Amino Acids (1997) 13: 115–130

Amino
Acids

Analogues of taurine as inhibitors of the phosphorylation of an ~20K molecular weight protein present in a mitochondrial fraction of the rat retina

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Accepted August 23, 1996

Summary. It has been previously demonstrated that taurine inhibits the phosphorylation of an ~20K apparent molecular weight protein present in the mitochondrial fraction of the rat retina (Lombardini, 1991). In the present studies, various analogues of taurine were tested for their inhibitory activity on the phosphorylation of this ~20K protein. The most potent analogues were (±)*trans*-2-aminocyclopentanesulfonic acid (TAPS) and 1,2,3,4-tetrahydroquinoline-8-sulfonic acid (THQS) which were approximately 21 and 7 times more potent than the parent compound, taurine. Median-effect plots were used to calculate the inhibitory median effect and combination index values for the combined effects of taurine and taurine analogues. From these studies, it was determined that the inhibitory taurine analogues were antagonistic to taurine when used in combination with taurine to inhibit the phosphorylation of the ~20K apparent molecular weight protein. It was also concluded that: 1) the distance between the nitrogen and sulfur atoms in the taurine structure was important for inhibitory activity; 2) if the nitrogen atom is either within or attached to an unsaturated ring structure the inhibitory potency was significantly decreased, and 3) if both the sulfur and nitrogen atoms are present within the ring structure the analogue has no activity.

Keywords: Amino acids – Taurine – Taurine analogues – Protein phosphorylation – Rat retina

Introduction

While it is well documented that all mammalian tissues contain high concentrations of taurine (2-aminoethanesulfonic acid) and also that taurine is involved in a myriad of physiological actions in excitable tissues such as the retina, brain, and heart (Huxtable, 1992), the mechanism of action for this

amino sulfonic acid remains unknown. It has also been established that taurine is a necessary component of the visual system in a variety of species, including the rat, cat, monkey, and man (Lombardini, 1991). However, while pathologies due to taurine deficiency have been recorded, again the exact mechanism(s) as to the function of taurine is (are) elusive. There is considerable speculation that taurine may be a neurotransmitter or a neuromodulator, a modifier of membrane function or a membrane stabilizer by interacting with membrane phospholipids, a modulator of calcium ion fluxes, and a regulator of protein phosphorylation (Huxtable, 1992). With respect to this last possibility, i.e., a regulator of protein phosphorylation, our laboratory and others have been interested in the effects of taurine on the phosphorylation of specific proteins found in the retina (Lombardini, 1985; Liebowitz et al., 1988; Liebowitz et al., 1989; Lombardini, 1992ab; Lombardini, 1993), brain (Li and Lombardini, 1990, 1991ab; Sturman and Gargano, 1990), and heart (Lombardini and Liebowitz, 1989; Schaffer et al., 1990; Lombardini, 1992b; Lombardini, 1994).

The objective of the present study was to investigate analogues of taurine, specifically cyclic analogues, with regards to conformational requirements essential to their function as inhibitors of the phosphorylation of a specific ~20K apparent molecular weight (M_r) protein present in a mitochondrial fraction of the rat retina. We previously reported (Lombardini, 1993) that a taurine concentration of 34.2 ± 2.1 mM is required to inhibit the phosphorylation of this specific protein by 50% (IC_{50}). However, since the structure of taurine has no conformational restrictions, the preferred conformation that taurine assumes when it exerts its inhibitory effect is unknown. The two carbon atoms in the methylene component of taurine plus the amino and sulfonic acid moieties can rotate thereby potentially allowing taurine to assume either an extended configuration, a cyclic configuration, or a configuration somewhere between the two extremes. Because of the lack of rigidity in the taurine molecule various analogues of taurine and especially cyclic analogues of taurine that have restricted mobility of the amino and sulfonic acid moieties have been tested for inhibitory activity.

Materials and methods

Chemicals

[γ-^{32}P]ATP (30 Ci/mmol) was purchased from New England Nuclear Corporation. Taurine (TAU), alanine (ALA), β-alanine (BALA) and 3-amino-1-propanesulfonic acid (APrS) were purchased from Sigma Chemical Co. α-Sulfo-β-alanine (ASBA) was obtained from United States Biochemical Corporation. Aminomethanesulfonic acid (AMS), 2-aminobenzenesulfonic acid (ABS), and pyridine-3-sulfonic acid (PyS) were purchased from Aldrich Chemical Company and recrystallized from water. Isethionic acid (ISA) was obtained from Eastman Kodak Co. Quinoline-8-sulfonic acid (QS) was purchased from Lancaster Synthesis, LTD and recrystallized from water. Guanidinoethanesulfonic acid (GES) was synthesized according to the procedure of Morrison et al. (1958); (±)piperidine-3-sulfonic acid (PiP) was synthesized according to the procedure of Lombardini and Liebowitz (1990); 1,2,3,4-tetrahydroquinoline-

8-sulfonic acid (THQS) was synthesized according to the procedure of Lombardini et al. (1989); (±)*trans*-2-aminocyclopentanesulfonic acid (TAPS) was synthesized according to the procedure of Liebowitz et al. (1987); 2-aminoethylmethylsulfone (AEMS), (±)3-aminotetrahydrothiophene-1,1-dioxide hydrochloride (ATS), and thiomorpholine-1,1-dioxide hydrochloride (TMS) were synthesized according to the procedures of Liebowitz et al. (1989). All chemical solutions were made up in deionized-distilled water.

