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SULFONE ANALOGUES OF TAURINE AS MODIFIERS OF CALCIUM UPTAKE AND PROTEIN PHOSPHORYLATION IN RAT RETINA

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Abstract—The syntheses of five sulfone analogues of taurine are described: 2-aminoethylmethyl sulfone (AEMS), thiomorpholine-1,1-dioxide (TMS), *N*-methylthiomorpholine-1,1-dioxide (M-TMS), (±)3-aminotetrahydrothiopyran-1,1-dioxide (APS), and (±)3-aminotetrahydrothiophene-1,1-dioxide (ATS). When these compounds were evaluated in the rat retina as modulators of ATP-dependent calcium ion uptake at low calcium ion concentrations (10 μM), AEMS, ATS, and APS were found to be more potent stimulators of ATP-dependent calcium ion uptake than taurine. TMS and M-TMS had no effect. At high concentrations of calcium ions (1.44 mM), taurine, AEMS, ATS, APS, and TMS inhibited ATP-independent calcium ion uptake; AEMS, ATS, and APS were more potent inhibitors than taurine. ATS was the only compound tested (including taurine) that inhibited ATP-dependent calcium ion uptake at high calcium ion concentrations. The effects of the sulfone analogues of taurine on the incorporation of phosphate into retinal proteins were also studied. Taurine, AEMS, ATS, APS, and TMS were equipotent inhibitors of phosphate incorporation (30–45%). M-TMS had no effect.

The role of taurine in the central nervous system is unknown. However, there are numerous suggestions that, in the brain, taurine is either a neurotransmitter [1–4] and/or a neuromodulator and may affect (1) interactions (uptake and release) with neurotransmitters or their receptors [5–7], (2) suppression of neuronal activity by stabilizing excitable membranes [8], or (3) regulation of intraterminal calcium levels [5, 6, 9–11]. In the retina, specifically in the amacrine cells, it has been proposed that taurine may be an inhibitory neurotransmitter released by light or depolarizing stimuli [12]. Taurine also appears to be important for retinal membrane integrity by preventing both lipid peroxidation in photoreceptors and membrane destabilization [13, 14].

The necessity for dietary taurine for normal retinal morphology and function has been demonstrated in a number of animal species including human. Taurine deficiency has resulted in severe retinal dysfunction in the cat [15], rat [16], and monkey [17]. In addition, Geggel and associates [18] have reported that children demonstrate abnormal electroretinograms when receiving long-term parenteral nutrition deficient in taurine.

At the molecular level taurine appears to influence the binding and transport of calcium ions in retinal membrane preparations. Taurine stimulates ATP-dependent calcium ion uptake in bicarbonate buffers at micromolar concentrations of calcium [19–21], whereas at millimolar concentrations of calcium taurine inhibits the ATP-independent uptake of calcium [22].

Taurine also inhibits the phosphorylation of specific retinal proteins [23]. Analogues of taurine that stimulate ATP-dependent calcium ion uptake inhibit protein phosphorylation [23]; analogues that have no effect on calcium ion uptake likewise have no effect on protein phosphorylation [23]; and analogues that inhibit calcium ion uptake stimulate protein phosphorylation [24, 25]. These results suggest a causal relationship between protein phosphorylation and calcium ion uptake; inhibition of membrane phosphorylation may be a mechanism by which taurine affects ATP-dependent calcium ion uptake [26] or vice versa. In addition, the hypothesis that taurine may be a membrane stabilizer, initially proposed by Huxtable and Bressler [27] and restated by Huxtable and Sebring [28], is further supported by the recent report that taurine affects the transition temperature and activation energy of Arrhenius profiles of ATP-dependent calcium ion uptake [29].

The aim of the present study was to examine a series of sulfone analogues since it has been reported that 6-(aminomethyl)-3-methyl-4*H*-1,2,4-benzothiadiazine-1,1-dioxide (TAG) is a taurine antagonist [30]. In this study five sulfone analogues of taurine were compared to taurine with regard to their abilities to affect calcium ion uptake at low and high calcium concentrations and for their effects on protein phosphorylation.

MATERIALS AND METHODS

Materials. Taurine was purchased from the Sigma Chemical Co. ^{45}Calcium chloride (25 mCi/mg) was

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purchased from New England Nuclear. [γ-^{32}P]ATP (25 Ci/mmol) was purchased from ICN Radiochemicals.

Preparation of membrane homogenate. Retinas obtained from adult Wistar rats were homogenized in bicarbonate buffer-A [$NaHCO_3$ (50 mM), KCl (50 mM), NaCl (50 mM), KH_2PO_4 (1.2 mM), $MgCl_2$ (2 mM), and ouabain (50 μM) (pH 7.4)] for calcium ion uptake experiments at low calcium ion concentration (10 μM) as described previously by Lombardini [23]. For calcium ion uptake experiments utilizing high calcium ion concentrations (1.4 mM), bicarbonate buffer-B [NaCl (118 mM), KCl (4.7 mM), $MgSO_4$ (1.4 mM), KH_2PO_4 (1.2 mM), $NaHCO_3$ (25 mM), glucose (5.6 mM), gassed with 95% O_2/5% CO_2, (pH 7.4)] was utilized. Protein was determined by the procedure of Lowry *et al.* [31] with bovine serum albumin as the standard.

Calcium ion uptake assay. In general, the uptake of $^{45}Ca^{2+}$ by the retinal membrane preparation was measured as previously described [23]. The incubation system contained either bicarbonate buffer-A or bicarbonate buffer-B (depending upon the calcium ion concentration), $^{45}Ca^{2+}$ (0.5 μCi), calcium chloride (10 μM or 1.4 mM), ± ATP (1.2 mM), and retinal preparation (~0.07 to 0.2 mg protein). The reaction was started after a 2-min preincubation period (37°) with the addition of the membrane preparation, incubated for a specified time period (usually 1 to 1.5 min), and then stopped by collection of the membrane homogenate which contained the $^{45}Ca^{2+}$ on Whatman GF/B filters.

