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Pharmacological characterization of the effects of taurine on calcium uptake in the rat retina

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Summary. Taurine is known to increase ATP-dependent calcium ion (Ca^{2+}) uptake in retinal membrane preparations and in isolated rod outer segments (ROS) under low calcium conditions (10μM) (Pasantes-Morales and Ordóñez, 1982; Lombardini, 1991). In this report, ATP-dependent Ca^{2+} uptake in retinal membrane preparations was found to be inhibited by 5μM cadmium (Cd^{2+}), suggesting the involvement of cation channel activation. The activation of cGMP-gated cation channels, which are found in the ROS, is a crucial step in the phototransduction process. An inhibitor of cGMP-gated channels, LY83583, was found to inhibit taurine-stimulated ATP-dependent Ca^{2+} uptake but had no effect on ATP-dependent Ca^{2+} uptake in the absence of taurine, indicating that taurine may be increasing ATP-dependent Ca^{2+} uptake through a mechanism of action involving the opening of cGMP-gated channels. The activation of cGMP-gated channels with dibutyryl-cGMP and with phosphodiesterase inhibition using zaprinast caused an increase in ATP-dependent Ca^{2+} uptake in isolated ROS, but not in taurine-stimulated ATP-dependent Ca^{2+} uptake. LY83583 had the same effects in isolated ROS as in retinal membrane preparations. Another inhibitor of cGMP-gated channels, Rp-8-Br-PET-cGMPS, produced the same pattern of inhibition in isolated ROS as LY83583. Thus, there appears to be a causal link between taurine and the activation of the cGMP-gated channels in the ROS under conditions of low calcium concentration, a connection that suggests an important role for taurine in the visual signalling function of the retina.

Keywords: Amino acids – Calcium uptake – Taurine – Rod outer segments – cGMP-gated channels

Introduction

Taurine (2-aminoethanesulfonic acid) is a free amino acid found in high concentrations in all tissue types studied (for review, see Lombardini,

1991). Taurine depletion causes retinal degeneration and abnormalities in electroretinogram (ERG) measurements, suggesting an important role in vision. This role is possibly played through the effects taurine has on calcium ion (Ca^{2+}) flux in the retina. Taurine causes an increase in Ca^{2+} uptake in the retina under conditions of low calcium concentration (10–100μM) and in the presence of ATP and sodium bicarbonate (Pasantes-Morales and Ordóñez, 1982; Lombardini, 1983). On the other hand, taurine was found to be inhibitory at high Ca^{2+} concentrations (up to 2.5 mM) (López-Colomé and Pasantes-Morales, 1981; Liebowitz et al., 1989). These effects were observed in whole retinal homogenates from rat and in isolated frog rod outer segments (ROS), the photosensitive portion of the photoreceptor cell in the retina. The nature of the Ca^{2+} uptake measured in the retina and the biphasic effects of taurine are not fully understood, but both are probably multifactorial involving different Ca^{2+} uptake systems. Understanding the mechanism of action behind biphasic effects of taurine will shed light as to the actual physiologic role taurine plays in the retina. This report details experiments performed to characterize the stimulatory effects of taurine using pharmacological agents.

In the retina, the most important Ca^{2+} uptake system is the cation channel activated by guanosine-3′,5′-cyclic monophosphate (cGMP) found in the plasma membrane of the ROS (for review, see Finn et al., 1996). In the absence of light stimulus, these channels allow for the movement of sodium and Ca^{2+} into the ROS, completing a sodium current, called the standing dark current, which Na^+/K^+ ATPase activity creates in the rod inner segment (for review, see Baylor, 1996). During photoexcitation, these channels are closed after a cascade of events causes the levels of cGMP to fall, and intracellular Ca^{2+} levels decrease due to the continued extrusion of Ca^{2+} by the Na^+/Ca^{++}-K^+ exchanger. The disruption of the dark current hyperpolarizes the cell, resulting in the modulation of transmitter release at the synaptic terminal and the production of a signal to the brain. As Ca^{2+} levels drop, cGMP production increases and the reopening of the channel allows for the influx of sodium and Ca^{2+} back into the rod outer segments, thereby reestablishing the standing dark current. The reestablishment of the standing dark current is crucial for continued phototransduction in the retina. Given the drastic and rapid fluctuations in Ca^{2+} levels during phototransduction, the biphasic effects of taurine may prove to be an important factor in the regulation of Ca^{2+} levels in the retina.

