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TAURINE REGULATION OF VOLTAGE-GATED CHANNELS IN RETINAL NEURONS

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Abstract

Taurine activates not only Cl^- -permeable ionotropic receptors, but also receptors that mediate metabotropic responses. The metabotropic property of taurine was revealed in electrophysiological recordings obtained after fully blocking Cl^- -permeable receptors with an inhibitory “cocktail” consisting of picrotoxin, SR95531, and strychnine. We found that taurine’s metabotropic effects regulate voltage-gated channels in retinal neurons. After applying the inhibitory cocktail, taurine enhanced delayed outward rectifier K^+ channels preferentially in Off-bipolar cells, and the effect was completely blocked by the specific PKC inhibitor, GF109203X. Additionally, taurine also acted through a metabotropic pathway to suppress both L- and N-type Ca^{2+} channels in retinal neurons, which were insensitive to the potent GABA_B receptor inhibitor, CGP55845. This study reinforces our previous finding that taurine in physiological concentrations produces a multiplicity of metabotropic effects that precisely govern the integration of signals being transmitted from the retina to the brain.

INTRODUCTION

Taurine, like GABA and glycine, activates ionotropic receptors that produce an inhibitory effect on neurons by promoting an influx of Cl^- . It is also capable of activating metabotropic receptors that regulate a wide range of mechanisms through intracellular second messenger and G-protein sensitive pathways. Although molecular evidence of a taurine-sensitive metabotropic pathway has not been uncovered, the metabotropic effects of taurine have been reported in brain tissues (Wu et al., 2005). Indeed, taurine has been found to regulate intracellular protein interaction, various aspects of mitochondrial function, and Ca^{2+} release from internal stores, resulting in changing activities of glutamate receptors and $\text{Na}^+/\text{Ca}^{2+}$ exchange, etc. (Li and Lombardini, 1991; El Edrissi A, Trenkner E. 1999, Wu & Prentice,

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2010). Many of these effects are believed to result from activation of metabotropic pathways via receptor-coupled G-proteins (Wu & Prentice, 2010).

In the vertebrate retina, taurine acts as a neurotransmitter as well as a modulator of neuronal activity. Many neurotransmitters serve to regulate voltage-gated channels (Parnas & Parnas, 2010), and thereby control cell excitability and neurotransmitter release. In a previous study from this laboratory, using Ca^{2+} imaging and whole-cell patch-clamp recordings of retinal neurons, we showed that taurine provides a dose-dependent regulation of Ca^{2+} -permeable glutamate receptors and voltage-gated Ca^{2+} channels. Moreover, we found that this regulatory activity was via CaMKII- and PKA-dependent intracellular pathways (Bulley & Shen, 2010). In addition, the regulation of Ca^{2+} -dependent synaptic release by taurine was reported in earlier studies of amphibian, rabbit and ox retinas (Burkhardt, 1970; Cunningham & Miller, 1980, DiGiorgio et., al 1977). Currently we have discovered that taurine also can suppress spontaneous synaptic release from inner retinal neurons. Clearly, taurine plays a key role in retinal synaptic transmission and modulation. In this report we will present evidence showing that taurine regulates voltage-gated K^{+} and Ca^{2+} channels in retinal bipolar cells and third-order neurons.

In many earlier studies, the effects of taurine were generated by application in concentrations of considered to be of no relevance physiologically. However, in the course of our studies we discovered that taurine produces metabotropic regulation at much lower concentrations, and that the effects of taurine were insensitive to the antagonists of GABA and glycine receptors. Interestingly, this taurine action leads to a specific modulation of voltage-gated K^{+} channels and Ca^{2+} channels, critical components of cell excitability and synaptic transmission.

1.2 METHODS

All procedures were performed in accordance with the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and approved by the University's Animal Care Committee.