The buffer systems utilized in the following experiments did not contain ethyleneglycolbis(aminoethylether)tetra acetate (EGTA), a calcium ion chelator that has been used to maintain the free calcium ion concentration at a contrast level. However, in a preliminary experiment, we compared the specific activity of the retinal preparation for its ability to take up calcium ions in the presence and absence of EGTA. The quantity of EGTA required to maintain the free calcium ion concentration at 10 μM was calculated by the method of Katz *et al.* [32]. The results indicated that taurine stimulates calcium ion uptake in buffer-A by approximately 2-fold regardless of the presence or absence of EGTA. However, in the presence of EGTA, the ATP-dependent calcium ion uptake activity was approximately 35% lower than in its absence (1.24 ± 0.02 vs 1.95 ± 0.08 nmol/mg/min). Similarly, the ATP-dependent taurine-stimulated activity was reduced by 35% in the presence of EGTA (2.51 ± 0.03 vs 3.87 ± 0.07 nmol/mg/min).

Phosphorylation of proteins in the membrane preparation. The incorporation of [$^{32}PO_4$] into retinal membrane proteins was determined as previously described [23].

Synthesis of (±)3-Aminotetrahydrothiophene-1,1-dioxide hydrochloride (ATS). A degassed solution of sulfolene (10.8 g, 91.4 mmol) in dry methanol (102 ml) at 0° was saturated with ammonia (22.7 g, 2.34 mol). The vessel was capped, and the solution was heated to 55° and stirred for 14 days. Concentration of the reaction mixture *in vacuo* produced a semi-solid material (12 g, 98% crude). One gram of the crude material was eluted on 50 g silica (methylene chloride:methanol, 30:1) which after concentration under high vacuum (0.01 mm Hg) for 24 hr yielded 0.95 g (95%) of pure amine: m.p. 79–80°. ^{1}H NMR (D_2O) δ2.35 (m,1H,CH_2), 2.80 (m,1H,CH_2), 3.20–3.90 (m,4H,CH_2S), 4.20 (m,1H,HCN); IR (KBr) 3420 (N—H), 1320 (sulfone), 1120 (sulfone) cm^{-1}. HCl salt appears as white needles from ethanol: m.p. 218–219° [lit. 33, m.p. 220°].

Synthesis of thiomorpholine-1,1-dioxide hydrochloride (TMS). A solution of 70% H_2O_2 (1.5 g, 31.2 mmol) in acetic acid (5 ml) was added dropwise with stirring to a cooled (0°) solution of thiomorpholine hydrochloride (1.45 g, 10.4 mmol) in acetic acid (10 ml). The mixture was heated gradually to 100° while stirring continued. After the complete disappearance of sulfoxide as detected by thin-layer chromatography, the reaction mixture was concentrated *in vacuo*. The resultant viscous oil was triturated in ethanol to produce a white precipitate. Recrystallization from ethanol produced 1.6 g (90%) of TMS as white needles: m.p. 240° (dec) [lit. 34, m.p. 239° (dec)]. ^{1}H NMR (D_2O) δ3.63 (m,4H,CH_2N), 3.70 (m,4H,CH_2S); IR (KBr) 3440 (N—H), 3200–3400 (2° amine salt), 1590 (2° N—H), 1295 (sulfone), 1120 (sulfone) cm^{-1}.

Synthesis of N-methylthiomorpholine-1,1-dioxide hydrochloride (M-TMS). A solution of commercial grade *N*-methylthiomorpholine-1,1-dioxide (1.49 g, 10.0 mmol) in methylene chloride (100 ml) at 0° was treated with a stream of dry HCl gas with stirring. The resultant white precipitate was filtered, dried *in vacuo*, and recrystallized from methanol to produce 1.8 g (97%) of the product as white needles, m.p. 260° (dec) [lit. 35, m.p. 262° (dec)]. IR (KBr) 3400 (N—H), 2700–2300 (3° amine salt), 1310 (sulfone), 1150 (3° C—N), 1115 (sulfone) cm^{-1}.

Synthesis of (±)3-aminotetrahydrothiopyran-1,1-dioxide hydrochloride (APS). Neat tetrahydrothiopyran (14.9 g, 0.146 mol) was cooled (0°) with stirring while 30% H_2O_2 (16.5 g, 0.146 mol) was added dropwise. The slurry was stirred overnight. The resultant solution was concentrated *in vacuo* and vacuum distilled to produce 16.2 g (94%) of tetrahydrothiopyran-1-oxide: b.p. 85°, 0.005 mm Hg; m.p. 45–46° (lit. 36, m.p. 44–46°). ^{1}H NMR ($CDCl_3$) δ1.65 (m,2H,CH_2), 2.20 (m,2H,CH_2), 2.85 (m,4H,CH_2S); IR (neat) 2920 (C—H), 1440 (sulfoxide), 1025 (sulfoxide) cm^{-1}; MS, *m/e* calcd. for $C_5H_{10}OS$ (M^+) 118, found 118.

A solution of the tetrahydrothiopyran-1-oxide (1.0 g, 8.5 mmol) in acetic acid (4 ml) at 110° was treated with acetic anhydride (3 ml, 25.5 mmol) dropwise with stirring over a period of 30 min. After complete disappearance of sulfoxide, as detected by thin-layer chromatography, the reaction mixture was diluted with ether (50 ml) and the excess acetic anhydride and acetic acid were neutralized with 10% sodium carbonate. The neutral ether layer was dried ($MgSO_4$), cautiously concentrated *in vacuo*, and the residue vacuum distilled to produce 0.8 g (94%) of 4*H*-2,3-dihydrothiopyran as a clear oil: b.p. 65–66° at 57 mm Hg (lit. 37, b.p. 66° at 57 mm Hg). ^{1}H NMR ($CDCl_3$) δ2.10 (m,4H,CH_2), 2.90 (m,2H,CH_2S), 5.80 (m,1H,CH), 6.10 (d,1H,CHS); IR (neat) 2930 (C—H), 1610 (C=C), 1440, 1285, 945 cm^{-1}.

