

GABA_B Receptors Regulate Extrasynaptic GABA_A Receptors

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Tonic inhibitory GABA_A receptor-mediated currents are observed in numerous cell types in the CNS, including thalamocortical neurons of the ventrobasal thalamus, dentate gyrus granule cells, and cerebellar granule cells. Here we show that in rat brain slices, activation of postsynaptic GABA_B receptors enhances the magnitude of the tonic GABA_A current recorded in these cell types via a pathway involving G_{i/o} G proteins, adenylate cyclase, and cAMP-dependent protein kinase. Using a combination of pharmacology and knockout mice, we show that this pathway is independent of potassium channels or GABA transporters. Furthermore, the enhancement in tonic current is sufficient to significantly alter the excitability of thalamocortical neurons. These results demonstrate for the first time a postsynaptic crosstalk between GABA_B and GABA_A receptors.

Introduction

The action of GABA upon postsynaptic GABA_A receptors can be categorized into two general forms, namely phasic and tonic inhibition. Phasic inhibition is the short-lasting increase in synaptic inhibitory conductance generated in response to vesicular release of GABA. Tonic inhibition, on the other hand, is produced via activation of high affinity receptors by ambient GABA (Farrant and Nusser, 2005).

GABA_A receptor-mediated tonic inhibition plays a diverse and important role in CNS function. For example, the level of tonic inhibition modifies synaptic integration in cerebellum (Chadderton et al., 2004), regulates anxiety-related behaviors and seizure susceptibility in the dentate gyrus (Maguire et al., 2005), and is necessary for the expressions of spike and wave discharges in thalamocortical networks that are associated with typical absence epilepsy (Cope et al., 2009).

It is well established that presynaptic GABA_B receptors regulate the release of GABA. Furthermore, postsynaptic GABA_B receptors produce a slow IPSP resulting from the activation of G protein-coupled, inwardly rectifying potassium (GIRK) channels (Bowerly et al., 2002). However, here we show for the first time that GABA_B receptors can also regulate tonic inhibitory tone by modulating GABA_A receptors via a postsynaptic mechanism.

Materials and Methods

Whole-cell voltage clamp. Wistar rats, B6.129-Gabrd^{tm1Geh/J} (δ knockout, $\delta^{-/-}$) mice and BALB/c-Gabab1^{tm1Bbe} (GABA_B knockout, GABA_B^{-/-}) mice (postnatal days 20–30, of either sex) were anesthetized with isoflurane and decapitated in accordance with the United Kingdom Animals (Scientific Procedures) Act of 1986 and local ethical committee approval. As described previously (Cope et al., 2005), brains were rapidly removed and 300 μ m-thick horizontal ventrobasal (VB) thalamus and dentate gyrus and coronal (cerebellum) slices were cut in continuously oxygenated neuroprotective sucrose aCSF. Slices were incubated for at least 1 h before being transferred to the recording chamber where they were continuously perfused (\sim 2 ml/min) with warmed (32–34°C) oxygenated recording aCSF containing the following (in mM): 125 NaCl, 2.5 KCl, 25 NaHCO₃, 1.25 NaH₂PO₄, 1 MgCl₂, 2 CaCl₂, 25 glucose, 3 kynurenic acid, and 0.0005 tetrodotoxin. Neurons were visualized using a Nikon Eclipse E600FN microscope equipped with a 40 or 60 \times immersion lens and a video camera (Hamamatsu). Whole-cell patch-clamp recordings were made from neurons held at -70 mV using pipettes (resistance, 2–4 M Ω) containing the following (in mM): 130 CsCl, 2 MgCl₂, 4 Mg-ATP, 0.3 Na-GTP, 10 Na-HEPES, and 0.1 EGTA, pH 7.25–7.30 (osmolality, \sim 295 mOsm). Gramicidin-perforated patch recordings were performed as described by Connelly and Lees (2010). To calculate the reversal potential, the gabazine-sensitive current was fit with a Goldman–Hodgkin–Katz flux equation (Hille, 2001). Voltage clamp was performed with a Multiclamp 700B preamplifier (Molecular Devices). Series resistance was compensated by 70–80% and was monitored regularly during recordings. Data were discarded if the series resistance was over 20 M Ω or changed by $>$ 20%. Experimental data were filtered at 3–6 kHz, digitized at 20 kHz (Digidata 1322A; Molecular Devices), and acquired using pClamp 10 software (Molecular Devices). Only a single experiment was performed on each slice, after which the slice was discarded. For experiments involving the infusion of intracellular G-protein signaling modulators/blockers, four neurons were patched simultaneously. Two neurons were selected and patched with drug-containing intracellular solution, while two were patched with drug-free solution to serve as the control. All drugs and reagents used were obtained from Sigma-Aldrich, Tocris Bioscience, or Abcam.