One possibility is that the stimulatory effects of taurine on Ca^{2+} uptake may be due to the opening of the cGMP-gated channels. Recent experiments demonstrated that the effect of taurine on ATP-dependent Ca^{2+} uptake in the retina is independent of ATPase activity (Militante and Lombardini, 1998), suggesting that active uptake is not involved and leaving channel opening as an alternative mechanism of action. To test the possibility that taurine causes its effects by modulating Ca^{2+} channel opening, cadmium (Cd^{2+}) was used to non-selectively block Ca^{2+} channels in the retinal membrane preparations. The involvement of cGMP-gated channels was also demonstrated using specific channel blockers.

Materials and methods

Chemicals

$^{45}CaCl_2$ was obtained from New England Nuclear, Boston, MA. LY83583 was purchased from RBI, Natick, MA. Rp-8-Br-PET-cGMPS was purchased from Biolog Life Science Institute, La Jolla, CA. BCA protein assay reagent was obtained from Pierce Chemicals, Rockford, IL.

Preparation of retinal membrane homogenate and isolated rod outer segments

For preparation of the retinal membrane homogenate, adult Wistar rats were euthanized and the eyes were immediately removed from the animal. The eyes were then stored at −80°C until used. The eyes were thawed and retinal tissue was teased out of the eye cup in 0.32 M sucrose while on ice. All subsequent procedures were done on ice to maintain a 2°C temperature. The tissue was centrifuged for 15 minutes at 16,000 × g, washed in 20 mM bicarbonate, recentrifuged as before and then washed in sodium-bicarbonate buffer [$NaHCO_3$, 50 mM, NaCl, 50 mM; KCl, 50 mM; KH_2PO_4, 1.2 mM; $MgCl_2$, 2 mM (Kuo and Miki, 1980)] with $CaCl_2$ added to a final concentration of 10 μM. The tissue was recentrifuged, resuspended in sodium bicarbonate-$CaCl_2$ buffer and gently homogenized.

For the isolation of rod outer segments (ROS), 0.3 M mannitol was used instead of 0.32 M sucrose. Retinal tissue was dissected out as before and the ROS were removed by vortex-mixing for 6s, allowing the tissue to settle, and then decanting the supernatant which contained the ROS. The supernatant was centrifuged at 16,000 × g for 15 minutes and the pellet was then suspended in sodium-bicarbonate-$CaCl_2$ buffer. The remaining tissue components were discarded.

ATP-dependent Ca^{2+} uptake assay

The incubation system used sodium-bicarbonate buffer and was kept in ice until the start of the reaction. Reagents such as ATP and taurine were added in the appropriate concentrations, including identical amounts of $^{45}CaCl_2$ (400,000–500,000 dpm) in a final concentration of 10 μM $CaCl_2$. The reaction tubes were preincubated in a shaking water bath set at 37°C for 2 minutes. Retinal homogenate (100–300 μg) or ROS (30–100 μg) was added to start the reaction, making a final incubation volume of 250 μl, and the mixture was then incubated for an additional 2 minutes. The reaction was terminated by adding 3 ml of ice-cold sodium-bicarbonate-$CaCl_2$ buffer and immediately filtering on a Millipore glass fiber filter. The filter was washed three times with 3 ml of the above sodium-bicarbonate-$CaCl_2$ buffer and then counted for rdioactivity with Aquasol scintillation fluid. The amount of ^{45}calcium taken up by the retinal tissue was determined by subtracting the counts retained on the filter after a zero-time incubation.

Protein assay

Protein concentrations were assayed using the bicinchoninic acid (BCA) method (Pierce Chemical Co.).

Statistical analysis

Each data point N was a measurement derived from an independent experiment. Statistical analyses were performed using the GraphPad Prism and InStat software. Data were analyzed using the one-way analysis of variance (ANOVA) or linear regression analysis. Post-hoc analysis was accomplished using the Duncan's multiple range test.