A solution of potassium peroxymonosulfate (Oxone®) (30.4 g, 49.5 mmol, 3 mEq of oxidant) in water (120 ml) was added dropwise with stirring to a cooled (0°) solution of 4*H*-2,3-dihydrothiopyran (3.3 g, 33 mmol) in methanol (120 ml). The stirred mixture was warmed to 25°. After the complete disappearance of intermediate sulfoxide was determined by thin-layer chromatography, the precipitate was filtered and washed with methanol. The methanol was removed *in vacuo* and the resultant solution was extracted with methylene chloride. The combined organic extracts were washed with brine (1 × 30 ml), dried ($MgSO_4$), and concentrated *in vacuo* to produce 4.0 g (92%) of 4*H*-2,3-dihydrothiopyran-1,1-dioxide as a white solid: m.p. 45–46° (lit. 38, m.p. 44–46°).

A solution of 4*H*-2,3-dihydrothiopyran-1,1-dioxide (1.0 g, 7.6 mmol) in dry ethanol (20 ml) was degassed in a Parr reactor vessel and subjected to ammonia gas (100 psi) for 14 days. After complete disappearance of olefin as determined by thin-layer chromatography, the reaction mixture was filtered and concentrated *in vacuo*. The crude amine was taken up in dry ether (100 ml), cooled to 0°, and treated with dry HCl with stirring. The resultant white precipitate was filtered, washed with dry ether, and recrystallized from ethanol to produce 1.2 g (85%) of (±)3-aminotetrahydrothiopyran-1,1-dioxide hydrochloride as white needles: m.p. 228–230°. ^1H NMR (D_2O) δ1.78 (m,1H,CH_2), 2.06 (m,1H,CH_2), 2.25 (m,1H,CH_2), 2.35 (m,1H,CH_2), 3.30 (m,2H,CH_2S), 3.45 (m,1H,CH_2S), 3.65 (m,1H,CH_2S), 3.89 (m,1H,CHN); IR (KBr) 3400 (N—H), 3200–2600 (1° amine salt), 1600, 1500, 1315 (sulfone), 1130 (sulfone) cm^{-1}. Anal. Calcd. for $C_5H_{12}ClNO_2S$: C,H,N (see Appendix I).

Synthesis of 2-aminoethylmethyl sulfone hydrochloride (AEMS). 2-Chloroethylmethyl sulfide (2.70 g, 24.3 mmol) was added to a stirred slurry of freshly prepared potassium phthalimide (5.0 g, 27 mmol) in dimethylformamide (13 ml). The mixture was heated to 110° and stirred until the complete disappearance of starting chloride was determined by thin-layer chromatography. The reaction mixture was cooled, diluted with water (15 ml), and extracted with methylene chloride. The combined organic extracts were washed with brine (1 × 30 ml), dried ($MgSO_4$), concentrated *in vacuo*, and the resultant crude solid recrystallized from ethanol to yield 3.9 g (75%) of 2-phthalimidoethylmethyl sulfide as white crystals: m.p. 88–89°. ^1H NMR ($DMSO_{d^6}$) δ2.08 (s,3H,CH_3), 2.78 (t,2H,CH_2S), 3.81 (t,2H,CH_2N), 7.91 (s,4H,ArH); IR (KBr) 1707 (C=O), 1615 (amide), 719 (C—S) cm^{-1}. Anal. Calcd. for $C_{11}H_{11}NO_2S$: C,H,N (see Appendix I).

A solution of 2-phthalimidoethylmethyl sulfide (0.5 g, 2.26 mmol) in 5 ml acetic acid was degassed with nitrogen. To this solution 70% hydrogen peroxide (4 Eq, 9.04 mmol) was added dropwise. The mixture was refluxed for 1 hr, cooled, quenched with dry acetone, taken to 0°, and the resultant precipitate filtered. Recrystallization from ethanol afforded 0.54 g (94%) of pure 2-phthalimidoethylmethyl sulfone: m.p. 174–175°. ^1H NMR ($DMSO_{d^6}$) δ3.06 (s,3H,CH_3), 3.50 (t,2H,CH_2S), 4.05 (t,2H,CH_2N), 7.90 (s,4H,ArH); IR (KBr) 1705 (C=O), 1303 (S—O), 1131 (S—O) cm^{-1}. Anal. Calcd. for $C_{11}H_{11}NO_4S$: C,H,N (see Appendix I).

Hydrazine hydrate (85%) (0.95 g, 25.0 mmol) was added to a solution of 2-phthalimidoethylmethyl sulfone (6.33 g, 25.0 mmol) in 60 ml methanol. The mixture was refluxed for 1 hr and then cooled, 35 ml of water was added, and the methanol was removed *in vacuo*. Concentrated HCl (35 ml) was added and the mixture was refluxed for 1 hr. The reaction mixture was taken to dryness *in vacuo* and the resultant precipitate was recrystallized twice from ethanol to produce 3.35 g (84%) of AEMS: m.p. 169–170°. ^1H NMR ($DMSO_{d^6}$) δ3.13 (s,3H,CH_3), 3.22(t,2H,CH_2S), 3.58 (t,2H,CH_2N), 8.5 (broad,3H,$NH_2 \cdot HCl$); IR (KBr) 3430 (N—H), 1595 (N—H), 1285 (sulfone), 1135 (sulfone) cm^{-1}. Anal. Calcd. for $C_3H_{10}ClNO_2S$: C,H,N (see Appendix I).

RESULTS

The structures and abbreviations of the taurine analogues used in this study are shown in Fig. 1.

Figure 2 shows the uptake of calcium ions at low calcium ion concentration (10 μM) as a function of the protein concentration (A) and time (B). In all subsequent experiments utilizing 10 μM calcium ion concentration, an incubation period of 1 min and approximately 0.07 mg of protein were used.

The effects of the sulfone taurine analogues on ATP-dependent calcium ion uptake at low calcium ion concentration (10 μM) are shown in Fig. 3. Three of the sulfone derivatives (AEMS, ATS, and APS) stimulated ATP-dependent calcium ion uptake to a greater extent than did taurine. ATS was the most potent of the analogues, whereas TMS and M-TMS lacked activity.