Dynamic clamp. Dynamic clamp was performed using the CED Power1401 system. The frequency component of the tonic current was calculated by subtracting the power spectrum of the whole-cell current in

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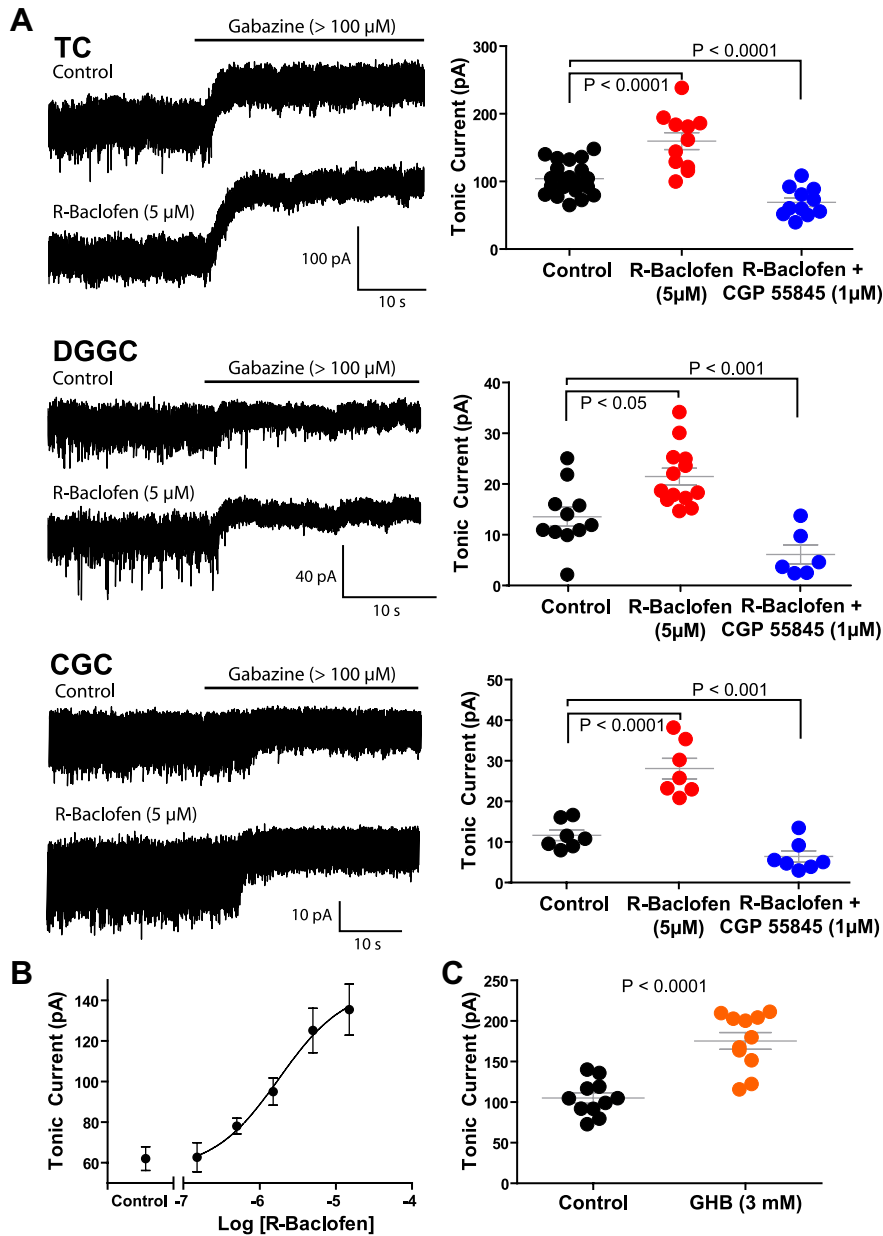