1.2.1 Retinal Slice Preparation

Larval tiger salamanders (*Ambystoma tigrinum*) purchased from the Charles Sullivan (Nashville, TN, USA) and Kons Scientific (Germantown, WI, USA) were used in this study. The animals were kept in aquaria at 13 C under a 12 h dark–light cycle with continuous filtration. The retinas were collected from animals kept at least 6 h in the dark. After the animals were decapitated and double-pithed, the eyes were enucleated. Retinal slices were prepared in a dark room, under a dissecting microscope equipped with powered night-vision scopes (BEMeyer Co., Redmond, WA, USA), an infrared illuminator (850 nm), an infrared camera and a video monitor. The retina was removed from the eyecup in Ringer's solution consisting of (in mM): NaCl (111), KCl (2.5), CaCl_2 (1.8), MgCl_2 (1.0), HEPES (5.0) and dextrose (10), pH = 7.7. It was then mounted on a piece of microfilter paper (Millipore, Billerica, MA, USA) with the ganglion cell layer downward. The filter paper with retina was vertically cut into 250 μm slices using a tissue slicer (Stoelting Co., Wood Dale, IL, USA).

1.2.2 Cell Dissociation

Retinas were removed from the eyecup in Ringer's solution. The tissue was then digested in a freshly prepared enzymatic solution containing 50µl papain (12 U/ml) in 500µl of Ringer's solution to which was added 5 mM L-cysteine and 1mM EDTA (pH=7.4), and bathed for 20–35 minutes at room temperature. The papain-treated retinas were washed and mechanically triturated through a flame polished Pasteur pipette into the standard Ringer's solution. The dissociated cells were seeded on 18mm glass cover slips coated with Lectin and allowed to set for 20 minutes. The cover slip was then moved into the recording chamber, and superfused with oxygenated Ringer's solution.

1.2.3 Whole-Cell Patch Clamp Recording

Tissues (retinal slices or isolated cells) were placed in a recording chamber on the stage of an Olympus BX51WI microscope equipped with a CCD camera linked to a monitor. Whole-cell patch-clamp recordings were performed on bipolar cells, amacrine and ganglion cells in retinal slices as well as isolated cells using an EPC-10 amplifier and Pulse software (HEKA Instruments Inc., Bellmore, NY, USA). Patch electrodes (5–8MΩ) were pulled with an MF-97 microelectrode puller (Sutter Instrument Co., Novato, CA, USA). Recordings were obtained 5–10 minutes after membrane rupture in order to allow the cells to stabilize after dialysis of the electrode solution. Data were analyzed and plotted using Pulse and Igor/Excel software (WaveMetrics, Inc., Lake Oswego, OR, USA).

A gravity-driven perfusion system was used to superfuse all external solutions. The perfusion tube was placed 3mm away from the retinal slice and was manually controlled for delivering drugs during the experiments. All of the chemicals used in this study were purchased from Sigma (St. Louis, MO) and Tocris Bioscience (Ellisville, MO, USA).

1.2.4 Extracellular and Intracellular Solutions

To study the metabotropic effect of taurine on delayed outward K^+ (K_v) channel currents, the Ringer solution contained an inhibitory “cocktail” of Cd^{2+} (100µM), picrotoxin (100µM) and strychnine (10µM), thereby blocking ionotropic membrane receptors.

To study taurine's metabotropic regulation of voltage-dependent Ca^{2+} channels, the modified Ringer solution contained tetraethyl ammonium chloride (TEA, 40mM), tetrodotoxin (TTX, 1µM), and barium chloride ($BaCl_2$, 10mM). To adjust for the excess concentration of Cl^- ions in the TEA and Ba^{2+} -Ringer's solution, the concentration of NaCl was reduced accordingly.