Preparation of the mitochondrial subcellular fraction

The mitochondrial fraction of the retinas was prepared as previously described (Lombardini, 1985). Briefly, 12 adult Wistar rats (175–225 g) were killed by decapitation. The eyes were immediately removed and placed in ice-cold 0.3 M mannitol, pH 7.4. The retinal tissue, maintained at ice temperatures for this and all subsequent procedures, was teased out of the eye cup and placed in 10 ml of the mannitol solution. The rod outer segments (ROSs) were removed by vortex-mixing the retina for 6 sec, allowing the tissue to settle, and then decanting (and discarding) the supernatant which contained the ROS. The remaining tissue components (contained in 10 ml of the mannitol solution) were gently hand-homogenized with 10 up-and-down strokes in a Potter-Elvehjem homogenizer and centrifuged at 150 g for 15 min to remove cell debris. The resulting supernatant was centrifuged at 12,500 g for 15 min and the pellet was suspended in the mannitol solution (2 ml). The tissue preparation was then layered on a discontinuous Ficoll gradient (8, 16, and 20% in 0.3 M mannitol) and centrifuged at 63,000 g for 1 hr in a swinging bucket rotor. The pellet at the bottom of the gradient contained mitochondria which were suspended in a bicarbonate buffer ($NaHCO_3$, 50 mM; NaCl, 50 mM; KCl, 50 mM; KH_2PO_4, 1.2 mM; $MgCl_2$, 2 mM; $CaCl_2$, 10 μM, pH 7.4) as described by Kuo and Miki (1980).

Phosphorylation assay and polyacrylamide gel electrophoresis (PAGE)

The incubation mixture containing bicarbonate buffer (above), mitochondrial fraction (~0.1 mg protein), and taurine or taurine analogue when indicated was preincubated for 2 min in a shaking water bath at 37°. The phosphorylation reaction was started by the addition of [γ-^{32}P]ATP (20 μCi, 10 μM). The system was incubated for 6 min which was determined to be in the linear time range for phosphorylation and the optimal time for the inhibitory effect of taurine. The reaction was then stopped by adding 0.3 ml of gel electrophoresis sample buffer [60 mM Tris-HCl (pH 6.8), 2% sodium dodecyl sulfate (SDS), 10% glycerol, 2 mM mercaptoethanol, and 0.00125% bromophenol blue] and immediately boiling for 5 min. Aliquots of the incubation mixture were subjected to PAGE on 12% gels according to the method of Laemmli (1970). The gels were dried and exposed to X-ray film to visualize the incorporation of radioactive phosphate into the various proteins. Quantitation of the amount of radioactive phosphate incorporated into the ~20 K M_r protein was determined by densitometry (BioRad scanning densitometer Model 1650).

Dose-effect analysis and the combined effects of analogues of taurine on the phosphorylation of an ~20 K M_r protein present in a mitochondrial fraction of the rat retina

The median-effect equation and its plot (Chou and Talalay, 1984, 1987) were used to calculate the median dose-effect of individual analogues of taurine and various analogues of taurine in combination with taurine:

$$\frac{fa}{fu} = \left(\frac{D}{D_m}\right)^m$$

or

$$\log(fa/fu) = m \log D - m \log D_m \tag{1}$$

where *fa* and *fu* are the decimal fractions of activities of the phosphorylation of an ~20 K M_r protein affected and unaffected by a dose (D) of a drug [For example: if a taurine analogue inhibits the phosphorylation of the ~20 K M_r protein by 20%, $fa = 0.2$ while $fu = 0.8$ ($fu = 1 - fa$)]. D_m is the median-effect dose (e.g., IC_{50}) and m signifies the shape of the dose-effect curve ($m = 1$, >1, and <1 indicate hyperbolic, sigmoidal, and negative sigmoidal curves, respectively). A plot (the median-effect plot) of $y = \log(fa/fu)$ versus $x = \log(D)$ gives a slope of m, and the antilog of the x-intercept gives the D_m value. The m and D_m parameters for each drug and their mixtures are substituted into the multiple drug effect equation of Chou and Talalay (1984; 1987) for mutually exclusive inhibitors:

$$\frac{(fa)_{1,2}}{(fu)_{1,2}} = \frac{(D)_1}{(D_x)_1} + \frac{(D)_2}{(D_x)_2} \tag{2}$$

where $(D_x)_1$ and $(D_x)_2$ are the doses of drug 1 and drug 2 that produce x% inhibition and at which $(D)_1$ and $(D)_2$ in combination also inhibit by x%. By definition, $fa + fu = 1$, ($fu = 1 - fa$). In the special circumstance in which $(fa)_{1,2} = 0.50$, the equation simplifies to the following equation:

$$\frac{(fa)_{1,2}}{(fu)_{1,2}} = \frac{(D)_1}{(D_m)_1} + \frac{(D)_2}{(D_m)_2} \tag{3}$$

