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Stimulatory Effect of Taurine on Calcium Ion Uptake in Rod Outer Segments of the Rat Retina Is Independent of Taurine Uptake[1]

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ABSTRACT

Taurine stimulates ATP-dependent Ca^{2+} uptake in the rat rod outer segments (ROS). This stimulation has been linked to the function of the cyclic nucleotide-gated cation channel, implying an important physiologic role for taurine in visual signal transduction. Calmodulin (CaM) has been reported to affect taurine transport in the choroid plexus and also to inhibit the cyclic nucleotide-gated channel; thus, the effects of the competitive CaM inhibitors trifluoperazine (TFP) and *N*-(8-aminooctyl)-5-iodonaphthalene-1-sulfonamide (J-8) were studied on Ca^{2+} and taurine uptake in the rat ROS. Pretreatment of the ROS preparation with TFP and J-8 for 5 min before measurement of Ca^{2+}-uptake activity produced inhibition of the effects of taurine on ATP-dependent Ca^{2+} uptake. Both TFP and J-8 also were effective in inhibiting high-affinity taurine uptake. In both uptake systems, inhibition by TFP was noncompetitive. These data initially suggested that the stimulatory effects of taurine on ATP-dependent Ca^{2+} uptake are dependent on taurine uptake. However, competitive inhibition of taurine uptake by guanidinoethane sulfonate did not produce any effect on the stimulatory effects of taurine. Previous studies have proposed that taurine binds directly to the plasma membrane, and our study demonstrated that TFP inhibits taurine binding to the ROS. In addition, our study demonstrated that taurine uptake is unaffected by varying the concentration of Ca^{2+} and that the effects of TFP are independent of Ca^{2+}, suggesting that TFP acts through a CaM-independent mechanism.

Taurine is a free amino acid found in high concentrations in mammalian tissues (Huxtable, 1989). Its function has been extensively studied in the heart, kidney, liver, and eye, to name a few tissues. Although taurine has been linked to many physiologic functions, such as osmoregulation, protein phosphorylation, and calcium metabolism, its exact mode of action is still unclear. Taurine appears to perform a protective buffering function in many cell types relative to Ca^{2+} transport, being inhibitory under conditions of high Ca^{2+} concentration and stimulatory under conditions of low Ca^{2+} concentration (for review, see Huxtable, 1989).

Taurine modulation of calcium flux in the retina is particularly interesting. In a model of experimental regeneration of goldfish retina, a system for the study of central nervous system regeneration (Landreth and Agranoff, 1979), taurine was demonstrated to stimulate neuritic growth by increasing calcium influx (Lima et al., 1988, 1993). Taurine is known to produce stimulation of Ca^{2+} uptake in the whole-rat retina and in isolated rod outer segments (ROS) under conditions of low micromolar Ca^{2+} concentrations (for review, see Lombardini, 1991). In these previous studies, stimulation by taurine was observed to be concentration-dependent, up to a concentration of 32 mM (Militante and Lombardini, 1998a). The effect of taurine is assumed to be dependent on binding to the plasma membrane with or without the subsequent uptake into the cell, although the mechanism of action behind the effects of taurine is currently unclear (Fig. 1).

The stimulation of Ca^{2+} uptake in the retina by taurine is ATP-dependent and is antagonized by pharmacologic agents that specifically block cyclic nucleotide-gated (CNG) cation channels (Fig. 1), suggesting that taurine may be modulating the function of these channels in the retina (Militante and Lombardini, 1998b). The CNG channels are essential components of the signal transduction system found in the ROS of the photoreceptor layer in the retina (Finn et al., 1996). The CNG channels also are activated under conditions of low intracellular Ca^{2+}, thus suggesting a physiologic significance for the participation of taurine in their modulation (for review, see Baylor, 1996). Ca^{2+} and calmodulin (CaM) participate in the same signal transduction system by exerting an inhibitory effect on the opening of the CNG channel (for

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ABBREVIATIONS: ROS, rod outer segments; CNG, cyclic nucleotide-gated; CaM, calmodulin; TFP, trifluoperazine; J-8, *N*-(8-aminooctyl)-5-iodonaphthalene-1-sulfonamide; GES, guanidinoethane sulfonate; KRB, Krebs-Ringer-bicarbonate; BCA, bicinchoninic acid.