1.2.5 Identification of Cell Types and Subtypes

On- and Off-bipolar cells in retinal slices were identified by their neuronal morphology after intracellular dialysis with the fluorescent dye Lucifer Yellow, introduced during whole-cell recording. The axonal loci of most On- and Off-bipolar cells are within sublamina *b* and *a* of the inner plexiform layer (IPL), respectively. However, many of the Off-bipolar cells were ‘displaced’, with somas in the outer nuclear layer (Maple et al. 2004). Their electrical signature and unique location allowed us to identify the Off-bipolar cells

Isolated third-order neurons (amacrine and ganglion cells) and bipolar cells often were distinguished based on physiological criteria; i.e., their distinctive transient Na^+ currents in whole-cell recording. Unlike bipolar and horizontal cells, which show extremely large inward rectifier currents, ganglion cells typically generate large Na^+ currents (exceeding 500 pA), and display very small inward rectifier currents at negative voltages, whereas amacrine cells have relatively small transient Na^+ currents as well as small inward rectifier currents. Because there are many different types of amacrine cells in salamander retina, these criteria are less than ideal, but did not significantly influence the results.

1.3 RESULTS

1.3.1 Taurine-enhanced K_v Currents in Off-Bipolar Cells

Retinal bipolar cells are interneurons that convey photoreceptor signals to amacrine and ganglion cells via mono- or multi-synaptic axon terminals located in the synaptic IPL. In general, they can be divided into On- and Off-bipolar cells by their depolarizing and hyperpolarizing responses to photic stimuli. However, as noted above, the two types can also be distinguished morphologically by the location of their terminal endings within the IPL. On-bipolar cells end in the more proximal portion (sublamina *b*) of the IPL, whereas the OFF-bipolar cells terminate in the distal portion (sublamina *a*) of the IPL.

Immunocytochemical analysis of the amphibian retina indicated that both taurine and the taurine transporter are present in photoreceptors and Off-bipolar cells (Bulley & Shen, 2010), suggesting that these neurons probably release taurine. To study the metabotropic effects of taurine the tissues were bathed in an extracellular solution that contained an inhibitory “cocktail” consisting of Cd^{2+} (100 μM) which blocks voltage-gated Ca^{2+} channels as well as Ca^{2+} dependent K^+ channels and Cl^- receptors were inhibited by picrotoxin (100 μM) and strychnine (5 μM). This solution is referred to as Cd(I) throughout the paper.

Voltage-gated channels in bipolar cells in retinal slices were activated by a series of 25 ms voltage steps ranging from -100 to $+60\text{mV}$. Figure 1A shows an example of currents elicited in an On- and Off- bipolar cell by this protocol with the tissue bathed in the Cd(I) solution (black traces). The large sustained rectifying K^+ currents appeared at voltages more positive than 0mV . Since voltage-gated Ca^{2+} channels in bipolar cells were blocked in Cd(I) solution, it is likely that Ca^{2+} dependent K^+ (K_{Ca}) channel currents were abolished. Therefore, the large sustained K^+ currents were mediated by outward rectifier K_v channels. Interestingly, taurine (80 μM) in Cd(I) solution greatly enhanced K_v current preferentially in Off-bipolar cells; whereas taurine slightly suppressed K_v currents in On-bipolar cells (red traces, On- and Off-bipolar cell). The K_v currents recovered to control levels within 1–2 minutes after taurine was withdrawn. The enhancement by taurine of K_v currents was via a metabotropic effect, since Cd(I) blocked taurine’s ionotropic action on Cl^- permeable receptors. Notice that the currents at very negative voltages, which were commonly observed in bipolar cells, were not affected by taurine; these were inward rectifier K^+ (K_{ir}) channel currents, activated at -100mV .

The percentage increase and decrease of K_v currents by taurine were quantified at $+60\text{mV}$ from each recorded cell. As depicted in Figure 1B, the statistical analysis indicates that

taurine enhancement of K_v currents varied between 10 – 80% from cell to cell. On average, taurine caused $44.37 \pm 8.5\%$ increase in K_v currents in Off-bipolar cells ($n = 21$; $p < .001$). Taurine inhibition of K_v currents in On-bipolar cells varied by 10 – 20% of control with an average inhibition of $8.32\% \pm 2.3$ ($n = 11$; $p < .01$). The differing degree of regulation for K_v channels in On- and Off-bipolar cells suggests that different receptors or different intracellular metabotropic pathways might mediate taurine's actions. Interestingly, we found that the enhancement in Off-bipolar cells by taurine persisted even after the axons were severed, indicating that taurine probably activates receptors in the somatodendritic areas of the cells. Since taurine is naturally found in high concentrations in the outer retina, these findings suggest that taurine could provide metabotropic regulation of Off-bipolar cells.

