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THE MODULATORY ROLE OF TAURINE IN RETINAL GANGLION CELLS

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Abstract

Taurine (2-aminoethylsulfonic acid) is present in nearly all animal tissues, and is the most abundant free amino acid in muscle, heart, CNS and retina. Although it is known to be a major cytoprotectant and essential for normal retinal development, its role in retinal neurotransmission and modulation is not well understood. We investigated the response of taurine in retinal ganglion cells, and its effect on synaptic transmission between ganglion cells and their pre-synaptic neurons. We find that taurine-elicited currents in ganglion cells could be fully blocked by both strychnine and SR95531, glycine and GABA_A receptor antagonists, respectively. This suggests that taurine-activated receptors might share the antagonists with GABA and glycine receptors. The effect of taurine at micromolar concentrations can effectively suppress spontaneous vesicle release from the pre-synaptic neurons, but had limited effects on light-evoked synaptic signals in ganglion cells. We also describe a metabotropic effect of taurine in the suppression of light-evoked response in ganglion cells. Clearly, taurine acts in multiple ways to modulate synaptic signals in retinal output neurons, ganglion cells.

1.1 INTRODUCTION

In the retina, taurine is found primarily in glutamatergic neurons, i.e., in photoreceptors and bipolar cells in the eyes of goldfish, amphibians, murines, and Cynomolgus monkeys (Marc et al., 1995; Kalloniatis et al., 1996; Omura and Inagaki, 2000; Militante and Lombardini, 2002). However, in early development other retinal cells, such as amacrine and ganglion cells, Muller cells and pigmentary epithelium cells have also been shown to take up taurine (Orr et al., 1976; Kennedy and Voaden, 1976; Lake et al., 1978; Pow and Crook, 1994). There is a strong link between taurine deficiency and visual dysfunction in retinal development, disorders that can be reversed through taurine dietary supplementation (Lombardini, 1991). The previous study showed that application of taurine to cultured rat

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retinas promotes rod photoreceptor production (Altshuler et al., 1993). However, application of glycine or GABA to the culture media did not have the same effect as taurine to promote photoreceptor growth, although the molecular structures of taurine, glycine and GABA are remarkably similar, suggesting that taurine activated different receptors (Renteria et al., 2004). Recent studies indicate that the ability of taurine to promote rod photoreceptor differentiation could be through the activation of the glycine receptor subtype GlyR α 2, although glycine seems unlikely to be the ligand for triggering the events in rod photoreceptor development (Young and Cepko, 2004). These studies, while demonstrating the importance of taurine in retinal neurodevelopment, pose an unsolved question as to whether taurine activates a specific receptor other than the glycine and GABA receptors.

The molecular structure of taurine, glycine and GABA are similar and all capable of activating ionotropic receptors that are permeable to Cl⁻. In each case it results in an inhibitory neuronal response. Because glycine and GABA are widely accepted as major inhibitory neurotransmitters in retinas, less attention has been paid to taurine, despite the fact that the endogenous taurine levels in retinas are much higher than either GABA or glycine. In fact, both glycine and GABA receptors have been cloned and their receptor pharmacology is well defined. Two ionotropic (GABA_A and GABA_C) and one metabotropic (GABA_B) receptors have been characterized in the retina, with distinctive pharmacology and dose-dependent response properties. Moreover, the strychnine-sensitive ionotropic glycine receptor has been well studied in the CNS, and the use of specific agonists and antagonists of these receptors has enabled study of their function and mechanism of action in the retina. In contrast, neither the pharmacology nor molecular evidence of a taurine-specific receptor has been elucidated. Thus, the role of taurine in neurotransmission and modulation is not well understood.

