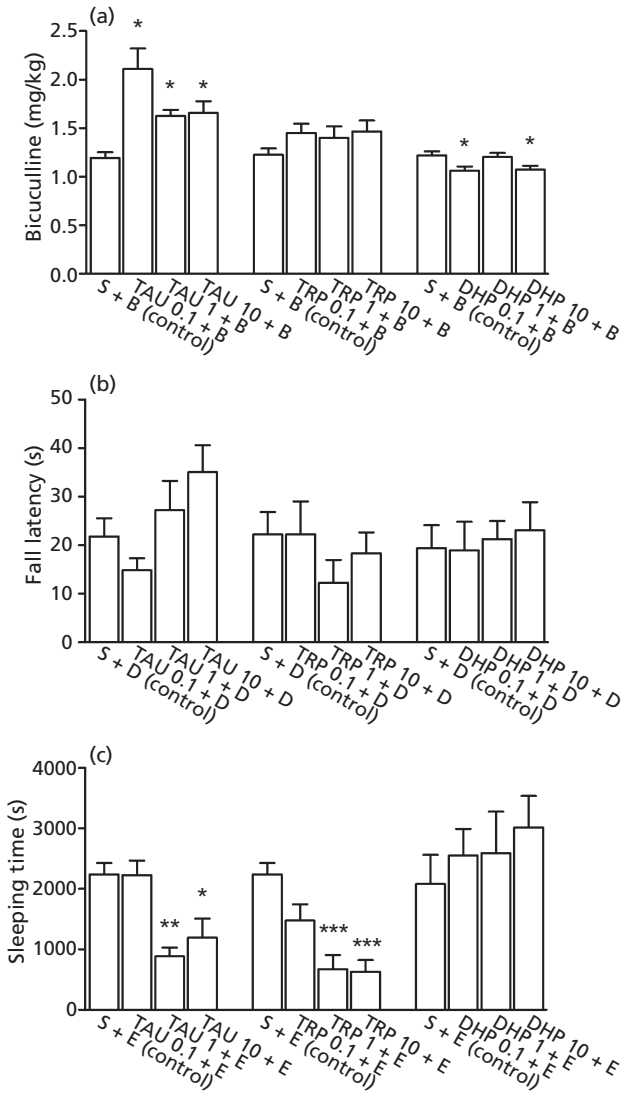

Figure 4 Behavioural tests. Influence of taurine (TAU), tauropyrone (TRP) and 1,4-dihydropyridine (DHP) on behavioural effects in mice caused by GABA-A receptor ligands. (a) Bicuculline-induced seizures. (b) Diazepam-induced myorelaxing effect in the rota-rod test. (c) Ethanol-induced sleeping time. TAU, TRP and DHP at doses 0.1, 1 and 10 mg/kg were administered intraperitoneally to mice 60 min before bicuculline (B; 0.1 mg/ml, i.v.) and ethanol (E; 4.0 g/kg 20% w/v, i.p) injections, and 60 min before the rota-rod test; diazepam (D; 5.0 mg/kg, i.p.) was administered 30 min after injection of test compounds. Control animals received saline (S) instead of TAU, TRP and DHP. The values are mean ± SEM ($n = 8$–10). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, significantly different compared with the corresponding control.

that taurine (0.1–10 mM) does not affect the MPT-induced hydroxyl radical production in isolated mitochondria,[40] and thus cannot scavenge classic reactive oxygen species directly.[41] Therefore, on the basis of the present work and other studies,[4–5,15,42] one may consider that mitochondria are not the target for taurine. It seems that complexing a DHP moiety to taurine (tauropyrone) did not change taurine properties in this respect. The latter observation is consistent with our previous data, which have demonstrated the inability of tauropyrone (up to 10 μM) to prevent free radical formation in cerebellar granule cells if MPP^+ is used as neurotoxic agent capable of damaging the mitochondrial complex I.[43]