When the calcium ion concentration was varied over a range from 0.01 to 2.0 mM, a sharp increase

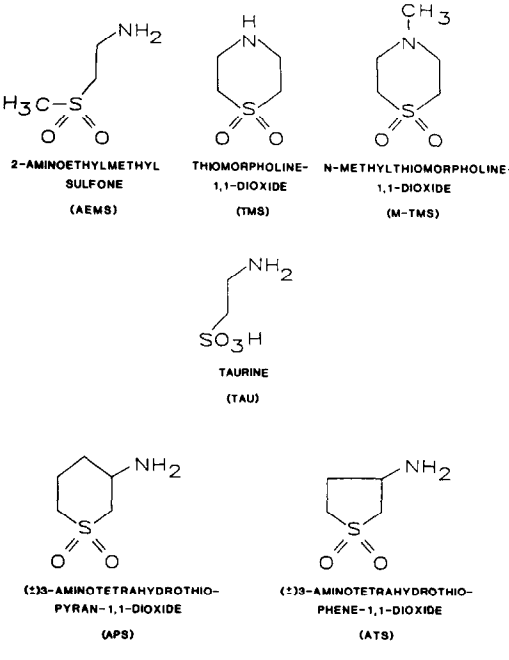

Fig. 1. Structural formulas of taurine and taurine analogues.

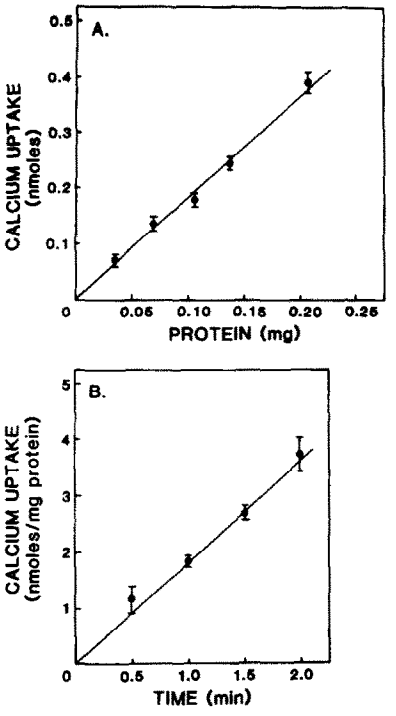

Fig. 2. Calcium ion uptake in rat retinal membrane preparations as a function of the quantity of protein and time at low calcium ion concentration (10 μM). Incubations were performed in the presence of buffer-A and 1.2 mM ATP. (A) Time of incubation was 1 min. (B) Amount of protein was approximately 0.07 mg.

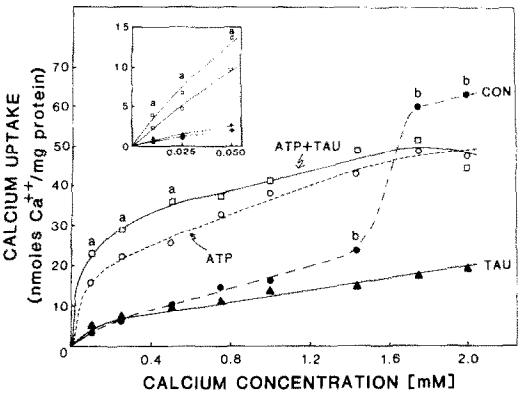

Fig. 4. Calcium ion uptake in rat retinal membrane preparations as a function of various calcium ion concentrations (0.01 to 2.0 mM). The incubation system contained bicarbonate buffer-B; (●) = no additions (CON); (▲) = 20 mM taurine (TAU); (○) = 1.2 mM ATP; (□) = 1.2 mM ATP plus 20 mM taurine (TAU). All data points represent the mean of five to seven experiments performed in duplicate. Analysis of variance and Duncan's multiple-range test were used to determine significant differences ($P < 0.05$). Means with superscript "a", calcium ion uptake in the presence of both 1.2 mM ATP and 20 mM taurine, are significantly different from calcium ion uptake in the presence of 1.2 mM ATP. Means with superscript "b", control, are significantly different from calcium ion uptake in the presence of 20 mM taurine.

in calcium ion uptake was observed between 1.44 and 1.75 mM (Fig. 4). Taurine (20 mM) inhibited calcium ion uptake at calcium ion concentrations of 1.44 mM and above. Calcium ion uptake was stimulated by ATP (1.2 mM) at calcium ion concentrations up to and including 1.44 mM. Taurine (20 mM) further stimulated the ATP-dependent calcium ion uptake at calcium ion concentrations from 0.01 to 0.5 mM. In these experiments in which calcium ion uptake was measured as a function of the calcium ion concentration, a discontinuity was observed under control conditions, but was not demonstrated subsequent to the addition of ATP, taurine, or ATP plus taurine. We have no explanation as to the cause of this discontinuity.

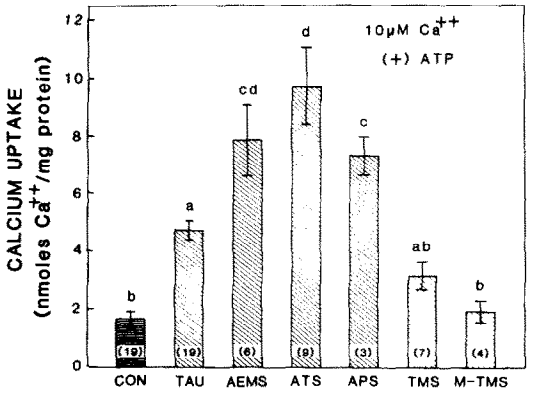

Fig. 3. Effects of taurine (TAU) and taurine analogues on ATP-dependent calcium ion uptake in rat retinal membrane preparations. The incubation system contained bicarbonate buffer-A, 10 μM calcium chloride, 1.2 mM ATP, and 20 mM taurine or taurine analogue. Data are expressed as means ± SEM. The numbers of experiments are shown in the parentheses. Analysis of variance and Duncan's multiple range test were used to describe significant differences between the groups (means with different superscripts are different; $P < 0.05$).

