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Analogues of Taurine as Stimulators and Inhibitors of ATP-Dependent Calcium Ion Uptake in Rat Retina: Combination Kinetics

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SUMMARY

Taurine is an amino acid that plays important roles in maintaining both the structural integrity and function of the retina. Thus, the effects of taurine, taurine analogues, and their combinations were studied in the ATP-dependent calcium ion uptake system at low calcium ion concentrations (10 μM) in a rat retinal membrane preparation. (±)-(*trans*)-2-Aminocyclopentanesulfonic acid (TAPS), a cyclic taurine analogue previously determined to inhibit ATP-dependent calcium ion uptake was demonstrated to be noncompetitive (K_i = 0.055 mM) with respect to taurine, that is, the values for the half-saturation concentrations calculated from varying concentrations of taurine compared with varying concentrations of taurine in the presence of a fixed concentration of TAPS (80 μM) did not change. However, the values for the maximal rates of change were significantly different. 1,2,3,4-Tetrahydroquinoline-8-sulfonic acid (THQS), a less potent inhibitor of ATP-dependent calcium ion uptake than TAPS, was also shown to be noncompetitive with taurine, with an inhibition constant (K_i) of 23.8 mM. Thus, it is presumed that both compounds (TAPS and THQS) are acting at receptor site(s) other than the taurine binding site. When TAPS and THQS were tested in a mixture that maintains a ratio (fixed ratio mixture) of 1 part TAPS and 25 parts THQS (by concentration, in mM), varied over a wide range of concentrations, and were then analyzed by median-effect plots and equation, the inhibitory effects are strongly synergistic, as shown by the combination index and the dose-reduction index. The parallel nature of the median-effect plots of TAPS and THQS indicates that the two inhibitors have a similar mode of action, that is, mutually exclusive. (±)-3-Aminotetrahydrothiophene-1,1-dioxide (ATS) and (±)-piperidine-3-sulfonic acid (PSA) are an agonist and partial agonist that demonstrated stimulatory effects on ATP-dependent calcium ion uptake. When tested in combination (1:1) with taurine, they were also determined to be mutually exclusive. It was demonstrated that ATS and taurine induced the same maximal rates of change of calcium ion uptake; however, PSA was less potent than taurine. The combination of taurine plus ATS was additive, whereas the combination of taurine plus PSA was synergistic. Structure-activity relationships of the taurine analogues and their topological relationships are discussed.

Taurine (2-aminoethanesulfonic acid) is the most abundant free amino acid in the retina, with concentrations ranging from 40 to 100 mM depending upon the species and area of the retina (1, 2). However, the role of taurine in the retina is unclear. It has been postulated that taurine is a neurotransmitter (3, 4), a necessary component for the structural integrity of the retina involved in preventing lipid peroxidation and membrane destabilization (5–7), a modifier of calcium ion fluxes (6, 8–11), and an inhibitor of protein phosphorylation (11–14).

Taurine appears to be an essential component of the diet of cats, because its absence results in abnormal electroretinograms and degenerative changes in retinal structure and function, leading ultimately to blindness (15, 16). However, in other animals including rats (17–21), monkeys (22, 23), and humans (24), taurine can be considered a conditionally essential nutrient under unusual situations of abnormal nutrition or pharmacological intervention (25). The taurine transport inhibitor guanidinoethane sulfonate has been used extensively to deplete

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ABBREVIATIONS: TAG, 6-(aminomethyl)-3-methyl-4*H*-1,3,4-benzothiadiazine-1,1-dioxide; THQS, 1,2,3,4-tetrahydroquinoline-8-sulfonic acid; PSA, (±)-piperidine-3-sulfonic acid; ATS, (±)-3-aminotetrahydrothiophene-1,1-dioxide; TAPS, (±)-(*trans*)-2-aminocyclopentanesulfonic acid; EGTA, [ethylenebis(oxyethylenenitrilo)]tetraacetic acid; ISA, isethionic acid; TAHS, (±)-(*trans*)-aminocyclohexanesulfonic acid; CAHS, (±)-(*cis*)-2-aminocyclohexanesulfonic acid; CAPS, (±)-(*cis*)-2-aminocyclopentanesulfonic acid; AEMS, 2-aminoethylmethylsulfone; APS, (±)-3-aminotetrahydrothiopyran-1,1-dioxide; DRI, drug reduction index.

rats of their taurine stores and, thus, demonstrates the deleterious effects of taurine depletion (17–21).

Previous investigations in our laboratories have attempted to determine the configuration of the taurine molecule needed for its biological activity as a known stimulator of ATP-dependent calcium ion uptake in the rat retina (13, 14, 26, 27). The strategy was to synthesize analogues that contain the taurine structure within a ring configuration. The analogues that were initially synthesized to explore the structural requirements for taurine were a series of aminocycloalkanesulfonic acids (13, 26). In addition, it has been reported that a compound (TAG) that contains a sulfone moiety rather than a sulfonic acid moiety was a taurine antagonist in a number of different biological systems (28–31). Thus, a series of cyclic sulfone analogues of taurine were also synthesized and tested for biological activity in the ATP-dependent calcium ion uptake system (14, 27).

The present investigation was designed to examine the effects of cyclic analogues, both sulfonic acid and sulfone derivatives, on ATP-dependent calcium ion uptake, in combination with taurine, to determine their kinetic relationships. Also the cyclic analogues were tested in combination with taurine or each other to determine whether the effects, i.e., inhibition or stimulation, on calcium ion uptake are additive, antagonistic, or synergistic. The syntheses of two new cyclic analogues of taurine are also reported.

Experimental Procedures

Materials. Taurine was purchased from Sigma Chemical Co. (St. Louis, MO). $^{45}CaCl_2$ (25 mCi/mg) was purchased from New England Nuclear (Boston, MA).

