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RAPID REPORT

Taurine activates delayed rectifier K_V channels via a metabotropic pathway in retinal neurons

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Key points

- Although taurine is one of the most abundant amino acids in human tissues, and serves a number of important functions ranging from cell development to cytoprotection, its precise mode of action is often obscure.
- Here we present evidence that, in the vertebrate retina, taurine regulates voltage-gated potassium (K_V) channels that are sensitive to the inhibitors of K_V1, K_V2 and K_V4 subunits.
- Taurine's effect was shown to be a metabotropic response, involving a G-protein linked, PKC-dependent intracellular pathway.
- Noteworthy was the finding that responses to taurine were blocked by a specific antagonist of 5-HT_{2A} receptors. Taurine activation of 5-HT_{2A} receptors was further confirmed in HEK cells that expressed recombinant 5-HT_{2A} receptors.
- Taurine has been shown to be beneficial in the management of a number of brain disorders. Its interaction with serotonergic pathways suggests that taurine may also play a role in various cognitive functions of the CNS.

Abstract Taurine is one of the most abundant amino acids in the retina, throughout the CNS, and in heart and muscle cells. In keeping with its broad tissue distribution, taurine serves as a modulator of numerous basic processes, such as enzyme activity, cell development, myocardial function and cytoprotection. Despite this multitude of functional roles, the precise mechanism underlying taurine's actions has not yet been identified. In this study we report findings that indicate a novel role for taurine in the regulation of voltage-gated delayed rectifier potassium (K_V) channels in retinal neurons by means of a metabotropic receptor pathway. The metabotropic taurine response was insensitive to the Cl^- channel blockers, picrotoxin and strychnine, but it was inhibited by a specific serotonin 5-HT_{2A} receptor antagonist, MDL11939. Moreover, we found that taurine enhanced K_V channels via intracellular protein kinase C-mediated pathways. When 5-HT_{2A} receptors were expressed in human embryonic kidney cells, taurine and AL34662, a non-specific 5-HT_2 receptor activator, produced a similar regulation of K_{IR} channels. In sum, this study provides new evidence that taurine activates a serotonin system, apparently via 5-HT_{2A} receptors and related intracellular pathways.

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Abbreviations AP, action potential; GFAP, glial fibrillary acidic protein; HEK, human embryonic kidney; IPL, inner plexiform layer; K_V channel, voltage-gated potassium channel; MgTX, margatoxin; PCR, polymerase chain reaction; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; ScTx-1, stromatoxin-1; TTX, tetrodotoxin.

Introduction

Taurine (2-aminoethanesulfonic acid), is often referred to as a 'non-essential' amino acid because it is the only amino acid not involved in protein synthesis. This is clearly a misnomer considering its functional significance in cell development and survival. Indeed, taurine depletion leads to severe cardiomyopathy, renal dysfunction, pancreatic β-cell malfunction and to the loss of retinal photoreceptors (Heller-Stilb *et al.* 2002; Yamori *et al.* 2010; Zulli *et al.* 2011). Because a taurine-specific receptor has not yet been identified, it has been suggested that taurine may work as an agonist of chloride-permeable GABA and glycine receptors (Hussy *et al.* 1997; Jia *et al.* 2008). However, in many cases the effects of taurine are not mimicked by either GABA or glycine (Medina & De Robertis, 1984; Young & Cepko, 2004), implying that another class of taurine-sensitive receptor may exist. Interestingly, it has been reported that taurine acts on metabotropic $GABA_B$ receptors in the cerebral cortex (Kontro & Oja, 1990) and at similar sites in the cerebellum (Smith & Li, 1991), but the intracellular pathway(s) involved in the metabotropic response is largely unknown.

Taurine concentration in retinal tissues is extremely high, particularly in glutamatergic neurons (Cohen *et al.* 1973; Marc *et al.* 1995; Fletcher & Kalloniatis, 1996). Although taurine uptake has been observed in many retinal cell types at early stages of development (Kennedy & Voaden, 1976; Orr *et al.* 1976; Young & Cepko, 2004), its cellular distribution in the adult retina is far from uniform. Cohen and co-workers found that taurine exceeds by >10-fold the concentration of each of the other amino acids in the mouse retina, and a study of goldfish retina confirmed that the concentration of taurine is almost 20 times higher than that of glutamate, and as much as 25 times higher than GABA (Marc *et al.* 1995). Not surprisingly, animals that do not produce taurine metabolically experience severe degenerative changes in their photoreceptors and retinal pigment epithelium when deprived of dietary taurine (Pasantes-Morales *et al.* 1986; Heller-Stilb *et al.* 2002).

It is apparent that retinal cells provide an ideal venue in which to study the activity of taurine and, in the present study, we examined the action of taurine on the voltage-gated potassium channels (K_V channels) that are critical for the generation of action potentials (APs) in the retina and CNS. Most significant was the effect of taurine on 5-HT_{2A} receptors that regulate delayed rectifier K_V channels via a metabotropic intracellular pathway involving protein kinases C and A (PKC and PKA).

Methods

All procedures were performed in accordance with the guidelines of National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Electrophysiological recording

Whole-cell patch-clamp recordings were performed on acutely isolated neurons from Larval tiger salamander (*Ambystoma tigrinum*) retina (Shen & Slaughter, 1999; Bulley & Shen, 2010), using an EPC-10 amplifier and HEKA Patchmaster software (HEKA Instruments Inc., Lambrecht/Pfalz, Germany). Low-resistance (5–10 MΩ) electrodes were pulled from borosilicate glass, and filled with a high K^+ solution containing (in mM): potassium-gluconate, 100; $MgCl_2$, 1; EGTA, 5; Hepes, 5; and an ATP regenerating cocktail consisting of (in mM): ATP, 20; phosphocreatine, 40; creatine phosphokinase, 2; pH 7.4. The Ringer solution contained the following (in mM): NaCl, 111; KCl, 2.5; $CaCl_2$, 1.8; $MgCl_2$, 1.0; Hepes, 5; dextrose, 10; pH 7.7. Cells were recorded 5–10 min after membrane rupture in order to allow the cells to stabilize after dialysis of the electrode solution. The isolated cells were constantly superfused with Ringer solution or drug solutions via a DAD-VM automated superfusion system (ALA Scientific Instruments, Farmingdale, NY, USA). All drugs were purchased from Tocris Bioscience (Minneapolis, MN, USA) and Sigma-Aldrich Co (St Louis, MO, USA), except AL34662 (a 5-HT_2 receptor agonist), which was purchased from the Caymen Chemicals Company (Ann Arbor, MI, USA).

