Brief Communications

GABA Transporters Regulate a Standing $GABA_C$ Receptor-Mediated Current at a Retinal Presynaptic Terminal

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At the axon terminal of goldfish retinal bipolar cells, $GABA_C$ receptors have been shown to mediate inhibitory reciprocal synaptic currents. Here, we demonstrate a novel standing GABAergic current mediated exclusively by $GABA_C$ receptors. Selective inhibition of GAT-1 GABA transporters on amacrine cells increases this tonic current and reveals a specific functional coupling between GAT-1 transporters and $GABA_C$ receptors. We propose that this $GABA_C$ receptor-mediated standing current serves to regulate synaptic gain by shunting depolarizing potentials that can produce Ca^{2+}-dependent action potentials at the bipolar cell terminal. Furthermore, we find that the amount of $GABA_C$ receptor-mediated reciprocal feedback between bipolar cell terminals and amacrine cells is greatly increased when GAT-1 transporters are specifically blocked by NO-711 (1-[2-[[(diphenylmethylene)imino]oxy]ethyl]-1,2,5,6-tetrahydro-3-pyridinecarboxylic acid hydrochloride). The involvement of GAT-1 transporters in regulating this standing (or tonic) $GABA_C$ current implicates them in a novel role as major determinants of presynaptic excitability.

Key words: **bipolar cell; retina; GAT-1; GABA transporters; tonic inhibition; presynaptic excitability**

Introduction

In the retina, both $GABA_A$ and $GABA_C$ receptors mediate inhibitory chloride currents. $GABA_C$ receptors, which have a smaller single channel conductance and higher affinity for GABA, have been shown to impact retinal signaling at multiple levels (Lukasiewicz et al., 2004). Their direct mechanism of inhibition, however, has not been completely described at many retinal synapses.

Several lines of evidence point to $GABA_C$ receptor activation as an important regulator of bipolar cell output in the inner retina. In electroretinograms, $GABA_C$ receptor inhibition (Kapousta-Bruneau, 2000) or knock-out (McCall et al., 2002) has been shown to alter the b-wave associated with bipolar cell function. Interestingly, selective $GABA_C$ receptor inhibition has been shown to potentiate escape behavior in frogs (Ishikane et al., 2005), a finding that was correlated with increased oscillatory discharges in dimming-detector ganglion cells. Also, inhibiting $GABA_C$ receptors can prolong the light responses of postsynaptic ganglion and amacrine cells and may abolish part of their capacity for transient ON–OFF signaling in the salamander retina (Dong and Werblin, 1998; but see Bieda and Copenhagen, 2000). In a similar manner, $GABA_C$ receptors on rabbit bipolar cells can shorten the duration and size of excitatory ganglion cell light responses, thus making an important contribution to their center-surround organization (Flores-Herr et al., 2001). At amacrine cells in the mouse retina, inhibiting $GABA_C$ receptors reveals an NMDA receptor-mediated component of EPSCs (Matsui et al., 2001). Together, these results suggest that $GABA_C$ receptors may act to limit the extent of glutamate release from bipolar cell terminals.

GABA transporters have also been implicated in regulating the output of bipolar cells in the inner retina. In particular, inhibiting the GAT-1 subtype of the GABA transporter has been shown to increase the size of bipolar cell $GABA_C$ IPSCs and diminish light responses at ganglion cells in the salamander retina (Ichinose and Lukasiewicz, 2002). Here, we use voltage-clamp recordings from isolated bipolar cell terminals in the goldfish retinal slice to reveal a novel tonic current mediated by $GABA_C$ receptors. This tonic current is selectively regulated by GAT-1 GABA transporters, which also limit the reciprocal activation of $GABA_C$ receptors. In concert with GAT-1 transporters, this tonic $GABA_C$ receptor-mediated inhibitory conductance can establish a powerful shunt to depolarizations at the bipolar cell terminal. These results suggest a novel role for GABA transporters in regulating presynaptic excitability and the gain of tonic inhibition at a retinal nerve terminal and provide a potential mechanism for $GABA_C$ receptor-mediated inhibition of glutamate release.