Synthetic procedures for TAPS and ATS were reported previously (14, 26).

Synthesis of PSA. Pyridine-3-sulfonic acid was purchased from Aldrich Chemical Co. (Milwaukee, WI) and recrystallized from water. The melting point was above 300° as described in Beilstein (**22**:387). Pyridine-3-sulfonic acid (1.0 g, 6.28 mmol) was placed in 50 ml of water that contained 1.5 g of 5% rhodium on alumina and was then hydrogenated at room temperature in a Parr minireactor, with 100 atm of H_2, for 6 hr. The catalyst was filtered and the mixture reduced *in vacuo*. The product (0.92 g, 89%) was recrystallized from water; m.p. > 300° (Beilstein, E III/IV, **22**:3453; m.p. 320–330°).

Synthesis of THQS. Quinoline-8-sulfonic acid (1.0 g, 4.78 mmol) was placed in 50 ml of water that contained 1.5 g of 5% rhodium on alumina and was then hydrogenated at room temperature in a Parr minireactor, with 100 atm of H_2, for 6 hr. The catalyst was filtered and the mixture was reduced *in vacuo*. The product (0.20 g, 87%) was recrystallized from water; m.p. > 300° (Beilstein, **22**:388; m.p. 240–242°, decomposed).

Preparation of membrane homogenate. Weanling rats (70–100 g) were used in all experiments. Procedures for obtaining retinas and preparing the membrane homogenate were previously described (26).

Calcium ion uptake assay. The incubation system contained retinal preparation (~0.2 mg), ATP (1.2 mM), 0.5 μCi of $^{45}Ca^{2+}$, and bicarbonate buffer (50 mM $NaHCO_3$, 50 mM KCl, 50 mM NaCl, 1.2 mM KH_2PO_4, 2 mM $MgCl_2$, 25 μM $CaCl_2$, 50 μM ouabain, pH 7.4) that included EGTA (18 μM) to buffer calcium ion concentrations so that the final free Ca^{2+} was 10 μM (32). The reaction was initiated, after a 2-min preincubation period (37°), with the addition of the membrane preparation, incubated for an additional 1 min, and then stopped by collection of the membrane homogenate, which contained the accumulated $^{45}Ca^{2+}$, on Whatman GF/B filters by rapid vacuum filtration.

Dose-effect analysis and the combined effects of inhibitors or stimulators. Dose-effect analysis was carried out by the median-effect equation and its plot (33, 34):

$$\frac{f_a}{f_u} = \left(\frac{D}{D_m}\right)^m \quad \text{or} \quad \log(f_a/f_u) = m \log D - m \log D_m \tag{1}$$

where f_a and f_u are the decimal fractions of activities of ATP-dependent calcium ion uptake affected and unaffected by a dose (D) of a drug. 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 $y = \log(f_a/f_u)$ 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 (33, 34) for mutually exclusive inhibitors:

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

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, $f_a + f_u = 1$, ($f_u = 1 - f_a$).

In the special circumstance in which $(f_a)_{1,2} = 0.50$, the equation simplifies to the following equation.

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

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% inhibition or 50% maximum stimulation). 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

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

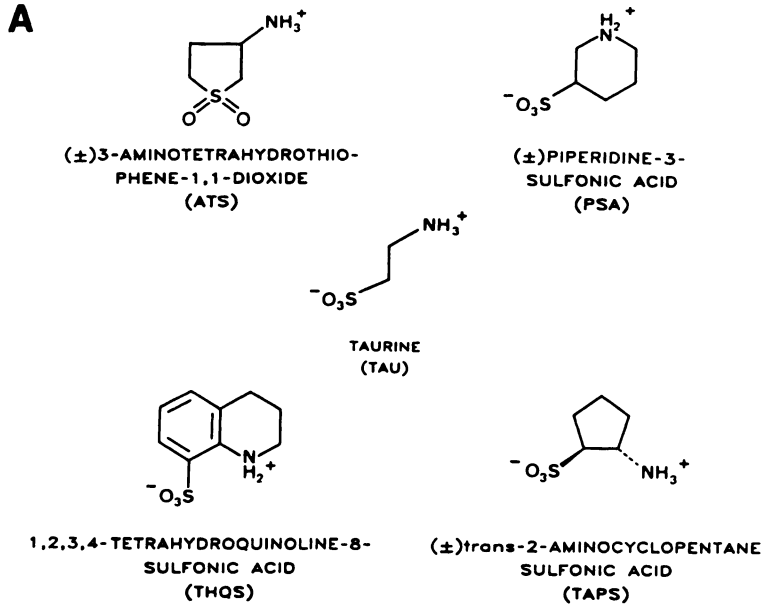
Thus, when the parameters D_m and m are determined from the median-effect plot (see above), the dose (D or D_x) required for any degree of effect (f_a) can be calculated. These calculated doses are then used in Eq. 5 for the calculation of CI to determine synergism/antagonism. Computer software (36) has been used for automated analysis.

Protein estimations. Protein concentrations were determined by the method of Lowry *et al.* (35), with bovine serum albumin being utilized as the standard.

Results

Structures and abbreviations of the taurine analogues used in this study are shown in Fig. 1A. Dreiding stereomodels of the compounds are shown in Fig. 1B.

Preparations of rat retinas that exhibit ATP-dependent calcium ion uptake are markedly stimulated by taurine, as shown in Fig. 2. In Fig. 2, the activity of calcium ion uptake is reported as the stimulation by taurine above the activity obtained in the presence of 1.2 mM ATP.