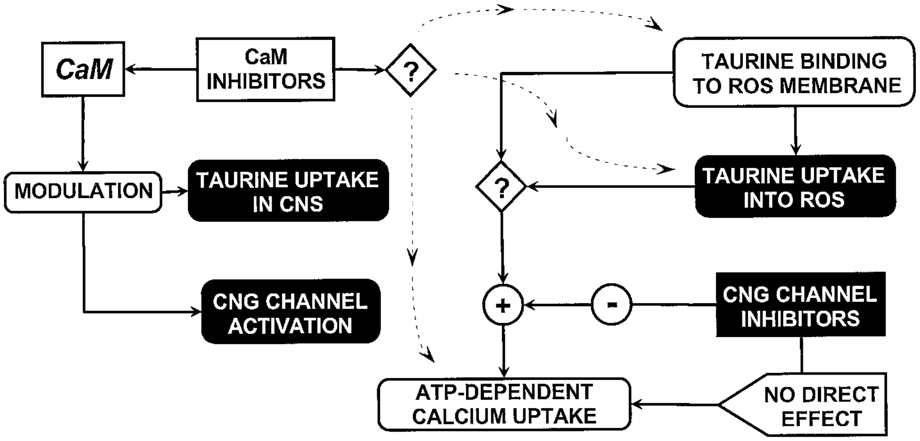

Fig. 1. Schematic diagram of the experimental design. Solid arrows represent effects on systems or physiological events that are well established. Broken arrows point to the variables being studied.

review, see Koch, 1995; Molday, 1996). In our study, the effect of CaM inhibitors on taurine modulation of Ca^{2+} uptake in the ROS was studied (Fig. 1).

Taurine is known to be transported in the retina through two saturable uptake systems, one system exhibiting high affinity with taurine and the other low affinity (Militante and Lombardini, 1999). However, only the high-affinity uptake system appears to be functional in the isolated ROS. The effects of taurine on ATP-dependent Ca^{2+} uptake in the ROS may thus be dependent on the function of this transporter. Because taurine transport also has been linked to CaM activity (Fig. 1) in rat cerebral cortical slices (Law, 1994, 1995), in the rat choroid plexus (Keep and Xiang, 1996), and in a human retinal pigment epithelial cell line (Ramamoorthy et al., 1994), the modulation of taurine uptake by CaM inhibitors is reported herein. In addition, CaM activity is dependent on Ca^{2+} (Persechini et al., 1989; Niki et al., 1996); thus, the effect of Ca^{2+} on taurine uptake also was studied.

The high-affinity transport system in the ROS exhibits a Michaelis-Menten (K_m) constant of 140 ± 8 μM and is clearly saturated at taurine concentrations <1.0 mM (Militante and Lombardini, 1999). Thus, the stimulatory effect of high taurine concentrations (i.e., up to 32 mM) on ATP-dependent Ca^{2+} uptake cannot be accounted for by an increase in taurine transport, suggesting that the effects of taurine on ATP-dependent Ca^{2+} uptake may not be dependent on taurine transport. However, taurine may exert its effects by binding to and modifying phospholipid membranes (Huxtable and Sebring, 1986), thereby modulating the function of membrane-bound proteins. In this context, taurine is known to increasingly bind to membranes up to 30 mM (for review, see Huxtable, 1989). Lombardini and Prien (1983) described two binding sites in the whole-rat retinal preparations, one with $K_D = 7.6$ μM and the other with $K_D = 334$ μM, although the highest concentration of taurine used in the binding assay was 1 mM, perhaps precluding the detection of a low- or lower-affinity binding site. Thus, taurine binding in the isolated ROS and the modulation of taurine binding by CaM antagonists also were studied herein to determine whether taurine stimulation of Ca^{2+} uptake is mediated through taurine binding (Fig. 1).

Experimental Procedures

Materials. Taurine and the CaM antagonist trifluoperazine (TFP) were purchased from Sigma Chemical Co. (St. Louis, MO) and *N*-(8-aminooctyl)-5-iodonaphthalene-1-sulfonamide (J-8), also a CaM antagonist, was obtained from Alexis Corp. (San Diego, CA). [1,2-Bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid acetoxy-methyl ester] was a generous gift from Dr. Tina Machu (Department of Pharmacology, Texas Tech University Health Sciences Center). Guanidinoethane sulfonate (GES), a taurine transport inhibitor, was synthesized according to the procedure of Morrison et al. (1958). ^{45}Calcium chloride and [^{3}H]taurine were purchased from New England Nuclear (Boston, MA). Ahlstrom glass fiber filter paper was obtained from Fisher Scientific (Pittsburgh, PA). Bincinchoninic acid was purchased from Pierce (Rockford, IL).

Isolation of ROS. Adult rats (Sprague-Dawley) were anesthetized with CO_2 and sacrificed through cervical dislocation. The eyes were removed and placed in 0.3 M mannitol (2°C). The cornea was cut open and the lens was extracted. The retina was teased off of the sclera and collected in ~40 ml of the mannitol solution. The isolated retinae were pooled and vortexed for 10 to 20 s and allowed to stand until the retinae settled. The supernatant, which contained the ROS, was collected and the procedure was repeated to maximize ROS yield. The supernatant was then centrifuged for 15 min at 16,000*g* and the pellet was resuspended in Krebs-Ringer-bicarbonate (KRB) buffer (118 mM NaCl, 1.2 mM KH_2PO_4, 4.7 mM KCl, 10 μM $CaCl_2$, 1.17 mM $MgSO_4$, 25 mM $NaHCO_3$, 5.6 mM glucose) for Ca^{2+} and taurine uptake experiments. For some experiments with KRB buffer, $CaCl_2$ was omitted or was added in varying concentrations. KRB buffer was aerated with 5% CO_2/95% oxygen for 15 min and the pH of the solution adjusted to 7.4 with 6 M HCl buffer. For taurine-binding experiments, Krebs-Tris HCl buffer (118 mM NaCl, 1.2 mM KH_2PO_4, 4.7 mM KCl, 10 μM $CaCl_2$, 1.17 mM $MgSO_4$, 26 mM Tris base) was used. The Krebs-Tris HCl buffer was prepared by adjusting the pH to 7.4 with 6 M HCl. The ROS were suspended in the appropriate buffer by passing the suspension through a 25-gauge needle. The ROS preparation was kept on ice until use.