Figure 1. GABA_B receptor activation increases the magnitude of the tonic GABA_A current. **A**, Application of baclofen to activate GABA_B receptors enhances the magnitude of the tonic current recorded in thalamocortical, dentate gyrus, and cerebellar granule cells. Application of the GABA_B antagonist CGP 55845 reduced the tonic current below control levels. **B**, Dose–response curve of baclofen enhancement of on tonic current in thalamocortical neurons. **C**, The weak GABA_B agonist GHB also enhances the tonic current in thalamocortical neurons.

the presence of gabazine from the control power spectrum. These data could be fit with the sum of two functions (a high-frequency and low-frequency component) given by $S(f) = 2D\tau^2/(1 + (2\pi f\tau)^2)$. The variables D and τ were used to create a noisy conductance based on an Ornstein–Uhlenbeck process such that the noise generated had the same power spectrum as that experimentally measured. The injected noise was generated by the equation $dG_t/dt = (G_{base} - G_t)/\tau + \sqrt{D}\chi$ where G_t is the conductance at any given sample, G_{base} is the mean conductance value of the tonic conductance, and χ is a random value obeying Gaussian statistics with a mean of 0 and standard deviation of 1 (Destexhe et al., 2001). D and τ for the high and low-frequency components were calculated across 20 cells, and an average was generated. Thus, to mimic the basal tonic current, $G_{base} = 1.4$ nS, $D_1 = 0.06$ nS², $\tau_1 = 10.2$ ms, $D_2 = 1.8$ nS², and $\tau_2 = 0.51$ ms. To mimic the baclofen-enhanced tonic current, the values were $G_{base} = 2.0$ nS, $D_1 = 0.12$ nS², and $D_2 = 3.6$ nS², while τ_1 and

τ_2 were not changed. Excitatory postsynaptic conductances had a 10–90% rise time of 2 ms and a decay time constant of 10 ms to mimic corticothalamic input (Hsu et al., 2010). I/O curves generated from dynamic clamp data were fit with a modified Boltzmann curve, with the equation $P_{(G)} = 1/(1 + \exp((E - G)/S))$, where $P_{(G)}$ is the probability of firing an action potential at EPSP conductance G . E is the E_{G50} , that is, the excitatory conductance where cells fire an action potential on 50% of trials, and S is the slope of the curve at the E_{G50} .

Data analysis. To measure the amplitude of the tonic current, an all points histogram was constructed for pre-gabazine and post-gabazine application, and the size of the tonic current was calculated as the difference between the modes. The magnitude of the tonic current did not correlate with whole-cell capacitance ($p = 0.8$, Pearson’s test), so it was not adjusted for capacitance. Across our dataset, the magnitude of the tonic current in control conditions was not normally distributed ($p < 0.0001$, $n = 51$; D’Agostini test); however, the logarithm of the data was Gaussian ($p = 0.15$). Furthermore, manipulations that altered the magnitude of the tonic current also altered the variance of the sample (e.g., across all VB neurons, the tonic current had a standard deviation of 69 pA, but in the presence of CGP this dropped to 24 pA; $F_{(50, 38)} = 7.2$, $p < 0.0001$). However, after taking the logarithm of the data, homoscedasticity was preserved ($F_{(50, 38)} = 1$, $p = 0.9$). Therefore, we took the \log_{10} of all tonic currents and then subjected them to an unpaired Student’s t test or ANOVA that, if significant, was followed up with a Dunnett’s post-test. Data are presented as mean ± SEM. Significance was set at $p < 0.05$ for all statistical tests.