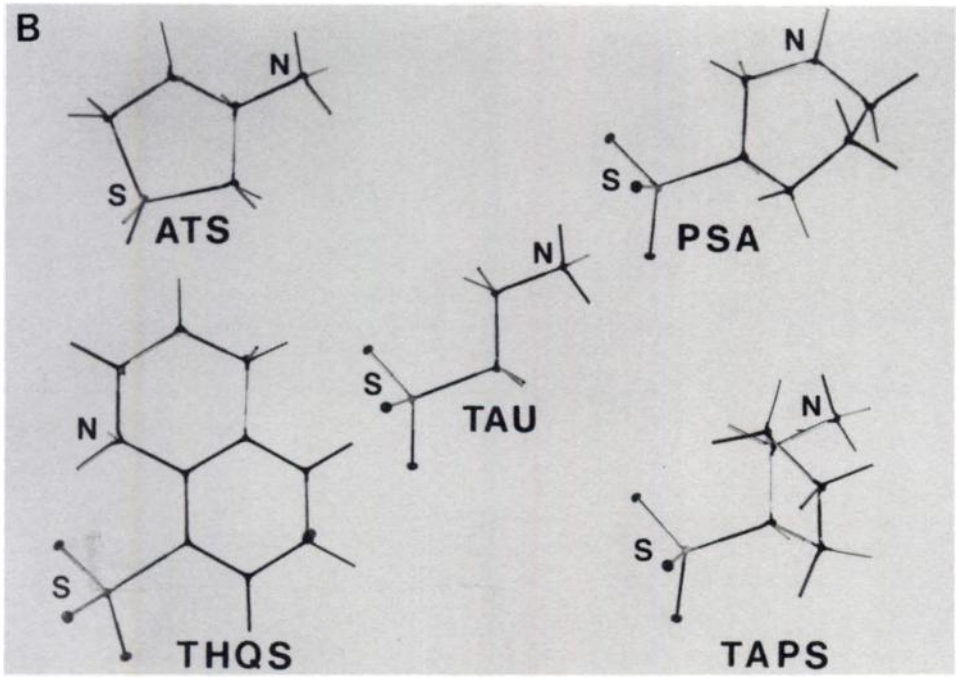


Fig. 1. Names and structures (A) and dreiding stereomodels (B) of taurine (*TAU*) and taurine analogues.

In view of our previous observations that TAPS was a potent inhibitor of ATP-dependent calcium ion uptake rather than a stimulator of activity, as was observed for taurine (13, 25), efforts were made to study the relationship between the two effectors when they were simultaneously present in the same system. Varying the concentration of taurine in the presence of a fixed concentration (80 μM) of TAPS demonstrates that the kinetic relationship of TAPS to taurine is noncompetitive, with an inhibition constant (K_i) of 0.055 mM (Fig. 2).

The effects of a second cyclic inhibitor of ATP-dependent calcium ion uptake are shown in Fig. 3. THQS, although considerably less potent than TAPS, also demonstrates a noncompetitive kinetic relationship with taurine, with a K_i of 23.8 mM.

Effects on ATP-dependent calcium ion uptake of a wide range of concentrations of ATS, a sulfone analogue of taurine, plus a combination of ATS and taurine in a fixed-ratio mixture of 1:1 are shown in Fig. 4. The effects of varying taurine concentrations are shown for comparison purposes. ATS is a more potent stimulator than taurine and also demonstrates a biphasic effect on ATP-dependent calcium ion uptake, i.e., it is a stimulator at low concentrations but an inhibitor at high concentrations, which is not observed when the effects of various concentrations of taurine are tested. The combination of taurine plus ATP also demonstrates the biphasic stimulatory-inhibitory effect. Both drugs and their combination showed an isoeffective dose of 60 mM (Fig. 4).

In Fig. 5, the effects of varying concentrations of PSA,

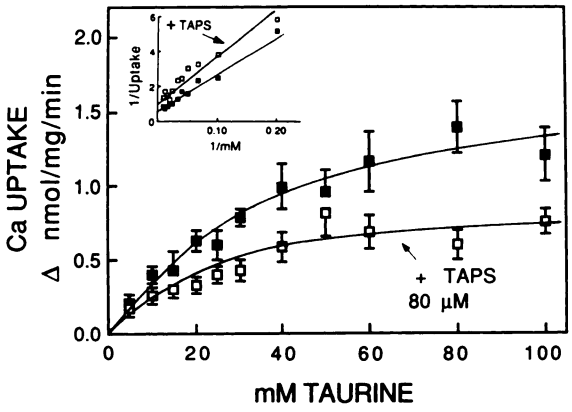


Fig. 2. Effects of varying concentrations of taurine on ATP-dependent calcium ion uptake in rat retina in the presence and absence of a fixed concentration of TAPS (80 μM). *Inset*, the double-reciprocal plot of the data. The inhibition constant (K_i) is calculated from the following equation (37).

$$K_i = \frac{[\text{Inhibitor conc.}]}{\frac{(y\text{-intercept})_i}{(y\text{-intercept})_0} - 1}$$

Details of the calcium ion uptake assay are described in Experimental Procedures. Data are presented as the change in stimulation of calcium ion uptake (Δ nmol/mg/min) by taurine above the quantity of calcium accumulated in the presence of ATP (1.2 mM). Data are presented as means ± standard errors and represent five experiments. The *curves* were plotted and drawn by GraphPad, using a nonlinear regression fit of the data to a formula for a rectangular hyperbola [$y = ax/(B + x)$] and assuming Michaelis-Menten kinetics.