Cell selection

During the isolation process, cells often lost their typical morphological features, although photoreceptors exhibited their unique structure, and dissociated ganglion cells could be identified by the long axonal processes that extend from their cell somas. When amacrine and bipolar cells were not distinguishable, it was necessary to rely on physiological criteria, i.e. their distinctive transient Na^+ currents in whole-cell recording. Unlike bipolar cells, which show extremely large inward rectifier currents, amacrine cells have relatively small transient Na^+ currents as well as small inward rectifier currents. Because there are many types of amacrine cells in salamander retina, these criteria are less than ideal, but did not significantly influence the results. Nor can we exclude the possibility

that some isolated cells we recorded might be bipolar cells, as some possess $Na^{\pm}$ channels (Ichinose *et al.* 2005).

Construction and expression of a 5-HT_{2A}-GFP fusion protein

The complete coding region of the mouse 5-HT_{2A} receptor (cDNA clone 40047362; GenBank accession number NM-172812.2) was obtained from Thermo Scientific Open Biosystems MGC (Pittsburgh, USA). Polymerase chain reaction (PCR) primers 5′-CCC AAG CTT CGC CAC C ATG GAA ATT CTC TGT G-3′ (forward) and 5′-CGC GGA TC C CA CAC ACA GCT AAC CTT TTC AT-3′ (reverse) were used to amplify cDNA, and both *Hind*III and *Bam*HI restriction sites were introduced to the ends of the PCR products for in-frame insertion. The 1.4 kb PCR product was digested with *Hind*III and *Bam*HI, and inserted into pEGFP-N1 (CLONTECH, Mountain View, USA). The orientation of the insert was confirmed by digestion with restriction enzyme, as well as sequenced to ensure that the 5-HT_{2A}-GFP fusion plasmid was in frame.

Human embryonic kidney (HEK)293 cells were cultured in growth medium composed of Dulbecco's modified Eagle's medium (DMEM) and 10% fetal bovine serum in a 37°C, 5% CO_2 incubator. Cells were grown to 65–70% confluency on coverslips in 12-well plates. Sixteen hours after plating, the cells were transfected with 1.5 mg 5-HT_{2A}-GFP and 4.5 ml Lipofectamine LTX (Invitrogen, Grand Island, USA) in 1 ml medium according to the manufacturer's instructions. Transfected cells, identified by the expression of GFP, were incubated for 16–24 h before use.

Immunocytochemistry

To detect cells that express 5-HT_{2A} receptors, retinal sections and transfected HEK293 cells were washed twice with PBS and fixed with 4% paraformaldehyde for 20 min at room temperature. The cells were permeabilized in 1% Triton-X100 in PBS for 10 min, and blocked in 5% goat serum–PBS for 1 h. HEK293 cells or retinal sections adherent to cover slips were immersed in either polyclonal anti-5-HT_{2A} (Abcam, Cambridge, MA, USA) or polyclonal anti-glial fibrillary acidic protein (GFAP; Sigma-Aldrich Co.) at 1:500 and 1:80 dilution in 5% goat serum, respectively, and incubated overnight at 4°C. After washing three times in PBS, the tissues were incubated with goat-anti-rabbit Cy-3-conjugated secondary antibody at a concentration of 1:600 for 1 h in darkness. Immunostained cells were visualized with a Zeiss LSM 700 confocal microscope system (Munich, Germany).

Western blot assay

This assay is fairly well standardized (cf. Kurien *et al.* 2011). In brief, retinal tissues were lysed in a 2× Laemmli buffer, and the total protein content was obtained from homogenates. Protein concentrations were calibrated using a BCA™ Protein Assay Kit (Pierce, Rockford, IL, USA). Equal amounts of samples were loaded onto 3–8% Tris-acetate gels, and retinal proteins were separated by electrophoresis (100 V for 1.5 h). The proteins were transferred to a Hybond-ECL Nitrocellulose membrane (GE Healthcare Bio-Sciences Corp., Piscataway, NJ, USA), and then immersed in a block solution with 5% dry milk in PBS for 1.5 h at room temperature. 5-HT_{2A} was detected with the specific antibody used at a concentration of 1:5000 in 5% milk solution and incubated overnight at 4°C. After washing in a Tris-buffered saline with 0.1% Tween (TBS-T) buffer and incubated for 45 min with a secondary antibody (horseradish peroxidase-conjugated goat anti-mouse IgG; 1:5000), positively stained bands were detected by a chemiluminescent blot assay with the ECL Plus Western blot reagent.

Results

Evidence that taurine regulates K_V channels via a metabotropic mechanism

Figure 1*A* and *B* shows examples of the voltage–current relationship of an isolated amacrine cell in whole-cell recording. The cell was held at −60 mV and activated by brief (25 ms) voltage steps from −100 mV to +65 mV at 15 mV increments. The typical properties of amacrine cells are evident in response to the brief voltage steps, i.e. small, transient inward Na^+ currents and large outward K^+ currents at depolarizing voltages. When 100 μM Cd^{2+} combined with 1 μM tetrodotoxin (TTX), a Na^+ channel blocker, was applied, the transient inward Na^+ currents were almost completely suppressed, and the outward K^+ currents for voltage steps greater than −10 mV were reduced by the Cd^{2+} (Fig. 1*Ab*). This is due to the fact that Cd^{2+} blocks Ca^{2+} influx through the voltage-gated Ca^{2+} channels, thereby also inhibiting Ca^{2+}-mediated K_{Ca} channels (Sah & Davies, 2000).