Calcium-Uptake Assay. The ROS were incubated in a 37°C water bath in a final volume of 250 μl in the presence of $^{45}CaCl_2$ (~1.0 μCi), as described in Militante and Lombardini (1998a). Reagents and the KRB buffer were added to the incubation tubes in the appropriate concentrations and the mixture was warmed in the water bath for 2 min before the reaction was initiated by the addition of the ROS (50–150 μg; no preincubation). The reaction was terminated after 2 min by the addition of 3 ml of ice-cold buffer and then immediately filtering through a Millipore apparatus. The glass fiber filter paper was washed three times with 3 ml of ice-cold buffer; the radioactivity bound to the paper was counted in a scintillation counter. For certain experiments, the ROS were added to the incubation tube in the absence of $^{45}Ca^{2+}$ and exposed to TFP in a 37°C water bath for 5 min (preincubation) before the reaction was initiated by the addition of 10 μM $^{45}Ca^{2+}$ (~1.0 μCi). The reaction was then terminated after 2 min as described above. Blanks were measured by filtering the mixture at zero time after initiating the reaction.

Taurine-Uptake Assay. The taurine-uptake assay was described in Militante and Lombardini (1999). Briefly, the reaction was carried out in a 37°C water bath in the presence of 50 μM [^{3}H]taurine (~1 μCi) and equal amounts of ROS in a final volume of 250 μl. For some experiments, the amount of [^{3}H]taurine added was varied. The reaction mixture was warmed for 2 min in the water bath, and the reaction was initiated by the addition of the ROS (50–150 μg/tube). The reaction was terminated after 5 min through filtration on a Millipore apparatus as described for the Ca^{2+}-uptake assay, and the glass fiber filter paper was washed three times with 3 ml of ice-cold buffer. Radioactivity remaining on the filter paper was then measured in the scintillation counter. Blank measurements were performed by measuring uptake at 2°C (on ice).

Taurine-Binding Assay. The taurine-binding assay was performed at 22°C (room temperature) and in a final volume of 250 μl, following procedures described in Lombardini and Prien (1983). Equal amounts of the ROS (50–150 μg) were added to the incubation mixture. Total [^{3}H]taurine (~2 μCi) concentration ranged from 50 μM to 5 mM. The taurine-binding reaction was started with the addition of the ROS to the mixture and was terminated after 60 min through filtration as with the Ca^{2+}-uptake assay. The bound radioactivity was counted in a scintillation counter. Taurine-nonspecific binding was determined by using high concentrations of taurine (100 mM) and was subtracted from total binding to calculate the taurine-specific binding.

Protein Measurement. Protein concentrations were assayed with the bicinchoninic acid (BCA) method. Briefly, aliquots of tissue suspensions were incubated with the BCA reagent (50 parts BCA solution:1 part 4% copper II sulfate) for 30 min in a 37°C water bath and the color reaction was measured in a spectrophotometer. BSA was used as the standard.

Statistical Analysis. Data were analyzed for statistical significance with Student's *t* test, one-way ANOVA, or linear regression analysis. Post hoc analysis was accomplished with the Duncan's multiple range test. Regression analyses were performed with GraphPad Prism software.

Results

Stimulation of Ca^{2+} Uptake in the ROS. Ca^{2+} uptake is stimulated in the isolated ROS by 1.2 mM ATP; this effect is potentiated by 32 mM taurine under conditions of low Ca^{2+} concentration (10 μM) (Fig. 2). Taurine (32 mM) alone did not significantly stimulate Ca^{2+} uptake. In previous reports, taurine stimulated ATP-dependent Ca^{2+} uptake in a concentration-dependent manner up to 32 mM (Militante and Lombardini, 1998a).

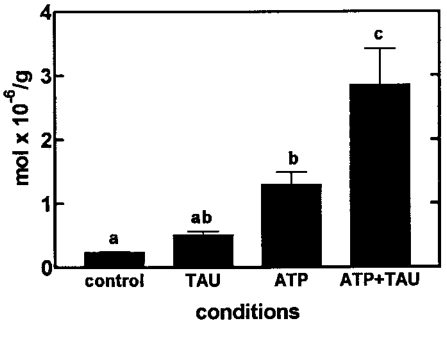

Fig. 2. Ca^{2+}-uptake activity determined in isolated ROS under different treatment conditions. Data are reported as means ± S.E. (N = 3–4). Different letters denote significant differences determined by ANOVA and Duncan's post hoc analysis ($p < .05$). TAU = 32 mM taurine; ATP = 1.2 mM ATP.