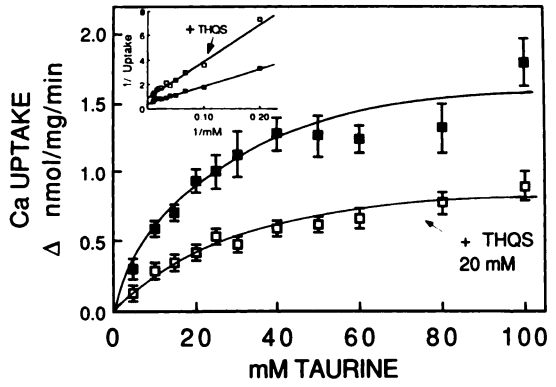


Fig. 3. Effects of varying concentrations of taurine on ATP-dependent calcium ion uptake in rat retina in the presence and absence of a fixed concentration of THQS (20 mM). *Inset*, the double-reciprocal plot of the data. Details of the calcium ion uptake assay and presentation of the data are described in Experimental Procedures and in the legend to Fig. 2. Data represent six experiments.

taurine, and a fixed-ratio mixture of taurine and PSA (1:1) on ATP-dependent calcium ion uptake are compared. PSA is a less potent stimulator of ATP-dependent calcium ion uptake than taurine. PSA and the combination of taurine plus PSA also exhibit a biphasic effect, as was observed for ATS. Of additional interest is the observation that the unsaturated analogue of PSA, pyridine-3-sulfonic acid, has no effect on calcium ion uptake (data not shown).

The kinetic constants (half-saturation concentrations and maximal rates of change) for the effects of taurine and taurine analogues (TAPS, THQS, ISA, ATS, and PSA) on ATP-dependent calcium ion uptake for the stimulatory phase are reported in Table 1. In confirmation of earlier findings from

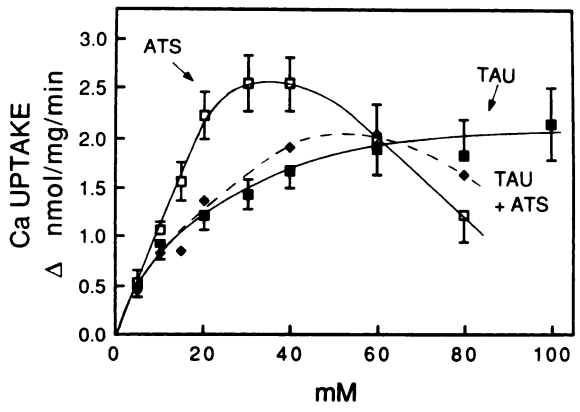


Fig. 4. Effects of varying concentrations of taurine (*TAU*), ATS, and an equimolar combination of taurine plus ATS on ATP-dependent calcium ion uptake in rat retina. Details of the calcium ion uptake assay and presentation of the data are described in Experimental Procedures and in the legend to Fig. 2. In the experiments utilizing equimolar combination of taurine plus ATS, the sum of the concentrations of taurine plus ATS is used for the combination dose on the *x*-axis. Thus, the data for a concentration of 40 mM denoted in the *x*-axis of the figure (as an example), signify 20 mM taurine and 20 mM ATS. Data represent seven experiments of taurine and ATS and five experiments for the combination taurine plus ATS. The standard error values, which vary from 0.10 to 0.40 nmol/mg/min for the taurine-ATS combinations, are omitted because of limited space. The data values only for the increasing part of the titration curve were used in the analyses with GraphPad and thus the kinetic constants are estimated values. Means for ATS compared with taurine at 20, 30, and 40 mM are significantly different, as determined by the Student's *t* test ($p < 0.05$). Computer analyses suggest that the stimulatory effects of taurine and ATS are nearly additive (see Table 3).

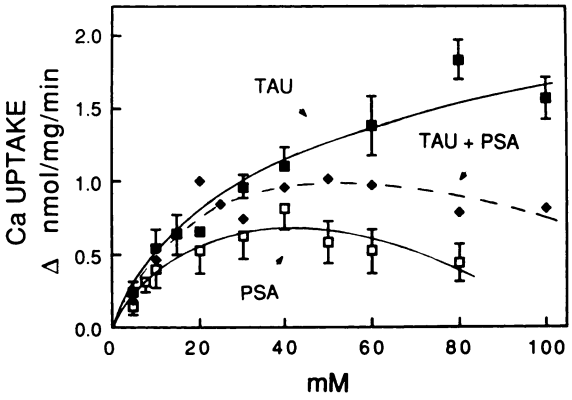


Fig. 5. Effects of varying concentrations of taurine (*TAU*), PSA, and an equimolar combination of taurine plus PSA on ATP-dependent calcium ion uptake in rat retina. Details of the calcium ion uptake assay and presentation of the data are described in Experimental Procedures and in the legend to Fig. 2. In the experiments utilizing equimolar combinations of taurine and PSA, the data for a concentration of 40 mM denoted on the *x*-axis of the figure (as an example) signify 20 mM taurine and 20 mM ATS. Data represent four experiments. The standard error values, which vary from 0.05 to 0.23 nmol/mg/min for the taurine-PSA combinations, are omitted because of limited space. The *curves* were plotted and drawn by GraphPad, using a nonlinear regression fit of the data to a formula for a rectangular hyperbola [$y = ax/(B + x)$] and assuming Michaelis-Menten kinetics. The data values only for the increasing part of the titration curve were used in the analyses with GraphPad and thus the kinetic constants are estimated values. Computer analyses suggest that the stimulatory effects of taurine and PSA are synergistic (see Table 3).

TABLE 1

Kinetic constants for the effects of taurine and taurine analogues (TAPS, THQS, ISA, and ATS) on ATP-dependent calcium ion uptake

Data are presented as means ± standard error. The number of experiments is noted in parentheses. Statistical differences for each set of subgroups were determined by the Student's *t* test or analysis of variance and Duncan's multiple range test as appropriate.