Results and discussion

Inhibition of Ca^{2+} uptake in retinal membrane preparations

It is well-established that under conditions of low Ca^{2+} concentrations, ATP causes a significant increase in Ca^{2+} uptake above baseline in rat retinal membrane preparations and that taurine potentiates this increase (Pasantes-Morales and Ordóñez, 1982; Lombardini, 1983). In the presence of both ATP and taurine, two Ca^{2+} uptake systems were observed, one a low-affinity type and the other high-affinity type (Pasantes-Morales and Ordóñez, 1982; Lombardini, 1983). Recent experiments exclude the modulation of ATPase activity as a mechanism for action for the effects of taurine (Militante and Lombardini, 1998). To test for the involvement of Ca^{2+} channels in the observed taurine effects, Cd^{2+} was used to inhibit Ca^{2+} uptake. In neurons, Cd^{2+} causes a non-selective block of Ca^{2+} currents at micromolar concentrations (2–20μM) (Carbone and Swandulla, 1990). In Fig. 1, the increase in ATP-dependent Ca^{2+} uptake due to exogenous taurine is shown to be inhibited by 5μM cadmium while ATP-dependent Ca^{2+} uptake (in the absence of taurine) is not affected. At very high Cd^{2+} concentration (100μM), both ATP-dependent and taurine-stimulated ATP-dependent Ca^{2+} uptake were inhibited. These data suggest that the effects of taurine on ATP-dependent Ca^{2+} uptake are dependent on the opening of a Ca^{2+} channel that Cd^{2+} blocks. Exactly what this channel, or channels, may be is uncertain as the tissue preparation contains all the cell types found in the retina.

In terms of the role taurine may play in the visual signalling process, the possible effect of taurine on the cGMP-gated channel, among all the other types of Ca^{2+} channels, holds the most importance. LY83583 (6-anilino-5, 8-quinolinedione) has been shown to potently block cGMP-gated channels in olfactory receptor neurons, causing inhibition of cGMP-dependent currents

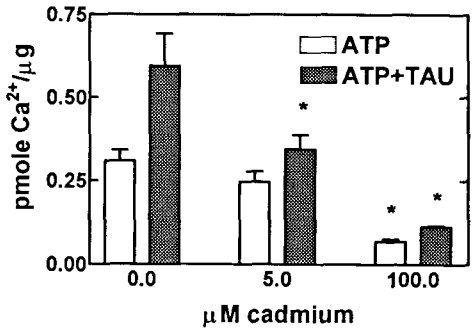

Fig. 1. The effect of Cd^{2+} on ATP-dependent Ca^{2+} uptake in rat retinal membrane preparations in the presence of 1.2 mM ATP, with or without 32 mM taurine. An asterisk (*) indicates a significant difference from their respective control (0μM Cd^{2+}) values ($P < 0.05$) calculated by one-way ANOVA and the Duncan's multiple range test (mean ± SEM, N = 4–5, each N being a determination from an independent experiment)

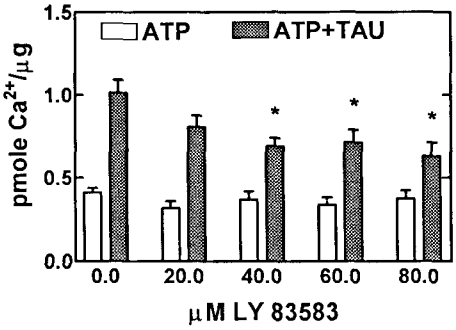

Fig. 2. The concentration-response graph for the effects of LY83583 on ATP-dependent Ca^{2+} uptake in rat retinal membrane preparations in the presence of 1.2 mM ATP, with or without 32 mM taurine. Linear regression analyses indicated that the slope for taurine-potentiated ATP-dependent Ca^{2+} uptake was significantly different from zero, sloping downward ($P < 0.05$), while the slope for ATP-dependent Ca^{2+} uptake was essentially equal to zero. An asterisk (*) indicates a significant difference from their respective control (0μM LY83583) values ($P < 0.05$) by one-way ANOVA and the Duncan's multiple range test (mean ± SEM, N = 5–8, each N being a determination from an independent experiment)

at concentrations as low as 1μM (Leinders-Zufall and Zufall, 1995). LY83583 appears to act both directly on the channel and on soluble guanylyl cyclase, the enzyme that produces cGMP in olfactory receptor neurons. This compound was thus used to inhibit the increase of Ca^{2+} uptake due to taurine. Figure 2 shows the effect of LY83583 on Ca^{2+} uptake in retinal membrane preparations. LY83583 has no effect on ATP-dependent Ca^{2+} uptake but has a significant inhibitory effect on taurine-potentiated Ca^{2+} uptake, albeit much less potently when compared to patch recording experiments with olfactory receptor neurons (Leinders-Zufall and Zufall, 1995). Though there is no direct way to correlate patch recordings with the Ca^{2+} uptake measured in these experiments, the data suggest that the effect of taurine is at least partially dependent on the open state of the cGMP-gated channel which is allowing Ca^{2+} flow into the cell.