Figure 5 shows the uptake of calcium ions at high calcium ion concentration (1.44 mM) as a function of the protein concentration (A) and time (B). In all subsequent experiments utilizing 1.44 mM calcium ion concentration, an incubation period of 1.5 min and approximately 0.2 mg of protein were used.

The effects of the sulfone analogues of taurine on calcium ion uptake at high calcium ion concentrations (1.44 mM) and in the absence and presence of 1.2 mM ATP are shown in Fig. 6. In the absence of ATP, taurine and TMS inhibited calcium ion uptake by 26 and 28% respectively. AEMS, ATS and APS had a significantly greater inhibitory effect than taurine (55–62%), whereas M-TMS had no effect. In the presence of 1.2 mM ATP, only ATS significantly inhibited calcium ion uptake.

When the effects of taurine (20 mM) and the taurine analogues (20 mM) on phosphate incorporation into retinal proteins were studied (Fig. 7), taurine, AEMS, ATS, APS, and TMS were all inhibitory. M-TMS had no effect on phosphate incorporation.

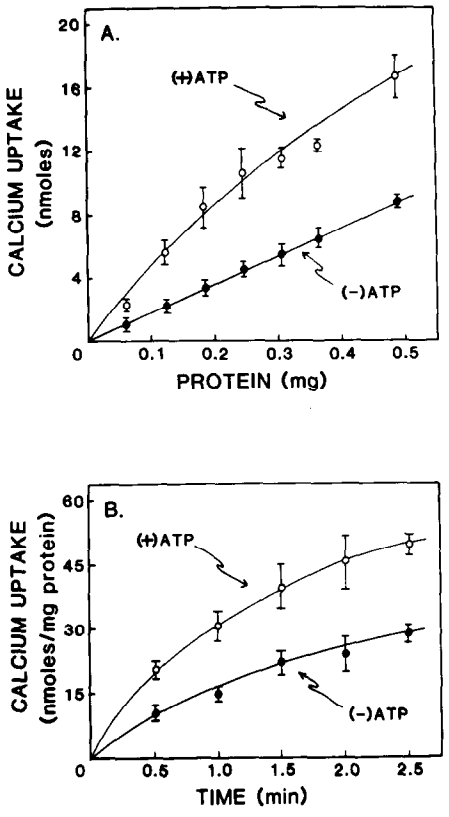

Fig. 5. Calcium ion uptake in rat retinal membrane preparations as a function of the quantity of protein and time at high calcium ion concentration (1.44 mM). Incubations were performed in the presence of buffer-B. (+)ATP equals 1.2 mM. (A) Time of incubation was 1.5 min. (B) Amount of protein was approximately 0.2 mg.

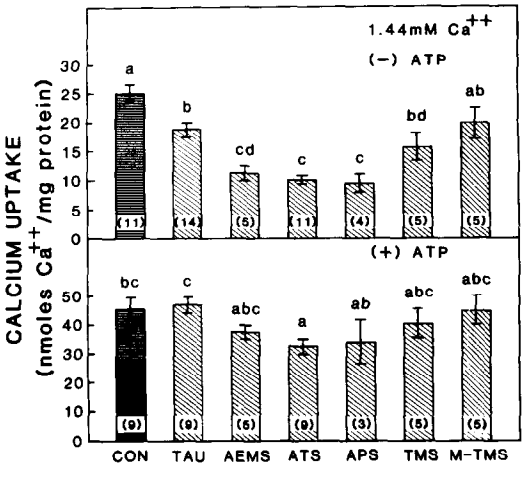

Fig. 6. Effects of taurine and taurine analogues on calcium ion uptake in rat retinal membrane preparations. The incubation system contained bicarbonate buffer-B, 1.44 mM calcium chloride, ±1.2 mM ATP, and 20 mM taurine or taurine analogue. Data are presented as means ± SEM. The numbers in parentheses equal the number of experiments. Analysis of variance and Duncan's multiple-range test were used to determine significant differences (means with different superscripts are different; $P < 0.05$).

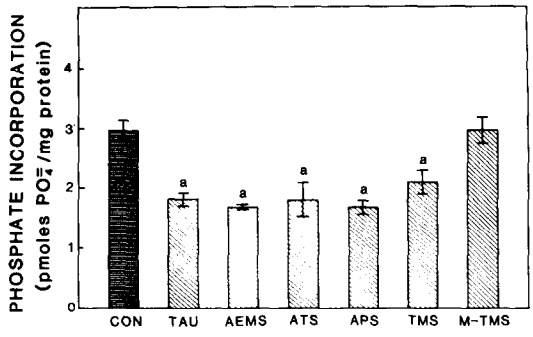

Fig. 7. Effects of taurine and taurine analogues on phosphate incorporation in rat retinal membrane preparations. The incubation system contained bicarbonate buffer-A, 10 μM calcium chloride, 3.2 μM ATP (20 μCi), and 20 mM taurine or taurine analogue. Values are the means of four experiments ± SEM. Analysis of variance and Duncan's multiple-range test were used to determine significant differences ($^aP < 0.05$).

The effects of ATS (20 mM) on phosphorylation of specific retinal membrane proteins are demonstrated in the autoradiogram of a sodium dodecyl sulfate (SDS)-polyacrylamide gel (Fig. 8). Autoradiography revealed that numerous proteins were phosphorylated in the absence of the taurine analogue (CON). However, in the presence of ATS, at least five proteins with molecular weights ranging from 20,000 to 48,000 (labeled a–e) incorporated less radioactive phosphate than the control.

DISCUSSION

This study was designed to examine the effects of a series of analogues of taurine, which were not sulfonic acids but sulfone derivatives, on calcium ion uptake in rat retinal membrane preparations. It is now well established that taurine has a stimulatory effect on ATP-dependent calcium ion uptake at low concentrations of calcium ions [19–21] and an inhibitory effect on ATP-independent calcium ion uptake at high concentrations of calcium ions [22].