Results

In the presence of TTX (500 nM) to minimize any potential presynaptic effect of drugs, application of the GABA_A receptor antagonist gabazine to the recording bath (>100 μM) revealed a tonic GABA_A-mediated current in rat thalamocortical (TC) cells, dentate gyrus granule cells (DGCCs), and cerebellar granule cells (CGCs) (TC: 104 ± 5 pA, $n = 21$; DGCC: 14 ± 2 pA, $n = 11$; CGC: 12 ± 1 pA, $n = 7$) (Fig. 1A). Application of the GABA_B receptor agonist *R*-baclofen (5 μM) for ~5 min before gabazine addition significantly enhanced the tonic current (TC: 160 ± 13 pA, $n = 11$; DGCC: 21 ± 2 pA, $n = 13$; CGC: 28 ± 3 pA, $n = 7$; $p < 0.05$, Dunnett’s post-test) (Fig. 1A). This enhancement could be blocked by the simultaneous application of the GABA_B receptor antagonist CGP 55845 (1 μM). Furthermore, in line with published findings (Cope et al., 2009), concurrent application of CGP 55845 with baclofen reduced the tonic current to below control levels, indicating a basal level of activity in this pathway in our slices (TC: 69 ± 6 pA, $n = 11$; DGCC: 6 ± 2, $n = 6$; CGC: 6 ± 1, $n = 7$; $p < 0.001$, Dunnett’s post-test; Fig. 1A). A concentration–response curve to *R*-baclofen showed that the EC_{50} for enhancing the tonic current in TC cells was 1.8 ± 0.2 μM, similar to that

reported for other neuronal actions of *R*-baclofen *in vitro* (Newberry and Nicoll, 1985) (Fig. 1*B*). The weak GABA_B receptor agonist γ -hydroxybutyrate (GHB; 3 mM) enhanced the tonic current in TC cells by a similar magnitude (control: 105 ± 6 pA; GHB: 175 ± 10 pA; $n = 11$, $p < 0.0001$, t test) (Fig. 1*C*). To investigate whether the GABA_B-mediated enhancement was specific to the extrasynaptic receptors that mediate the tonic current, we also investigated whether synaptic currents were altered by measuring miniature inhibitory postsynaptic currents (mIPSCs) in TC neurons. Baclofen had no effect on the amplitude or weighted decay of mIPSCs (control: -44 ± 1 pA, 2.4 ± 0.1 ms; baclofen: -45 ± 1 pA, 2.3 ± 0.2 ms; $p > 0.05$, Kolmogorov–Smirnov test), although it did reduce their frequency (control: 3.4 ± 0.5 Hz; baclofen: 1.6 ± 0.3 Hz; $p < 0.05$, Kolmogorov–Smirnov test; data not shown). This demonstrates the well established fact that baclofen can presynaptically regulate GABA release while having no effect on synaptic GABA_A receptors (e.g., Le Feuvre et al., 1997).

We further confirmed that baclofen was acting through GABA_B receptors by using knockout mice (GABA_B^{-/-}). In wild-type littermate animals, baclofen still significantly enhanced the tonic current (control: 64 ± 14 pA; baclofen: 146 ± 41 pA; $n = 8$, $p = 0.007$, t test) (Fig. 2*A*). However, baclofen had no effect on GABA_B^{-/-} mice (control: 82 ± 14 pA; baclofen: 66 ± 17 pA; $n = 8$ –12, $p = 0.4$, t test). Interestingly, it did not appear that GABA_B^{-/-} mice had smaller tonic currents under control conditions compared to wild-type animals, indicating a potential upregulation in extrasynaptic GABA_A receptor function to compensate for the loss of the GABA_B receptor function ($p = 0.5$, t test) (Fig. 2*A*).

To confirm that the baclofen-induced increase in tonic current required expression of functional δ -containing GABA_A receptors, we used δ -subunit knockout (δ ^{-/-}) mice. δ ^{-/-} mice did not show a baclofen-induced enhancement in tonic current (control: 5 ± 7 pA; baclofen: 1 ± 3 pA; $n = 7$ –8, $p = 0.3$, t test) (Fig. 2*B*). We next began to explore the possible mechanisms that may underpin the effect of GABA_B receptor activation on tonic currents. It is established that inhibiting GABA transporter (GAT) function can alter the magnitude of tonic current (Nusser and Mody, 2002) and that GABA_B receptors can modulate the function of GATs (Gomez et al., 1994). Therefore, we applied saturating concentrations of NO-711 (10 μ M) and SNAP 5114 (20 μ M) to block the activity of GAT-1 and GAT-3 (the only GAT subtypes present in the VB thalamus; De Biasi et al., 1998). However, when the activity of GABA transporters was removed, baclofen still produced significant enhancement of the tonic current (control: 117 ± 10 pA; baclofen: 193 ± 17 pA; $n = 9$ –10, $p = 0.001$, t test) (Fig. 2*C*). Another classical action of GABA_B receptors is the activation of GIRK potassium channels. To exclude the possibility that GIRK activation was responsible for the GABA_BR-mediated enhanced tonic current, we applied a cocktail of potassium channel blockers including Ba²⁺ (2 mM), 4-AP (2 mM), and tetraethylammonium (TEA) (10 mM) to com-