and, therefore

$$\frac{(D)_1}{(D_m)_1} + \frac{(D)_2}{(D_m)_2} = 1 \tag{4}$$

Eq. 4 is the classical isobologram for ED_{50}, where $(D)_1$ and $(D)_2$ are fractions of the median-effect dose (50% of inhibition). For a general case, the combination index (CI) is defined by:

$$CI = \frac{(D)_1}{(D_x)_1} + \frac{(D)_2}{(D_x)_2} \tag{5}$$

where CI = 1, <1, and >1 indicates summation, synergism, and antagonism, respectively. The calculation of CI is carried out as follows. Rearrangement of Eq. 1 gives

$$D = D_m\left[fa/(1 - fa)\right]^{1/m} \tag{6}$$

Thus, when the parameters D_m and m are determined from the median-effect plot (see above), the dose (D or D_x) required for any degree of effect (fa) can be calculated. These calculated doses are then used in Eq. 5 for the calculation of CI to determine summation, synergism or antagonism. [Note: All the above equations and derivations have been developed by Chou and Talalay (1984, 1987)].

Results and discussion

A representative autoradiogram obtained from 1-dimensional PAGE demonstrates the inhibitory effects of various concentrations of taurine on the

phosphorylation of the ~20K M_r protein present in the mitochondrial subcellular fraction of the rat retina (Fig. 1).

Inactive analogues of taurine

Analogues of taurine that have demonstrated no inhibitory activity on the phosphorylation of the ~20K M_r protein present in the mitochondrial fraction of the rat retina are listed in Table 1 along with their structures. These compounds were tested at a maximum concentration of 60mM.

Active analogues of taurine

The concentrations of taurine and taurine analogues required to inhibit the phosphorylation of the ~20K M_r protein by 50% are reported in Table 2 (structures are shown in Fig. 2). Semi-reciprocal plots shown in Fig. 3 were used to calculate the concentration required to inhibit the phosphorylation of the ~20K M_r protein by 50% (IC_{50}). The IC_{50} for taurine was determined to be 33.2 ± 4.1mM. Aminomethanesulfonic acid (AMS), an analogue of taurine that has only a one carbon bridge between the amino and sulfonic acid

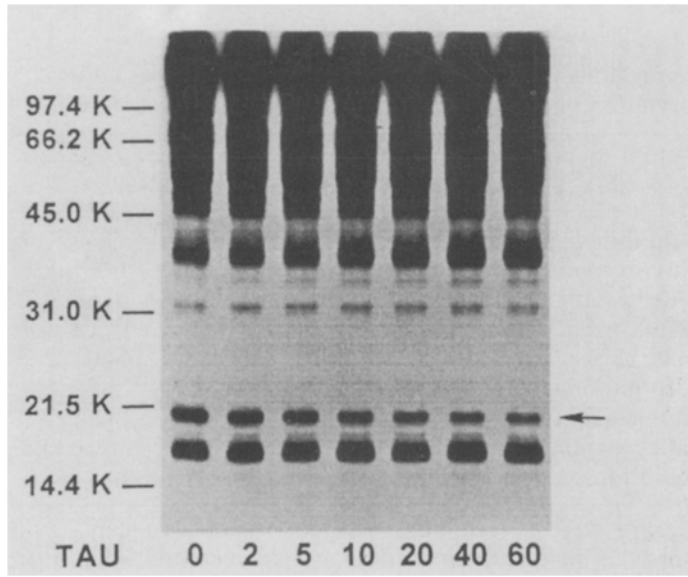
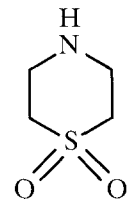


Fig. 1. Autoradiogram of the effects of taurine on the phosphorylation of an ~20K M_r protein present in a mitochondrial fraction of the rat retina. SDS-polyacrylamide gel electrophoresis and autoradiography of the phosphorylated proteins are described in the Materials and methods section. Arrow designates the location of the ~20K M_r protein whose phosphorylation is inhibited by taurine. Marker proteins with molecular weights ranging from 14,400 to 97,400 are indicated. *TAU* taurine; concentration range = 0–60mM. The results presented represent a single typical experiment of a total of 7 experiments

Table 1. Analogues of taurine that have no or only minimal effect on the phosphorylation of the ~20 K M_r protein present in a mitochondrial fraction of the rat retina

Compound	Structure
Alanine (ALA)	$H_2N\text{-}\underset{\displaystyle CH_3}{\underset{\vert}{CH}}\text{-}COOH$
β-Alanine (BALA)	$H_2N\text{-}CH_2\text{-}CH_2\text{-}COOH$
Isethionic acid (ISA)	$HO\text{-}CH_2\text{-}CH_2\text{-}SO_3H$
Guanidinoethanesulfonic acid (GES)	$H_2N\text{-}\overset{\displaystyle NH}{\overset{\Vert}{C}}\text{-}NH\text{-}CH_2\text{-}CH_2\text{-}SO_3H$
3-Aminopropanesulfonic acid (APrS)	$H_2N\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}SO_3H$
α-Sulfo-β-alanine (ASBA)	$H_2N\text{-}\underset{\displaystyle SO_3H}{\underset{\vert}{CH}}\text{-}CH_2\text{-}COOH$
Thiomorpholine-1,1-dioxide (TMS)	

Analogues were tested at a maximum concentration of 60 mM.