To determine the structural requirements for activity in the calcium ion uptake systems, our laboratories have been actively involved in both the synthesizing and the testing of a number of new taurine analogues. Various compounds which contain the taurine structure in a restricted ring configuration have been synthesized. Interestingly, two compounds, (±)*trans*-2-aminocyclopentane sulfonic acid and (±)*trans*-2-aminocyclohexane sulfonic acid, are potent inhibitors of ATP-dependent calcium ion uptake rather than stimulators, whereas (±)*cis*-2-aminocyclohexane sulfonic acid is a stimulator, although weaker than taurine [24]. A fourth compound in this series, (±)*cis*-2-aminocyclopentane sulfonic acid, is a weak inhibitor of ATP-dependent calcium ion uptake [25].

It has been reported that TAG, a sulfone containing structure, antagonizes the taurine-induced inhibition of the spontaneous firing of rat cerebellar Purkinje cells [30]. Thus, analogues of taurine con-

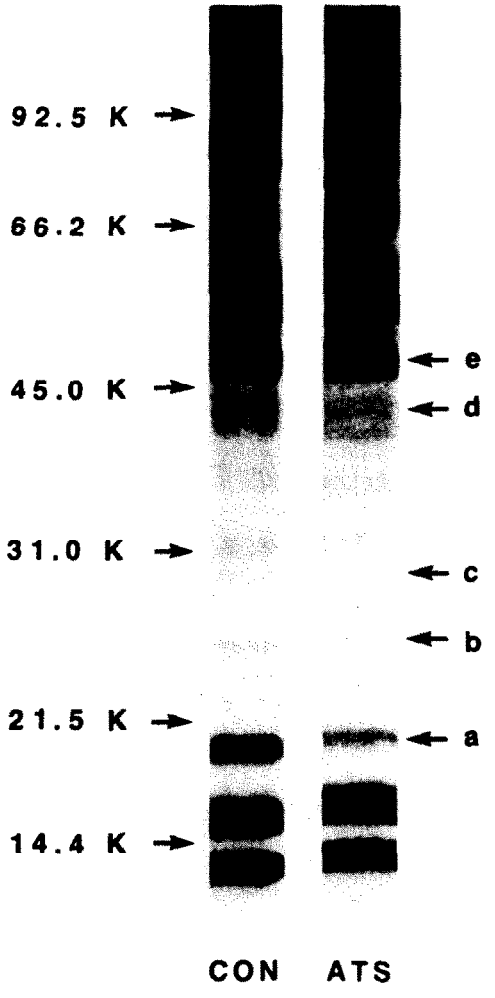

Fig. 8. SDS-polyacrylamide gel electrophoresis and autoradiography of phosphorylated membrane proteins obtained from rat retinal homogenates. The phosphorylation reaction was carried out in incubation tubes containing 1.0 mg protein and 20 μCi [γ-^{32}P]ATP. The column designated ATS contained a 20 mM concentration of the sulfone analogue. Each well for the polyacrylamide gel electrophoresis contained equal quantities of radioactivity (9500 cpm), as determined by TCA precipitation, but unequal amounts of protein (CON = 59.8 μg; ATS = 138 μg). Marker proteins with molecular weights ranging from 14,400 to 92,500 are indicated. The regions in the vicinity of 20,000–48,000 molecular weight that contained proteins phosphorylated to a lesser degree in the presence of ATS are indicated by the letters a–e.

taining a sulfone moiety in lieu of a sulfonic acid moiety were synthesized. It has also been demonstrated that TAG antagonizes the hypotensive and bradycardic effects of taurine in the cat and by itself has hypertensive and tachycardiac effects when administered directly into the lateral ventricle of this species [39]. Furthermore, TAG has been shown to antagonize the enhancing effect of taurine on ethanol-induced sleep time in rats [40].

However, while TAG has no effect in the ATP-dependent calcium ion uptake system [24], we have recently synthesized and reported that AEMS is a more potent stimulator than taurine of ATP-dependent calcium ion uptake [41]. We now report that two additional aminosulfones, APS and ATS, also stimulate ATP-dependent calcium ion uptake to a greater extent than does taurine. Unlike AEMS, which has the same degrees of freedom as taurine, APS and ATS are conformationally restricted cyclic structures. TMS and M-TMS are also cyclic structures and, similar to APS and ATS, incorporate the sulfone moiety into the ring configuration. These two derivatives, however, lack activity. Biological inactivity of TMS and M-TMS may be the result of steric hindrance. The inactive derivatives, TAG, TMS, and M-TMS, are either secondary or tertiary amines, whereas the active derivatives, AEMS, APS and ATS, are all primary amines. The additional steric bulk about the nitrogen may prevent inactive derivatives from interacting with the tissue sites that result in biological activity. On this basis, the dimethylamino analogues of APS and ATS would be predicted to be less potent stimulators of ATP-dependent calcium ion uptake than APS and ATS respectively.

In the present study, additional sulfone derivatives of taurine were synthesized. In assessing the sulfone derivatives of taurine on ATP-dependent calcium ion uptake at low concentrations (10 μM) of calcium ions, we found AEMS, ATS, and APS to be more potent stimulators than taurine. In contrast, taurine and four of the five sulfone analogues (AEMS, ATS, APS, TMS) inhibited protein phosphorylation. These data further support our previous conclusion [23–25] that taurine analogues which stimulate calcium ion uptake also inhibit protein phosphorylation. However, whether the taurine effect on protein phosphorylation is the mechanism by which calcium ion uptake is affected or vice versa is presently not known.

At high concentrations (1.44 mM) of calcium ions, taurine, AEMS, ATS, APS, and TMS all inhibited ATP-independent calcium ion uptake. AEMS, ATS and APS were more potent inhibitors than taurine. However, in the ATP-dependent calcium ion uptake system at high calcium ion concentrations only ATS demonstrated inhibition.