Materials and Methods

Retinal slice preparation. Retinal slices were prepared from pieces of goldfish retina (for details, see Palmer et al., 2003). The slices (200 μm thick) were constantly perfused at 2–3 ml/min with 2.5 mM Ca^{2+} Ringer's solution for patch-clamp recording. Bipolar cell terminals with severed axons were identified in the inner plexiform layer (IPL) based on (1) single-exponential membrane time constant, (2) the presence of an L-type Ca^{2+} current and ΔC_m jump, and (3) Mb-shaped (bulbous) terminal morphology.

Electrophysiology. Isolated bipolar cell terminals in retinal slices were

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voltage clamped in the whole-cell mode using a HEKA Elektronik (Lambrecht/Pfalz, Germany) EPC-9 patch-clamp amplifier in conjunction with Pulse software running the X-chart extension (Pulse version 8.53). The Sine+DC technique was used for real-time measurements of membrane capacitance. Briefly, a 30 mV peak-to-peak 1 kHz sine wave was superimposed on the holding potential of the cells (−60 mV) and used by on-line analysis software to calculate time-resolved membrane capacitance. Standard external recording solutions contained (in mM) 120 NaCl, 2.5 KCl, 2.5 $CaCl_2$, 1.0 $MgCl_2$, 10 HEPES, and 12 glucose (pH 7.2; osmolarity, 260–265 mOsm). Patch pipettes were pulled from borosilicate capillary glass and coated with dental wax to reduce pipette capacitance. Internal pipette solutions, depending on the experiment, contained one of the following solutions (in mM): low chloride: 110 Cs-gluconate, 15 TEA-Cl, 25 HEPES, 3 Mg-ATP, 0.5 Na-GTP, and 0.5 EGTA; high chloride (125 mM): same as standard solution, except that 115 CsCl and 10 TEA-Cl were used. All internals were set to pH 7.2 with CsOH. APV, CNQX, and gabazine (SR95531) were obtained from Tocris (Bristol, UK). TTX was obtained from Alamone Laboratories (Jerusalem, Israel). All other chemicals were obtained from Sigma (St. Louis, MO).

Outside-out patches. Outside-out patches were excised from bipolar cell terminals in retinal slices using 4–6 MΩ borosilicate patch pipettes. Patches were pulled after break-in to the whole-cell mode and confirmation of terminal identity using the above stated criteria. GABA receptor agonists and antagonists were applied to patches using a borosilicate, two-barreled theta glass pipette (outer diameter, 1.5 mm; inner diameter, 1.0 mm; septum, 0.2 mm; Warner Instruments, Hamden, CT) pulled to a tip diameter of ~100–300 μm. For rapid translation, the theta glass pipette was attached to a piezo bending actuator (Piezo Systems, Cambridge, MA) driven by a constant voltage-isolated stimulator (Digitimer, Hertfordshire, UK) set to 11 V. This stimulator was triggered with the recording Pulse software (HEKA Elektronik). To reduce vibrations in the application system, a 1 MΩ resistor was placed in series with the piezo actuator. The time required for exchanging solutions was estimated by measuring the open-tip current resulting from a junction potential between normal extracellular solution and 3× concentrated extracellular solution. Open-tip current rise times were always <1 ms, and 10–90% rise times of the open-tip current typically ranged from 400 to 600 μs. For details of this setup, see Jonas (1995). Patch currents were recorded with a low-chloride internal solution (above) at a holding potential of 0 mV, or in a high-chloride internal solution at a holding potential of −60 mV. Long GABA applications designed to measure receptor desensitization were therefore performed in patches shown to have only one type of GABA receptor ($GABA_A$ or $GABA_C$).

Analysis. Off-line data analysis was performed using IgorPro software (version 5.03; Wavemetrics, Lake Oswego, OR). Reciprocal feedback was quantified by integrating the total current during a depolarization. This method provides a measure of the net charge transfer associated with I_{Ca} plus GABAergic feedback that can be compared across subsequent depolarizations and has been used previously for quantifying relative changes in reciprocal feedback at this synapse (Vigh et al., 2005). To avoid any potential influence of feedback increases as a result of amacrine cell metabotropic glutamate receptor activation, the first depolarization-evoked control response was not considered (Vigh et al., 2005). Only the second feedback response, obtained after at least 30 s, was considered as the control feedback to be compared with feedback after 1-[2-[[(diphenylmethylene)imino]oxy]ethyl]-1,2,5,6-tetrahydro-3-pyridinecarboxylic acid hydrochloride (NO-711) application.