At this juncture it is important to consider the structural and functional properties of the vertebrate retina, essentially an outgrowth of the CNS that is responsible for detecting environmental light and translating the visual scene into a series of electrochemical signals. The structural components and cellular organization of the retina are highly conserved among vertebrate species. In general, retinal neurons are classified into five major types (including subtypes): photoreceptors (rods and cones), bipolar cells (On-bipolar cells and Off-bipolar cells), horizontal cells, amacrine cells and ganglion cells. They communicate within two synaptic layers: the outer plexiform layer (OPL) and inner plexiform layer (IPL), as shown in the schematic image of Figure 1. The laminar organization of the retina generates two streams of visual information: a vertical pathway from photoreceptors to ganglion cells via bipolar cells, and a lateral pathway that comprises local feedback from horizontal cells and amacrine cells in the OPL and IPL, respectively. The vertical pathway is directly involved in sending signals to the brain, whereas the feedback circuits adjust the gains of pre- and post-synaptic neurons to optimize signal transmission within the vertical pathway. Receiving the integrated signals from bipolar and amacrine cells are the ganglion cells, output neurons that process the information and relay it to their first station (lateral geniculate nucleus) in the CNS. Because ganglion cells are the sole output neurons in the retina, their activity represents a critical stage in the visual pathway.

The amphibian retina is often used as a model system for studying retinal physiology, since this tissue has been well characterized as to both its neural structure and its signal pathway (Fig. 1). More importantly, the amphibian retina shares the same functions and mechanisms of neurotransmissions with higher vertebrate animals, but is accessed much more easily for electrophysiological recordings owing to the fact that its cells are larger in size and the overall structure of the network is more compact. Our previous study of the amphibian retina has shown that taurine is primarily present in photoreceptors and bipolar cells (Bulley and Shen, 2010), and a similar finding has been reported in a study of the mammalian retina (Pow et al., 1994). Photoreceptors and bipolar cells are both glutamatergic neurons, and it is likely that glutamate and taurine are released from both cell types to regulate the activities of second-order (horizontal and bipolar cells) and third-order neurons (amacrine and ganglion cells). Indeed, we found that taurine suppresses glutamate-induced $[Ca^{2+}]_i$ changes in amacrine and ganglion cells (Bulley and Shen, 2010).

In this study we examined the regulatory effects of taurine on the spontaneous firing of ganglion cells, and on the light-evoked synaptic transmission in the neuronal network.

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 Kons Scientific (Germantown, WI) and Charles Sullivan (Nashville, TN), were used in this study. The animals were kept in aquaria at 13°C under a 12-hour dark-light cycle with continuous filtration. The retinas were collected from animals kept at least six hours in the darkness. Briefly, the animals were decapitated, double-pithed and the eyes were enucleated. Retinal slices were prepared in a dark room under a dissecting microscope equipped with powered Night-Vision scopes (BE Meyer Co., Redmond, WA), an infrared illuminator (850nm), an infrared camera and a video monitor. The retina was removed from an eyecup in Ringer's solution and mounted on a piece of microfilter paper (Millipore, Bedford, MA), with the ganglion cell layer downward. The filter paper with retina was vertically cut into 250nm slices using a tissue slicer (Stoelting Co, IL). A single retinal slice was mounted in a recording chamber and superfused with oxygenated 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. The recording chamber was placed on an Olympus BX51WI microscope equipped with a CCD camera linked to a monitor.

1.2.2 Cell dissociation

Retinas were removed from the eyecups into a Ringer's solution. The tissue was then digested in a freshly prepared enzymatic solution containing 50µl papain (12 U/ml) in 0.5ml 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 dissociated through a flame polished Pasteur pipette into the standard Ringer's solution. The dissociated cells were seeded on 18mm glass-cover slips freshly coated with lectin and allowed to set for 20 minutes before use. The cover slip was then placed in a recording chamber on an Olympus BX51WI microscope equipped for electrophysiological recording, and viewed with a CCD camera linked to a monitor. After superfusion with Ringer's solution, recordings were made at room temperature and within a few hours after cell dissociation.

