Amino Acids (2000) 19: 561–570

Amino
Acids
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Printed in Austria

Stabilization of calcium uptake in rat rod outer segments by taurine and ATP

J. D. Militante[1] and **J. B. Lombardini**[1,2]

[1] Department of Pharmacology, Texas Tech University Health Sciences Center, Lubbock, Texas, U.S.A.
[2] Department of Ophthalmology & Visual Sciences, Texas Tech University Health Sciences Center, Lubbock, Texas, U.S.A.

Accepted January 31, 2000

Summary. Calcium ion (Ca^{2+}) uptake was measured in rod outer segments (ROS) isolated from rat retina in the presence of varying concentrations of $CaCl_2$ in the incubation buffer (1.0–2.5 mM). It is known that taurine increases Ca^{2+} uptake in rat ROS in the presence of ATP and at low concentrations of $CaCl_2$ (Lombardini, 1985a); taurine produces no significant effects when $CaCl_2$ concentrations are increased to 1.0 and 2.5 mM. With the removal of both taurine and ATP, Ca^{2+} uptake in rat ROS increased significantly in the presence of 2.5 mM $CaCl_2$. Taurine treatment in the absence of ATP was effective in decreasing Ca^{2+} uptake at the higher levels of $CaCl_2$ (2.0 and 2.5 mM). Similar effects were observed with ATP treatment. The data suggest that taurine and ATP, alone or in combination, limit the capacity of the rat ROS to take up Ca^{2+} to the extent that a stable uptake level is achieved under conditions of increasing extracellular Ca^{2+}, indicating a protective role for both agents against calcium toxicity.

Keywords: Amino acids – Calcium uptake – Rod outer segments – Rat retina – ATP – Taurine

Introduction

Taurine (2-aminoethanesulfonic acid) is a free amino acid that has been described as an important modulator of mammalian cell function (reviewed in Huxtable, 1992). Taurine is found almost ubiquitously in mammalian tissues, usually in the μmole/g wet weight range, and is considered to be a regulatory factor in the maintenance of osmotic pressure, in calcium ion (Ca^{2+}) flux and in the phosphorylation of proteins. However, the exact mechanism of action of taurine has not been elaborated. The regulation of retinal function is one of the most interesting roles that has been proposed for taurine (reviewed in Lombardini, 1991). In experimental models using cats and rhesus monkeys,

taurine deficiency has been demonstrated to result in visual dysfunction and cellular damage in the retina. Changes in cellular morphology and in the electroretinogram pattern were also observed in rats that were taurine deficient.

Taurine is found in concentrations as high as 79 mM in rat eyes, specifically in the photoreceptor layer, the light sensitive cell layer of the retina (Voaden et al., 1977). The high concentration suggests that taurine may be involved in the phototransduction process that permits the perception of light to occur. Phototransduction is a process that is largely Ca^{2+}-dependent (Baylor, 1996) and taurine is known to modulate Ca^{2+} uptake in retinal membranes (reviewed in Lombardini, 1991). In fact, there is evidence that taurine modulates the uptake of Ca^{2+} through cGMP-gated channels in the retinal rod outer segments (ROS) (Militante and Lombardini, 1998), cation channels which figure prominently in the regulation of the phototransduction process (reviewed in Finn et al., 1996). Specifically, the effect of taurine to stimulate Ca^{2+} uptake in the ROS is inhibited by competitive antagonists of cGMP-gated channels. Thus, the modulation of Ca^{2+} uptake in the ROS by taurine may be a crucial event in the physiology of vision and in the function of the retina in general.

Taurine is known to increase Ca^{2+} uptake in the retina in an ATP-dependent manner under conditions of low Ca^{2+} concentration (10–100 μM) and to inhibit Ca^{2+} uptake under conditions of high Ca^{2+} concentration (1.4–2.5 mM) (reviewed in Lombardini, 1991). These effects have been demonstrated in isolated frog ROS (López-Colomé and Pasantes-Morales, 1981; Pasantes-Morales, 1982; Pasantes-Morales and Ordóñez, 1982) and in crude rat retinal membrane preparations (Lombardini, 1983; Liebowitz et al., 1989). With chick retinal tissue, the inhibitory effect of taurine in the presence of 2.5 mM $CaCl_2$ was greatest in isolated ROS as compared to synaptosomal and nuclear fractions (Pasantes-Morales et al., 1979). Ca^{2+} uptake in isolated rat ROS was increased with taurine and ATP in the presence of 10 μM $CaCl_2$ (Lombardini, 1985a; Militante and Lombardini, 1998) but, interestingly, was not significantly inhibited by taurine in the presence of 1.4 mM $CaCl_2$ (Lombardini and Liebowitz, 1990). This paper presents data from experiments designed to study the effects of both taurine and ATP on Ca^{2+} uptake in isolated rat ROS under conditions of high Ca^{2+} concentration (1.0–2.5 mM) and compares the data with findings from experiments using whole rat retina and animal models other than the rat.