Specific Inhibition of Taurine Effects by CaM Antagonists. TFP and J-8 are CaM antagonists that act by binding to the CaM molecule with approximately equal potencies (IC_{50} = 4 and 3 μM, respectively; MacNeil et al., 1988; Craven et al., 1996). TFP and J-8 produced no significant effects on ATP-dependent Ca^{2+} uptake, either in the presence or absence of 32 mM taurine, when exposure to either CaM antagonist occurred only after the initiation of Ca^{2+} uptake (no preincubation; see *Experimental Procedures*) (Figs. 3A and 4A). However, TFP was previously reported to produce its effects on taurine transport when preincubation for 10 min was performed (Keep and Xiang, 1996); thus, in our study, ROS were preincubated (5 min) with TFP and J-8 before initiating ATP-dependent Ca^{2+} uptake. Under these preincubation conditions, TFP exposure resulted in the total inhibition of the effects of taurine on ATP-dependent Ca^{2+} uptake (Fig. 3B). However, no effects were observed on ATP-dependent Ca^{2+} uptake with TFP preincubation in the absence of taurine (Fig. 3B) as was the observed result when no preincubation was used (Fig. 3A). TFP also was observed to produce a concentration-dependent stimulatory effect on Ca^{2+} uptake in the absence of ATP and taurine (Fig. 5; data are not significant with one-way ANOVA and the Student's *t* test, but they are significant with linear regression analysis), perhaps as a result of CaM disinhibition of the CNG channel. Preincubation of the ROS with J-8 produced similar effects on taurine-stimulated ATP-dependent Ca^{2+} uptake, although the inhibition was not as marked as with TFP (Fig. 4B; significant with linear regression analysis). The inhibiting effects of TFP could not be overcome by increasing the

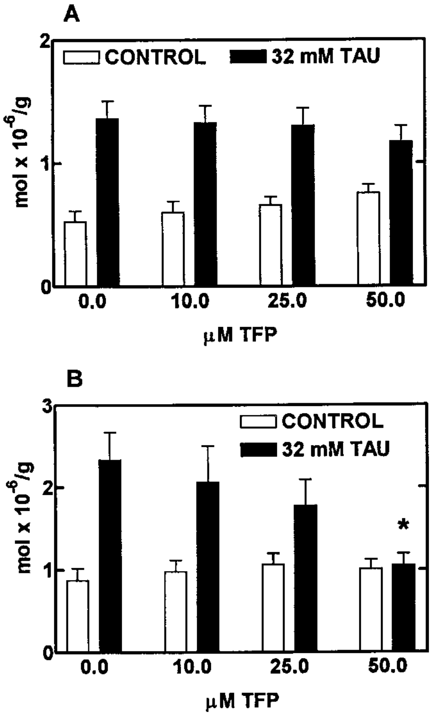

Fig. 3. ATP-dependent Ca^{2+}-uptake activity determined in isolated ROS incubated in the presence of varying concentrations of TFP. All tubes contained 1.2 mM ATP. Data are reported as means ± S.E. (N = 3). A, uptake in isolated ROS exposed to TFP only after the initiation of the uptake reaction. B, uptake in isolated ROS preincubated with TFP for 5 min before the initiation of the uptake reaction. Significant difference (*) compared with 0 TFP was determined by ANOVA and Duncan's post hoc analysis ($p < .05$).

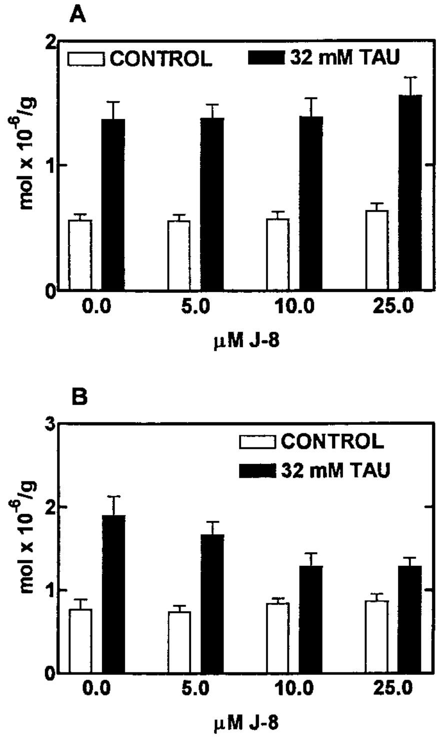

Fig. 4. ATP-dependent Ca^{2+}-uptake activity determined in isolated ROS incubated in the presence of varying concentrations of J-8. All tubes contained 1.2 mM ATP. Data are reported as means ± S.E. ($N = 3$). A, uptake in isolated ROS exposed to J-8 only after the initiation of the uptake reaction. B, uptake in isolated ROS preincubated with J-8 for 5 min before the initiation of the uptake reaction. There is no significant difference between groups with the one-way ANOVA and Student's *t* test. However, linear regression analysis revealed a significant concentration-dependent inhibition caused by J-8 in the presence of 32 mM taurine.