	Half-saturation concentration	Maximal rate of change in calcium ion uptake
	mM	*Δ nmol/mg/min*
Taurine	33.6 ± 5.5 (5)	1.96 ± 0.34 (5)
Taurine + TAPS (80 μM)	25.1 ± 9.0 (5)	0.97 ± 0.21 (5)[a]
Taurine	29.0 ± 4.9 (6)	2.23 ± 0.27 (6)
Taurine + THQS (20 mM)	30.8 ± 5.7 (6)	1.06 ± 0.14 (6)[a]
Taurine	30.9 ± 8.7 (4)	2.04 ± 0.37 (4)
Taurine + ISA (20 mM)	40.8 ± 4.4 (4)	2.12 ± 0.23 (4)
Taurine	23.9 ± 6.3 (7)	2.69 ± 0.48 (7)
ATS[b]	25.5 ± 5.3 (7)	4.49 ± 0.70 (7)
Taurine + ATS (1/1)[b]	22.8 ± 5.3 (5)	2.73 ± 0.47 (5)
Taurine	40.5 ± 8.9 (4)	2.28 ± 0.30 (4)
PSA[b]	21.5 ± 7.1 (4)	1.15 ± 0.20 (4)[a]
Taurine + PSA (1/1)[b]	17.1 ± 4.3 (4)	1.32 ± 0.14 (4)

[a] $p < 0.05$.

[b] The kinetic constants for ATS, PSA, and the fixed combinations of taurine and ATS and of taurine and PSA were calculated from data values that included only the increasing part of the titration curve. Data values for the inhibtory part of the response curve at high concentrations of ATS and PSA were not entered into the graphical program (GraphPad) or the computer software of Chou and Chou (36). Thus these kinetic constants are estimated values.

this laboratory, we reaffirm that not all sulfonic acids affect ATP-dependent calcium ion uptake (11). Thus, we report that a fixed concentration (20 mM) of ISA, the hydroxy analogue of taurine (although not zwitterionic in nature), does not have any effect on the stimulatory effects of varying concentrations of taurine on ATP-dependent calcium ion uptake activity. These values were calculated from the data depicted in Figs. 2–6. In each group of experiments, the values for the half-saturation concentrations for taurine and taurine plus a fixed concentration of analogue are not statistically different. (The values for the half-saturation concentrations for taurine in all the various subgroups vary from 23.9 ± 6.3 to 40.5 ± 8.9 mM and are not significantly different by analysis of variance). Also, the values for the maximal rates of change for taurine, which vary from 1.96 ± 0.34 to 2.69 ± 0.48 nmol/mg/min in the various subgroups, are not significantly different by analysis of variance. However, the values for maximal rates of change between taurine and the combination of taurine plus a fixed amount of TAPS (80 μM) are significantly different (1.96 ± 0.34 versus 0.97 ± 0.21 nmol/mg/min), thus confirming the noncompetitive relationship between TAPS and taurine that was graphically displayed in Figure 2.

When the effects on ATP-dependent calcium ion uptake of varying concentrations of taurine and a fixed quantity of THQS (20 mM) are compared with varying concentrations of taurine alone, the values for the maximal rates of change (2.23 ± 0.27 versus 1.06 ± 0.12 nmol/mg/min) are also statistically different (Table 1; Fig. 3). A fixed concentration of ISA (20 mM) in the presence of varying concentrations of taurine had no effect on the value for the maximal rate of change for ATP-dependent calcium ion uptake (2.04 ± 0.37 versus 2.12 ± 0.23 nmol/mg/min), thus ruling out the possibility that the inhibitory effect was due to increased ionic strength (Table 1).

ATS, the sulfone analogue of taurine, is a stimulator of ATP-dependent calcium ion uptake. In this series of experiments, it was determined that the value for the maximal rate of change for ATS (4.49 ± 0.70 nmol/mg/min) is approximately 65% higher than the value for taurine (2.69 ± 0.48 nmol/mg/min) (Table 1). However, although the values for the maximal rates of change for taurine and ATS are not significantly different, indicating that ATS is a full agonist, velocities for ATP-dependent calcium ion uptake determined for specific concentrations of taurine or taurine analogue do demonstrate differences. Thus, as observed in Fig. 4, ATS is a more potent stimulator of ATP-dependent calcium ion uptake than taurine at concentrations of 20, 30, and 40 mM.

The values for the maximal rates of change calculated for PSA and taurine from the data shown in Fig. 5 are significantly different from each other (2.28 ± 0.3 versus 1.15 ± 0.2 nmol/mg/min), indicating that PSA is a partial agonist (Table 1). However, the values for the half-saturation concentrations are not significantly different.

Median-effect plots of the effects on ATP-dependent calcium ion uptake of the simultaneous presence of TAPS and THQS in a fixed ratio of 1:25 are shown in Fig. 6. Utilizing the median-effect equation and the multiple drug effect equation for the quantitative analysis of the combined use of effectors of biological activity, we determined that the two inhibitors were highly synergistic, 20–60%, as demonstrated by combination index values of 0.43–0.78 at ED_{30}–ED_{90}, which are substantially less than unity (Table 2).

Statistical treatment of the data in Table 2 is denoted by the *r* value (linear correlation coefficient). The *r* value indicates the conformity of the data to the method (the median-effect principle and plot) used. This diagnostic plot is incorporated in the computer software (36) and serves as a statistical prerequisite for all the data analyses. (For details of the statistical treatment, see reviews in Refs. 33 and 34.)