Materials and methods

Chemicals

Taurine and β-alanine were purchased from Sigma Chemical Co. (St. Louis, MO). Guanidinoethanesulfonic acid (GES) 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). Bicinchoninic acid was purchased from Pierce Chemical Co. (Rockford, IL).

Isolation of rod outer segments

Adult rats (Sprague-Dawley strain) were anesthetized with CO_2 and killed though decapitation. The eyes were dissected out and stored at −80°C. The frozen eyes were thawed and placed in 0.3 M mannitol (2°C). The cornea was cut open, the lens was removed and the retina was teased off of the sclera. The retinae were pooled and vortexed for 10–20 seconds 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 minutes at 16,000 g and the pellet was resuspended in Krebs-bicarbonate-Ringer (KBR) buffer (NaCl 118 mM, KH_2PO_4 1.2 mM, KCl 4.7 mM, $MgSO_4$ 1.17 mM, $NaHCO_3$ 25 mM, glucose 5.6 mM) with various concentrations of $CaCl_2$. KRB buffer was aerated with 5% CO_2/95% oxygen for 15 minutes and the pH of the solution adjusted to 7.4 with 6 M HCl. The ROS were suspended in the KRB buffer and tissue clumps were broken up by passing the suspension through a 25-gauge needle. The ROS preparation was kept on ice until use.

Calcium-uptake assay

For the Ca^{2+} 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 previously (Militante and Lombardini, 1998). Reagents were added to the incubation tubes in the appropriate concentrations and the mixture was warmed in the water bath for 2 minutes before the reaction was initiated by the addition of the ROS (50–150 μg). The reaction was terminated after 2 minutes by the addition of 3 ml of ice cold buffer and then immediately filtered through a Millipore apparatus. The glass fiber filter paper was washed 3 times with 3 ml of ice-cold buffer; the radioactivity bound to the paper was counted in a scintillation counter. Blanks were measured by filtering the mixture at 0 time after initiating the reaction.

Protein measurement

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

Statistical analysis

Data were analyzed for statistical significance using one-way analysis of variance (ANOVA) and post-hoc analysis was accomplished using the Duncan's multiple range test.

Results and discussion

Effects of taurine in the presence of ATP

Taurine stimulated Ca^{2+} uptake in isolated rat ROS when 1.2 mM ATP was present and the buffer contained 10 μM $CaCl_2$ (Fig. 1A) (Lombardini, 1985a), similar to observations with rat retinal membrane preparations in buffer containing less than 500 μM $CaCl_2$ (Lombardini, 1983). This phenomenon has also been demonstrated in isolated frog ROS with 20 and 100 μM $CaCl_2$