Average standing conductances were calculated from average standing currents using Ohm's law, and the terminal surface area was calculated using the measured capacitance of each terminal and assuming a specific membrane capacitance of 1 $\mu F/cm^2$. Statistics were calculated using Prism (version 4; GraphPad Software, San Diego, CA) with two-tailed *t* tests for comparing data sets. Data are reported as mean ± SEM.

Results

$GABA_C$ receptor-mediated tonic current

Both $GABA_A$ and $GABA_C$ receptors mediate chloride currents at bipolar cell terminals in goldfish retinal slices (Vigh et al., 2005). Under high internal pipette chloride (125 mM), we observed that a standing (or tonic) current was present at isolated bipolar cell terminals (severed axons) (Fig. 1). Application of the $GABA_A$ receptor antagonist bicuculline-methiodide (BMI; 20 μM) did not reduce this standing current (Fig. 1*A*). Past work has shown that GAT-1 transporters limit the synaptic activation of $GABA_C$ receptors in the salamander retina (Ichinose and Lukasiewicz, 2002). We thus tested the GAT-1-selective antagonist NO-711 to see whether transporters could also limit the activation of this standing current. Application of NO-711 (3 μM) dramatically increased this standing current, and the selective $GABA_C$ receptor antagonist (1,2,5,6-tetrahydropyridin-4-yl)methylphosphinic acid (TPMPA) (150 μM) completely blocked the standing current. Importantly, unlike other GABA transporter antagonists such as nipecotic acid (Keros and Hablitz, 2005), NO-711 is not a substrate for GAT-1 and does not produce a depolarizing membrane current (Dong et al., 1994; Yang, 1998). As shown in Figure 1*B*, TPMPA also blocks the standing current in the absence of NO-711 and can prevent the NO-711-induced increase in this current. Although TPMPA can act as a weak antagonist of $GABA_A$ receptors at high concentrations (Jones et al., 2001), our experiments were designed to avoid this complication by applying BMI before TPMPA, as shown in Figure 1*A* and in the latter part of Figure 1*B*. These results thus suggest that a subset of $GABA_C$ receptors is tonically open and solely responsible for mediating the bipolar cell terminal standing current.

To measure the influence of this standing $GABA_C$ current at the bipolar cell terminal, we have quantified it under several pharmacological conditions in terms of its specific conductance