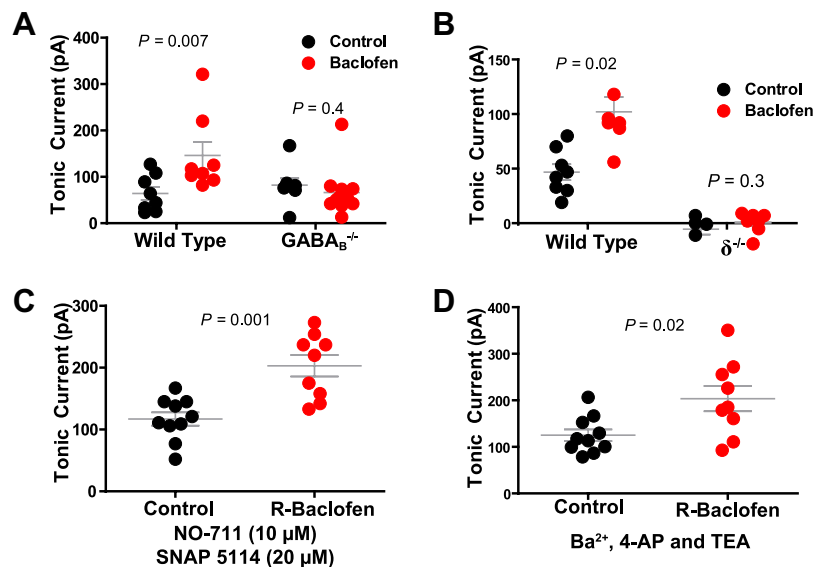


Figure 2. Mechanistic dissection of GABA_B receptor-mediated enhancement of tonic inhibition. *A*, Baclofen does not enhance the tonic current of TC neurons in slices from GABA_B^{-/-} mice. *B*, The baclofen-induced enhancement of tonic current is dependent on δ -containing GABA_A receptors, as baclofen has no effect on tonic currents in TC neurons of δ ^{-/-} mice. *C*, In the presence of GAT-1 and GAT-3 blockade, baclofen is able to enhance the tonic current recorded in rat TC neurons. *D*, In the presence of general potassium channel blockade (2 mM Ba²⁺, 2 mM 4-AP, and 10 mM TEA), baclofen still enhanced the tonic current recorded in rat TC neurons.

ment the intracellular block provided by our Cs⁺-containing pipette solution. However, in the presence of this range of potassium channel blockers, baclofen still produced an enhancement in tonic current amplitude (control: 125 ± 13 pA; baclofen: 204 ± 27 pA; $n = 10$ –9, $p = 0.015$, t test) (Fig. 2*D*).