To study the metabotropic taurine effect on K_V channels, an inhibitory 'cocktail' containing (in μM): picrotoxin, 100; strychnine, 10; Cd^{2+}, 100; TTX, 1; was added to the Ringer solution. These agents eliminated the effect of taurine on Cl^--permeable GABA and glycine receptors, but did not affect outward K^+ channels. Figure 1*Ba* shows the steady state K^+ currents in the presence of the Cd^{2+}-containing inhibitory 'cocktail', referred to throughout the paper as Cd(I). When

taurine was applied with Cd(I), the outward currents were increased (Fig. 1*Bb*). Because taurine-sensitive Cl^--permeable receptors were blocked by the inhibitory cocktail, we attribute the enhancement by taurine to a metabotropic effect, involving an intracellular pathway that regulates K^+ channels.

Because taurine had been shown previously to act on metabotropic $GABA_B$ receptors (Smith & Li, 1991), we added CGP55845, a potent $GABA_B$ receptor antagonist, to the taurine + Cd(I) solution to test whether the taurine effect was mediated by this class of GABA receptor. Despite blocking $GABA_B$ receptors, the K_V currents were

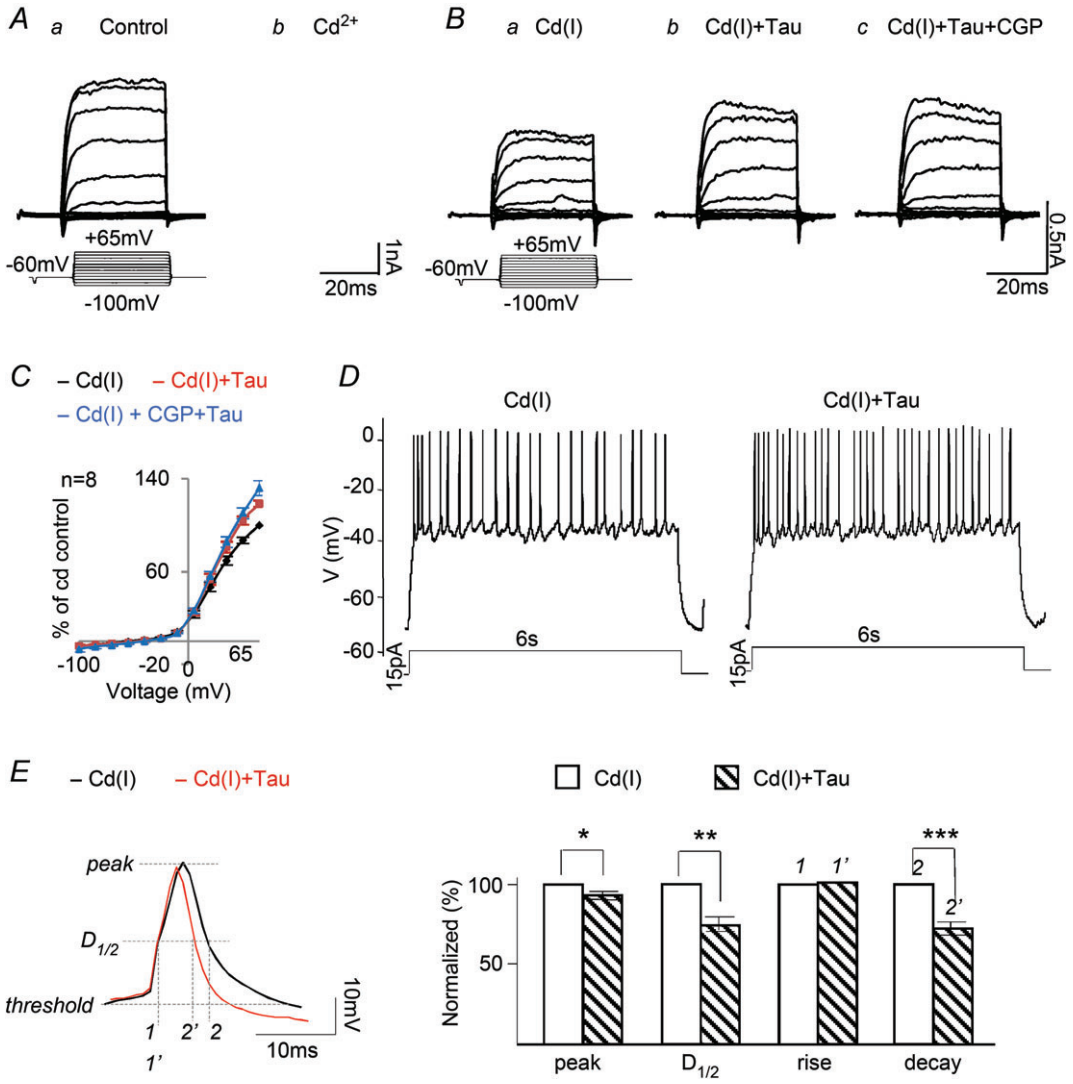

Figure 1. Taurine regulates K^+ currents and APs in isolated amacrine and ganglion cells
Aa and *Ab*, whole-cell recording of voltage-dependent currents from an amacrine cell in Ringer solution,100 μM Cd^{2+} + 1 μM TTX reduced both the K^+ and Na^+ currents. *Ba*, the addition of 100 μM picrotoxin and 10 μM strychnine, referred to as a Cd^{2+} inhibitory cocktail Cd(I), had little effect on the outward K^+ currents. *Bb*, taurine markedly enhanced the K^+ current amplitudes. *Bc*, the effect of taurine was insensitive to 10 μM CGP55845, a $GABA_B$ antagonist. *C*, the *I–V* curves measured and plotted from 8 cells in each of the test solutions. *D*, taurine increases the firing rate of APs generated by current injection (15 pA) in a ganglion cell superfused with the Cd(I) solution. Similar results were obtained from 7 of the 15 ganglion cells tested. The other 8 cells, in which injecting depolarizing currents generated spikes, rapidly accommodated and stopped firing within 1 s; these cells were not tested further. *E*, an expanded spike recording to indicate the points at which measurements were made of the peak, half-duration ($D_{1/2}$), rise and decay times of APs in Cd(I) and with taurine (left). Bar graphs (right panel) show that taurine reduced the peak by 7.4 ± 2% (*$P > 0.02$, $n = 189$), $D_{1/2}$ by 26.2 ± 7.2% (**$P > 0.01$, $n = 189$) and decay time by 27.1 ± 8.3% (***$P > 0.01$, $n = 189$).

still increased by taurine (Fig. 1*Bc*), indicating that the action of taurine was *not* mediated by metabotropic $GABA_B$ receptors. Voltage-dependent curves were plotted from eight cells in each of the test solutions used in Fig. 1*B*. The outward rectifying *I–V* curves derived from these 8 cells (Fig. 1*C*) confirm the fact that CGP55845 had no significant effect on the response of taurine.