1.2.3 Electrophysiological recording

Whole-cell recordings were performed on amacrine and ganglion cells (third-order retinal neurons) in dark-adapted retinal slices or after dissociation. Patch electrodes (5–8 M Ω) were pulled with an MF-97 microelectrode puller (Sutter Instrument Co.), and filled with a high K⁺ solution containing (in mM): K-gluconate (100), MgCl₂ (1), EGTA (5) and HEPES (5), and an ATP regenerating cocktail consisting of (in mM): ATP (20), phosphocreatine (40) and creatine phosphokinase (2); (pH 7.4). An EPC-10 amplifier and HEKA Pulse software (HEKA Co., Germany) were used for data acquisition, and analysis was performed with Igor Pro software (WaveMetrics).

A gravity-driven perfusion system was used to superfuse all external solutions in the retinal slice preparation. The perfusion tube was placed 3 mm away from the retinal slice and was manually controlled for delivering drugs during the experiments. All recordings were performed under 850nm infrared illumination to avoid exposure to visible light. A red LED light source (660nm peak emission) was focused directly upon the retinal slice, and the brief (3 sec) light stimuli were controlled by the output of the HEKA amplifier.

The isolated cells were constantly superfused with Ringer's solution or drug solutions via a DAD-VM automated superfusion system (ALA Scientific Instruments, Farmingdale, NY). All the chemicals were purchased from Tocris Bioscience (Minneapolis, MN) and Sigma-Aldrich Co. (St. Louis, MO).

1.2.5 Cell selection

The third-order neurons in retinal slices were identified by their localization and their light-evoked response patterns. On the other hand, isolated cells often were not so readily identified. During the isolation process many cells lost their typical morphological features, although dissociated ganglion cells could frequently be identified by the long axonal processes that extended from their cell somas. However, amacrine and bipolar cells often were not distinguishable, and it was necessary to rely 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 500pA), 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 Pharmacology of the ionotropic taurine response in ganglion cells

We noted earlier that taurine activates ionotropic receptors permeable to Cl^- . In that case, the reversal potential for taurine-generated currents would be expected to be near the Cl^- equilibrium potential (E_{Cl}). We used a low Cl^- and high K^+ intracellular solution to measure the E_{Cl} in ganglion cells with whole-cell voltage-clamp recording. Figure 2 shows the current responses to a puff of 350 μM taurine when a ganglion cell (in a retinal slice) was voltage-clamped at various potentials (−80mV, −70mV, −65mV and −60mV) near the E_{Cl} . Taurine generated inward and outward currents that reversed at or near −70mV, indicating that taurine activated Cl^- permeable receptors in ganglion cells with a reversal potential at the E_{Cl} .

To determine the sensitivity of retinal ganglion cells to taurine we determined their dose response function. Taurine was applied at various concentrations (10 μM , 100 μM , 500 μM , 1mM, 2mM and 5mM) to ganglion cells in the retinal slice preparation, and taurine-elicited currents were recorded at −60mV (Fig. 2B). The taurine dose-response curve shows that on average the maximum current response was generated by 2mM taurine, and the EC_{50} (concentration producing a half maximal response) was around 350 μM ($n=7$). Note that 10 μM taurine did not produce a measurable current in ganglion cells ($n=21$, Fig. 2B, lower panel). Similar dose-dependent responses could be obtained from isolated ganglion cells (unpublished data).

Several studies of neurons in the CNS have reported that taurine acts like a weak agonist on GABA and glycine receptors based on the observation that antagonists for GABA and glycine receptors can block the taurine response. It is unknown whether the ganglion cell response to taurine is also sensitive to GABA and glycine receptor antagonists. To test this we applied 350 μM taurine (the EC_{50} concentration) to isolated ganglion cells in which retinal network inputs were eliminated. Ganglion cells were voltage-clamped at a potential negative to the E_{Cl} and the inward current was elicited in response to the taurine (Fig. 3A & 3B). The taurine current was fully blocked by 2 μM strychnine, a glycine receptor specific antagonist (Fig. 3A). Taurine-elicited currents were also sensitive to the GABA_A receptor specific inhibitor, 10 μM SR95531 (Fig. 3B). These results suggest that taurine-activated receptors might share the same receptor antagonists as those of GABA_A and glycine receptors. It seems less likely that taurine is activating both GABA and glycine receptors, since the effect of taurine could be separately blocked by either the GABA_A antagonist or the glycine receptor antagonist.