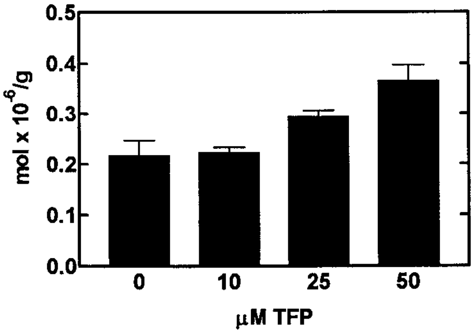

Fig. 5. Ca^{2+}-uptake activity determined in ROS exposed to varying concentrations of TFP in the absence of both taurine and ATP. Data are reported as means ± S.E. ($N = 4$). The ROS were preincubated with TFP for 5 min before the initiation of the uptake reaction. There is no significant difference between groups with the one-way ANOVA and Student's *t* test. However, linear regression analysis revealed a significant concentration-dependent stimulation caused by TFP.

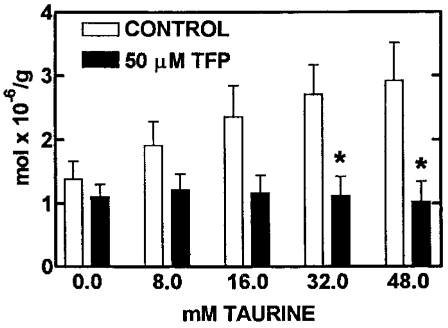

Fig. 6. ATP-dependent Ca^{2+}-uptake activity determined in isolated ROS exposed to 50 µM TFP and varying concentrations of taurine. Data are reported as means ± S.E. ($N = 6$). The ROS were preincubated with TFP for 5 min before the initiation of the uptake reaction. All tubes contained 1.2 mM ATP. Significant differences (*) of varying concentrations of TAU compared with respective control were determined by the Student's *t* test ($p < .05$).

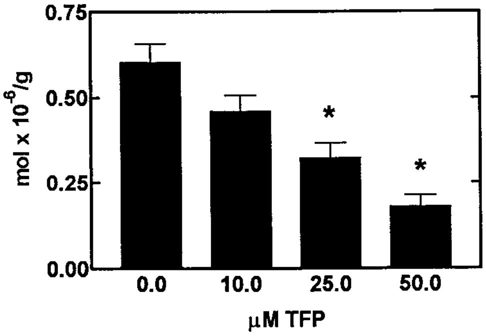

Fig. 7. Taurine-uptake activity determined in isolated ROS in the presence of varying concentrations of TFP. Data are reported as means ± S.E. ($N = 4$). Significant differences (*) of varying concentrations of TFP compared with 0 TFP were determined by ANOVA and Duncan's post hoc analysis ($p < .05$).

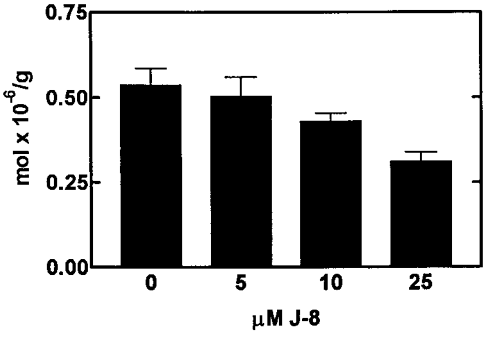

Fig. 8. Taurine-uptake activity determined in isolated ROS in the presence of varying concentrations of J-8. Data are reported as means ± S.E. ($N = 3$). No significant differences between groups are reported with the one-way ANOVA and Student's *t* test. However, linear regression analysis revealed a significant concentration-dependent decrease in taurine uptake with J-8 treatment.

concentration of taurine, suggesting a noncompetitive mechanism for TFP inhibition in the ATP-dependent Ca^{2+}-uptake system (Fig. 6).

Inhibition of Taurine Uptake by CaM Inhibitors. TFP treatment produced significant inhibition of taurine uptake at 50 µM (Fig. 7), whereas J-8 produced similar effects but to a lesser degree (Fig. 8; significant with linear regression analysis). TFP inhibition of taurine uptake also was measured through a range of taurine concentrations (10–250 µM). Eadie-Hofstee transformation of the data revealed that the inhibition was noncompetitive (Fig. 9), similar to TFP inhibition of the effects of taurine on ATP-dependent Ca^{2+} uptake (Fig. 6).