In addition, due to the synergistic combination, DRIs from 1.39- to 2.5-fold for TAPS and 3.03- to 25.6-fold for THQS were calculated for 30 to 90% inhibition (Table 2). The DRIs quantitatively indicate the amount that the dose of each inhibitor can be reduced when tested in combination and still achieve the same effect when tested singly. The two inhibitors TAPS and THQS are also mutually exclusive (i.e., have a similar mode of action) in their effects on ATP-dependent calcium ion uptake, as suggested by the median-effect plot for the combi-

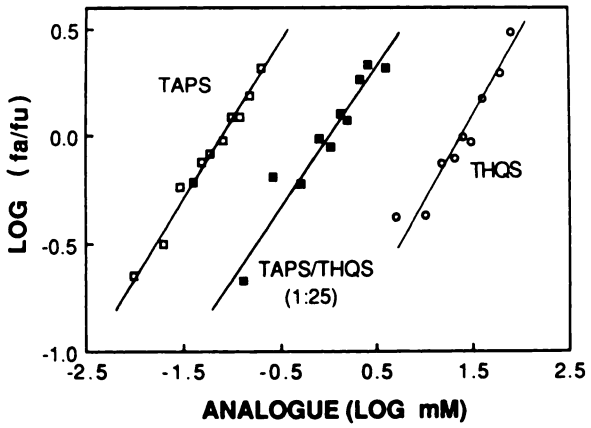


Fig. 6. Median-effect plots of the effects of TAPS, THQS, and a combination of TAPS plus THQS (1:25) on ATP-dependent calcium ion uptake in rat retina. Details of the calcium ion uptake assay and median-effect plots are described in Experimental Procedures. Data are presented as the means of five experiments.

TABLE 2

Inhibitory effect of TAPS and THQS on ATP-dependent calcium ion uptake in rat retina and their synergistic interaction upon combination

Compounds	Median-effect plot parameters[a]			Combination index values[b]				DRI[c]			
	Median-effect concentration (D_m)	Slope (m)	Linear correlation coefficient (r)	30% inhibition	50% inhibition	70% inhibition	90% inhibition	30% inhibition	50% inhibition	70% inhibition	90% inhibition
	mM										
TAPS	0.080	0.726	0.992					2.50	2.12	1.80	1.39
THQS	24.1	0.728	0.964					3.03	25.6	21.7	16.6
TAPS + THQS (1:25)	0.977	0.637	0.960	0.433	0.510	0.601	0.781				

[a] The median-effect plot parameters, D_m, m, and r signify the potency, shape of dose-effect curve, and the applicability of the median-effect principle, respectively, as described by Chou and Talalay (33, 34). These values were obtained using a computer program developed by Chou and Chou (36). The values were calculated from pooled data obtained from five experiments.

[b] Combination index values <1, =1, and >1 indicate synergism, summation, and antagonism, respectively. The calculations are based on the classical isobologram equation (33, 34), using a computer program developed by Chou and Chou (36).

[c] DRI indicates how many folds of dose reduction for each drug for a given effect are due to the synergistic combination. DRI values are calculated by using a computer program (36) based on the median-effect equation and the multiple drug-effect equation (34). $(Dx)_1/(D)_1$ and $(Dx)_2/(D)_2$ are the DRIs for drug 1 and drug 2, respectively.

nation of inhibitors (slope = 0.637), which parallels the plots for each of the component inhibitors (slope for TAPS = 0.726; slope for THQS = 0.728). The slopes (m values) of less than unity indicate that the dose-effect curves are negatively sigmoidal.

Similar calculations for analyzing the combined effects of taurine and ATS were also performed. Determination of the concentrations required to stimulate the calcium ion uptake by 50% (median effects) for each compound separately and in a fixed combination ratio of 1:1 and subsequent substitution of the values into the median-effect equation suggest that the mixture is nearly additive, as demonstrated by combination index values approximating unity: 1.21, 1.08, and 0.92 at 50, 70, and 90% of saturation (Table 3). The slopes of the data for taurine (0.808), ATS (0.956), and the combination (1:1) of taurine plus ATS (0.978) are all parallel, as determined in the median-effect plots, thus suggesting that the stimulators are mutually exclusive with a slope close to unity (i.e., Michaelis-Menten-type hyperbolic saturation curves or first-order kinetics).

When a fixed ratio mixture of PSA and taurine (1:1) was tested for its effects on ATP-dependent calcium ion uptake, the combination index values calculated from the median-effect equation indicated that the mixture was synergistic (Table 3). Furthermore, as shown in Fig. 7, the slopes of the data for taurine (1.03), PSA (0.780), and the combination (0.924) are parallel, thus indicating that the two compounds are also mutually exclusive, with a slope close to unity, in their effects on ATP-dependent calcium ion uptake.

Discussion

Recently, our laboratories have been interested in determining the structure-activity relationships of taurine that enable it to be a stimulator of ATP-dependent calcium ion uptake at low calcium ion concentrations in a rat retinal membrane preparation. Our goal was to synthesize cyclic compounds that contained taurine within a ring structure and, thus, the mobility of the amino and sulfonic acid moieties would be restricted, compared with the free mobility in taurine. Towards this objective, a series of aminocycloalkanesulfonic acid analogues of taurine were synthesized, TAPS, CAPS, TAHS, and CAHS (13, 26). Surprisingly, three of these compounds were inhibitors of ATP-dependent calcium ion uptake, whereas only one was a stimulator. Thus, we have previously reported that the *trans* configurations of both the cyclopentane and cyclohexane analogues of taurine, TAPS and TAHS, are potent inhibitors, with TAPS being the more active (13, 26). In this series of analogues, CAPS was a weak inhibitor, whereas CAHS stimulated ATP-dependent calcium ion uptake but not nearly as effectively as taurine. From these data, it was concluded that the amino and sulfonic acid moieties of taurine and CAHS assume a *gauche* conformation, whereas differences in the position and binding

TABLE 3

Median-effect plot parameters and combination index values for taurine, ATS, and PSA on ATP-dependent calcium ion uptake in rat 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)	50% saturation	70% saturation	90% saturation
	mM					
Taurine	17.8	0.808	0.936			
ATS	26.0	0.956	0.920			
Taurine + ATS (1:1)	25.6	0.978	0.963	1.21	1.08	0.92
Taurine	33.9	1.03	0.944			
PSA	20.7	0.780	0.936			
Taurine + PSA (1:1)	15.2	0.924	0.937	0.59	0.55	0.52

[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 (33, 34). These values were obtained using a computer program developed by Chou and Chou (36). The values were calculated from pooled data obtained from five experiments for taurine, ATS, and taurine plus ATS and from four experiments for taurine, PSA, and taurine plus PSA.