1.3.2 Role of Protein Kinases in Taurine- Enhanced K_v Currents

To confirm that a metabotropic pathway was involved in taurine regulation of K_v channels in bipolar cells, we tested the effects of specific inhibitors of PKA and PKC, the major intracellular signaling pathways in metabotropic regulation. We recorded K_v current changes with and without taurine using a single voltage step protocol to activate K_v channels in Off-bipolar cells. K_v currents were elicited by a 25ms voltage step from -60mV to $+60\text{mV}$ in control Cd(I) solution, both with and without taurine. As illustrated in Fig. 2A, the K_v current was significantly enhanced by taurine, and the histogram in figure 2B shows that, on average, the K_v currents (measured at the time indicated by the dotted line) were enhanced by approximately 80%. When GF109203X (10 μM), a membrane permeable, PKC-specific inhibitor, was added to the control solution, it suppressed the sustained portion of the K_v currents in Off-bipolar cells (Fig. 2C, black trace), and interestingly, it completely suppressed the enhancing effect of taurine on the K_v currents (red trace). When PKC was inhibited pharmacologically, taurine only produced on average a 3.7% increase in the K_v currents of Off-bipolar cells (Fig. 2D, $n=4$). Although this result strongly suggests that the internal pathway activated by taurine is PKC-specific, the result was confounded by the fact that the taurine enhancing effect was still observed when we dialyzed PKI, a PKA specific antagonist, into the cell during whole-cell recording (data not shown). It is therefore not possible to conclude that a PKA-mediated pathway is involved in taurine's metabotropic action on Off-bipolar cells

1.3.3 Taurine Shapes the Voltage Response of Off-bipolar Cells

A physiological role for the regulation of K_v channels in Off-bipolar cells was addressed by recording bipolar cell membrane changes in response to a transient depolarization pulse that mimicked the physiological response of the cells to light offset. Although bipolar cells do not normally generate spike potentials, Off-bipolar cells typically show a transient "overshoot" depolarization at light offset due to the fact that they receive large, multi-vesicular phasic signals at the termination of a light pulse. Outward rectifying K_v channels play an important role in the membrane repolarization in "overshoot"; they are activated at positive voltages, which allow the outward flow of K^+ ions, and thus effectively controlling the repolarization rate.

In current-clamp mode, a protocol was developed to mimic the phasic signal received by Off-bipolar cells. Extracellular Cd(I) was still applied to block the network effects of bath

applied taurine, and a brief (5ms) current injection was used to depolarize the cell to +20mV from its resting level. After depolarization the cell repolarized via multiple mechanisms, including K_v channel activation. To confirm that K_v channels are critical for establishing the repolarization rate in Off-bipolar cells, we employed the broad spectrum K^+ channel blocker tetraethylammonium (TEA, 20mM). As expected, with the cell in control solution the rate of repolarization following current injection was rapid, and described a single exponential decay function with a time constant of $\tau = 32\text{ms}$ (Fig. 3A). This was in stark contrast to the slower repolarization rate ($\tau = 272\text{ms}$) when TEA was used to block K^+ channels (including K_v). This is obviously not an ideal state in which to encode multiple large and transient signals. It seems likely that the long, delayed repolarization with TEA reflects the ability of K_v channels to ease the cell back to its resting level following brief, intense stimuli.