We examined the influence of taurine, tauropyrone and DHP on the three ligands affecting different GABA-A receptor sites: bicuculline (GABA site competitive antagonist), diazepam (benzodiazepine site agonist) and ethanol (modulatory site ligand). In these studies, we used small doses of taurine and tauropyrone, such as 0.1, 1.0 and 10.0 mg/kg, which we considered more physiologically relevant than those used previously for taurine (100–200 mg/kg on average).[12–14,44] Previously, we found the active doses of tauropyrone to be 0.5, 5,[20] and 1 mg/kg.[23] The present study demonstrates for the first time that taurine, even at low doses such as 0.1 and 1 mg/kg, shows an anticonvulsant activity by antagonising bicuculline (Figure 4a), which is consistent with the ability of taurine to bind to the GABA-A receptor GABA site *in vitro*. These data, at least in part, indicate that low doses of taurine may penetrate the blood–brain barrier, contrary to previous studies[45,46] demonstrating rapid transport of taurine across the blood–brain barrier only at high doses. As for tauropyrone, which has no binding affinity for GABA-A receptor GABA site *in vitro*, it did not alter the effect of bicuculline (Figure 4a). DHP, which also did not bind to GABA-A receptor at doses of 0.1 and 10.0 mg/kg, showed only slight bicuculline-potentiating activity by reducing the bicuculline-convulsive dose, but this mechanism (convulsogenic) remains unclear.

In the rota-rod test, where diazepam was used as a myorelaxing test drug, taurine, tauropyrone and DHP did not alter the diazepam effect (Figure 4b), however, at a dose of 10.0 mg/kg, taurine showed a tendency to prolong the fall latency. It seems that a low ability (50 mM) to bind with the benzodiazepine receptor[47] may explain the lack of influence of taurine on the diazepam effect. A similar explanation could be attributed to tauropyrone. Although the pharmacological effects of taurine have been well studied, there remain contradictory findings related to its diazepam-like action. For instance, there are reports that taurine may produce a pronounced anxiolytic-like effect in the elevated plus-maze test in mice after a single or a repeated administration of a dose of 60 mg/kg, or doses of 14, 42 and 126 mg/kg (i.p.) in different behavioural tests.[13] In contrast, more recent data indicate that taurine does not affect behaviour in mice and hence do not support claims for the anxiolytic, antidepressant or stimulant activities of taurine.[48]

Contradictory data also exist in the literature about the influence of taurine on the effects of ethanol. On one hand, it was demonstrated that taurine administration reduces acute ethanol consumption by approximately 25–40% at doses of 50, 100 and 200 mg/kg,[49] which coincided with taurine's (10–40 mg/kg) beneficial influence on impaired motor coordination in alcohol-treated animals.[50] On the other hand, it has been reported that taurine (which alone does not induce sleep) injected intracerebroventricularly (7.5, 14.0 and 25.0 μmol/kg) in rats increases the duration of ethanol-sleep time,[51] while when injected peripherally, it attenuates ethanol sedating effects.[49]

Our data show for the first time that taurine at doses of 1.0 and 10 mg/kg (i.p), which are lower than those used

previously,[49] shortened ethanol-sleeping time by about 2 times (Figure 4c). A similar 'anti-ethanol' effect was found by tauropyrone, which at doses 1.0 and 10 mg/kg reduced the ethanol sleeping time by about 3–4 times (Figure 4c). The DHP moiety itself was inactive. The pronounced 'anti-ethanol' effect of taurine and tauropyrone at comparatively low doses is the most important finding arising from the present study, however the mechanism of action is still unclear. It could be suggested that these drugs interact with different ethanol-sensitive sites that are identified not only in heterogenic GABA-A receptor protein, but also in glutamatergic NMDA,[52,53] glycine,[54] 5-HT subtype 3A,[55] nicotinic acetylcholine,[56] and opiate[57] receptor proteins. It is not excluded that taurine and tauropyrone may influence calcium channels in an alternative manner directed at the ethanol-induced blocking of voltage-dependent calcium channels.[58]

Conclusions

A comparison of taurine and tauropyrone effects has revealed that the DHP moiety in the tauropyrone structure has qualitatively changed the action profile of taurine. First, unlike taurine, tauropyrone did not display binding affinity to the specific GABA-A receptor binding site. Second, taurine at low doses (0.1, 1.0 and 10 mg/kg), but not tauropyrone, was able to produce an anticonvulsant (anti-bicuculline) effect. Third, taurine and tauropyrone did not influence processes of mitochondrial bioenergetics, suggesting that mitochondria are not a target for their action. Fourth, both compounds showed a similar effect on behaviour induced by ligands of GABA-A receptor benzodiazepine and ethanol sites, such as diazepam and ethanol. The data indicate that the DHP ring added to taurine hinders its targeting of specific GABA receptor sites, and hence does not improve its pharmacological efficacy at the level of GABA-A receptor sites.

Declarations

Conflict of interest

The Authors declare that they have no conflicts of interest to disclose.

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