Effect of Taurine Transport Inhibition on ATP-Dependent Ca^{2+} Uptake. The involvement of taurine transport in the stimulation of ATP-dependent Ca^{2+}uptake in the ROS can be studied through the use of GES, a taurine analog that competitively inhibits taurine transport in the retina (Lake and Cocker, 1983; Quesada et al., 1984). In previous studies, GES was demonstrated to inhibit taurine

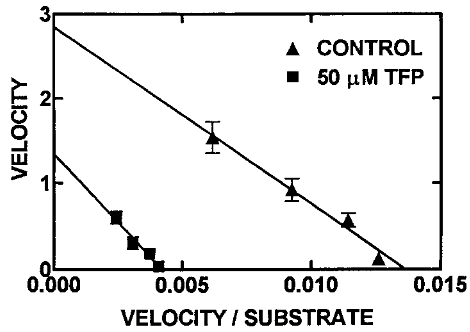

Fig. 9. Eadie-Hofstee transformation of data from taurine-uptake experiments performed at taurine concentrations from 10 to 250 μM and in the absence and presence of 50 μM TFP. Data are reported as means ± S.E. ($N = 3$). (Velocity = picomoles of taurine per microgram protein; Substrate = micromolar total taurine).

uptake specifically in ROS (Militante and Lombardini, 1999). If the stimulatory effects of taurine are dependent on taurine transport, then treatment with GES should antagonize the effects of taurine. GES at 32 mM produced no significant inhibition of the effects of taurine at 8, 16, and 32 mM (Fig. 10), suggesting that taurine transport is not involved in the stimulation of ATP-dependent Ca^{2+} uptake.

Effect of TFP on Taurine Binding. Taurine has been suggested to bind to phospholipid membranes to produce cellular effects (Huxtable and Sebring, 1986; Huxtable, 1990). Thus, the effect of TFP on taurine binding was studied to search for an alternative mechanism of action behind the stimulatory effects of taurine on ATP-dependent Ca^{2+} uptake. Taurine is known to bind to membranes with both high- and low affinity (for review, see Huxtable, 1989). TFP at 50 μM produced significant inhibition of taurine binding at 50 μM to 5.0 mM taurine (Fig. 11).

CaM Independence of TFP Effects. Interestingly, TFP modulation of taurine uptake appears to be Ca^{2+}-independent, suggesting that its effect is not mediated through CaM modulation. To study the Ca^{2+} dependence of taurine uptake, Ca^{2+} was excluded from the buffer and intracellular Ca^{2+} was eliminated by incubating the ROS in a 37°C water bath for 10 min in 100 μM 1,2-bis(2-aminophenoxy)ethane-*N,N,N′,N′*-tetraacetic acid acetoxymethyl ester, a Ca^{2+} chelator that is membrane-permeable (Tsien, 1981). Under these Ca^{2+}-depleted conditions, TFP produced the same inhibitory effects on taurine uptake (Fig. 12). Taurine uptake also was measured in the presence of increasing concentrations of Ca^{2+} (0–1000 μM); no changes in taurine uptake were observed by varying the Ca^{2+} concentrations (data not shown), similar to findings observed in Militante and Lombardini (1999). Thus, the effects of TFP on taurine transport in the ROS are probably not dependent on its effects on CaM.

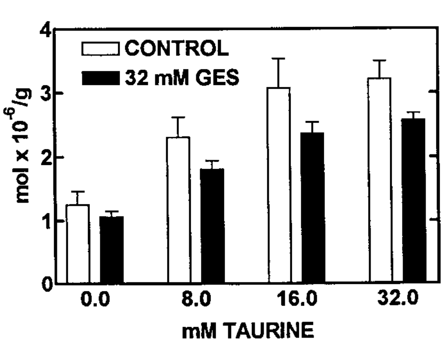

Fig. 10. ATP-dependent Ca^{2+}-uptake activity determined in isolated ROS exposed to 32 mM GES and varying concentrations of taurine. Data are reported as means ± S.E. ($N = 3$). No significant differences were observed in uptake activity with GES compared with respective control; data analyzed by Student's *t* test ($p > .05$).

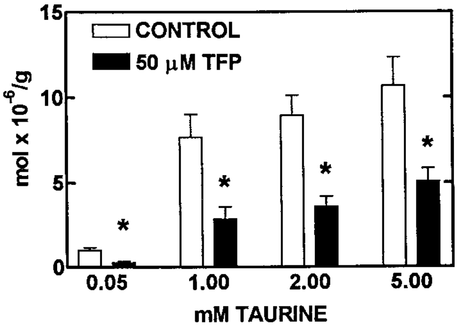

Fig. 11. Taurine-binding activity determined in isolated ROS in the presence of 50 μM TFP and varying concentrations of taurine. Data are reported as means ± S.E. ($N = 3–5$). Significant differences (*) of binding activity in presence of TFP compared with respective control were determined by the Student's *t* test ($p < .05$).