GABA_B receptors are expressed on both presynaptic and postsynaptic elements of neurons in the CNS, as well as on glia. We tested the possibility of a direct postsynaptic link between GABA_B receptors and GABA_A receptors by intracellular application of drugs via the patch pipette that modulate GABA_B receptor G protein-coupled signaling pathways. In these experiments we simultaneously recorded from four TC neurons with two cells containing drugs and two drug-free cells to act as internal controls. By infusing cell-impermeable GDP- β -S (1 mM) into individual TC neurons and thus blocking G-protein-mediated signaling in only that cell, we were able to confirm that GABA_B receptor-dependent enhancement of the tonic current is expressed postsynaptically. We found that GDP- β -S completely occluded the baclofen-induced enhancement in tonic current (baclofen: 118 ± 27 pA; baclofen + GDP: 57 ± 10 pA; $n = 12$ –15, $p = 0.02$, t test) and, importantly, GDP- β -S did not reduce the size of the tonic current recorded in the presence of CGP 55845 (CGP: 41 ± 3 pA; CGP + GDP: 43 ± 6 pA; $n = 12$ –14, $p = 0.9$) (Fig. 3*A, B*). By continuing to apply drugs intracellularly, we investigated the pathway by which baclofen enhances the tonic current. Infusion of pertussis toxin (500 ng/ml) to block all G_{i/o}-dependent G-protein signaling also prevented the baclofen-induced enhancement in tonic current (baclofen: 100 ± 15 pA; baclofen + pertussis: 62 ± 7 pA; $n = 16$ –14, $p = 0.04$, t test) (Fig. 3*C*). Pertussis toxin does not block the tonic current directly, as it did not reduce the amplitude of the tonic current when applied together with CGP 55845 (CGP) (CGP: 58 ± 6 pA; CGP + pertussis: 52 ± 7 pA; $n = 12$ –14, $p = 0.5$, t test) (Fig. 3*D*). The inhibitor of adenylate cyclase, SQ 22536 (SQ) (10 μ M), mimicked the effect of baclofen and enhanced the tonic current (control: 68 ± 15 pA; SQ: 120 ± 21 pA; $n = 14$ –17, $p = 0.03$) (Fig. 3*E*). Infusion of the