Table 2. Concentrations of taurine and taurine analogues required to inhibit the phosphorylation of the ~20 K M_r protein present in the rat retina by 50%

Compound	IC_{50}	(N)
Taurine (TAU)	33.2 ± 4.1^{a}	(6)
Aminomethanesulfonic acid (AMS)	43.6 ± 7.3^{ab}	(5)
Aminoethylmethylsulfone (AEMS)	47.4 ± 8.5^{b}	(5)
3-Aminotetrahydrothiophene-1,1-dioxide (ATS)	20.5 ± 3.7^{c}	(4)
(±)Piperidine-3-sulfonic acid (PiP)	19.1 ± 1.9^{c}	(7)
Pyridine-3-sulfonic acid (PyS)	44.0 ± 2.7^{ab}	(4)
1,2,3,4-Tetrahydroquinoline-8-sulfonic acid (THQS)	4.9 ± 1.0^{dc}	(4)
Quinoline-8-sulfonic acid (QS)	14.9 ± 2.0^{cc}	(7)
(±)*trans*-2-Aminocyclopentanesulfonic acid (TAPS)	1.6 ± 0.2^{d}	(6)
2-Aminobenzenesulfonic acid (ABS)	40.5 ± 5.6^{ab}	(4)

Statistical differences were determined by ANOVA and Duncan's multiple-range test ($P < 0.05$). Means with different superscripts are significantly different from each other. *N* number of experiments.

moieties, is a less effective (though not significantly different) inhibitor than taurine with an IC_{50} greater than 40 mM. Aminoethylmethylsulfone which has the sulfonic acid moiety of taurine replaced with a sulfone moiety is also less effective in inhibitory potency than taurine ($IC_{50} > 40$ mM).

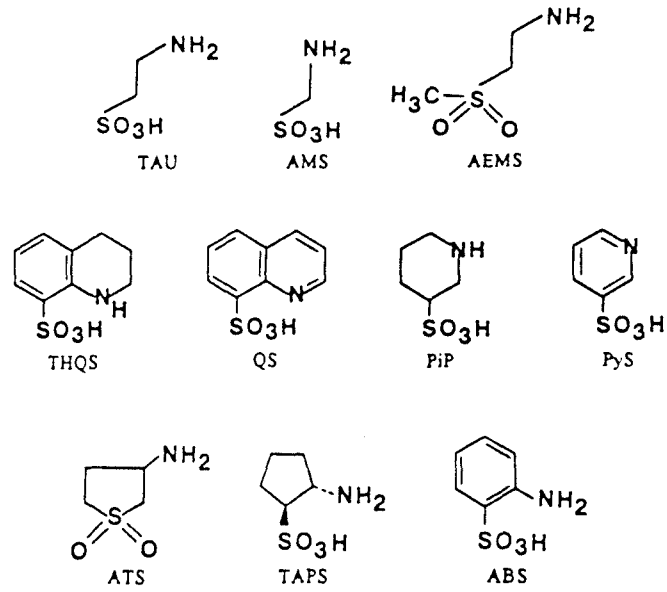


Fig. 2. Structures of taurine and the taurine analogues

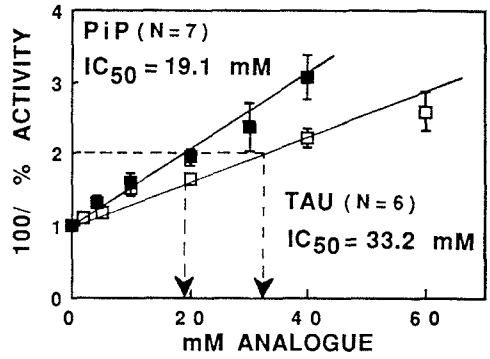


Fig. 3. Quantitation of the inhibitory effects (IC_{50}) of taurine and PiP on the phosphorylation of the ~20K M_r protein present in a mitochondrial fraction of the rat retina. Experimental conditions are described in the Materials and methods section. Data are presented as means ± S.E.M. Number of experiments is indicated in the parentheses

However, 3-aminotetetrahydrothiophene-1,1-dioxide (ATS), a cyclic sulfone analogue of taurine containing a primary amine and the sulfone moiety within a 5-membered ring structure, is more potent (IC_{50} = 20.5 ± 3.7mM) than the unrestricted sulfone analogue of taurine, 2-aminomethylsulfone (AEMS).

Cyclic sulfonic acid analogues of taurine have also been designed (Lombardini et al., 1989; Lombardini and Liebowitz, 1990) in which the nitrogen atom resides within the ring structure, i.e., a secondary amine, in either a saturated six-membered ring or an unsaturated six-membered ring. Two pairs of these analogues have been tested for their effects on the phosphorylation of the ~20K M_r protein. The analogues which contain the

aminoethane portion of taurine within their ring structure and are thus semi-rigid compared to free taurine are the following: (±)piperidine-3-sulfonic acid (PiP) and pyridine-3-sulfonic acid (PyP); 1,2,3,4-tetrahydroquinoline-8-sulfonic acid (THQS) and quinoline-8-sulfonic acid (QS). (±)Piperidine-3-sulfonic acid (PiP), the saturated taurine analogue (IC_{50} = 19.1 ± 1.9 mM), is approximately twice as potent as pyridine-3-sulfonic acid (PyS), the unsaturated analogue (IC_{50} = 44.0 ± 2.7 mM).