1.3.2 The effects of micromolar taurine on spontaneous and light-evoked responses in ganglion cells

Ganglion cells receive glutamate inputs from bipolar cells, and GABA and glycine inputs from amacrine cells. In the dark, spontaneous vesicular release of glutamate, GABA and glycine from the pre-synaptic neurons produce spontaneous excitatory postsynaptic currents (sEPSCs) and inhibitory postsynaptic currents (sIPSCs) in ganglion cells. To determine whether taurine affects these local vesicle release events, we recorded sEPSCs and sIPSCs

from ganglion cells in dark adapted retinal slices. To minimize the direct ionotropic effect of taurine caused membrane conductance changes in ganglion cells, we used a sufficiently low concentration of taurine (10 μ M) that did not significantly change the membrane conductance in ganglion cells as shown in the dose-response curve in figure 2b.

sEPSCs in ganglion cells were recorded at -70 mV where GABAergic and glycinergic currents were close to zero. Figure 4A shows an example of sEPSCs that were continuously recorded from ganglion cells in dark-adapted retinal slices, first in control, then in the presence of taurine, and lastly after washout of taurine. In darkness, there is a high frequency discharge with a combination of large and small amplitudes sEPSCs, which reflect the rate of vesicle release events from bipolar cell terminals. Application of taurine largely reduced the frequency and amplitude of the sEPSCs, effects that were totally eliminated within a minute after withdrawal of taurine.

The effect of taurine on the amplitude and frequency of sEPSCs in ganglion cells was analyzed with the Minianalysis Program (Synaptosoftware). Figure 4B shows quantitative analysis of sEPSCs frequency and amplitude changes in control, with taurine and after taurine washout, sampled from the recordings in Fig. 4A. The histograms indicate that taurine effectively eliminated the sEPSCs with amplitudes greater than 100pA and frequencies less than 30 events/min, but it had limited effect on the high frequency sEPSCs with amplitudes less than 40pA. Also, taurine largely reduced the frequencies of sEPSCs that had amplitudes ranging from 40–100pA. The analysis showed that the average frequencies of the sEPSCs were 7.63Hz in control, 3.35 Hz with taurine, and 9.51 Hz after washout. The large reduction in the frequencies of sEPSCs implies that taurine suppressed the presynaptic release events. The effect of taurine on sEPSC amplitude was further analyzed from measurements of the decay time constants of a series of single events, since the decay time constants of sEPSCs may represent the properties of glutamate receptors on postsynaptic neurons, ganglion cells. Figure 4C shows the quantitative analyses of the decay time constants of individual sEPSCs in the control, in the presence of taurine, and after taurine withdrawal. The decay phases of each sEPSCs with an amplitude greater than 20pA were fitted with a double-exponential curve, and the decay time constants were obtained by calculating the time to decay to 37% of the peak amplitudes. The histogram bars show that the distribution patterns of decay time constant of sEPSCs with and without taurine. The average decay time constant for sEPSCs was around 17ms in control, and was not affected by taurine, although the number of the events/minute (frequency) was reduced by taurine. This suggests that taurine may affect the spontaneous pre-synaptic release of glutamate in the bipolar cells; but had a much leaser effect on the decay time constants of glutamate receptors on the ganglion cells. Taken together, taurine at this low concentration reduced the frequencies of large sEPSCs in ganglion cells; we speculate that this effect might result from the suppression of presynaptic sites on bipolar cell terminals. Note that the frequencies of the large sEPSCs were increased in ganglion cells after taurine was withdrawn. This was commonly observed in ganglion cells, and could be due to an accumulation of synaptic vesicles in the release pools of bipolar cell terminals during taurine application, and their discharge once the suppressive effect of taurine was removed.