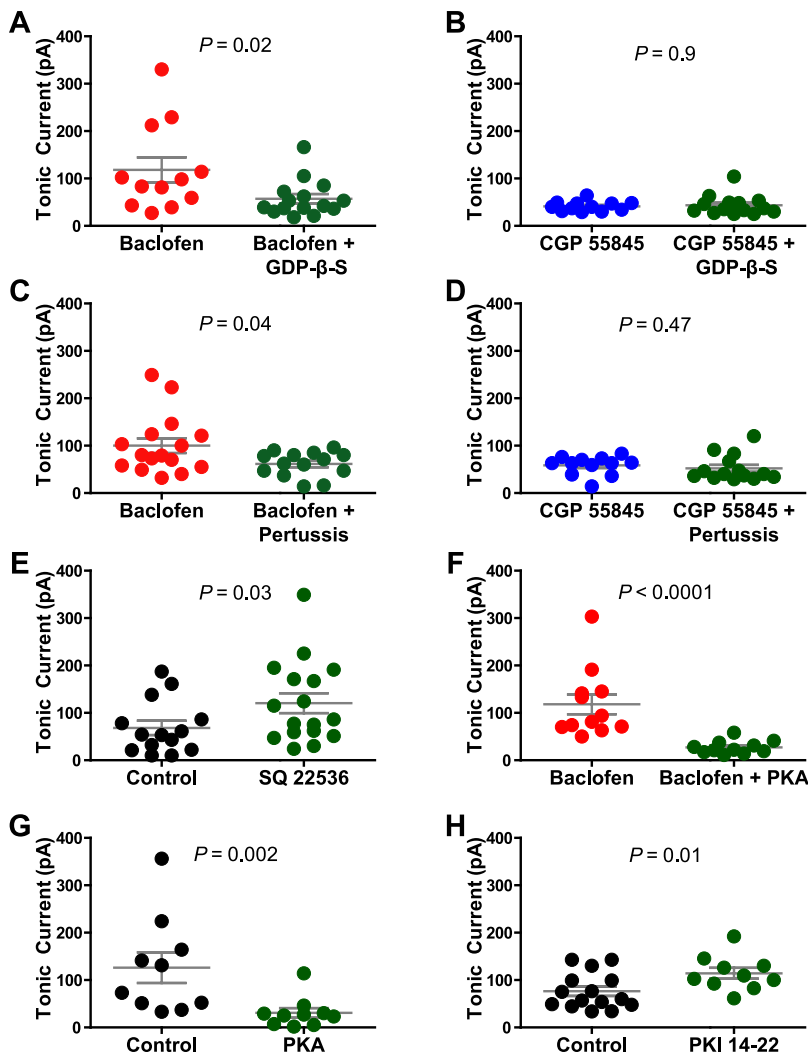


Figure 3. Recordings from TC neurons shows that baclofen enhances tonic GABA_A-mediated currents through a G_{i/o} G-protein, adenylate cyclase, and PKA-dependent pathway. **A**, Blockade of all G-protein signaling with GDP-β-S (1 mM) prevented the baclofen-induced enhancement in tonic current. **B**, GDP-β-S (1 mM) does not block the tonic current directly, as in the presence of CGP 55845 (1 μM) GDP-β-S has no effect on the magnitude of the tonic current. **C**, Blocking all G_i dependent G-protein signaling with pertussis toxin (500 ng/ml) prevented the baclofen-induced enhancement in tonic current. **D**, Pertussis toxin does not block the tonic current directly, as it did not reduce the amplitude of the tonic current when applied together with CGP 55845 (1 μM). **E**, The inhibitor of adenylate cyclase SQ 22536 (10 μM), mimicked the effect of baclofen and enhanced the tonic current. **F**, Infusion of the active catalytic subunit of PKA (10 IU/ml) blocked the enhancement of the tonic current caused by baclofen. **G**, PKA (10 IU/ml) significantly reduced the tonic current. **H**, The PKA inhibitor PKI 14-22 (50 ng/ml) mimicked the effect of baclofen and produced significant enhancement of GABA_A receptor-mediated tonic current.

active catalytic subunit of protein kinase A (PKA) (10 IU/ml) was able to block the baclofen-induced enhancement in tonic current (baclofen: 118 ± 21 pA; baclofen + PKA: 27 ± 4 pA; *n* = 12–11, *p* < 0.0001) (Fig. 3F). PKA by itself significantly reduced the tonic current to a level equivalent to baclofen + PKA (control: 126 ± 32, PKA: 31 ± 10; *n* = 10, *p* = 0.002, *t* test) (Fig. 3G). Conversely, the PKA inhibitor PKI 14-22 (50 ng/ml) significantly enhanced the tonic current (control: 76 ± 10 pA; PKI: 114 ± 11 pA; *n* = 15–10, *p* = 0.01, *t* test) (Fig. 3H).

Having defined a mechanistic pathway for the modulation of GABA_A receptors by GABA_B receptors, we next investigated how this modulation of tonic inhibition altered the excitability of TC neurons. To avoid complications arising from modulation of GIRKs and voltage-gated Ca²⁺ channels that would result from pharmacological activation of GABA_B receptors, we addressed this problem by using the dynamic clamp technique. TC neurons

were patched and depolarized by current injection to approximately -55 mV, and thereafter the level of holding current was not changed. We then injected excitatory postsynaptic conductances and measured the probability of action potential generation, thus creating an input/output (I/O) curve. To accurately mimic the tonic current with dynamic clamp, we needed to know its reversal potential. Thus, we performed gramicidin-perforated patch recordings and applied a slow voltage ramp between -70 and -30 mV. Then we applied gabazine and performed the ramp again. The difference in the transmembrane current between these two ramps gave us the I/V relationship of the tonic current (Fig. 4). We found that the average reversal potential was -76 ± 2 mV (*n* = 4; in line with previously published values; Ulrich and Huguenard, 1997). As described, TC neurons had a mean tonic current of 104 pA, and in the presence of baclofen this increased to 160 pA; thus, the basal tonic conductance was set to 1.4 nS, and to mimic the effect of baclofen it was set to 2.0 nS. The model of the tonic conductance was noisy and had spectral characteristics to match those seen *in vitro* (see Materials and Methods). The addition of gabazine (>100 μM) shifted the I/O curve to the left by 1.2 nS, in line with the measured tonic GABA_A conductance of 1.4 nS (Fig. 4A, C). By injecting this conductance, we almost perfectly recapitulated the control condition (control: EG₅₀ = 11.9 ± 1.3 nS; slope = 0.34 ± 0.01; dynamic clamp tonic conductance, EG₅₀ = 11.7 ± 1.2 nS; slope = 0.41 ± 0.02; *n* = 6). We then increased the tonic conductance to 2.0 nS to reproduce the baclofen-induced enhancement of the GABA_A system. This significantly shifted the I/O curve to the right by 0.32 ± 0.2 nS (*p* < 0.001), but had no effect on the slope (0.38 ± 0.02;

p = 0.3) (Fig. 4C). Thus, by mimicking the effect of baclofen on the tonic current, both in terms of increasing the magnitude of the conductance as well as the power of its noise, we were able to significantly alter the excitability of TC neurons.