The finding that aminosulfone analogues of taurine were modulators of calcium ion uptake and protein phosphorylation, rivaling and even exceeding taurine in potency, is of considerable interest. The sulfonic acid moiety of taurine is exceedingly more acidic (estimates of the pK_a of taurine range from -1.0 to 1.5) [42, 43] than methyl sulfones (the hydrogens on the methyl group adjacent to the sulfur have an approximate pK_a of 31.0) [44]. At physiological conditions sulfonic acids are ionized, whereas methylsulfones are un-ionized. While the aminosulfone analogues of taurine had the same effect on calcium ion uptake (stimulation) and protein phosphorylation (inhibition), it is not clear if these effects are exerted through the same mechanism.

Sulfonic acids and sulfones are not considered to be classical isosteric functional groups. However, it has been suggested that sulfones are isosteric to carbonyls and not sulfonic acids because of their similar steric and electronic configurations [45]. Recently [46], sulfate sequestered in the sulfate bind-

ing protein of *Salmonella typhimurium* has been reported to be bound solely by hydrogen bonds. No positively charged residues, cations or water molecules are reported to be within van der Waal's distance of the sulfate. This indicates that the sulfate charge is not neutralized by the formation of any salt bridges. These findings further suggest that strong acids, e.g. sulfonic acids, can be stabilized within a protein by nonionic bonds, i.e. hydrogen bonding or dipole–dipole interactions. The calcium mediating effects exerted by taurine and aminosulfone derivatives may possibly be elicited via the same binding site. Further studies to identify the commonality of the binding sites will be necessary to address this question.

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APPENDIX I: ELEMENTAL ANALYSES

		Calculated	*Found*
$C_5H_{12}ClNO_2S$	C	32.35	32.41
	H	6.51	6.76
	N	7.54	7.42
$C_{11}H_{11}NO_2S$	C	59.71	59.66
	H	5.01	5.06
	N	6.33	6.30
$C_{11}H_{11}NO_4S$	C	52.17	52.19
	H	4.38	4.38
	N	5.53	5.52
$C_3H_{10}ClNO_2S$	C	22.57	22.92
	H	6.31	6.15
	N	8.77	8.89

REFERENCES

1. Davison AN and Kaczmarek LK, Taurine a possible neurotransmitter. *Nature* **234**: 107–108, 1971.
2. Kaczmarek LK, A comparison of the evidence for taurine and GABA as neurotransmitters. In: *Taurine* (Eds. Huxtable R and Barbeau A), pp. 283–302. Raven Press, New York, 1976.
3. Oja SS and Kontro P, Neurotransmitter actions of taurine in the central nervous system. In: *Taurine and Neurological Disorders* (Eds. Barbeau A and Huxtable RJ), pp. 181–200. Raven Press, New York, 1978.
4. Taber KH, Lin C-T, Lin J-W, Thalmann RH and Wu J-Y, Taurine in hippocampus: Localization and postsynaptic action. *Brain Res* **386**: 113–121, 1986.
5. Kuriyama K, Muramatsu M, Nakagawa K and Kakita K, Modulating role of taurine on release of neurotransmitter and calcium transport in excitable tissues. In: *Taurine and Neurological Disorders* (Eds. Barbeau A and Huxtable RJ), pp. 201–216. Raven Press, New York, 1978.
6. Namima M, Okamoto K and Sakai Y, Modulatory action of taurine on the release of GABA in cerebellar slices of the guinea pig. *J Neurochem* **40**: 1–9, 1983.
7. Medina JH and De Robertis E, Taurine modulation of the benzodiazepine-γ-aminobutyric acid receptor complex in brain membranes. *J Neurochem* **42**: 1212–1217, 1984.
8. Huxtable RJ and Sebring LA, Cardiovascular actions of taurine. In: *Sulfur Amino Acids: Biochemical and Clinical Aspects* (Eds. Kuriyama K, Huxtable RJ and Iwata H), pp. 5–37. Alan R. Liss, New York, 1983.
9. Izumi K, Butterworth RF and Barbeau A, Effect of taurine on calcium binding to microsomes isolated from rat cerebral cortex. *Life Sci* **20**: 943–950, 1977.
10. Nakagawa K and Kuriyama K, Increased association of calcium with brain mitochondria in the presence of taurine. *Jpn J Pharmacol* **29**: 309–313, 1979.
11. Pasantes-Morales H and Gamboa A, Effect of taurine on $^{45}Ca^{2+}$ accumulation in rat brain synaptosomes. *J Neurochem* **34**: 244–246, 1980.
12. Cunningham R and Miller RF, Electrophysiological analysis of taurine and glycine action on neurons of the mudpuppy retina. I. Intracellular recording. *Brain Res* **197**: 123–138, 1980.
13. Pasantes-Morales H and Cruz C, Taurine: A physiological stabilizer of photoreceptor membranes. In: *Taurine: Biological Actions and Clinical Perspectives* (Eds. Oja SS, Ahtee L, Kontro P and Paasonen MK), pp. 371–381. Alan R. Liss, New York, 1985.
14. Pasantes-Morales H, Domínguez L, Campomanes MA and Pacheco P, Retinal degeneration induced by taurine deficiency in light-deprived cats. *Exp Eye Res* **43**: 55–60, 1986.
15. Hayes KC, Carey RE and Schmidt SY, Retinal degeneration associated with taurine deficiency in the cat. *Science* **188**: 949–951, 1975.
16. Pasantes-Morales H, Quesado O, Cárabez A and Huxtable RJ, Effects of the taurine transport antagonist, guanidinoethane sulfonate, and β-alanine on the morphology of rat retina. *J Neurosci Res* **9**: 135–143, 1983.
17. Sturman JA, Wen GY, Wisniewski HM and Neuringer MD, retinal degeneration in primates raised on a synthetic human infant formula. *Int J Dev Neurosci* **2**: 121–129, 1984.
18. Geggel HS, Ament ME, Heckenlively JR, Martin DA and Kopple JD, Nutritional requirement for taurine in patients receiving long-term parenteral nutrition. *N Engl J Med* **312**: 142–146, 1985.
19. Kuo C-H and Miki N, Stimulatory effect of taurine on Ca-uptake by disc membranes from photoreceptor cell outer segments. *Biochem Biophys Res Commun* **94**: 646–651, 1980.
20. Pasantes-Morales H and Ordóñez A, Taurine activation of a bicarbonate-dependent, ATP-supported calcium uptake in frog rod outer segments. *Neurochem Res* **7**: 317–328, 1982.
21. Lombardini JB, Effects of ATP and taurine on calcium uptake by membrane preparations of the rat retina. *J Neurochem* **40**: 402–406, 1983.
22. Pasantes-Morales H and Cruz C, Possible mechanisms involved in the protective action of taurine on photoreceptor structure. In: *Sulfur Amino Acids: Biochemical and Clinical Aspects* (Eds. Kuriyama K, Huxtable RJ and Iwata H), pp. 263–276. Alan R. Liss, New York, 1983.
23. Lombardini JB, Effects of taurine on calcium ion uptake and protein phosphorylation in rat retinal membrane preparations. *J Neurochem* **45**: 268–275, 1985.
24. Liebowitz SM, Lombardini JB and Salva PS, Cyclic taurine analogs: Synthesis and effects on ATP-dependent Ca^{2+} uptake in rat retina. *Biochem Pharmacol* **36**: 2109–2114, 1987.
25. Liebowitz SM, Lombardini JB and Allen CI, Effects of aminocycloalkanesulfonic acid analogs of taurine on ATP-dependent calcium ion uptake and protein phosphorylation. *Biochem Pharmacol* **37**: 1303–1309, 1988.
26. Lombardini JB, Inhibition by taurine of the phosphorylation of rat retinal membranes. In: *Taurine: Biological Actions and Clinical Perspectives* (Eds. Oja SS, Ahtee L, Kontro P and Paasonen MK), pp. 383–393. Alan R. Liss, New York, 1985.