The metabotropic effect of taurine was further investigated on the train of ganglion cell APs generated by injecting depolarizing currents in current-clamp mode. Figure 1*D* shows typical recordings from a ganglion cell with a resting potential of about −70 mV. Injecting 15 pA currents for 6 s evoked a train of APs in the cell exposed to the control Cd(I) inhibitory cocktail, and the firing rate of APs was increased with the addition of taurine. This suggests that taurine may increase the repolarization rate of APs by increasing K^+ efflux through K_V channels. The peak, half-duration ($D_{1/2}$), rise and decay time of APs were compared in Cd(I) with and without taurine from seven cells, using the Minianalysis program (Synaptosoftware, Fort Lee, USA). A representative recording of an AP is shown on an expanded time scale to indicate the points at which kinetic measurements were made (Fig. 1*E*, left), and the histograms illustrate that taurine reduced the peak, $D_{1/2}$ and decay time of APs, but had no effect on the rise time (Fig. 1*E*, right).

Pharmacological evidence that taurine responses were derived from various subtypes of delayed rectifier K_V channels

To determine the specific subtypes of K_V channels that are sensitive to taurine regulation, selective channel blockers for delayed rectifier K_V channel subtypes were utilized. Margatoxin (MgTX) is a selective inhibitor of $K_V1.1$ and $K_V1.3$ channel complexes on retinal neurons (Koeberle & Schlichter, 2010), whereas stromatoxin-1 (ScTx-1) specifically inhibits channels formed by combinations of K_V2 and K_V4 subgroup-mediated channels (Guan *et al.* 2011). These antagonists allowed us to pharmacologically distinguish the subtypes of K_V channels present in amacrine and ganglion cells that are regulated by taurine. Figure 2*Aa* shows that the sustained K_V current evoked by a 25 ms voltage pulse in the Cd(I) solution (black trace) was largely suppressed by MgTX (50 nM), but it had little effect on the initial peak (grey trace); the addition of taurine had no effect on the currents (Fig. 2*Ab*). These findings were confirmed in recordings from five additional amacrine cells. Subtracting the control response from the current in MgTX revealed that the MgTX-sensitive currents resembled the slowly activated delayed rectifier K_V current (Fig. 2*Ac*), thus indicating that $K_V1.1$ and/or $K_V1.3$ channels are enhanced by taurine.

Figure 2*Ba* and *Cc* shows that ScTx-1 (50 nM) also suppressed delayed rectifier K_V channels in amacrine and ganglion cells, although to a lesser extent than MgTX. Here too the addition of taurine following the blockage of K_V2 and K_V4 channel subtypes had no detectable effect on the K_V currents (Fig. 2*Bb*), evidence that taurine also regulates K_V channels formed by K_V2 subtypes. Subtracting the control from the ScTx-1 current revealed that ScTx-1-sensitive K_V currents had a much slower activation rate compared with MgTX-sensitive currents (Fig. 2*Bc*). Clearly, the various subtypes of delayed rectifier K_V channels exhibit distinct channel kinetics, and it appears