Stimulation of Ca^{2+} uptake in the ROS

In our experimental system, the majority of the cGMP-gated channels are assumed to be closed as the retinal sample is exposed to ambient light. In theory, the opening of these channels should result in increased ATP-dependent Ca^{2+} uptake, an effect best seen in isolated ROS, as the cGMP-gated channels are primarily found in the ROS. In patch clamp experiments, cGMP is known to activate the channel with a dissociation constant K_d (for channel opening) of 17–30μM (Pugh and Lamb, 1990). Dibutyryl-cGMP, a cell-permeant analogue of cGMP, stimulated ATP-dependent Ca^{2+} uptake, but the increase was minimal for all concentrations of the agonist below

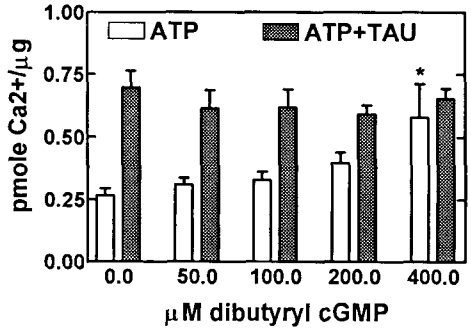

Fig. 3. The concentration-response graph for the effects of dibutyryl cGMP on ATP-dependent Ca^{2+} uptake in rat rod outer segments in the presence of 1.2 mM ATP, with or without 32 mM taurine. Linear regression analyses indicated that the slope for ATP-dependent Ca^{2+} uptake was significantly different from zero, sloping upward ($P < 0.01$), while the slope for taurine-potentiated ATP-dependent Ca^{2+} uptake was essentially equal to zero. An asterisk (*) indicates a significant difference from their respective control (0 μM dibutyryl cGMP) values ($P < 0.05$) calculated by one-way ANOVA and the Duncan's multiple range test (mean ± SEM, N = 6–7, each N being a determination from an independent experiment)

400 μM (Fig. 3). There is an obvious difference in potency when these effects of dibutyryl-cGMP are compared to its effects in patch clamp studies, but this is probably another manifestation of the lack of direct correlation between channel studies involving patch clamp techniques and actual Ca^{2+} uptake measurements in ROS isolates. It is also possible that, within the experimental time period of 2 minutes, dibutyryl-cGMP did not diffuse quickly enough through the cell membrane to raise the internal cGMP level to a level that would result in adequate channel opening and, in turn, stimulation of ATP-dependent Ca^{2+} uptake.

In contrast, no effect was observed when taurine-stimulated ATP-dependent Ca^{2+} uptake was measured in the presence of dibutyryl-cGMP. However, it is known that without proper depletion protocols, some level of endogenous cGMP remains in experimentally prepared dissociated ROS (Cote and Brunnock, 1993), providing low levels of endogenous agonist for channel activation. Thus, it is possible that cGMP-gated channels were maximally opened in the presence of taurine, through a mechanism of action that makes use of endogenously present cGMP, making ineffectual the addition of exogenous agonist.

Zaprinast, otherwise known as M&B 22,948, is a potent inhibitor (IC_{50} = 160 nM) of the cGMP-binding, cGMP-specific phosphodiesterase (PDE) found in the rod photoreceptor (Gillespie and Beavo, 1988). This compound was used to inhibit the degradation of endogenous cGMP in the ROS, potentially elevating, or at least maintaining, cGMP levels and theoretically causing greater activation of the cGMP-gated channels. Linear regression analyses of the data indicated a significant increasing trend ($P < 0.01$) in ATP-dependent Ca^{2+} uptake with zaprinast treatment (Fig. 4), although the absolute change