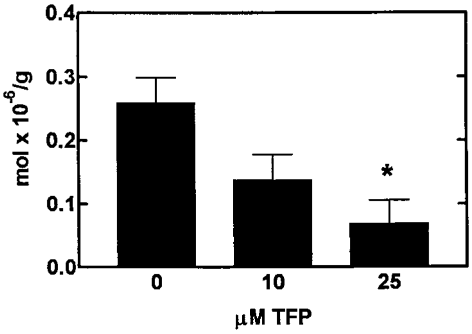

Fig. 12. Taurine-uptake activity determined in isolated ROS in the presence of varying concentrations of TFP after preincubation in the absence of Ca^{2+}. Data are reported as means ± S.E. ($N = 3$). Significant difference (*) of varying concentrations of TFP compared with 0 TFP was determined by ANOVA and Duncan's post hoc analysis ($p < .05$).

Discussion

The role that taurine plays in retinal physiology is interesting, primarily because of the reversible blindness or visual deficiencies discovered in mammalian models of taurine depletion (for review, see Lombardini, 1991). Consequences such as visual abnormalities may be expected because taurine levels are extremely elevated in retinal tissue, particularly in the photoreceptor layer wherein concentrations have been measured as high as 79 mM, and depletion would thus result in a drastic change in the physiologic milieu of the retinal cells. In fact, taurine depletion has been reported to cause gross damage and death of photoreceptor cells in the retina (Lake and Malik, 1987). However, few studies have addressed the issue of the mechanism of action of blindness due to taurine depletion.

The discovery of the possible link between taurine and CNG channels presents a significant addition to the knowledge of the mechanism of action of taurine in the retina (Militante and Lombardini, 1998b). These channels are involved in the phototransduction process that converts light signals into neural impulses, specifically by allowing for the inward movement of cations, including Na^+ and Ca^{2+}, into

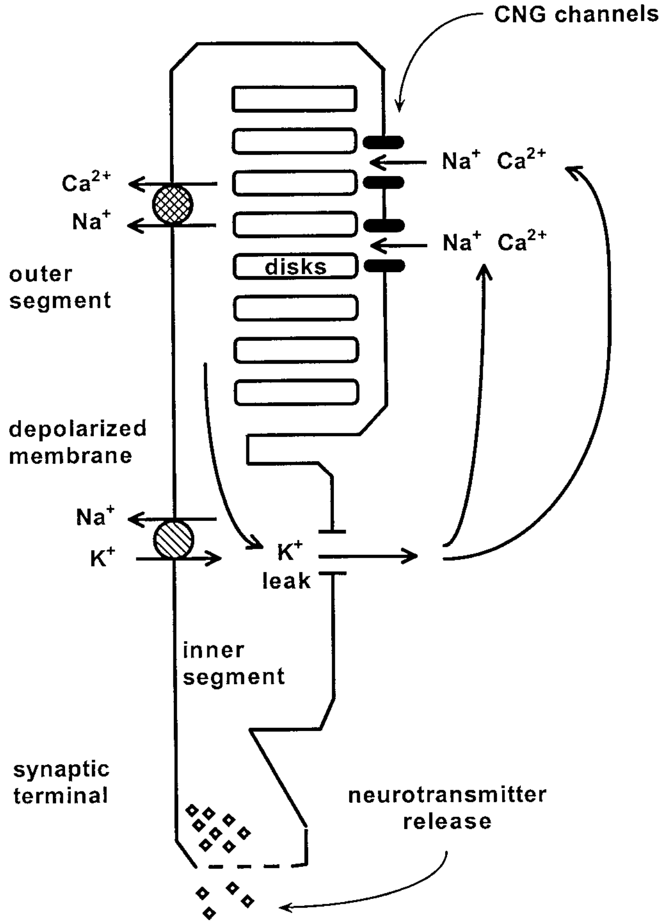

Fig. 13. Schematic diagram of the photoreceptor cell showing the ROS, the rod inner segment, and the Na^+-dependent standing dark current in the absence of light stimulation. The CNG channels are activated, allowing for the flow of cations into the cell, while intracellular Ca^{2+} is actively extruded. With light stimulus, the channels are closed but active extrusion of Ca^{2+} continues (see text).

the ROS, and in the process maintaining or reestablishing the "standing dark current" that is responsible for the depolarization of the ROS membrane (for review, see Finn et al., 1996) (Fig. 13). During light stimulus, the CNG channels are closed and cation levels drop. Specifically, Ca^{2+} levels decrease, mainly because of the continued active extrusion of Ca^{2+} through the sodium-calcium exchanger. The ROS membrane becomes hyperpolarized and a signal is then transmitted through the photoreceptor, altering neurotransmitter release onto nerve terminals. The depolarization of the ROS membrane before photostimulation must be restored within a brief time frame to allow for continued transmission of succeeding light signals (for review, see Pugh and Lamb, 1990). The restoration of the standing dark current occurs when the drop in intracellular Ca^{2+} concentration starts a series of events that ends with the reopening of the CNG channel (for review, see Baylor, 1996). The stimulatory effects of taurine under conditions of low Ca^{2+} concentration suggest that taurine might actually be essential for the rapid reopening of the CNG channels and the timely restoration of the standing dark current.