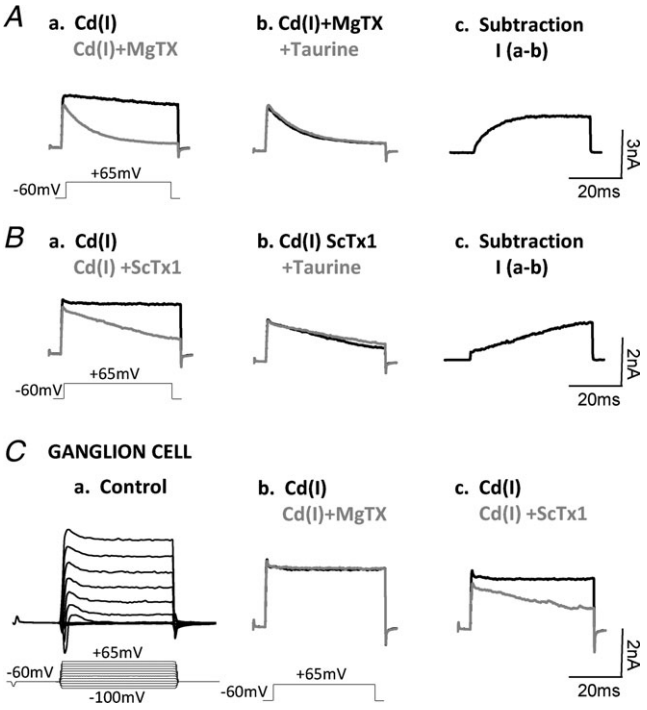

Figure 2. The effect of taurine on K_V current was inhibited by margatoxin (MgTX) and stromatoxin-1 (ScTx-1)
Aa, the K_V current evoked by a 25 ms voltage pulse of −60 mV to +65 mV in the Cd(I) solution (black trace); MgTX (50 nM) suppressed the majority of the sustained K_V currents with little effect on the initial peak (Grey trace); the addition of taurine had no effect on the currents (*Ab*). Taurine-sensitive $K_V1.1$ and/or $K_V1.3$ channel currents are revealed by subtracting the control response from the current in MgTX (*Ac*). *Ba*, ScTx-1 (50 nM) also suppressed delayed rectifier K_V channels in amacrine cells, although to a lesser extent than MgTX. *Bb*, the addition of taurine following the ScTx-1 had no detectable effect on the K_V currents. *Bc*, subtracting the control from the ScTx-1 current revealed that ScTx-1-sensitive K_V2 and K_V4 channels had a much slower activation rate compared with MgTX-sensitive currents. In approximately 50% of ganglion cells the K_V currents were only sensitive to ScTx-1, but not MgTX, as shown in (*Ca*) K^+ currents from a ganglion cell in Ringer solution; and the K^+ currents sensitive to ScTx-1, but not MgTX (*Cb* and *Cc*).

that these various subgroups probably coexist in most amacrine cells. In contrast, K_V channels in approximately 50% of the 25 ganglion cells tested were not blocked by MgTX (Fig. 2*Cb*), but were blocked by ScTx-1 (Fig. 2*Cc*), an indication that there is a subgroup of ganglion cells that express primarily K_V2 subunits.

Intracellular pathways involved in the metabotropic taurine response

An intriguing issue that is difficult to address is the identification of the intracellular pathway mediating the metabotropic taurine response. PKC and PKA pathways