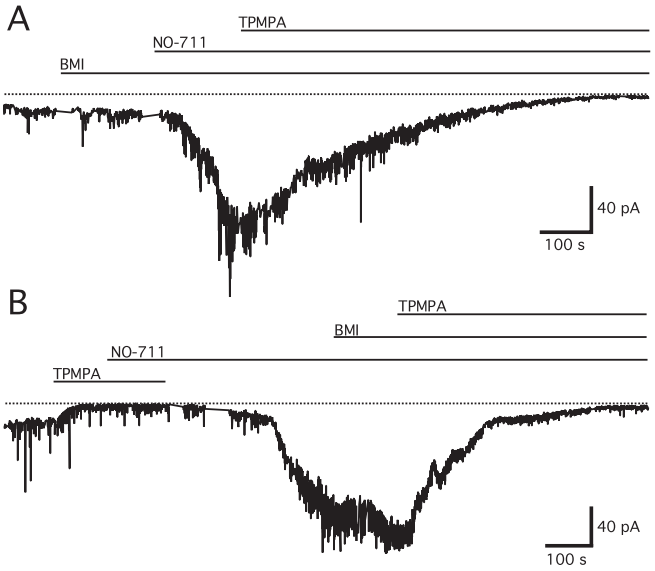

Figure 1. Bipolar cell terminals in retinal slices have tonic currents. ***A***, Whole-cell mode voltage-clamp recording from an isolated bipolar cell terminal (severed axon) in the retinal slice shows a standing current. BMI (20 μM) has no effect on this current, but NO-711 (3 μM) increases the standing current severalfold (−18.4 ± 3 pA in control vs −126 ± 15 pA in NO-711). This increase is completely reversed by TPMPA (150 μM), and the standing current is abolished (below control level, −18.4 ± 3 pA in control vs −5.3 ± 0.7 pA in TPMPA). Note also the current deflections that are superimposed on the tonic leak in NO-711. These are large and slow spontaneous $GABA_C$ IPSCs that sometimes occur in NO-711, and these TPMPA-sensitive current oscillations can exhibit periodicity (data not shown; see also Fig. 2*A*). ***B***, TPMPA also abolishes the standing current in the absence of NO-711 (−31.7 ± 6.9 pA in control vs 10.6 ± 3.0 pA in TPMPA) and prevents the NO-711-induced increase in the standing current until the TPMPA is washed out. BMI does not reduce the standing current in the presence of NO-711, but TPMPA still abolishes it. The occasional breaks (flat portions) in the current traces shown here and in Figure 2 are brief periods when data acquisition was interrupted to record Ca^{2+} currents. In this example, data are reported as ±SD.

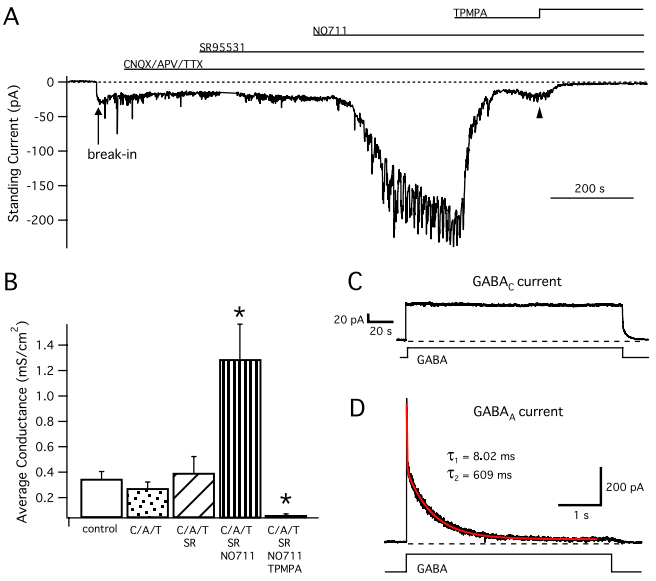

Figure 2. GABA$_C$-mediated standing currents are enhanced by the selective GAT-1 transporter inhibitor NO-711 (3 μM) and blocked by TPMPA (200 μM). ***A***, After break-in to the whole-cell mode under high internal chloride (125 mM), the terminal exhibited a standing current. This current was decreased slightly with a combination of CNQX, APV, and TTX (25, 50, and 1 μM, respectively) and was increased slightly in SR95531 (25 μM). NO-711 caused a dramatic increase in the standing current, whereas TPMPA completely reversed this increase and also essentially eliminated the original standing current (2 pA leak current in TPMPA). To fully reduce the standing current in this example, TPMPA was elevated from 150 to 200 μM (arrowhead). ***B***, Average standing conductances in each drug condition: control (0.34 ± 0.06 mS/cm^2, $n = 10$), CNQX/APV/TTX (0.27 ± 0.06 mS/cm^2, $n = 10$), CNQX/APV/TTX+SR95531 (0.39 ± 0.14 mS/cm^2, $n = 5$), CNQX/APV/TTX+NO-711 (1.28 ± 0.22 mS/cm^2, $n = 5$), CNQX/APV/TTX+TPMPA (0.056 ± 0.002 mS/cm^2, $n = 8$). Average conductances were calculated using Ohm's law, and the terminal surface area was calculated using the measured capacitance of each terminal and assuming a specific membrane capacitance of 1 μF/cm^2. The average conductance was significantly larger only in NO-711 ($p = 0.0006$) and significantly smaller in TPMPA ($p = 0.0010$) compared with control. ***C***, GABA$_C$ receptors did not desensitize in the presence of continuous GABA (10 mM). GABA was applied for 3–4 min to outside-out patches with no GABA$_A$ receptors (TPMPA blocked all current, no effect of SR95531), and in every case, GABA$_C$ currents remained at their peak amplitude throughout the GABA application (5 patches). ***D***, GABA$_A$ receptors quickly desensitize in the presence of continuous GABA (10 mM; 9 patches; red line is a double-exponential fit). Note the difference in timescales for ***C*** and ***D***.