The test was repeated in Off-bipolar cells in Cd(I) control with and without 80 μM taurine. Since the enhancement of K_v currents by taurine would cause more K^+ efflux and increase the membrane conductance, signals are expected to decay more rapidly as K^+ efflux increases, and the cell will quickly repolarize following depolarization. As shown in figure 3B, the control signal (*black trace*) was greater in response to depolarization and the decay rate was slower compared with results when taurine was in the bath (*red trace*). Taurine caused a fast signal decay and limited the duration of depolarization, indicating that taurine plays a role in encoding offset light “overshoot” signals in Off-bipolar cells, especially those receiving rapid, transient responses from cones.

1.3.4 Taurine Suppresses Voltage-gated Ca^{2+} Channels via a Metabotropic Pathway

To determine whether taurine directly influences synaptic vesicle release in retinal neurons, the effect of taurine was studied on voltage-gated Ca^{2+} channels in isolated neurons free of inputs from the retinal network. All control Ca^{2+} channel currents were recorded in a Ba^{2+} - and TEA-containing Ringer’s solution; Since Ca^{2+} channels are more permeable to Ba^{2+} than to Ca^{2+} , and TEA blocked all voltage-gated K^+ channels. In addition, taurine-sensitive Cl^- permeable channels were blocked with picrotoxin (100 μM) and strychnine (10 μM). TTX (1 μM) was used to block voltage-gated Na^+ channels. The combination of Ba^{2+} and the cocktail of inhibitors served as the control solution, referred to as Ba(I) throughout the paper.

Ca^{2+} channel currents were studied using a ramp protocol with voltages increasing from -100mV to +50mV over a period of 2 seconds. An inward Ca^{2+} channel current was generated by the ramp in Ba(I), and the addition of taurine (100 μM) caused a reduction in the current (Fig. 4); this suppression could be washed away after 1–2 minute in the control solution. Ca^{2+} channel currents could be fully blocked by bath application of Cd^{2+} (100 μM), a voltage-dependent Ca^{2+} channel blocker. In the Cd^{2+} containing Ba(I) solution, taurine had no further effect, indicating that the inhibitory effect of taurine was on voltage-gated Ca^{2+} channels. Taurine regulation of Ca^{2+} channels was further examined in the third-order neurons and some bipolar cells. We found that taurine suppressed Ca^{2+} channel currents rather consistently in most of the cells recorded, but the degree of suppression varied among the different types of neurons.

Several studies have reported that taurine activates metabotropic GABA_B receptors (Kontro & Oja, 1990 Smith & Li, 1991; Nicoll 2004). To investigate whether the GABA_B receptor was involved in mediating taurine regulation of Ca²⁺ channels, CGP55845 (10μM), a potent GABA_B receptor inhibitor, was used to block the GABA_B receptors on retinal neurons. We then tested the inhibitory effect of taurine on Ca²⁺ channel currents with and without presence of CGP55845. The results obtained in recordings from amacrine cells (known to express GABA_B receptors). Figure 5A and 5B show an example of recordings. In the Ba(I) control, taurine suppressed Ca²⁺ channel currents and the effect was recovered after taurine was removed (Fig. 5A). Then, CGP55845 was applied in Ba(I). We observed that CGP55845 itself had a suppressive effect on Ca²⁺ channel currents in some cells (Fig. 5B). With CGP55845 in the solution taurine still suppressed Ca²⁺ channel currents. Subtraction of the Ca²⁺ channel activation curves with and without taurine during control and in CGP55845 conditions revealed that the same amount of current was suppressed by taurine (see the lower panels in Fig. 5A & 5B), supporting that CGP55845 did not influence the effect of taurine. This finding indicates that taurine is not acting on the GABA_B receptor in amacrine cells.

1.3.5 Both L- and N-type Ca²⁺ Channel Currents are Regulated by Taurine

To determine which types of Ca²⁺ channels are regulated by taurine, the specific L-type Ca²⁺ channel blocker, nifedipine (100μM), and N-type Ca²⁺ channel blocker, ω-conotoxin (1μM) were used to block the individual Ca²⁺ channel subtype in third-order neurons.