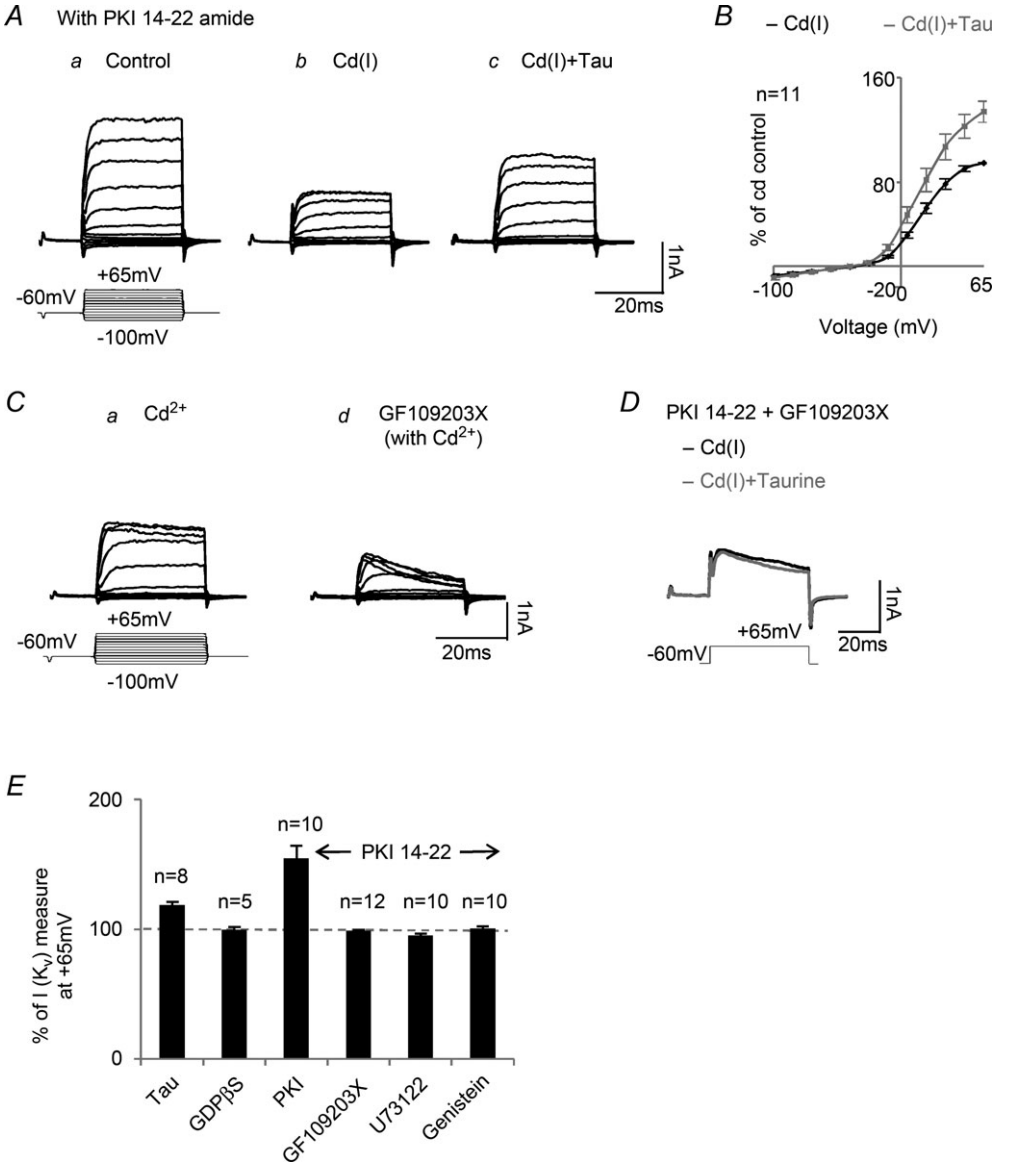

Figure 3. Potential intracellular pathways mediating taurine's metabotropic regulation of K_V channels
Aa and *Ab*, after intracellular dialysis of 10 μM PKI 14–22 amide (the PKA inhibitor), K^+ currents were recorded in control Ringer and the Cd(I) solution. *Ac*, taurine still enhanced K_V currents after blocking PKA. *B*, the *I–V* curves show that in the presence of PKI 14–22, taurine enhanced K_V currents from 11 cells. *Ca* and *Cb*, 10 μM GF109203X, a selective PKC inhibitor, blocked sustained K_V currents in amacrine cells. *D*, after blocking both PKA and PKC with PKI 14–22 and GF109203X, taurine no longer had an effect on K_V currents. *E*, the effects of various intracellular protein inhibitors on the metabotropic taurine regulation of K_V currents. The horizontal dashed line represents the control K_V current recorded in the Cd(I) solution in response to a +65 mV voltage step. On average, taurine increased the K_V currents by about 19% ($n = 8$); with PKI 14–22 to block PKA, taurine enhanced K_V currents by approximately 48% ($n = 11$). While PKA was blocked, inhibition of PKC (with GF109203X) or PLC (with U73122) or tyrosine kinase (with genistein) eliminated the effect of taurine on K_V currents. The effect of taurine was also blocked by intracellular application of $GDP_\beta S$, an inhibitor of G-proteins ($n = 6$).

are ubiquitous pathways that exist in retinal neurons (Gillette & Dacheux, 1996). Accordingly, we tested the effect of taurine on K_V currents when selective kinase inhibitors interrupted these intracellular pathways. The peptide PKI 14–22 amide (10 μM), the specifically PKA inhibitor, was introduced intracellularly through the whole-cell recording electrodes. Following a series of control current–voltage recordings in the PKI 14–22 solution (Fig. 3*Aa*), the K^+ currents were recorded following the addition of the Cd(I) cocktail (Fig. 3*Ab*); further addition of taurine greatly increased the K_V currents (Fig. 3*Ac*). The *I–V* curves ($n = 11$) shown in Fig. 3*B* are a clear indication that blocking PKA does not inhibit the taurine enhancement of K_V channel currents.

GF109203X, a membrane-permeable PKC inhibitor, was used to specifically block PKC and its related downstream pathways in amacrine and ganglion cells. After K_V currents were recorded in a Cd^{2+}-containing Ringer solution (Fig. 3*Ca*), superfusion of GF109203X (10 μM) greatly suppressed the K_V currents (Fig. 3*Cb*); the effect of PKC inhibition was consistent in all the recordings from amacrine cells and most of the ganglion cells ($n = 23$ total). This indicates that PKC is critical for K_V channel conductance in these neurons. Although suppression of PKA did not suppress the response to taurine, blocking both PKC and PKA pathways (applying PKI 14–22 with GF109203X) resulted in complete blockage of the current enhancement by taurine (Fig. 3*D*). The bar graphs of Fig. 3*E* illustrate the effects of various intracellular transduction pathway inhibitors on taurine enhancement of K_V currents. Only taurine and taurine with the PKA inhibitor PKI 14–22 enhanced the K_V currents; taurine increased the K_V currents by 19% ($n = 8$), whereas in the presence of the PKA inhibitor, taurine enhanced the K_V current by 48% ($n = 11$). Thus, blocking the PKA pathway significantly upregulated the taurine effect.

Because phospholipase C (PLC) is an upstream enzyme in the PKC cascade, it may be involved in the transduction pathway governing taurine regulation of K_V channels. U73122, a specific PLC inhibitor, was applied with PKI 14–22 via internal dialysis to block the PLC/PKA