(Fig. 2). When excitatory synaptic transmission and sodium channels were blocked (CNQX, APV, TTX), there was no significant change in this standing conductance (control: 0.34 ± 0.06 mS/cm^2, $n = 10$; CNQX/APV/TTX: 0.27 ± 0.06 mS/cm^2, $n = 10$; $p = 0.38$). These recordings show that non-evoked GABA release (i.e., independent of action potentials) is sufficient to maintain the standing current mediated by GABA$_C$ receptors. Notably, there was often a small increase in the standing conductance when GABA$_A$ receptors were blocked by BMI or SR95531 (25 μM) (Fig. 2*A*), but this increase was not statistically significant, on average (CNQX/APV/TTX+SR95531: 0.39 ± 0.14 mS/cm^2, $n = 5$; $p_{control} = 0.74$; $p_{cnqx/apv/ttx} = 0.34$). This small increase in the bipolar terminal standing conductance is consistent with a disinhibition of GABAergic amacrine cells (Zhang et al., 1997) that results in more GABA release and an increased activation of GABA$_C$ receptors in the presence of SR95531 or BMI. It does not, however, suggest a direct GABA$_A$ receptor-mediated standing current at the bipolar terminal, because the response to GABA$_A$ receptor antagonists was a small increase in the standing current.

When GAT-1 transporters were inhibited with NO-711, however, there was a 3.8-fold average increase in the standing conductance (1.28 ± 0.22 mS/cm^2, $n = 5$; $p_{control} = 0.0006$). This increase was completely reversible by blocking GABA$_C$ receptors with TPMPA. In fact, TPMPA not only reversed the NO-711-induced increase in the standing conductance but blocked nearly all of the standing conductance to a level significantly below the control level (Fig. 2*B*) (TPMPA: 0.056 ± 0.002 mS/cm^2, $n = 8$; $p_{control} = 0.0010$). The small remaining standing conductance (corresponding to −13.8 ± 3 pA) was insensitive to antagonists and likely constitutes a "leak current" resulting from an imperfect gigaseal after break-in to the whole-cell mode.

Presynaptic outside-out patches

GABA$_C$ receptors are ideally suited to mediate a standing current because they experience no desensitization in the presence of continuous GABA (Amin and Weiss, 1994). However, some fish GABA$_C$ receptors do desensitize (Han et al., 1997). We thus excised outside-out patches from the terminal and quickly applied 10 mM GABA to patches containing only GABA$_C$ currents (confirmed with SR95531, current was blocked by TPMPA for short 2.5 ms applications; data not shown) for 3–4 min. As shown in Figure 2*C*, the resulting GABA$_C$ currents remained at their peak for the entire duration of GABA application ($n = 5$). In comparison, GABA$_A$ receptors are known to desensitize considerably in the continuous presence of GABA, a finding we also observed in outside-out patches that contained only GABA$_A$ receptors (Fig. 2*D*). The decay of the GABA$_A$ current in the presence of GABA was fit by a double exponential with time constants: $\tau_1 = 7.59 \pm 1.22$ ms and $\tau_2 = 924 \pm 121$ ms ($n = 9$ patches).

GAT-1 transporters limit reciprocal activation of GABA$_C$ receptors

We next tested the effects of NO-711 on reciprocally evoked GABAergic currents. Depolarizing the bipolar cell terminal from −60 to 0 mV under low internal chloride (15 mM) produced an inward Ca^{2+} current with superimposed outward GABAergic reciprocal feedback (Fig. 3*A*, control) (Vigh et al., 2005). This reciprocally evoked GABAergic feedback was increased significantly after bath application of NO-711 (3 μM) (Fig. 3*A*). Importantly, NO-711 had no effect on membrane capacitance jumps (ΔC_m), indicating that Ca^{2+} currents had not changed during the recordings and that the exocytosis of glutamate responsible for reciprocal GABA release had not run down. In contrast with the total feedback, NO-711 had no effect on the pure GABA$_A$ receptor-mediated reciprocal feedback recorded in the presence of TPMPA (Fig. 3*B*). When GABA$_C$ receptor-mediated reciprocal feedback was isolated with SR 95531, however, NO-711 again produced a large increase (Fig. 3*C*). These changes in reciprocal feedback after bath application of NO-711 were quantified by integrating the net synaptic charge transfer during the depolarization [as performed by Vigh et al. (2005)], and the feedback increase attributed to GABA$_C$ receptors accounted for the entire increase in the feedback (Fig. 3*D*).