Taurine also strongly suppressed sIPSCs in ganglion cells. Figure 5 shows sIPSCs recorded from the same ganglion cell as in figure 4, but with the cell voltage-clamped at 0mV (the reversal potential for glutamate currents). At this voltage, sIPSCs in ganglion cells produced by GABA and glycine synaptic inputs were outward currents in the dark-adapted retina. The sIPSCs were almost abolished when taurine was applied, and they fully recovered within a minute after return to the control Ringer's solution. In general, the effect of taurine (10 μ M) was stronger on suppression of sIPSCs than sEPSCs in ganglion cells. We postulated that the strong suppression of sIPSCs by taurine could be due to the effects of both pre- and post-synaptic regulation by limitation of the pre-synaptic spontaneous releases of GABA and glycine, as well as down-regulation of GABA and glycine receptor activity in the post-synaptic ganglion cells.

Ganglion cells receive light-evoked signals from bipolar cells and amacrine cells in the network. Photic stimulation generates excitatory and inhibitory neurotransmitter release from bipolar and amacrine cells, which can be recorded from ganglion cells in retinal slice preparation. Figure 6A shows typical light-evoked excitatory currents in ganglion cells in 3 sec duration (dark trace). These large EPSCs, elicited at the onset and offset of the light stimulus, are the result of glutamatergic inputs from pre-synaptic On- and Off-bipolar cells. Taurine (10 μ M) had little effect on the amplitude of these light evoked currents. However, the miniature events riding on the light-evoked currents and after light offset were significantly reduced by taurine (Fig. 6A, light trace). On average, taurine suppressed the peak currents evoked by the onset and offset of the light stimulus by $7.8 \pm 3\%$ and $8.2 \pm 4\%$ (n=18), respectively (Fig. 6B).

The effect of taurine on light-evoked IPSCs in ganglion cells is shown in Figure 6C, recorded from the same cell as in Figure 6A. With the cell voltage-clamped at 0mV, taurine produced a small suppression of light-evoked IPSCs, but significantly reduced the fast frequency spontaneous events. Statistical analysis showed that taurine reduced the light-evoked IPSCs at the onset by $8 \pm 2\%$ and offset by $7.4 \pm 4\%$, (n=14, Fig. 6D). It is apparent that taurine reduced the concomitant miniature events on both the light-evoked excitatory and inhibitory responses in ganglion cells.

In summary, taurine (10 μ M) had a limited effect on light-evoked large synaptic signals, but suppressed miniatures responses in ganglion cells. It appears that taurine in the IPL may play a role in filtering out the synaptic noise spontaneous miniatures in ganglion cells.

1.3.3 Taurine suppresses light-evoked responses in ganglion cells via a metabotropic pathway

To further study the effect of taurine on light-evoked responses in ganglion cells, we used a higher concentration of taurine (100 μ M) with picrotoxin (100 μ M) and strychnine (5 μ M) to block taurine-sensitive, as well as GABA- and glycine-sensitive Cl⁻-permeable receptors in ganglion cells. Meanwhile, picrotoxin and strychnine would increase excitatory synaptic inputs in ganglion cells due to blocking the inhibitions in the network. In presence of picrotoxin and strychnine, light-evoked EPSCs were recorded from ganglion cells in dark-adapted retinal slices with the same protocol as used in figure 6. The addition of taurine (100 μ M) reduced the amplitude of the light-evoked onset and offset EPSCs (Fig. 7A). The

effect was present in all ganglion cells tested ($n=6$). To quantitatively analyze the effect of taurine on light-evoked onset and offset EPSCs in the six ganglion cells, we measured the amplitudes of the currents from the ganglion cells in the control (with picrotoxin and strychnine) and presence of taurine. On average, with picrotoxin and strychnine, taurine suppressed $48.7 \pm 6\%$ of the onset currents, and $41.3 \pm 4\%$ of the offset currents in the ganglion cells, shown in the histogram (Fig. 7B). Since taurine-sensitive Cl^- -permeable receptors were blocked by the antagonists, the taurine regulation of light-evoked EPSCs in ganglion cells should be via a metabotropic pathway. We speculated that the effect of taurine might be due to suppression of pre-synaptic glutamate release from bipolar cell terminals, since metabotropic regulation of Ca^{2+} -dependent glutamate release is a common mechanism present in neural systems.