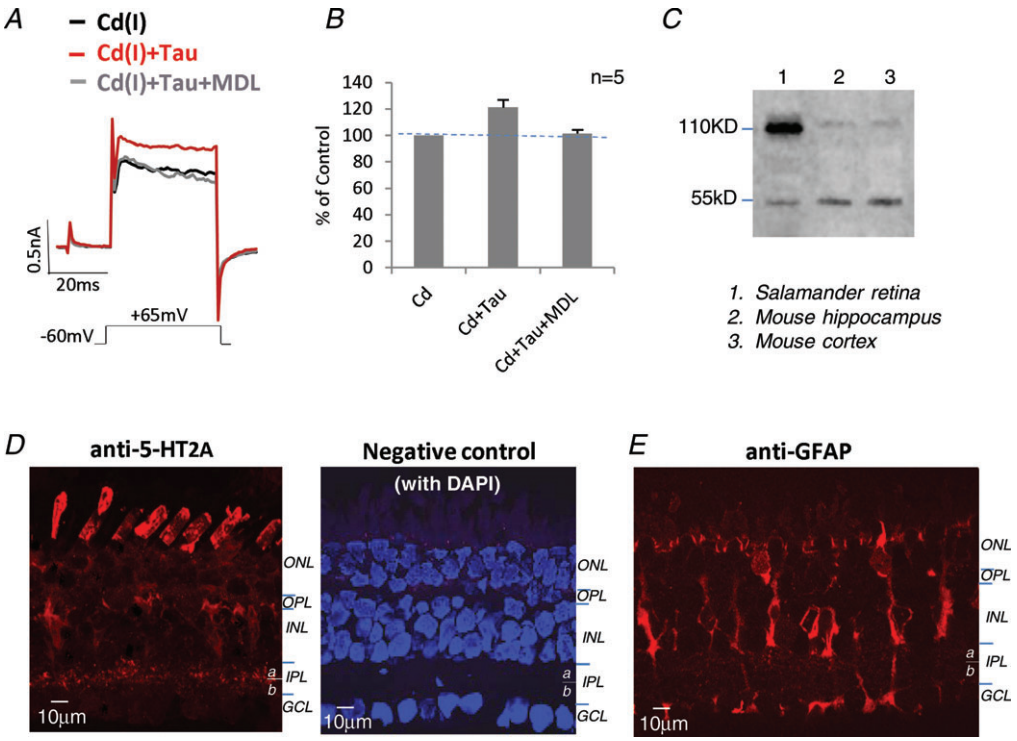

Figure 4. Taurine activates 5-HT_{2A} receptors in retinal neurons
A, taurine enhanced K_V currents in the Cd(I) solution, but the effect was blocked by 1 nM MDL11939, a selective 5-HT_{2A} receptor antagonist. *B*, the bar graphs show the summary results of the effect of MDL11939 on blocked taurine-enhanced K_V current amplitudes measured at +65 mV. *C*, Western blots show that the anti-5-HT_{2A} detected protein bands at the predicted MW of 53 kDa in samples from mouse brain and salamander retina. It is likely that protein bands near MW of 110 kDa are dimers. *D*, confocal image of anti-5-HT_{2A} labeling in a salamander retinal section, depicting the strong labeling in photoreceptors and within the inner plexiform layer (IPL); punctate labeling is also seen in the somas of the inner nuclear layer (INL) and ganglion cell layer (GCL); and the negative control (omitting 5-HT_{2A} antibody) shows no labeling in retinal sections (the cell nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI)). *E*, Muller cells in a retinal section were labeled by the anti-glial fibrillary acidic protein (GFAP). ONL. outer nuclear layer; OPL, outer plexiform layer.

pathway. The enhancement of K_V currents by taurine was completely blocked by 10 μM of the PLC inhibitor, $n = 10$ (Fig. 3*E*). This suggests that PLC is probably an important stage in the taurine transduction pathway.

There is evidence that activation of delayed rectifier K_V channels is regulated by tyrosine kinase phosphorylation (Strauss *et al.* 2002). Therefore, we applied genistein, a tyrosine kinase inhibitor, while PKA was inhibited with PKI 14–22. As shown in Fig. *3E*, this effectively blocked taurine enhancement of K_V currents, indicating that tyrosine kinase phosphorylation may also be involved in the taurine-activated transduction pathway.

GTP-binding protein (G-protein) often serves as an intracellular second messenger of neuronal metabotropic receptors. Indeed, this seems to be the case in regard to the taurine-induced activation of K_V channels. To block G-protein activity and its downstream pathways, $GDP_\beta S$ (1 mM) was applied through the whole-cell recording electrodes. After intracellular application of $GDP_\beta S$, taurine failed to enhance the K_V currents ($n = 5$; Fig. 3*E*), demonstrating that a G-protein-linked metabotropic pathway is involved in the taurine response.

The involvement of 5-HT_{2A} serotonin receptors

To investigate a potential site for taurine binding that subsequently triggers the intracellular metabotropic cascades that regulate K_V currents, several metabotropic receptor antagonists were tested. Among these, MDL11939, a potent and selective antagonist for serotonin receptor 5-HT_{2A} (Fox *et al.* 2010), was found to significantly block the effect of taurine (Fig. 4*A* and *B*).

To localize 5-HT_{2A} receptors in salamander retina, an antibody specific for 5-HT_{2A} receptors was used; the antibody specificity was tested by Western blots in salamander retina and in the regions of mouse brain. The results demonstrated that the antibody recognized proteins with the same molecular weight (53 kDa) in these tissues (Fig. 4*C*); the secondary bands at 110 kDa are probably dimers. This antibody was also used to identify cells expressing 5-HT_{2A} receptors by immunocytochemistry. Dense labeling was present in the proximal half (sublamina a) of the inner plexiform layer (IPL) where bipolar cell terminals, amacrine and ganglion cell processes are located. The antibody also lightly labeled some somas at the inner nuclear layer and ganglion cell layer, as well as photoreceptor outer-segments and possibly Muller cells (Fig. 4*D*, left). No positive labeling was observed in the negative control experiments performed using the same protocol, except the 5-HT_{2A} (primary antibody) was omitted. 4′,6-Diamidino-2-phenylindole (DAPI) was used as a fluorescence marker to stain cell nuclei and to depict the retinal structure (Fig. 4*D*, right). Figure 4*E* shows Muller cells in a retinal section labeled by the GFAP antibody. Comparing labeling patterns with GFAP and 5-HT_{2A} antibodies suggests that 5-HT_{2A} labeling in the IPL and ganglion cell layer is probably not associated with Muller cells.

The effect of taurine on HEK cells expressing 5-HT_{2A}

To further demonstrate that 5-HT_{2A} receptors mediate the effects of taurine, we transfected HEK cells with GFP-tagged 5-HT_{2A} receptors. Successful transfection was confirmed by 5-HT_{2A} antibody-labeling of the cells. As illustrated in Fig. 5*A*, confocal images showed the presence of GFP-tagged 5-HT_{2A} proteins (green) in both the cytosol and on the cell membrane. On the other hand, the 5-HT_{2A} antibody labeled the protein solely on the plasma membrane (red). Superposition of the two images confirms that 5-HT_{2A} receptors were expressed on the cell membranes of the HEK cells.