The GABA$_C$ standing current limits Ca^{2+}-dependent action potentials

To determine a physiological role for the standing GABA$_C$ receptor-mediated current and GAT-1 transporters, we investigated voltage responses to current steps from bipolar cell terminals under the current-clamp mode (Fig. 4). With a K-gluconate-based internal solution, terminals were held at a holding current (that varied from −20 to −40 pA) such that the resting membrane potential was −60 mV. On top of this holding current, current steps (from −20 to 50 pA, 10 pA per step) were applied and voltage responses were recorded (Fig. 4*A*). NO-711 reduced

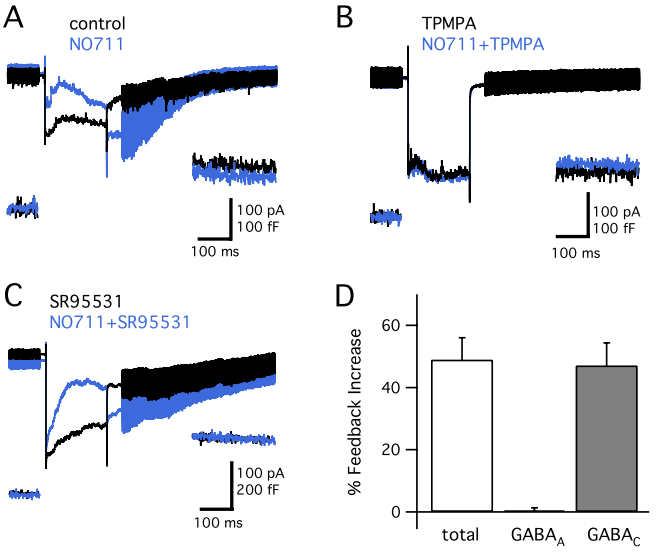

Figure 3. NO-711 selectively enhances $GABA_C$ reciprocal feedback. ***A***, Under low internal chloride (15 mM), depolarizing the bipolar terminal from −60 to 0 mV produces reciprocal GABAergic feedback from amacrine cells via both $GABA_A$ and $GABA_C$ receptors. After bath application of NO-711 (3 μM), this total GABAergic feedback is significantly potentiated. ***B***, Using TPMPA (200 μM) to isolate the pure $GABA_A$ receptor-mediated feedback, NO-711 had no effect on feedback currents. ***C***, Using SR95531 (SR; 25 μM) to isolate the $GABA_C$-mediated feedback, NO-711 dramatically potentiated the feedback currents. ***D***, Average potentiation of GABAergic currents in NO-711. Integration of feedback currents during the depolarization revealed a 49.0 ± 7.1% increase in the total feedback after bath application of NO-711 ($n = 7$). The pure $GABA_A$ receptor-mediated feedback was not potentiated (0 ± 1.3% change after NO-711 application; $n = 9$). The pure $GABA_C$ receptor-mediated feedback was potentiated 47.1 ± 1.2% after application of NO-711 ($n = 6$). Note that the potentiation in control terminals was not significantly different than the potentiation measured in terminals in which the pure $GABA_C$ receptor-mediated feedback was isolated ($p = 0.86$).

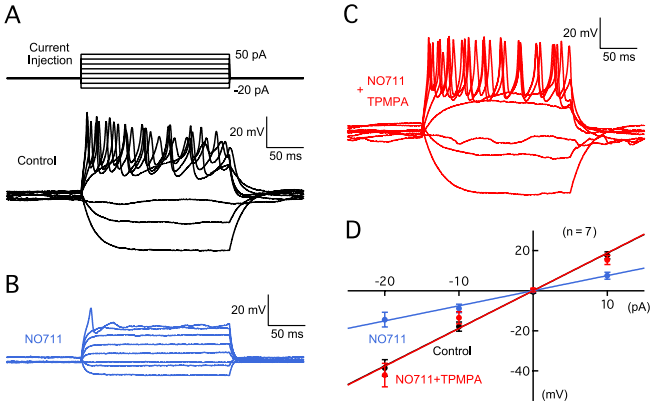

Figure 4. Blocking GAT-1 transporters decreased the excitability of bipolar cell terminals by reducing their input resistance. ***A***, Voltage response (bottom) of a bipolar cell terminal under the current-clamp mode to current steps (top). Notice the spikes for large positive current injections. ***B***, NO-711 (1 μM) reduced the voltage responses to all current steps, and most spikes were eliminated. ***C***, TPMPA (200 μM) reversed the effect of NO-711. Recordings in ***A–C*** are from the same terminal. ***D***, The *I–V* curve for nine bipolar cell terminals: NO-711 (1–3 μM) reduced the input resistance (slope), whereas TPMPA (200 μM) increased it approximately back to control levels, on average.