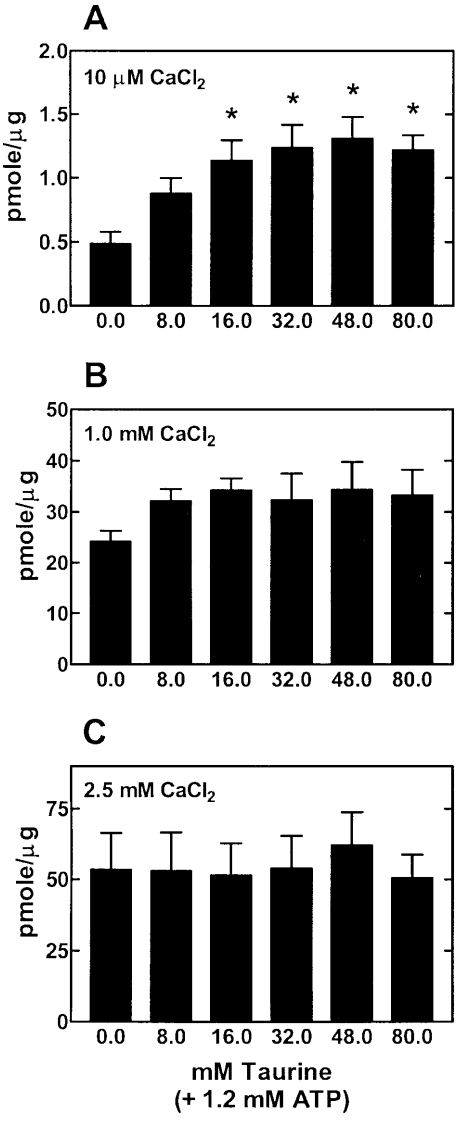

Fig. 1. The concentration-response graph for the effects of taurine on ATP-dependent Ca^{2+} uptake in rat rod outer segments in the presence of 1.2 mM ATP and **A** 10 μM $CaCl_2$, **B** 1.0 mM $CaCl_2$ and **C** 2.5 mM $CaCl_2$. An asterisk (*) indicates a significant difference from their respective control (0 mM taurine) values ($P < 0.05$) calculated by one-way ANOVA and the Duncan's multiple range test (mean ± SEM, N = 3–4, each N being a determination from an independent experiment)

(López-Colomé and Pasantes-Morales, 1981; Pasantes-Morales, 1982; Pasantes-Morales and Ordóñez, 1982), and in frog ROS disk membrane preparations with 10 μM $CaCl_2$ (Kuo and Miki, 1980). In the absence of ATP and under conditions of low $CaCl_2$, taurine has been reported to have no stimulatory effect on Ca^{2+} uptake in a rat retinal membrane preparation (Lombardini, 1983, 1985b; Liebowitz et al., 1989), in frog ROS (Pasantes-Morales and Ordóñez, 1982) and in frog ROS disk membranes (Kuo and

Miki, 1980). The dependence of the taurine stimulation of Ca^{2+} uptake in the frog and rat retina on ATP is unclear, but previous data suggest that in the rat retina, taurine modulation of ATPase activity is not the mechanism of action (Militante and Lombardini, 1998). Regardless, the data suggest that taurine acts to compensate for the lower levels of $CaCl_2$ by increasing uptake of Ca^{2+}.

The ATP-dependent stimulatory effect of taurine on Ca^{2+} uptake in the rat ROS was not observed under conditions of higher $CaCl_2$ concentrations. Taurine produced no significant change in Ca^{2+} uptake in the presence of 1.2 mM ATP and 1.0 or 2.5 mM $CaCl_2$ (Fig. 1B and 1C). This is similar to data gathered from rat retinal membrane experiments which demonstrated that taurine produced no effects in the presence of ATP at $CaCl_2$ concentrations $>500\,\mu$M (Liebowitz et al., 1989). Given the available data from rat experiments, it is clear that the stimulatory effect of taurine on retinal Ca^{2+} uptake in ROS is 1) dependent on the presence of ATP and 2) is lost as the level of $CaCl_2$ in the buffer increases. As expected, Ca^{2+} uptake increased in relation to the total amount of $CaCl_2$ present in the buffer.

In buffers containing 2.5 mM $CaCl_2$, taurine (25 mM) has been reported to inhibit Ca^{2+} uptake in chick retinal synaptosome, nuclear and ROS preparations in the presence of 1.0 mM ATP (Pasantes-Morales et al., 1979). The same effect was observed in frog ROS (López-Colomé and Pasantes-Morales, 1981). These finding contrast with data gathered from the aforementioned rat retina experiments (whole membrane and ROS preparations), however, the differences between species are unclear.

Effects of taurine in the absence of ATP

On the other hand, taurine produces significant inhibition of Ca^{2+} uptake in the ROS in the absence of ATP at 2.0 mM $CaCl_2$ concentrations (Fig. 2C). No effect was observed at lesser $CaCl_2$ levels (1.0 and 1.5 mM (Fig. 2A–B). The inhibition is more pronounced as the $CaCl_2$ level in the buffer is increased to 2.5 mM (Fig. 2D). The data concur with rat ROS data from previous experiments which indicate that taurine has no effect at 1.4 mM $CaCl_2$ concentration (Lombardini and Liebowitz, 1990). As with the stimulatory effects of taurine in the presence of ATP, these inhibitory effects appear to be dependent on the level of $CaCl_2$ present.

Similar findings were reported in rat retinal membrane preparations, although inhibition was observed to occur at a slightly lower $CaCl_2$ threshold (1.4 mM) (Liebowitz et al., 1989). The threshold $CaCl_2$ concentration (2.0 mM vs 1.4 mM for the ROS and the whole membrane preparation, respectively) is a significant difference between the effects of taurine in the whole retina and in the ROS. Perhaps, the effect of taurine in the ROS is more specific, occurring only at a higher range of Ca^{2+} concentration.