1.4 DISCUSSION

1.4.1 Taurine sensitive receptors

Although taurine has often been considered a neurotransmitter or neuromodulator in the CNS, the site of taurine's action is unclear, since a taurine-specific receptor has yet to be identified. One of the difficulties is the lack of a specific antagonist to differentiate the action of taurine from either glycine or GABA. A major question yet to be resolved is whether taurine acts on GABA or glycine receptors, or whether it activates a specific receptor that is also sensitive to the antagonists of GABA and glycine receptors. The evidence from this study is hardly conclusive, but it suggests that taurine-sensitive receptors might share the same receptor antagonists with GABA and glycine receptors.

Since glycine receptors and GABA_A receptors have been considered as the major Cl^- -permeable receptors co-existing in amphibian ganglion cells (Lucasiewicz & Werblin, 1990), less has been known about taurine sensitive receptors in the retinal neurons. Strychnine and SR95531 are widely used as the specific antagonists to block glycine and GABA_A receptors, respectively. The concentrations of strychnine ($2\mu\text{M}$) and SR95531 ($10\mu\text{M}$) used in our study were in the range that has been shown to specifically block each receptor and it should have minimum or no antagonist crosstalk to the other receptor. We showed that taurine currents could be completely blocked by either strychnine or SR95531 in isolated ganglion cells (see figure 3), implying that both antagonists might block the same site that was sensitive to taurine. The previous studies of taurine in photoreceptors show that taurine-mediated responses can not be mimicked by GABA and glycine (Renteria et al., 2004; Young and Cepko, 2004). Our results are consistent with the results of previous studies showing that taurine did not activate the same sites as either GABA or glycine. Although pharmacological studies demonstrate a discrepancy between taurine's action on GABA and glycine receptors in retinal neurons, the answer as to whether taurine activates a specific receptor type still awaits molecular evidence.

Much of what is known about taurine leads to the conclusion that taurine activates Cl^- -permeable receptors. With picrotoxin and strychnine to block ionotropic taurine responses, the metabotropic effect of taurine was revealed. This is the first time that a metabotropic effect of taurine has been implicated in regulation of a light-evoked response in ganglion cells, although metabotropic effects of taurine have been reported in brain neurons, where it

has been suggested that the effects might be via a GABA_B receptor (Kontro and Oja, 1990; Smith & Li., 1991). Nevertheless, the actual site of taurine binding, and the intracellular metabotropic pathways for the taurine effect still need to be elucidated.

1.4.2 Taurine modulates signal transmission in ganglion cells

Taurine is found in the outer nuclear layer (ONL), mainly in photoreceptors (rods and cones) and Off-bipolar cells in the amphibian retina (Bulley & Shen, 2010). Seemingly, Off-bipolar cells release glutamate in the IPL, and probably taurine as well. This study shows that taurine in the IPL performs multiple roles in the regulation of spontaneous and light-evoked synaptic transmissions in ganglion cells, which integrate these signals, and convert them into a train of spikes that carry both information and some unwanted noise to the CNS. In low micromolar concentrations, taurine preferentially suppressed spontaneous excitatory and inhibitory synaptic events (noise), but not the light-evoked synaptic signals in ganglion cells. Since taurine effectively reduced the frequencies of sEPSCs, the suppressive effects of taurine seem to be acting on presynaptic spontaneous vesicle release sites on bipolar cells. These results suggest that endogenous taurine released from bipolar cells may feedback to regulate spontaneous release from bipolar cells. The fact that the suppressive effect of taurine was seemingly much stronger on sIPSCs suggests that, in addition of reducing spontaneous vesicle releases from amacrine cells, taurine might also interact with GABA and glycine receptors and suppress these receptors on ganglion cells. Unquestionably, the effect of taurine on GABA and glycine receptors needs to be studied further.