27. Huxtable R and Bressler R, Effect of taurine on a muscle intracellular membrane. *Biochim Biophys Acta* **323**: 573–583, 1973.
28. Huxtable RJ and Sebring LA, Towards a unifying theory for the actions of taurine. *Trends Pharmacol Sci* **7**: 481–485, 1986.
29. Lombardini JB, Taurine effects on the transition temperature in Arrhenius plots of ATP-dependent calcium ion uptake in rat retinal membrane preparations. *Biochem Pharmacol* **34**: 3741–3745, 1985.
30. Girard Y, Atkinson JG, Haubrich DR, Williams M and Yarbrough GG, Aminomethyl-1,2,4-benzothiadizines as potential analogues of γ-aminobutyric acid. Unexpected discovery of a taurine antagonist. *J Med Chem* **25**: 113–116, 1982.
31. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
32. Katz AM, Repke DI, Upshaw JE and Polascik MA, Characterization of dog cardiac microsomes. Use of zonal centrifugation to fractionate fragmented sarcoplasmic reticulum, $(Na^+ + K^+)$-activated ATPase and mitochondrial fragments. *Biochim Biophys Acta* **205**: 473–490, 1970.
33. Fenyes JGE and Pera JD, U.S. Application 914250. Aminoalkylenephosphonic acids and their salts. *Belgium Patent* No. 869,794, 1978.
34. Arya VP, Synthesis of new heterocycles: Part XI. Syntheses of 2,3,5,6-tetrahydro-4*H*-1,4-thiazines and some related heterocycles from divinylsulfone. *Indian J Chem* **13**: 1262–1266, 1975.
35. Maillard J, Vincent M, Delanuay P, Rapin M, Vo-Van-Tri and Remond G, 4-Alkyl- and 4-acylmorpholines and thiomorpholines with anti-arrhythmic activity. *J Chim Ther* **4**: 80–88, 1969.
36. Tarbell DS and Weaver C, The condensation of sulfoxides with *p*-toluenesulfonamide and substituted acetamides. *J Am Chem Soc* **63**: 2939–2942, 1941.
37. Parham WE, Christensen L, Groen SH and Dodson RM, Reactions of enol ethers with carbenes. III. Vinyl sulfides and Δ^3-dihydrothiapyran. *J Org Chem* **29**: 2211–2214, 1964.
38. Fehnel EA, Thiapyran derivatives. III. The preparation, properties and reactions of Δ^2-dihydrothiapyran 1,1-dioxide. *J Am Chem Soc* **74**: 1569–1574, 1952.
39. Bousquet P, Feldman J, Bloch R and Schwartz J, TAG antagonizes the central cardiovascular effects of taurins. *Eur J Pharmacol* **98**: 269–273, 1984.
40. Ferko AP, Ethanol-induced sleep time: Interaction with taurine and a taurine antagonist. *Pharmacol Biochem Behav* **27**: 235–238, 1987.
41. Liebowitz SM, Lombardini JB and Allen CI, 2-Aminoethylmethyl sulfone (AEMS): A potent stimulator of ATP-dependent calcium uptake. *Eur J Pharmacol* **120**: 111–113, 1986.
42. Andrews S and Schmidt CLA, Titration curves of taurine and of cysteic acid. *J Biol Chem* **73**: 651–654, 1927.
43. Irving CS, Hammer BE, Danyluk SS and Klein PD, Coordination and binding of taurine as determined by nuclear magnetic resonance measurements on ^{13}C-labeled taurine. In: *Taurine in Nutrition and Neurology* (Eds. Huxtable RJ and Pasantes-Morales H), pp. 5–17. Plenum Press, New York, 1982.
44. Matthews WS, Bares JE, Bartmess JE, Bordwell FG, Cornforth FJ, Drucker GE, Margolin Z, McCallum RJ, McCollum GJ and Vanier NR, Equilibrium acidities of carbon acids. VI. Establishment of an absolute scale of acidities in dimethyl sulfoxide solution. *J Am Chem Soc* **97**: 7006–7014, 1975.
45. Schatz VA, Isosterism and bio-isosterism as guides to structural variations. In: *Medicinal Chemistry* (Ed. Burger A), 2nd Edn, pp. 72–88. Interscience, New York, 1960.
46. Pflugrath JW and Quiocho FA, Sulphate sequestered in the sulphate-binding protein of *Salmonella typhimurium* is bound solely by hydrogen bonds. *Nature* **314**: 257–260, 1985.