[b] Combination index values <1, =1, and >1 indicate synergism, summation, and antagonism, respectively. The calculations are based on the classical isobologram equation (33, 34), using a computer program developed by Chou and Chou (36).

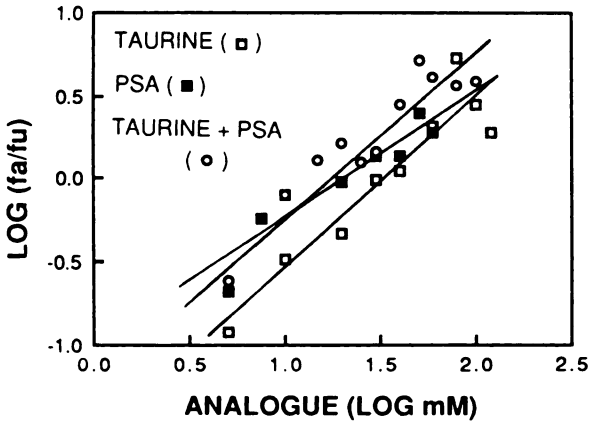


Fig. 7. Median-effect plots of the effects of taurine, PSA, and an equimolar combination of taurine plus PSA on ATP-dependent calcium ion uptake in rat retina. Details of the calcium ion uptake assay and median-effect plots are described in Experimental Procedures. Data are represented as the means of four experiments.

of the cycloalkyl moiety of TAPS, TAHS, and CAPS to the biological receptor (calcium ion uptake system) perhaps account for their inhibitory properties.

A second series of compounds, which contained a sulfone moiety rather than the sulfonic acid moiety, was also synthesized (14, 27). Interest in sulfone analogues of taurine was generated from the literature reports that TAG is a taurine antagonist (28–31), Thus, three classes of compounds containing the sulfone moiety were synthesized, 1) aliphatic, in which free rotation occurs about the methylene bridge between the amino and sulfonic moieties (AEMS); 2) heterocyclic (two heteroatoms), in which both the sulfone moiety and the nitrogen atom are incorporated within the ring structure, thus yielding secondary or tertiary amines [thiomorpholine-1,1-dioxide] and [*N*-methylthiomorpholine-1,1-dioxide]; and 3) heterocyclic (one heteroatom), in which the sulfone moiety is within the ring structure but the amino group is external, thus yielding primary amines (APS and ATS).

AEMS, ATS, and APS are all potent stimulators of ATP-dependent calcium ion uptake, with activity exceeding by approximately 2-fold that of taurine (14, 27). However, due to the vast differences in the electronic configuration between sulfonic acids and sulfone moieties, which are normally considered not to be bioisosteric functional groups, it is not known whether the stimulatory effects of taurine and the sulfone analogues on ATP-dependent calcium ion uptake in the rat retina are exerted through the same mechanism.

The syntheses of two new analogues of taurine are reported in these studies, along with their effects on ATP-dependent calcium ion uptake. THQS was observed to be an inhibitor, although considerably less potent than TAPS. PSA was found to be a stimulator of ATP-dependent calcium ion uptake, although less potent than taurine.

The studies reported herein were designed to examine the effects of the combined use of the taurine analogues or taurine plus a taurine analogue on ATP-dependent calcium ion uptake. An immediate question was whether inhibitors of ATP-dependent calcium ion uptake (TAPS and THQS) were acting at the same site as taurine. Although it is assumed that TAPS (and THQS) is acting at a different biological receptor than taurine because of opposing properties, there is no definitive proof (13, 26). In the experiments now reported, noncompetitive kinetics were observed for the combined use of either TAPS plus taurine or THQS plus taurine in a fixed concentration of inhibitor while the concentrations of taurine were varied, thus confirming the assumption that the inhibitory analogues of taurine are acting at different sites. The values for the half-saturation concentrations do not change, whereas the value for the maximal rate of change of the ATP-dependent calcium ion uptake in the presence of the taurine-inhibitor combination is significantly less (2-fold difference) than with taurine alone. Therefore, these inhibitors because of their noncompetitive nature do not offer any information as to the structural conformation of taurine at its biological site.

The median-effect plot and median-effect equation, as described by Chou and Talalay (33, 34), were utilized in the present study to quantitatively analyze 1) the potency (e.g., the median-effect dose or IC_{50}); 2) the shape of the dose-effect curve (e.g., the m value); 3) the conformity of the dose-effect relationship to the mass-action principle (i.e., r value); and 4) the combined effects of taurine and the taurine analogues in terms of synergism or antagonism. It was determined from the parallel nature of the median-effect plots of the data for TAPS and THQS that these inhibitors of ATP-dependent calcium ion uptake are mutually exclusive, i.e., they have similar modes of action. In addition, when the two compounds were used to inhibit calcium ion uptake in a fixed ratio of 1:25 (TAPS:THQS), the combination as calculated by the median-effect equation was determined to be synergistic.