1.5 CONCLUSION

We find that taurine activates Cl⁻-permeable receptors that may also be sensitive to strychnine and SR95331, specific antagonists for glycine and GABA_A receptors, respectively. Taurine applied in low micromolar concentrations reduced spontaneous vesicle release (noise), but not light-evoked signals (information) in ganglion cells, thereby acting as a negative control to enhance the signal/noise ratio of signal transmission. The light-evoked responses of ganglion cells were suppressed by taurine via a metabotropic pathway that has yet to be identified. Thus, taurine serves as a neuromodulator that regulates synaptic transmission at multiple sites in the inner retina.

Acknowledgments

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Abbreviations

OPL	outer plexiform layer
IPL	inner plexiform layer
ONL	outer nuclear layer
INL	inner nuclear layer
GCL	ganglion cell layer

CNS Central Nervous System

1.7 REFERENCES

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Figure 1. Retinal structure and cellular organization. Highlighted with dark coloring are the five major cell types: photoreceptors (rods and cones), bipolar cells (On-BC and Off-BC), horizontal cells (HC), amacrine cells (AC) and ganglion cells (GC). These neurons synapse within two synaptic layers: the outer plexiform layer (OPL) and inner plexiform layer (IPL).

Figure 2.

Whole-cell recording of the taurine current reversal potential and the taurine dose response in retinal ganglion cells. (A) 350 μ M taurine elicited currents at various voltages of -80 mV, -70 mV -65 mV and -60 mV (upper) and the current reversal potential (lower) was near -70 mV (n=6). (B) Taurine dose dependent currents recorded from a single ganglion cell (upper) and the dose-response curve generated from a number of cells (in parentheses).

Figure 3.

Taurine-elicited currents were blocked by glycine and GABA receptor antagonists. 350 μM taurine elicited currents that were fully blocked by either 2 μM strychnine, a specific glycine receptor antagonist (A), or 10 μM SR95531, a potent GABA_A receptor antagonist (B).

Figure 4.

The effect of taurine on spontaneous excitatory postsynaptic currents (mEPSCs) in ganglion cells in dark-adapted retinal slices. mEPSCs were recorded from a ganglion cell at -70mV . (A) Continuously recording of sEPSCs in control, with $10\mu\text{M}$ taurine and washout of taurine in a ganglion cell. (B) Histogram of the frequencies (time of the events) and amplitudes of the sEPSCs and (C) Histogram of the decay time constants of the spontaneous synaptic events with and without taurine.

Figure 5.

Taurine suppresses spontaneous inhibitory postsynaptic currents (sIPSCs) in ganglion cells. sIPSCs were recorded from a ganglion cell held at 0mV in a dark-adapted retinal slice. The arrows indicate the time points for application and withdrawal of 10 μ M taurine during the recording.

Figure 6.

The effects of taurine on light-evoked excitatory and inhibitory responses in ganglion cells. (A) Transit currents were evoked by the light onset and termination (offset) in a ganglion cell clamped at -70mV . Taurine ($10\mu\text{M}$) slightly reduced the amplitudes of the light-evoked EPSCs, and more effectively suppressed miniature currents. (B) Statistical analysis of the effect of taurine on the light-evoked current amplitudes from the 18 ganglion cells recorded. (C) The effect of taurine on light-evoked IPSCs recorded at 0mV and the (D) statistics of the current amplitudes in control and with taurine.

Figure 7.

Taurine suppresses light-evoked response in ganglion cells via a metabotropic mechanism. With picrotoxin (100μM) and strychnine (5μM) to block Cl⁻ permeable receptors, taurine (100μM) effectively reduced light-evoked EPSCs in a ganglion cell (A); also shown are the statistics of the effect of taurine on suppression of light-evoked currents in ganglion cells (B).