The quinoline analogues of taurine have the sulfonic acid on the unsaturated ring while the nitrogen atom is either in the adjacent saturated ring [1,2,3,4-tetrahydroquinoline-8-sulfonic acid (THQS)] or unsaturated ring [quinoline-8-sulfonic acid (QS)]. The inhibitory effect of THQS (IC_{50} = 4.9 ± 1.0 mM) is approximately 3 times more effective as a phosphorylation inhibitor than QS (IC_{50} = 14.9 ± 2.0 mM).

(±)*Trans*-2-aminocyclopentanesulfonic acid (TAPS), a five-membered cycloalkane analogue of taurine with both the amino and sulfonic acid moieties external to the ring is the most potent analogue yet tested (IC_{50} = 1.6 ± 0.2 mM) as an inhibitor of the phosphorylation of the ~20 K M_r protein. On the contrary, 2-aminobenzenesulfonic acid (ABS), an unsaturated six-membered ring analogue has very little inhibitory activity with an IC_{50} = 40.5 ± 5.6 mM.

Median-effect plots for combinations of taurine with various analogues

Median-effect plot parameters and combination index values were determined by analysis of the dose-effect relationships for the combined effects of taurine and a taurine analogue. These data calculated by the median-effect plot and accompanying formulas which were developed by Chou and Talalay (1984, 1987) are reported in Table 3. In this study, taurine was combined in a constant ratio with a taurine analogue that was shown to possess inhibitory activity (Table 2).

The only combination that was determined to be additive by the quantitative analysis of the dose-effect relationships was taurine plus THQS in the ratio of 8:1 [Fig. 4A (top panel) and 4B (bottom panel), Table 2]. The combination index value for the effect of 30, 50, and 70% saturation were 1.05, 1.04, and 1.05. Also, it was determined from the median-effect plot that the slopes for taurine, THQS, and taurine plus THQS were approximately 1 indicating that the inhibitors of the phosphorylation of the ~20 K M_r protein behave in agreement with Michaelis-Menton kinetics (i.e., hyperbolic plots or first-order kinetics). The parallel nature of the median-effect plots of taurine, THQS, and taurine plus THQS suggest that the two inhibitors are mutually exclusive, i.e., have a similar mode of action at their binding site and consequently are competitive with each other.

The graphical representations of the data for the combination of taurine plus ATS are shown in Fig. 5A and 5B. The combination index value (1.29) for taurine plus ATS (1:1) indicates that the combination is slightly antagonist at 30% saturation but becomes more antagonistic at 50 and 70% saturation (CI values = 1.36 and 1.44). The slopes for taurine, ATS, and the combination

Table 3. Median-effect plot parameters and combination index values for taurine and taurine analogues on the phosphorylation of the ~20K M_r protein present in a mitochondrial fraction of the retina and their interactions upon combination

Compounds	Median-effect plot parameters[a]			Combination index values[b]		
	Median-effect concentration (D_m)	Slope (m)	Linear correlation coefficient (r)	30% saturation	50% saturation	70% saturation
	mM					
Taurine	33.2	0.823	0.989			
TAPS	1.6	2.598	0.974			
Taurine + TAPS (10:1)	20.3	0.848	0.946	1.14	1.68	2.75
ABS	45.2	3.817	0.999			
Taurine + ABS (1:1)	Antagonistic – no inhibitory effect					
PiP	21.4	0.814	0.976			
Taurine + PiP (1:1)	46.8	0.953	0.940	2.08	1.80	1.56
PyS	52.0	1.197	0.959			
Taurine + PyS (1:1)	113	1.045	1.000	3.11	2.80	2.58
THQS	6.2	0.961	0.921			
Taurine + THQS (8:1)	23.4	0.872	0.993	1.05	1.04	1.05
QS	13.3	1.160	0.977			
Taurine + QS (2:1)	67.0	0.777	0.903	2.43	3.03	3.85
ATS	21.7	1.192	0.934			
Taurine + ATS (1:1)	35.8	0.952	0.930	1.29	1.36	1.44

[a] The median-effect plot parameters, D_m, m, and r, signify the potency, the shape of the dose-effect curve, and the applicability of the median-effect principle, respectively, as described by Chou and Talalay (1984, 1987). [b] Combination index values <1, =1, and >1 indicate synergism, summation, and antagonism, respectively.
The values were calculated from pooled data obtained from four or five experiments.