the voltage responses and eliminated most Ca^{2+}-dependent spikes (Fig. 4*B*), and these effects were reversed by TPMPA (Fig. 4*C*). In addition, TPMPA (200 μM) increased the input resistance, on average, to near the control level (Fig. 4*D*). Note that for our low-chloride internal solution, the reversal potential of chloride (E_{Cl}) was −55.4 mV. Thus, NO-711 caused only very small changes from the resting membrane potential of −60 mV. Similar results were obtained from nine isolated terminals (axon severed).

These experiments suggest that the standing $GABA_C$ conductance shown here can have a significant shunting capacity for depolarizations and the Ca^{2+}-dependent action potentials generated at the terminals of goldfish bipolar cells (Zenisek and Matthews, 1998; Palmer, 2006). Furthermore, GAT-1 transporters can regulate the level of this shunt, as shown by the application of NO-711 (Fig. 4*B*). This prediction is in good agreement with a previous finding that GABA receptors can significantly inhibit potassium-induced depolarizations and Ca^{2+} influx into isolated goldfish bipolar cell terminals (Matthews et al., 1994).

Discussion

Here, we have shown that a tonic current mediated by $GABA_C$ receptors persists when synaptic transmission is blocked with TTX, CNQX, and APV, indicating that spontaneous release is sufficient and necessary to maintain this tonic current. Furthermore, GAT-1 transporters tightly regulate the level of this $GABA_C$ receptor-mediated standing current. In addition, GAT-1 GABA transporters selectively limit the reciprocal activation of $GABA_C$ receptors. Thus, there is a functional coupling between $GABA_C$ receptors and GAT-1 transporters. This functional coupling likely serves to regulate the gain of transmission from bipolar cell terminals, as suggested by our current-clamp experiments that show $GABA_C$ receptors shunt depolarizations that produce Ca^{2+}-dependent action potentials. These experiments thus implicate GAT-1 transporters as a major determinant of presynaptic excitability at this axon terminal.

GAT-1 transporters on amacrine cells

We have shown that the GAT-1 GABA transporter has a selective effect in limiting the activation of bipolar cell $GABA_C$ currents. The GAT-1 subtype of the GABA transporter has previously been localized to the IPL in goldfish (Klooster et al., 2004), salamander (Yang et al., 1997), and rat (Johnson et al., 1996) retina. In addition, it has been shown that GAT-1 can limit conventional synaptic activation of bipolar cell $GABA_C$ receptors in the salamander retina and influence postsynaptic ganglion cell excitation (Ichinose and Lukasiewicz, 2002). In their study, Ichinose and Lukasiewicz (2002) demonstrated that NO-711 increases the size of puff-evoked GABA responses at bipolar terminals as well as IPSCs evoked by rapid kainate puffs to amacrine cells. They also showed that NO-711 reduced light responses in ganglion cells. Here, we extend these findings in several important ways. First, we have shown that GAT-1 selectively limits the reciprocal activation of $GABA_C$ receptors but has no effect on reciprocal $GABA_A$ currents. Second, we have shown that GAT-1 regulates a standing conductance mediated by $GABA_C$ receptors. This standing conductance was not significantly affected by the application of glutamate receptor antagonists and TTX, suggesting that there is enough spontaneous GABA release from amacrine cells to maintain continuous $GABA_C$ receptor activation. We thus suggest that GAT-1 transporters play a primary role in setting the concentration of extracellular GABA in the vicinity of bipolar cell $GABA_C$ receptors. In support of this idea, our current-clamp experiments suggest that a reduction in GABA transport will significantly increase the amount of current necessary to depolarize the bipolar cell terminal to its threshold for generating Ca^{2+}-dependent action potentials.