β-Alanine and guanidinoethanesulfonic acid (GES) are analogues of taurine. The inhibitory effect of taurine in the absence of ATP is mimicked by β-alanine with lesser potency but not by GES (Fig. 3) under conditions of higher

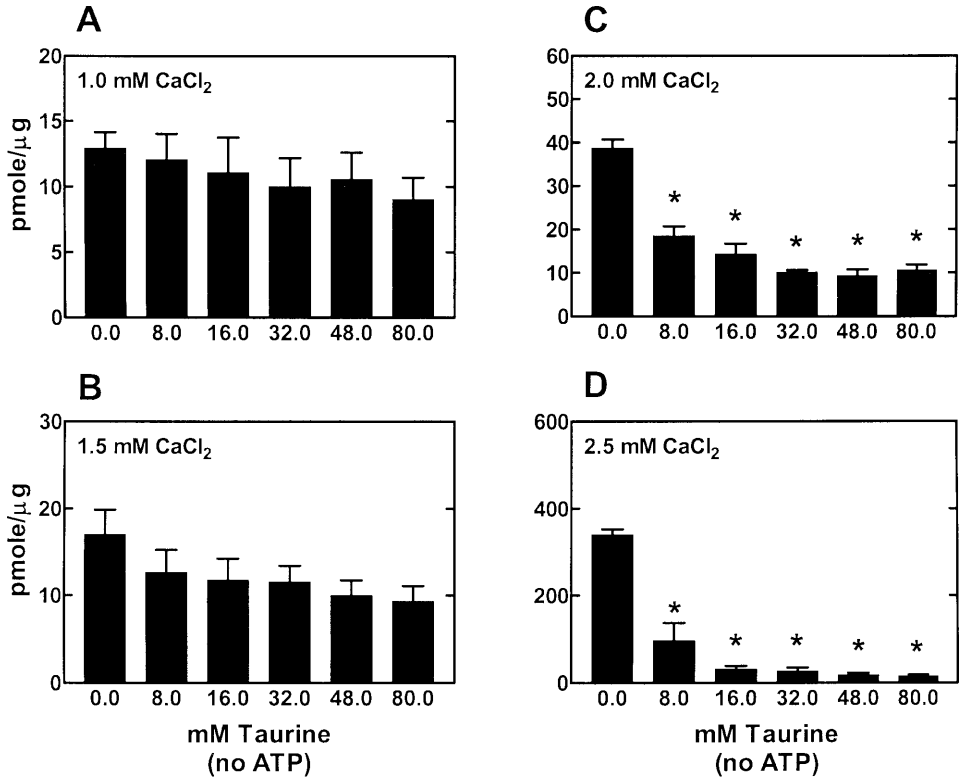

Fig. 2. The concentration-response graph for the effects of taurine on Ca^{2+} uptake in rat rod outer segments in the absence of ATP and in the presence of **A** 1 mM $CaCl_2$, **B** 1.5 mM $CaCl_2$, **C** 2.0 mM $CaCl_2$ and **D** 2.5 mM $CaCl_2$. An asterisk (*) indicates a significant difference from their respective control (0 mM taurine) values ($P < 0.05$) calculated by one-way ANOVA and the Duncan's multiple range test (mean ± SEM, N = 3–5, each N being a determination from an independent experiment)