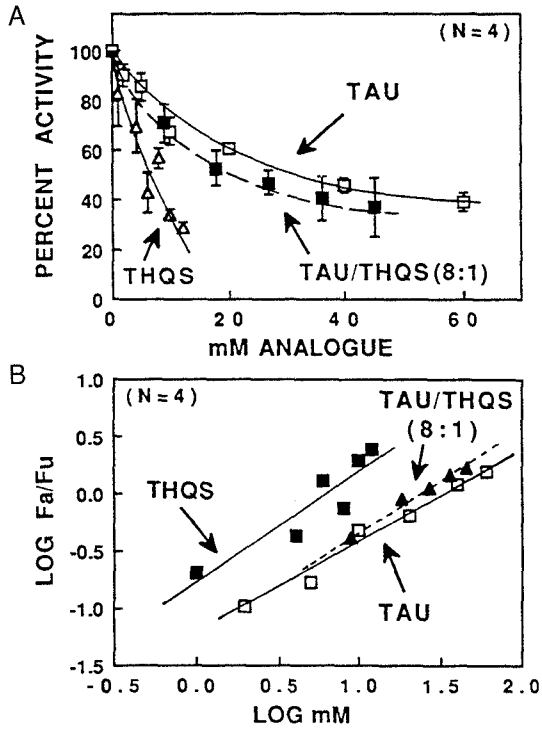


Fig. 4. Quantitation of the combined inhibitory effects of taurine plus THQS on the phosphorylation of the ~20K M_r protein present in a mitochondrial fraction of the rat retina. The top panel (**A**) indicates the concentration-response effects of taurine, THQS, and the combined effects of taurine plus THQS in a ratio of 8:1. The bottom panel (**B**) depicts the median-effect plots for quantitation of the combined effects of taurine plus THQS. Experimental conditions are described in the Materials and methods section. Data are presented as means ± S.E.M. Number of experiments is indicated in the parentheses

taurine plus ATS were observed to be approximately parallel indicating that these compounds most likely have a similar mechanism of action in inhibiting the phosphorylation of the ~20K M_r protein.

The combination of taurine and PiP (Fig. 6A and 6B) is highly antagonistic at 30% saturation (CI = 2.08), but the antagonism decreases as the saturation increases to 50 and 70% (CI = 1.80 and 1.56). On the contrary, antagonism increases with saturation (CI = 2.43, and 3.03, and 3.85) for the combination of taurine plus QS (Fig. 7A and 7B). Both analogues of taurine, PiP and QS, when used in combination with taurine appear to be mutually exclusive as demonstrated by the relatively parallel nature of the median-effect plots (Figs. 6B and 7B).

Aminobenzenesulfonic acid (ABS), while a very weak inhibitor of the phosphorylation of the ~20K M_r protein, has no effect (Fig. 8) when tested in combination with taurine (1:1 combination). Thus, combination index values cannot be calculated, but obviously this is an antagonistic combination with taurine. The slope for the inhibitory effects of ABS as determined from the median-effect plot is 3.817 which indicates that the kinetics of the inhibition

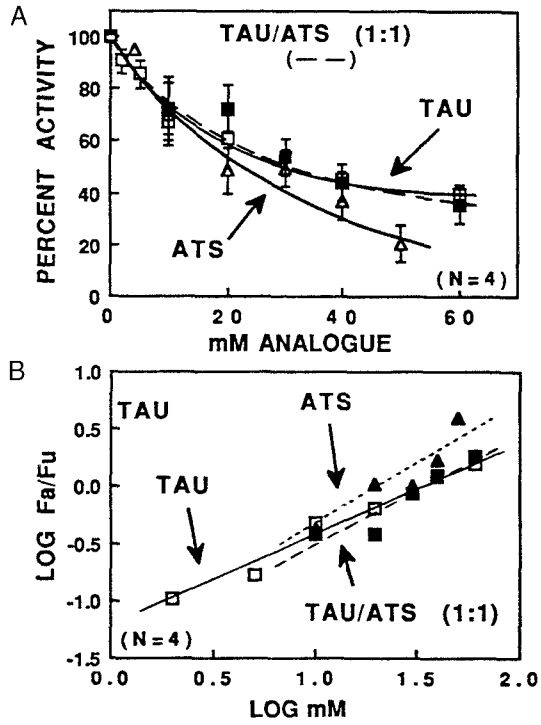


Fig. 5. Quantitation of the combined inhibitory effects of taurine plus ATS on the phosphorylation of the ~20K M_r protein present in a mitochondrial fraction of the rat retina. See legend to Fig. 4 for details

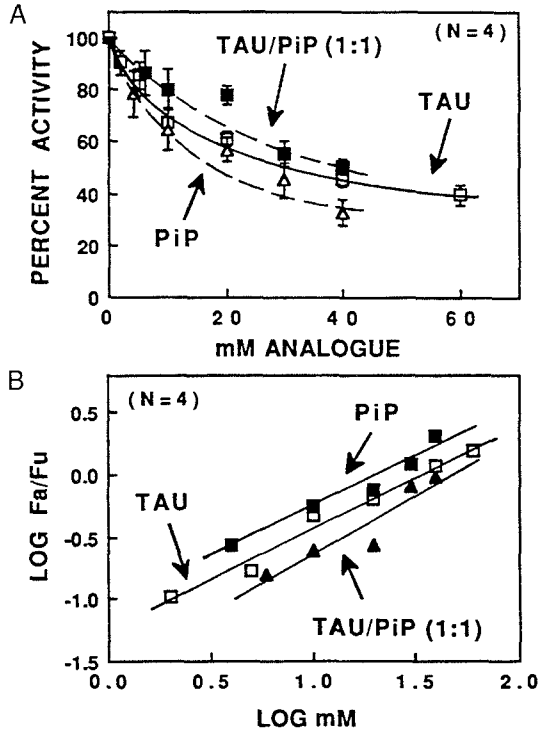


Fig. 6. Quantitation of the combined inhibitory effects of taurine plus PiP on the phosphorylation of the ~20K M_r protein present in a mitochondrial fraction of the rat retina. See legend to Fig. 4 for details