Voltage-gated Ca²⁺ channel currents were generated with a single depolarizing step from -60mV to 0 mV, the voltage that elicits a maximum Ca²⁺ channel current in neurons. Figure 6A shows that nifedipine partially suppressed the inward Ca²⁺ channel currents (30% at the beginning of the pulse and 70% near the end of the pulse), and with nifedipine to block L-type channels, taurine reduced approximately 50% of the Ca²⁺ channel current (measured near the end of the pulse (red trace)). On the other hand, application of the N-type channel blocker ω conotoxin reduced by almost 50% the Ca²⁺ channel currents. And in the presence of ω-conotoxin, taurine suppressed an additional 30% of the Ca²⁺ channel current. These examples show that taurine regulates both the L- and N-type Ca²⁺ channels in the retinal neurons. The histogram bars show that on average taurine suppressed 46% of nifedipine-insensitive Ca²⁺ channel currents (n=6) and 24% of ω-conotoxin-insensitive Ca²⁺ channel currents (n=6).

1.4 DISCUSSION

We report here the results of a series of experiments which strongly suggest that taurine has a metabotropic effect that is insensitive to GABA and glycine receptor antagonists. In addition, we have shown that taurine regulates voltage-gated K_v channels and Ca²⁺ channels via a metabotropic pathway. Also shown is the more ubiquitous role of taurine in the modification of synaptic transmission and cell excitability by its ability to modulate voltage-gated channels of retinal neurons. These properties may also apply to other brain regions and sensory systems in the CNS.

Although the exact site at which taurine acts in Off-bipolar cells is unknown, a recent study from this laboratory indicates that taurine enhances delayed rectifier K_v channels in third-order neurons by activating the serotonin receptor, $5HT_{2A}$ via a metabotropic pathway (paper in press). It is possible that taurine regulates K_v channels in Off-bipolar cells by the same receptor and same mechanism we find in third-order neurons. Our findings provide new information that (1) a novel metabotropic taurine response in retinal neurons is completely distinct from the earlier described metabotropic $GABA_B$ receptor, (2) the taurine response leads to the downstream activation of K_v channels and appears to act through a PKC-dependent pathway within Off-bipolar cells, (3) this activity is completely different in On-bipolar cells which apparently lack the receptor site for this PKC-dependent effect, and (4) since taurine increased K^+ efflux through K_v channels and increased the rate of cell repolarization, it may possibly shape the rapid “overshoot” signals in Off-bipolar cells. In fact, a transient “overshoot” depolarization at light offset occurs in Off-bipolar cells, but not in On-bipolar cells. Therefore, the effect of taurine on regulation of K_v channels may allow Off-bipolar cells to increase the encoding rate of light-induced signals.

In general, taurine’s ability to increase K_v channel activity may serve as a mechanism for protecting cells from over-excitation. As voltage-gated K^+ channels are endogenous suppressors of neuronal excitability, their modulation leads to potential therapeutic benefits by reducing neuronal hyperexcitability in stroke and epileptic patients. Delayed outward rectifier K_v channels are particularly important in somatodendritic excitability in hippocampal and cortical pyramidal neurons (Bekkers, 2000; Zhang et al., 2010; Korngreen & Sakmann, 2000). Taurine has been shown to exert a neuroprotective role on the brain when administered before or after a middle cerebral artery occlusion (Wang et al., 2007), and it prevents stroke in stroke-prone spontaneously hypertensive rats (Yamori et al., 2009). It is possible that its metabotropic effect is one of the potential mechanisms for neuroprotection.

1.5 CONCLUSION

We find that taurine at physiological concentrations (μM) generate metabotropic effects that are taurine specific, insensitive to the antagonists of GABA and glycine receptors. The metabotropic effects of taurine play a dual-function by enhancing voltage-gated, delayed rectifier K_v channels and suppressing high voltage-activated Ca^{2+} channels in retinal neurons.