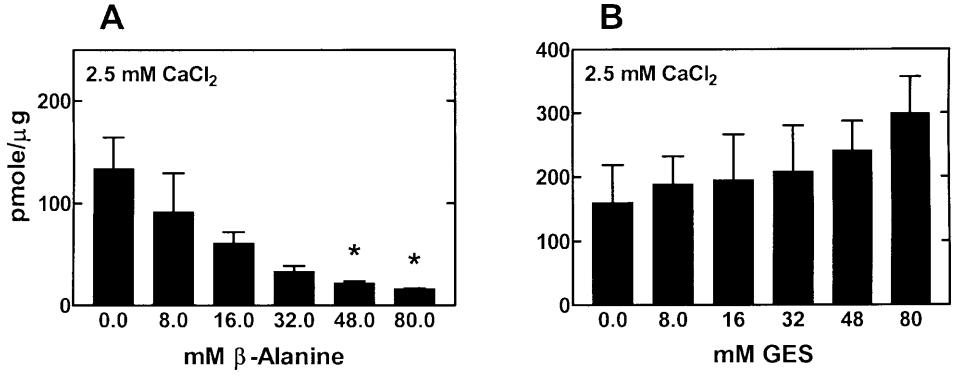

Fig. 3. The concentration-response graph for the effects of **A** β-alanine and **B** guanidinoethanesulfonic acid (*GES*) on Ca^{2+} uptake in rat rod outer segments in the absence of ATP and in the presence of 2.5 mM $CaCl_2$. An asterisk (*) indicates a significant difference from their respective control (**A**: 0 mM β-alanine; **B**: 0 mM GES) values ($P < 0.05$) calculated by one-way ANOVA and the Duncan's multiple range test (mean ± SEM, N = 3, each N being a determination from an independent experiment)

$CaCl_2$ concentration (2.5 mM). Clearly, the effect of taurine in both whole retina and isolated ROS from the rat is not due to a generalized disruption of membrane function due to changes in the molarity of the buffer. At 1.4 mM $CaCl_2$ concentration, β-alanine and GES did not produce any effect in preparations of whole rat retinae (Lombardini and Liebowitz, 1990), a finding difficult to evaluate relative to the present data due to the lower level of $CaCl_2$ in the buffer. β-Alanine produced an inhibitory effect of lesser potency than taurine when Ca^{2+} uptake in crude chick nuclear fractions was studied in the presence of 2.5 mM $CaCl_2$ and 1.0 mM ATP (Pasantes-Morales et al., 1979), but results are difficult to correlate as ATP is an additional factor and the treatment conditions are different.

As it was in the presence of 1.2 mM ATP (Fig. 1A–C, 0 mM taurine), Ca^{2+} uptake in the rat ROS increases in proportion to the amount of $CaCl_2$ in the buffer in the absence of ATP (Fig. 2A–D, 0 mM taurine). However, it is apparent that in the absence of both ATP and taurine, Ca^{2+} uptake at 2.5 mM $CaCl_2$ is much higher (Fig. 2D, 0 mM taurine). Also, taurine does not appear to produce inhibition to the same degree through the different levels of $CaCl_2$ used. Taurine at 16 mM concentration produces almost the same level of Ca^{2+} uptake (~10–20 pmoles/μg) regardless of the $CaCl_2$ concentration (Fig. 2A–D). Furthermore, at taurine concentrations of 32 mM and higher, Ca^{2+} uptake appears to stabilize to values around 5–10 pmoles/μg (Fig. 2A–D), which is roughly equal to Ca^{2+} uptake in the presence of 1.0 mM $CaCl_2$. Taurine, thus, produces a greater degree of inhibition as the total amount of $CaCl_2$ present increases. The data suggest that the effects of taurine on Ca^{2+} uptake in the rat ROS at high $CaCl_2$ concentrations may be more of a stabilizing nature than of an inhibitory nature.

Taurine may be decreasing the actual capacity of the ROS to bind or incorporate Ca^{2+}, and not merely modulating a single transport process. Taurine probably does not act directly with calcium ions but rather affects their binding to the membrane and subsequent transport (Huxtable, 1992). Huxtable suggests that taurine interacts with phospholipid structures to modulate Ca^{2+} transport processes (Huxtable, 1990). Taurine modification of membrane phospholipids may provide a mechanism behind the apparent decrease in the capacity of the rat ROS to bind and transport Ca^{2+}.

The effects of ATP in the absence of taurine

ATP alone appears to produce inhibition of Ca^{2+} uptake at the highest $CaCl_2$ concentration used (2.5 mM) (Fig. 1C v Fig. 2D, 0 mM taurine). A dose-response curve was thus accomplished for ATP alone under conditions of increasing $CaCl_2$ (1.0, 2.0 and 2.5 mM) (Fig. 4). ATP did not produce any significant effects at 1.0 mM $CaCl_2$ (Fig. 4A) but was inhibitory at the higher $CaCl_2$ concentrations (Fig. 4B–C). This effect has been demonstrated previously in rat retinal membrane preparations (Liebowitz et al., 1989). Similar to taurine, ATP produced greater inhibition of Ca^{2+} uptake as the $CaCl_2$ levels increased in the buffer, but only to essentially the same level (~10–20 pmole/