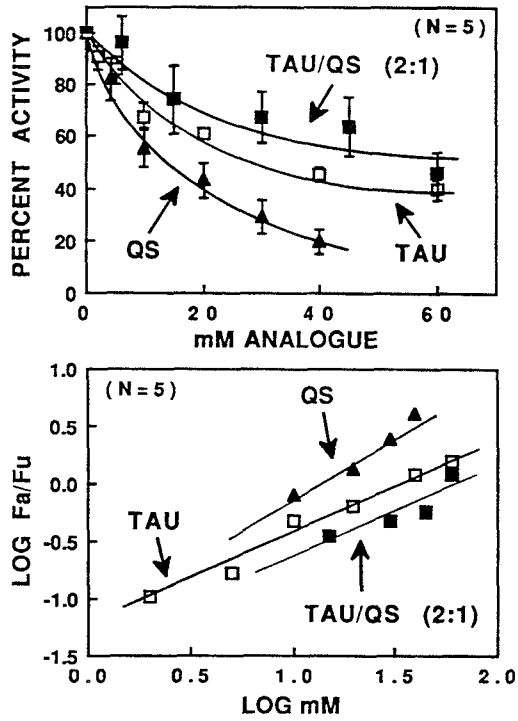


Fig. 7. Quantitation of the combined inhibitory effects of taurine plus QS on the phosphorylation of the ~20K M_r protein present in a mitochondrial fraction of the rat retina. See legend to Fig. 4 for details

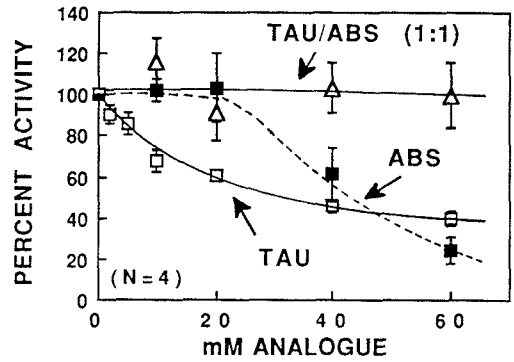


Fig. 8. Concentration-response effects of taurine, ABS and the combined inhibitory effects of taurine plus ABS on the phosphorylation of the ~20K M_r protein present in a mitochondrial fraction of the rat retina

do not behave according to Michaelis-Menten predictions ($m \neq 1$). Furthermore, since the slopes for taurine and ABS are not parallel mutual exclusivity (similar mode of action at the binding site, i.e., competitive relationship) and mutual nonexclusivity (actions independent of each other, i.e., noncompetitive relationship) can not be determined with the present data.

PyS, the non-hydrogenated (unsaturated-ring) analogue of PiP, was determined to be a weak inhibitor (Fig. 9A and 9B) with a median-effect (D_m) value of 52.0 mM. The combination index values for PyS and taurine are 3.11, 2.80, and 2.58 for 30, 50, and 70% saturation which indicate that the combination is highly antagonistic. The slopes for both PyS and the combination PyS plus taurine are approximately 1 and, therefore, parallel to the slope obtained for taurine. Consequently, the inhibitors, PyS and TAU, appear to have a similar mechanism of action (i.e. mutually exclusive).

The most potent analogue of taurine thus far tested for its inhibitor effect on the phosphorylation of the ~20K M_r protein present in the mitochondrial fraction of the rat retina is (±)*trans*-2-aminocyclopentanesulfonic acid (TAPS) (Fig. 10A and 10B). The median-effect plot shows a slope of 2.598 for TAPS (Fig. 10B) which is indicative of higher order kinetics and, therefore, not Michaelis-Menten or first-order kinetics. Also, since the slopes for TAPS and taurine as observed in the median-effect plot are not parallel while the slope for the combination taurine plus TAPS is parallel to taurine alone, it cannot be definitively determined whether the two compounds have similar mechanisms of action, i.e., whether they are mutually exclusive or mutually non-exclusive. It appears that at 30% saturation for the combination of taurine and TAPS at a ratio of 10:1 the relationship is probably additive (CI = 1.14)