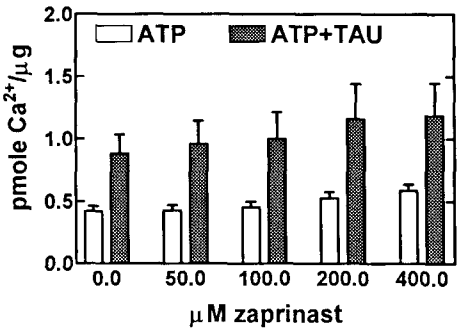

Fig. 4. The concentration-response graph for the effects of zaprinast on ATP-dependent Ca^{2+} uptake in rat rod outer segments in the presence of 1.2 mM ATP, with or without 32 mM taurine. Linear regression analyses indicated that the slope for ATP-dependent Ca^{2+} uptake was significantly different from zero, sloping upward ($P < 0.01$), while the slope for taurine-potentiated ATP-dependent Ca^{2+} uptake was essentially equal to zero. Data presented are means ± SEM, N = 6–7, each N being a determination from an independent experiment

above control (0 μM zaprinast) was found to be not significant using the one-way ANOVA. The data suggest that endogenous cGMP levels were sufficiently maintained to cause significant but not maximal channel opening. Perhaps, there was not enough time for endogenous cGMP to accumulate or that endogenous cGMP can only accumulate to a limited degree under these experimental conditions. Similar to the effects of dibutyryl-cGMP, zaprinast had no significant effect on taurine-stimulated ATP-dependent Ca^{2+}, also suggesting that cGMP-gated channels have been maximally stimulated already in the presence of taurine. Though the effects of dibutyryl-cGMP and zaprinast do not suggest an exact mechanism of action of taurine, the data still suggest that the stimulation of cGMP-gated channels is somehow involved in Ca^{2+} uptake measured in ROS.

Inhibition of ATP-dependent Ca^{2+} uptake in isolated rod outer segments

In theory, the effect of LY83583 with retinal membrane preparations should also be observed in isolated ROS. Figure 5 shows ATP-dependent Ca^{2+} uptake measured in isolated ROS and the inhibitory effect of LY83583 on taurine-stimulated ATP-dependent Ca^{2+} uptake, similar to the effects seen in retinal membrane preparations. The inhibition is not complete, indicating the involvement of other uptake systems or, perhaps, inefficient drug delivery. As in the homogenate preparation of the retinal membranes, LY83583 had no effect on ATP-dependent Ca^{2+} in the absence of taurine.

To verify the involvement of the cGMP-gated channel with the effects of taurine, a competitive antagonist of the channel was used to inhibit ATP-dependent Ca^{2+} uptake in the ROS. Rp-8-Br-PET-cGMPS is a cell-permeant cGMP derivative that has been found to inhibit cGMP-induced current with

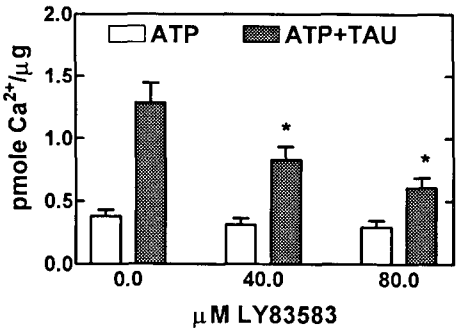

Fig. 5. The effect of LY83583 on ATP-dependent Ca^{2+} uptake in rat rod outer segment in the presence of 1.2 mM ATP, with or without 32 mM taurine. An asterisk (*) indicates a significant difference from their respective control (0μM LY83583) values ($P < 0.05$) calculated by one-way ANOVA and the Duncan's multiple range test (mean ± SEM, N = 4–7, each N being a determination from an independent experiment)

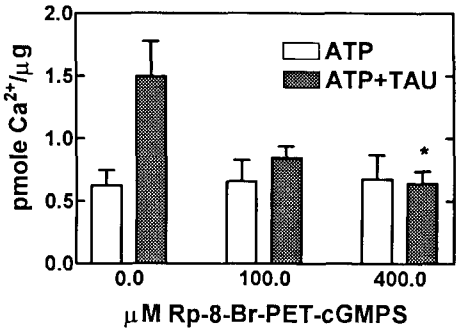

Fig. 6. The effect of Rp-8-Br-PET-cGMPS on ATP-dependent CA^{2+} uptake in rat rod outer segments in the presence of 1.2 mM ATP, with or without 32 mM taurine. An asterisk (*) indicates a significant difference from their respective control (0μM Rp-8-Br-PET-cGMPS) values ($P < 0.05$) calculated by one-way ANOVA and the Duncan's multiple range test (mean ± SEM, N = 4, each N being a determination from an independent experiment)

an IC_{50} of 25μM in excised patches (Wei et al., 1996). ATP-dependent Ca^{2+} uptake was not affected by Rp-8-Br-PET-cGMPS but taurine-stimulated uptake was inhibited (Fig. 6), demonstrating a certain level of specificity in the interaction of taurine and cGMP-gated channels.