Surprisingly, GAT-1 inhibition with NO-711 had no effect on $GABA_A$ receptor-mediated reciprocal currents at this synapse. In

cultured hippocampal neurons, NO-711 reduces the amplitude of $GABA_A$ receptor-mediated IPSCs and miniature IPSCs (mIPSCs), an effect attributed to increased $GABA_A$ receptor desensitization with elevated extracellular GABA (Overstreet et al., 2000). In contrast, there are several examples of central synapses that express GAT-1 but show no effect of transporter block on $GABA_A$ spontaneous or mIPSCs (Nusser and Mody, 2002; Overstreet and Westbrook, 2003). In these cases, however, GAT-1 antagonists can affect evoked $GABA_A$ currents, whereas our results show that NO-711 does not affect evoked $GABA_A$ currents. One likely explanation for our result is that $GABA_C$ receptors have a much higher affinity for GABA than $GABA_A$ receptors (Amin and Weiss, 1994). Therefore, the increase in extracellular GABA in the presence of NO-711 may simply not be enough to affect $GABA_A$ receptors. Also, previous work has suggested that $GABA_A$ and $GABA_C$ receptors reside on separate synapses on the bipolar cell terminal in the IPL of rat retina (Koulen et al., 1998). Therefore, $GABA_A$ receptor-containing synapses may not experience the same elevations in extracellular GABA as the $GABA_C$ receptor-containing synapses at the bipolar cell terminal. Importantly, it is known that both GAT-2 and GAT-3 GABA transporters are present along with GAT-1 in the IPL of goldfish retina (Klooster et al., 2004). Thus, these transporters may be colocalized with $GABA_A$ receptors and could serve to protect them from elevated extracellular GABA spillover during NO-711 application.

Modulation of GABA transporters

Our results suggest that changes in GABA transport activity could be a major factor in determining the level of tonic inhibition. In hippocampal neurons, levels of GAT-1 activity have been shown to be highly dynamic, with transporters redistributing to and from the plasma membrane on the timescale of minutes (Deken et al., 2003). Furthermore, this type of GAT-1 modulation can be triggered via activation of several types of G-protein-coupled receptors that are linked to protein kinase C (Beckman et al., 1999), or to GAT-1 agonists including GABA itself (Bernstein and Quick, 1999), or tyrosine phosphorylation (Law et al., 2000). The capacity to regulate GAT-1 activity in this manner raises the unusual and interesting possibility that tonic inhibition at the bipolar terminal could be adjusted with triggered changes in transporter activity (and/or presence on the plasma membrane) rather than changes in GABA release.

Tonic inhibitory currents at nerve terminals

In contrast with other synapses where $GABA_A$ receptors have been shown to mediate a tonic current (Semyanov et al., 2004), the bipolar cell terminal has apparently instituted a division of labor, with specialized, nondesensitizing $GABA_C$ receptors for standing currents. Our outside-our patch recordings confirm that the Mb-type bipolar cell $GABA_C$ receptors are nondesensitizing (Matthews et al., 1994), whereas the $GABA_A$ receptors desensitize rapidly. We suggest that the standing current mediated by $GABA_C$ receptors may serve to shunt depolarizing potentials at the bipolar cell terminal [Zenisek and Matthews (1998), their Fig. 7]. Interestingly, it has been observed that Mb bipolar cell Ca^{2+}-dependent action potentials are rare under light-adapted conditions (Protti et al., 2000), and we speculate that the underlying mechanism for this could result from increased amacrine cell GABA release in light-adapted conditions. We note, however, that the continuous chloride flux associated with this standing current could allow $GABA_C$ receptors to alter the chloride reversal potential (E_{Cl}) at the bipolar cell terminal (Billups and Attwell, 2002). Additional chloride influx could shift E_{Cl} to more positive potentials, possibly allowing GABA to be depolarizing under certain conditions. Therefore, future gramicidin-perforated patch recordings should be performed to assess E_{Cl} in the presence and absence of standing $GABA_C$ currents.

Finally, we note that Sagdullaev et al. (2006) have recently reported that $GABA_C$ knock-out mice have ON-type ganglion cells with a higher spontaneous firing rate. One explanation for this higher spiking rate is that the ON-type bipolar cells in the null mice lack a tonic $GABA_C$ receptor mediated current like the one we have described here.

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