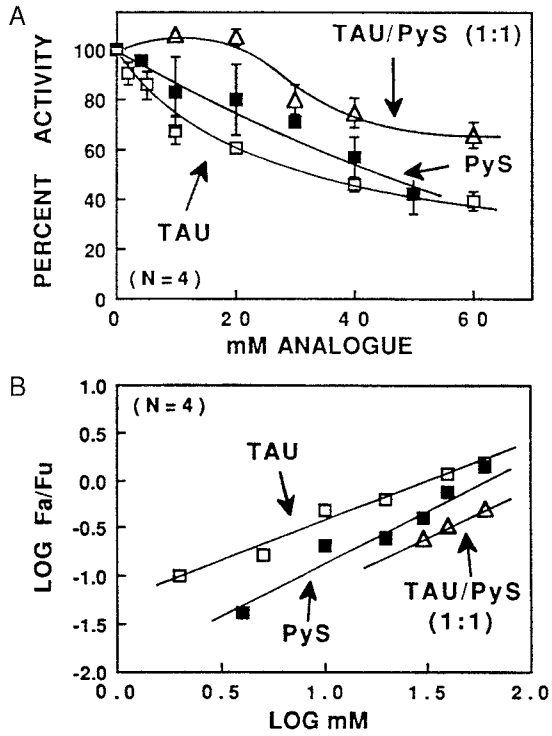


Fig. 9. Quantitation of the combined inhibitory effects of taurine plus PyS on the phosphorylation of the ~20K M_r protein present in a mitochondrial fraction of the rat retina. See legend to Fig. 4 for details

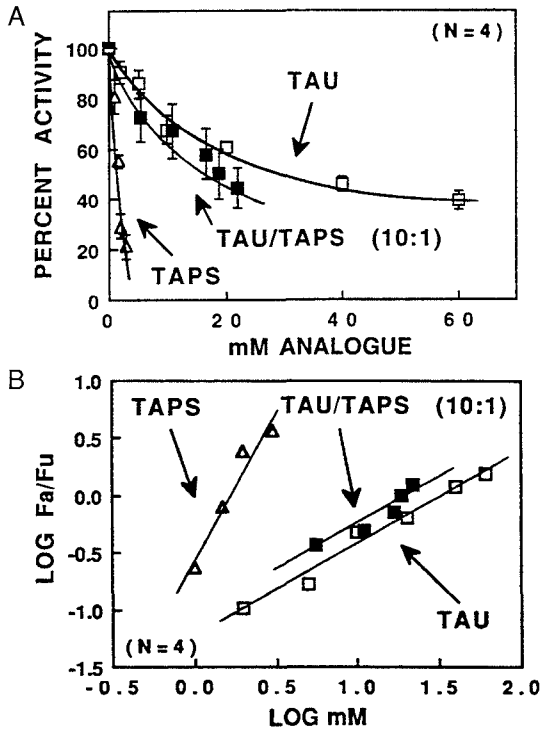


Fig. 10. Quantitation of the combined inhibitory effects of taurine plus TAPS on the phosphorylation of the ~20K M_r protein present in a mitochondrial fraction of the rat retina. See legend to Fig. 4 for details

whereas at 50 and 70% saturation the CI values increase to 1.68 and 2.75 which are clearly antagonistic.

The relative inhibitory potency of each of the semirigid cyclic analogues of taurine that were tested indicates three structural parameters that are important for inhibiting the phosphorylation of the ~20K M_r protein. First, the distance between the nitrogen and sulfur atom is critical. In Dreiding models of the structures of the taurine analogues, the distance in arbitrary units between the nitrogen and sulfur atoms of the two most potent compounds is 8.5 for TAPS and 7.8 for THQS. However, the distance between these two atoms in PiP and ATS, analogues which are considerably less potent, is 10.2 and 10.0. Second, if the nitrogen atom is either within or attached to an unsaturated ring, the inhibitory potency is significantly decreased such as is the case for the first compound in the following pairs of analogues: *QS* vs. THQS, *PyS* vs. PiP, and *ABS* vs. TAPS. The activity of the unsaturated-ring analogues QS and PyS is reduced by approximately 50% when compared to the saturated analogues THQS and PiP. ABS is approximately 28 fold less active than TAPS. (However, the comparison between ABS and TAPS is not completely valid in that ABS is a six-membered cyclic compound while TAPS is a 5-membered cyclic compound.) It should be noted that QS, PyS and ABS have a net negative charge at physiological pH and thus are quite different electronically than their saturated counterparts (THQS, PiP, and TAPS).

The results obtained with QS, PyS and ABS on the inhibition of the phosphorylation of the ~20K M_r retinal protein are considerably different from those obtained for cardiac tissue in which the phosphorylation of an ~44K M_r protein is stimulated by the unsaturated taurine analogues while inhibited by the saturated compounds (PiP and TAPS) (Lombardini, 1994). THQS, a taurine analogue which contains both a saturated and unsaturated ring, is biphasic in the cardiac system in that at low concentrations (4–10mM) it stimulated the phosphorylation of the ~44K M_r protein but at high concentrations (20–40mM) it was inhibitory. Third, if both the sulfur and nitrogen atoms are included within the ring structure, the inhibitory capabilities are negated. For example, thiomorpholine-1,1-dioxide (TMS) was demonstrated to have no activity in inhibiting the phosphorylation of the ~20K M_r protein (Table 1).

Finally, it can be concluded from the presented data that the analogues of taurine are, in general, antagonistic to taurine when used in combination with taurine to inhibit the phosphorylation of an ~20K M_r protein present in the rat retina.

Acknowledgements

Mrs. Josie L. Aleman and Ms. Tonia Borrego are gratefully acknowledged for their secretarial assistance. These studies were supported by grants from the RGK Foundation of Austin, Texas, and the Taisho Pharmaceutical Co., Ltd. of Tokyo, Japan.

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Received May 26, 1995