Taurine has long been thought to act at the level of the plasma membrane through two means: 1) interacting with protein receptors with high affinity and 2) binding with phospholipids with low affinity to alter the membrane environment (Huxtable and Sebring, 1986). The protein receptors are usually identified with taurine transport, although other types of membrane protein may bind taurine. The high-affinity taurine transporter has been cloned in the mouse brain (Liu et al., 1992) and retina (Vinnakota et al., 1997) and also in the rat brain (Smith et al., 1992). The specific taurine transporter in the rat ROS has not been cloned and may be different from the protein expressed in the rat brain. However, in human tissue, the high-affinity taurine transporter was cloned and was found to be identical in the retinal pigment epithelium, thyroid, and placenta (Miyamoto et al., 1996), suggesting that the same protein transporter is functional in different tissue types.

The high millimolar concentrations of taurine in mammalian tissues suggest a physiologic function(s) requiring low-affinity concentration interactions. The taurine-transport system in the ROS exhibits only high-affinity kinetics (Militante and Lombardini, 1999). Saturation of this transport system occurs at taurine concentrations of <1 mM, presenting a need for an alternate mechanism of action involving low-affinity interactions to explain the concentration-dependent stimulatory effects of taurine on Ca^{2+} uptake at concentrations >1 mM (8–48 mM) (Fig. 6). Correspondingly, the data presented herein provide evidence that the stimulatory effect of taurine is not dependent on the function of the taurine transporter and may instead be dependent on low-affinity binding of taurine to ROS. Particularly, TFP (50 μM) was demonstrated to inhibit both taurine uptake and taurine binding at a concentration that also inhibits taurine stimulation of Ca^{2+} uptake, whereas GES inhibition of taurine transport does not significantly affect the stimulation of Ca^{2+} uptake by taurine (Fig. 10).

Taurine transport has been closely associated with taurine binding to membranes, but distinguishing between these processes is difficult with commonly used retinal preparations. Low temperature and sodium-free conditions have been used to discriminate between taurine uptake and taurine binding (Salceda and Pasantes-Morales, 1982). In our study, taurine uptake was performed in a warm water bath (37°C) for 7 min and binding was done at room temperature (22°C) for 60 min. It is possible that the binding experiments in our study may not involve taurine binding exclusively, but rather a mixture of taurine binding and taurine uptake (Lombardini and Prien, 1983). The converse would be true for the taurine-uptake assays (i.e., these assays may contain a taurine-binding component) were it not for the short incubation period that would not allow time for proper equilibrium. Nevertheless, it can be concluded from the experiments reported herein that TFP also inhibits taurine binding to the ROS membrane, which probably is the mechanism behind TFP inhibition of taurine uptake. Consequently, inhibition of taurine binding also may be the mechanism behind TFP inhibition of the stimulation of ATP-dependent Ca^{2+} by taurine. However, because taurine binding was performed at taurine concentrations of ≤ 5 mM and the majority of taurine stimulation is observed at concentrations >8 mM (Fig. 6), this assumption still requires additional study.

The data suggest that the effect of TFP on taurine uptake is probably not mediated by the inhibition of CaM activity, mainly because the inhibitory effect of TFP on taurine uptake was preserved under Ca^{2+}-free conditions (Fig. 12). Also supportive of this idea are data that demonstrate taurine uptake is not modulated by changes in Ca^{2+} concentrations (0–1000 μM) (data not shown). It is possible that TFP directly interferes with taurine binding to the membrane to produce this effect on taurine uptake. It is interesting to note that the effects of TFP on taurine transport previously reported (Law, 1994, 1995; Ramamoorthy et al., 1994; Keep and Xiang, 1996) may not involve modulation of CaM activity.

The J-8 data indirectly support the idea that CaM activity is not involved in taurine stimulation of ATP-dependent Ca^{2+} uptake. The inhibitory effect of J-8 on both ATP-dependent Ca^{2+} uptake (Fig. 4) and taurine uptake (Fig. 8) is less marked than that of TFP, whereas TFP and J-8 are known to inhibit CaM-dependent processes with almost identical potency (MacNeil et al., 1988). That the two drugs produce similar effects on CaM activity but demonstrate dissimilar effects on stimulation of ATP-dependent Ca^{2+} uptake by taurine argues against CaM involvement. However, the data do not strictly preclude the involvement of CaM-dependent mechanisms in the inhibitory effects of these compounds on taurine-stimulated ATP-dependent Ca^{2+} uptake.

We conclude that taurine is stimulating the activation of CNG channels in the ROS through a mechanism that is not dependent on taurine uptake, but the taurine activation of the CNG channels may be dependent on low-affinity binding to the ROS membrane. Although the exact effect of taurine on membrane structure is unknown, the phospholipid environment of the channel may be altered to allow for increased activation of the CNG channel.

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