Acknowledgments

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ABBREVIATIONS

AMPA	α -amino-3-hydroxy-5-methyl-4- isoxazole-propionic acid
GABA	γ -aminobutyric acid
IPL	inner plexiform layer

ONL	outer nuclear layer
INL	inner nuclear layer
GCL	ganglion cell layer

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Figure 1.

Metabotropic effect of taurine on regulation of K_V currents in bipolar cells studied in whole-cell patch clamp recording. (A) Current responses were recorded from both On- and Off-bipolar Cells activated by multiple depolarizing steps from -100 to $+60$ mV with 10 mV increments. Large K_V currents were generated from bipolar cells at positive voltages in the Cd(I) solution; taurine ($80\mu\text{M}$) enhanced K_V currents in Off-bipolar cells, but not On-bipolar cells. (B) Taurine-induced percentage increase or decrease of K_V currents in Off- and On-bipolar cells, measured at $+60$ mV.

Figure 2.

Taurine enhanced K_v currents in Off-bipolar cells via a PKC pathway. (A) K_v currents in a displaced Off-bipolar cell, elicited by a single voltage step, were significantly enhanced by taurine measured at the dotted line. (B) Histogram depicting taurine enhancing K_v current about 80% in Off-bipolar cells. (C) K_v current of Off-bipolar cells was suppressed by the specific PKC inhibitor GF109203X (black trace), whereas taurine had no effect on the K_v currents in the presence of GF109203X. GF109203X also reduced the sustained K_v currents in the cells. (D) The taurine effect in Off-bipolar cells was completely eliminated ($p > 0.05$) compared to control when the PKC inhibitor GF109203X was applied;

Figure 3.

Voltage waveform in Off -bipolar cells is shaped via changes in K^+ conductance (A) Off Bipolar Cell under current clamp conditions were subjected to short 5ms current injections sufficient to depolarize the cell to +20 mV (black). After blocking K^+ conductance with TEA (20mM), an identical current injection caused a larger voltage increase with an increase in the time to decay. B, The voltage waveform is reduced in amplitude with taurine (80 μ M) with an increase in the decay rate as compared with control.

Figure 4.

Taurine suppression of voltage-gated Ca^{2+} channel currents in third-order neurons, recorded in the Ba(I) - modified Ringer solution. Voltage-activated Ca^{2+} channel currents were generated with a voltage ramp from -100mV to $+50\text{mV}$ within 2 seconds. Ca^{2+} channel currents were suppressed by taurine ($100\mu\text{M}$). Both Ca^{2+} channel currents and taurine inhibition were fully blocked by the voltage-gated Ca^{2+} channel blocker Cd^{2+} .

Figure 5.

Taurine suppressed Ca^{2+} channels via a receptor or pathway that was unrelated to GABA_B receptors. (A) Taurine reduced voltage-gated Ca^{2+} channel currents in control with external Ba(I) , and the suppression could be washed away (the light trace); the suppression was estimated by subtraction of taurine regulated Ca^{2+} current curve from control curve. (B) With CGP55845 applied, taurine still suppressed Ca^{2+} currents; the effect of taurine was derived by subtracting the two curves with and without taurine.

Figure 6.

Metabotropic effect of taurine regulates voltage-gated L-type and N-type Ca^{2+} channels. (A) Ca^{2+} channel currents were generated in a single voltage depolarization step from $-60mV$ to $0mV$ in a second. Nifedipine reduced most of sustained Ca^{2+} channel currents and had less effect on the transient Ca^{2+} channel currents, and taurine suppressed Ca^{2+} channel current with nifedipine. (B) ω -conotoxin suppressed both sustained and transient Ca^{2+} currents and taurine further reduced Ca^{2+} channel currents. (C) With nifedipine and ω -conotoxin, average suppressive effect of taurine on Ca^{2+} channel currents in the third-order neurons ($n=6$).