The action of taurine on the 5-HT_{2A} receptors in HEK cells was again tested in the Cd(I) solution. The inward rectifier K_{IR} channels, endogenously expressed in HEK cells (Fig. 5*B*, black trace), were suppressed by 80 μM taurine (Fig. 5*B*, red trace). Similarly, Fig. 5*C* shows that the taurine response could be mimicked

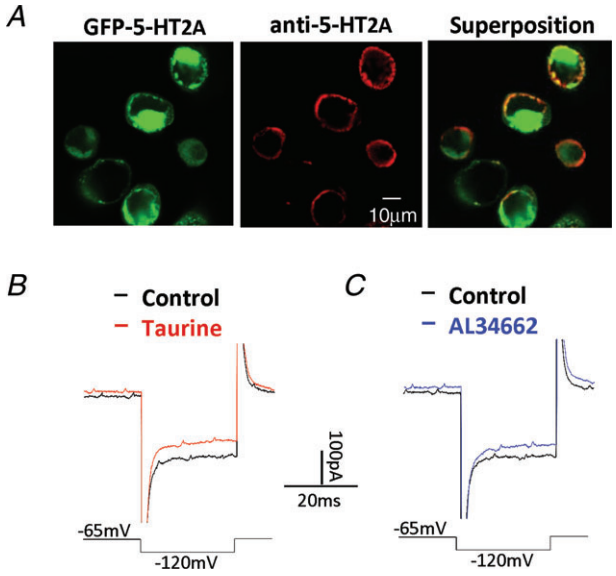

Figure 5. The effect of taurine on HEK cells expressing 5-HT_{2A} receptors
A, confocal images of HEK cells expressing GFP-tagged 5-HT_{2A} receptors (green), and labeled with anti-5-HT_{2A} receptors (red). Superposition of the images shows that 5-HT_{2A} receptors are expressed in the plasma membrane of the transfected HEK cells. *B*, whole-cell recording with 80 μM taurine in the Cd(I) solution partially suppressed the endogenous inward rectifier (K_{IR}) currents at −120 mV in a HEK cell transfected with 5-HT_{2A} receptors. *C*, AL34662 mimicked the effect of taurine on the HEK cell.

by the non-selective 5-HT_2 receptor agonist AL34662. Approximately 20% of the currents were suppressed by AL34662 ($n = 7$) and taurine ($n = 11$). In HEK cells in which the 5-HT_{2A} receptor was not expressed, neither AL34662 nor taurine affected the inward rectifier K_{IR} currents ($n = 16$, data not shown).

Discussion

This study shows for the first time that taurine activates 5-HT_{2A} receptors, which, via a metabotropic intracellular pathway, increases the currents through delayed rectifier K_V channels in retinal neurons. In the vertebrate retina, these channels are essential for the generation of APs in amacrine and ganglion cells. Although in some systems it appears that taurine acts on metabotropic $GABA_B$ receptors (Kontro & Oja, 1990; Smith & Li, 1991), the $GABA_B$ receptor antagonist CGP55845 had no effect on the response to taurine. In contrast, MDL11939, a potent and selective antagonist of the 5-HT_{2A} serotonin receptor, was found to significantly block the effect of taurine.

Overall, the pharmacological results indicate that the metabotropic taurine response is via an intracellular G-protein-mediated pathway involving PLC, PKC and tyrosine kinase. These features correspond to the regulatory pathway by which serotonin regulates K_V channels in pulmonary artery smooth muscle through 5-HT_{2A} receptors (Varghese *et al.* 2006). Although a PKC-sensitive cascade represents the major intracellular pathway for taurine enhancement of delayed rectifier K_V channels, we found that inhibition of PKA increases the taurine effect on the channel currents. Thus, a PKA-mediated pathway may represent a negative control mechanism for taurine regulation in amacrine and ganglion cells.

Mechanistic studies of channel regulation will contribute to our understanding of how the myriad K_V channels respond to physiological inputs in neural signaling. The K_V family, with 12 known subfamilies, is widely expressed in the CNS, including retina. Among these, the delayed rectifier K^+ channels, assembled by K_V1 and K_V2, are the major K^+ channels that directly influence the firing rates of APs. The responses to MgTX and ScTx-1 revealed that K_V1, K_V2 and K_V4 subtypes of delayed rectifier K_V channels are the principal sustained K^+ channels in amacrine and ganglion cells.

There is evidence that PKC phosphorylation of K_V1 and K_V2 channels is a feature of delayed rectifier K_V channels in various cell types (Cai & Douglass, 1993; Murakoshi *et al.* 1997; Boland & Jackson, 1999; Strauss *et al.* 2002), and that tyrosine kinase directly interacts with these channels (Huang *et al.* 1993; Holmes *et al.* 1996). Our findings show that these internal proteins are critical for the metabotropic regulation of K_V channels by taurine in retinal neurons. It is noteworthy that most amacrine cells have at least two subtypes of K_V channel that can be blocked by MgTX and ScTx-1. However, there are other ganglion cells that are sensitive only to ScTx-1, suggesting that different neuronal subtypes express a unique set of K_V channels, which probably subserve distinct physiological functions and allow the neurons to control their unique pattern of excitability (Leung, 2010).

As mentioned earlier, taurine has been shown to be a major cytoprotectant, and essential for the normal function of heart, kidney, pancreas and other organs. In addition, taurine supplementation has been found useful for various brain disorders, for example epilepsy and addiction (Wu *et al.* 2005; Junyent *et al.* 2009; Olive, 2010). Clearly, it would be of significant interest to know the mechanisms of the action of taurine in the CNS. The present findings suggest that its effects may be mediated by 5-HT_{2A} receptors, one of the various serotonin receptor subtypes that have been extensively studied as targets for the development of antipsychotic drugs.

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Author contributions

S.B., H.R. and W.S. were involved in the conception and design of the experiments, and in the collection and analysis of data; S.B. conducted the major experiments depicted in Figs 1–4. W.S. conducted the experiments in Figs 1*D*, 5*B* and *C*. Y.L. performed the experiments of Figs 4*C* and *D*, 5*A*. W.S. and H.R. wrote the manuscript. The manuscript was read and approved by all the authors. The experiments were conducted in the Department of Biomedical Science, Charles E Schmidt College of Medicine at Florida Atlantic University.

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