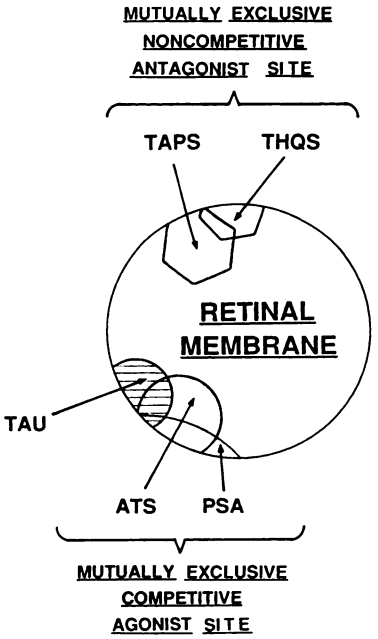


Fig. 8. Schematic interrelationships of ligand binding sites for taurine (*TAU*) and taurine analogues on the retinal membrane in the ATP-dependent calcium ion uptake system. Noncompetitive inhibition by TAPS or THQS toward taurine merely indicates that their binding site(s) are other than the taurine binding site, which includes nonspecific binding by the noncompetitive inhibitors. THQS and TAPS are synergistic, which suggests that THQS (or TAPS) may induce conformational changes that enhance the inhibitory binding of TAPS (or THQS). Similarly, PSA (a partial agonist) and taurine are synergistic and, thus, PSA may induce conformational changes on the binding site of taurine that enhance the stimulatory binding of taurine. The kinetic evaluation of the data for the combined usage of taurine plus ATS (an agonist) suggests an additive effect. The V_{max} for taurine equals the V_{max} for ATS but is greater than the V_{max} for PSA.

When the median-effect plots were used to analyze the stimulatory effects of taurine plus ATS and taurine plus PSA on ATP-dependent calcium ion uptake, it was also observed that the slopes were equal (i.e., parallel), suggesting that taurine, ATS, and PSA have similar modes of action. In addition, the analysis of the effects of the fixed combination of taurine plus ATS (1:1) indicates that the combination is additive, whereas the analysis of the fixed combination of taurine plus PSA (1:1) suggests synergism. The synergistic effect suggests that PSA may induce conformational changes on the receptor site(s) that favor further taurine binding.

We report here that PSA is a stimulator of ATP-dependent calcium ion uptake, although relatively weak when compared with taurine. This observation is of considerable interest because PSA is a secondary amine incorporating the nitrogen atom within the ring structure. In the sulfone series of taurine analogues that were previously tested for their stimulatory effects on ATP-dependent calcium ion uptake, ATS and APS have the sulfur atom within the ring structure; the amino moiety is free to rotate on the carbon atom in the 3 position of the ring (14). Sulfone analogues that have both the sulfur and the nitrogen atom in the ring, thiomorpholine-1,1-dioxide and *N*-methyl-thiomorpholine-1,1-dioxide (tertiary amine), have no effect. In these inactive analogues, the nitrogen atom is incorporated into the ring, similar to PSA, and the sulfur atom is incorporated into the ring, similar to ATS and APS. A pivotal binding moiety may be required for biological activity, whether it is a primary amine or a sulfonic acid moiety. Neither the sulfone or secondary amine provide pivotal binding. The active analogues ATS and APS have primary amino nitrogen atoms as the pivotal binding moiety, whereas PSA has a sulfonic acid moiety as the pivotal binding moiety. Thiomorpholine-1,1-dioxide and *N*-methyl-thiomorpholine-1,1-dioxide have no pivotal binding moiety and therefore are inactive.

It can be postulated from looking at the Dreiding stereomodels of the structures of taurine and the taurine analogues in Fig. 1B that the agonists (ATS and PSA) in the ATP-dependent calcium ion uptake system assume an extended configuration about the N-C-C-S (taurine) moiety that is common to each analogue. Taurine, because of the free rotation of the amino and sulfonic acid moieties about the methylene bridge, can also assume this extended configuration. In both ATS and PSA, the N-C-C-S moiety is relatively planar, although each compound has a different portion of the taurine molecule contained within the ring structure. However, in the antagonist analogue TAPS, the ring is perpendicular to the plane of the N-C-C-S moiety. In THQS, the amino and sulfonic acid moieties are not in an extended configuration. Furthermore, THQS contains an aromatic ring that may affect binding despite its possessing a pivotal binding moiety, i.e., the sulfonic acid moiety. These structural relationships may, therefore, account for the agonist activity of ATS and PSA and the noncompetitive antagonist activity of TAPS and THQS. A schematic interrelationship of the ligand binding sites for taurine and the taurine analogues on the retinal membrane is shown in Fig. 8. In this scheme, the effects of the analogues singly, in combination with taurine (ATS plus taurine, PSA plus taurine, TAPS plus taurine, THQS plus taurine), or in combination with each other (TAPS plus THQS) are summarized with respect to their synergistic, additive, or antagonistic actions, their competitive/noncompetitive inhibitions, and their mutually exclusive/nonexclusive effects on ATP-dependent calcium ion uptake activity. In addition, the scheme demonstrates that certain of the relationships between taurine and the analogues of taurine are noncompetitive and mutually exclusive.

In summary, we conclude from these studies that the two inhibitors, TAPS and THQS, act independently of taurine but each has a similar mode of action on ATP-dependent calcium ion uptake in a rat retinal membrane preparation. Both demonstrate noncompetitive kinetics with respect to taurine. ATS and PSA, stimulators of ATP-dependent calcium ion uptake, were both determined to be mutually exclusive with respect to taurine, when tested in combination with taurine in equimolar concentrations. It was calculated from the median-effect equation that the combination of taurine plus ATS is additive, whereas the combination of taurine plus PSA is synergistic.

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