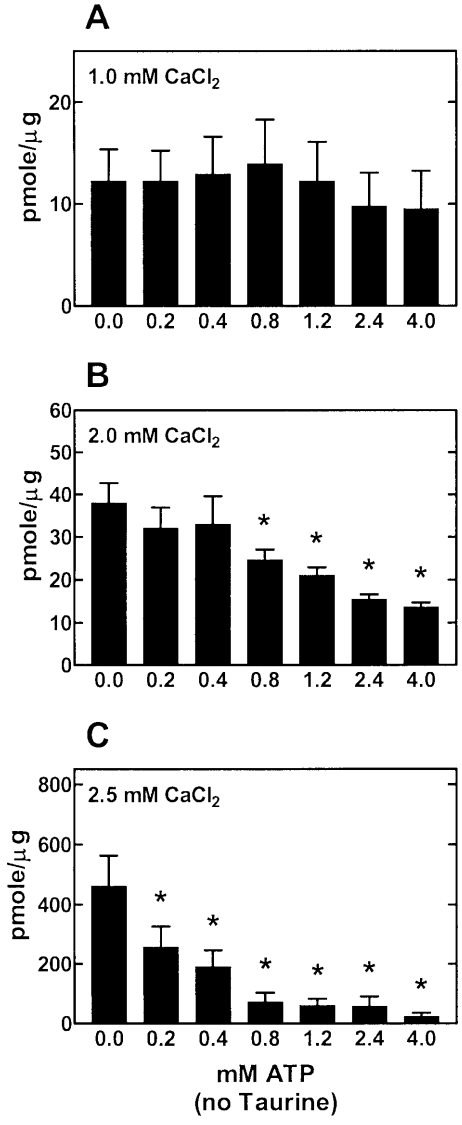

Fig. 4. The concentration-response graph for the effects of ATP on Ca^{2+} uptake in rat rod outer segments in the absence of taurine and in the presence of **A** 1 mM $CaCl_2$, **B** 2.0 mM $CaCl_2$ and **C** 2.5 mM $CaCl_2$. An asterisk (*) indicates a significant difference from their respective control (0 mM ATP) values ($P < 0.05$) calculated by one-way ANOVA and the Duncan's multiple range test (mean ± SEM, N = 3–4, each N being a determination from an independent experiment)

μg) and not any less. ATP may act in the same way as taurine to limit the capacity of the ROS to bind or incorporate Ca^{2+} and not merely affect a specific transport process.

A difference in species response is again evident relative to the effects of ATP. In buffers containing 2.5 mM $CaCl_2$, 1.0 mM ATP in the absence of taurine had no effect on Ca^{2+} uptake in chick retinal synaptosome and nuclear preparations (Pasantes-Morales et al., 1979), nor in frog ROS (López-Colomé

and Pasantes-Morales, 1981). In contrast, 1.2 mM ATP alone produced inhibition of Ca^{2+} uptake in both whole rat retina (Liebowitz et al., 1989) and in isolated rat ROS (Fig. 4C). It is interesting to note that in chick and frog retina, taurine produced inhibition of Ca^{2+} uptake in the presence of ATP (Pasantes-Morales et al., 1979; López-Colomé and Pasantes-Morales, 1981), while in rat retina, taurine produced no effects in the presence of ATP (Liebowitz et al., 1989; Fig. 1B–C).

Both ATP and taurine appear to be stabilizing factors as their efficacy in lowering Ca^{2+} uptake increases as the $CaCl_2$ levels increase, apparently with the end purpose of maintaining a constant level of Ca^{2+} movement into the ROS. In addition, the inhibitory effects of ATP and taurine do not appear to be additive in the rat retina, a premise that concurs with the idea that taurine and ATP act similarly to limit Ca^{2+} saturation in general. As the concentrations of taurine and ATP used in these experiments approximate physiologic levels (Voaden et al., 1977; Robinson et al., 1975; Carretta and Cavaggioni, 1976), it is reasonable to assume that physiologic control of Ca^{2+} flux in the rat retina involves the effects of both ATP and taurine described by these experiments.

Acknowledgement

This work was supported in part by grants from the RGK Foundation of Austin, TX, and the Taisho Pharmaceutical Co. Ltd. of Tokyo, Japan. Special thanks to Dr. James C. Hutson, Dr. Sandor Gyorke, Dr. John C. Fowler, Dr. Howard K. Strahlendorf, Dr. Yevgeniya Lukyanenko and Ms. Janet S. Koss for supplying us with rat eyes.

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Authors' address: John B. Lombardini, Ph.D., Department of Pharmacology, Texas Tech University Health Sciences Center, 3601 4th St., Lubbock, TX 79430, U.S.A.

Received January 25, 2000