It is important to note that in the absence of taurine, ATP-dependent Ca^{2+} uptake in the retina, specifically in the ROS, does not seem to involve the opening of the cGMP-gated channel, while in the presence of taurine it does. Thus, it is reasonable to assume that while ATP-dependent Ca^{2+} uptake in the absence of taurine probably involves a variety of different systems, taurine may specifically stimulate cGMP-gated channel opening to induce Ca^{2+} uptake in the ROS. Taurine could modulate channel opening by increasing the levels of cGMP, thereby increasing channel activation, or by increasing

the affinity of the channel for its agonist. More experiments are required to study these possibilities.

The idea that taurine modulates the opening of the cGMP-gated channel is a novel one and may present a very important function for taurine in the retina, specifically in the ROS. The decrease in Ca^{2+} level within the ROS is a crucial step in the process of photoexcitation. During this period of low Ca^{2+} concentration, the expected effect of taurine would be to stimulate Ca^{2+} uptake into the ROS, a potentially beneficial effect in the process of reestablishing the standing dark current. Taurine, in theory, stimulates the activation of the cGMP-gated channel by endogenous cGMP during this recovery period. Afterwards, as Ca^{2+} levels rise, taurine would lose this stimulatory effect through some feedback mechanism and would actually inhibit Ca^{2+} uptake, preventing Ca^{2+} overload. This inhibitory effect of taurine may or may not involved the function of cGMP-gated channels, and provides another interesting field for inquiry. In any case, this type of biphasic modulation of Ca^{2+} uptake by taurine could become a very significant consideration in the understanding of phototransduction in the ROS.

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References

Baylor D (1996) How photons start vision. Proc Natl Acad Sci USA 93: 560–565

Bean BP (1989) Classes of calcium channels in vertebrate cells. Annu Rev Physiol 51: 367–384

Carbone E, Swandulla D (1989) Neuronal calcium channels: kinetics, blockade and modulation. Prog Biophys Mol Biol 54: 31–58

Cote RH, Brunnock MA (1993) Intracellular cGMP concentration in rod photoreceptors is regulated by binding to high and moderate affinity cGMP binding sites. J Biol Chem 268: 17190–17198

Finn JT, Grunwald ME, Yau K-W (1996) Cyclic nucleotide-gated ion channels: an extended family with diverse functions. Annu Rev Physiol 58: 395–426

Gillespie PG, Beavo JA (1989) Inhibition and stimulation of photoreceptor phosphodiesterases by dipyridamole and M&B 22,948. Mol Pharmacol 36: 773–781

Kuo C-H, Miki N (1980) Stimulatory effect of taurine on Ca-uptake by disc membranes. Biochem Biophys Res Commun 94: 646–651

Leinders-Zufall T, Zufall F (1995) Block of cyclic nucleotide-gated channels in salamander olfactory receptor neurons by the guanylyl cyclase inhibitor LY 83583. J Neurophysiol 74: 2759–2762

Liebowitz SM, Lombardini JB, Allen C (1989) Sulfone analogues of taurine as modifiers of calcium uptake and protein phosphorylation in rat retina. Biochem Pharmacol 38: 399–406

Lombardini JB (1983) Effects of ATP and taurine on calcium uptake by membrane preparations of the rat retina. J Neurochem 40: 402–406

Lombardini JB (1991) Taurine: retinal function. Brain Res Rev 16: 151–169

López-Colomé AM, Pasantes-Morales H (1981) Effect of taurine on ^{45}Ca transport in frog retinal rod outer segments. Exp Eye Res 32: 771–780

Militante JD, Lombardini JB (1998) The effect of chelerythrine inhibition of calcium uptake and ATPase activity in the rat retina. Biochem Pharmacol 55: 557–565

Pasantes-Morales H, Ordóñez A (1982) Taurine activation of a bicarbonate-dependent, ATP-supported calcium uptake in frog rod outer segments. Neurochem Res 7: 317–328

Pugh EN Jr, Lamb TD (1990) Cyclic GMP and calcium: the internal messengers of excitation and adaptation in vertebrate photoreceptors. Vision Res 30: 1923–1948

Wei J-Y, Cohen ED, Tan Y-Y, Genieser H-G, Barnstable CJ (1996) Identification of competitive antagonists of the rod photoreceptor cGMP-gated cation channel: β-phenyl-1, N^2-etheno-substituted cGMP analogues as probes of the cGMP-binding site. Biochem 35: 16815–16823

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