Discussion

The tonic GABA_A current is observed across a range of neuronal cell types and has been implicated in a number of physiological and pathophysiological roles, including regulation of synaptic integration and modulation of anxiety-related behaviors and as a necessary factor in the expression of spike and wave discharges associated with typical absence epilepsy (Chadderton et al., 2004; Maguire et al., 2005; Cope et al., 2009). Here we show that GABA_B receptors are able to modulate the δ-subunit-containing GABA_A receptors responsible for generating this crucial tonic current in three important cell types, namely TC, DGGC, and CGC. More-

over, our data indicate that GABA_B receptor-mediated enhancement of the tonic current is sufficient to significantly alter the excitability of TC cells, showing that this source of modulation can be of functional importance for the electrical behavior of TC neurons. We are able to rule out that this effect is due to an alteration in the presynaptic release of GABA, as the GABA_B receptor-mediated enhancement of tonic current could be blocked or mimicked by infusing drugs solely into the postsynaptic cell. Likewise, the enhancement of tonic current is not due to GABA_B receptors reducing the uptake of GABA or altering potassium channel function, as baclofen still enhanced the tonic current in the presence of GAT and potassium channel blockade. This modulation appears to be selective for extrasynaptic GABA_A receptors, as the amplitude and kinetics of mIPSCs are not affected by GABA_B receptor activation. Thus, GABA_B receptors are capable of enhancing the action of extrasynaptic GABA_A receptors via a G protein-mediated postsynaptic mechanism.

There is a significant body of literature showing that PKA can phosphorylate $\beta 1$ and $\beta 3$ subunits of the GABA_A receptor and that phosphorylation at the $\beta 3$ subunit enhances GABA_A function, phosphorylation at the $\beta 1$ subunit inhibits it, and the $\beta 2$ subunit is not affected by PKA modulation (McDonald et al., 1998; Brandon et al., 2003; Tang et al., 2010). It is hard to conclude which β subunits are responsible for the tonic current in TC neurons. It seems clear that the major species is $\beta 2$, due to the sensitivity of thalamic tonic currents to etomidate and the reduction in the size of tonic currents in $\beta 2^{-/-}$ mice. It is often concluded that as tonic currents are still enhanced (albeit weakly) by etomidate in mice with the etomidate-insensitive $\beta 2$ mutation $\beta 2_{N265S}$, this indicates that $\beta 3$ subunits are responsible for the rest of the tonic current (Belelli et al., 2005). However, it is worth noting that $\beta 1$ subunits appear to be sensitive to the concentration of etomidate (3 μM) used by Belelli et al. (2005) (Hill-Venning et al., 1997). While some evidence shows a paucity of $\beta 1$ subunit expression in the VB thalamus (Wisden et al., 1992), other reports indicate that it is expressed in significant amounts (albeit less than $\beta 2$) (Huntsman et al., 1996; Pirker et al., 2000). If we assume that the PKA sensitivity of β subunits mentioned above is true across all subunit combinations, then our results indicate that $\alpha 4\beta 1\delta$ make up a significant fraction of extrasynaptic receptors. The other interpretation is that $\alpha 4\beta 2\delta$ may indeed be sensitive to negative-modulation by PKA. In DGGCs, it seems likely that the tonic current is mediated by mainly a mix of $\beta 1$ - and $\beta 2$ -containing GABA_A receptors, and hence our results are easy to reconcile with the molecular makeup of these receptors (Mtchedlishvili and Kapur, 2006; Herd et al., 2008). The mRNA for all β subunits is expressed by cerebellar granule cells, but we are not aware of any studies showing which β subunits are responsible for the tonic current in CGCs (Laurie et al., 1992). Hence, we believe it is likely that across these three different cell types, GABA_B receptor activation enhances tonic GABA_A receptor function by inhibiting cAMP and reducing the inhibitory effect of PKA at $\beta 1$ -containing

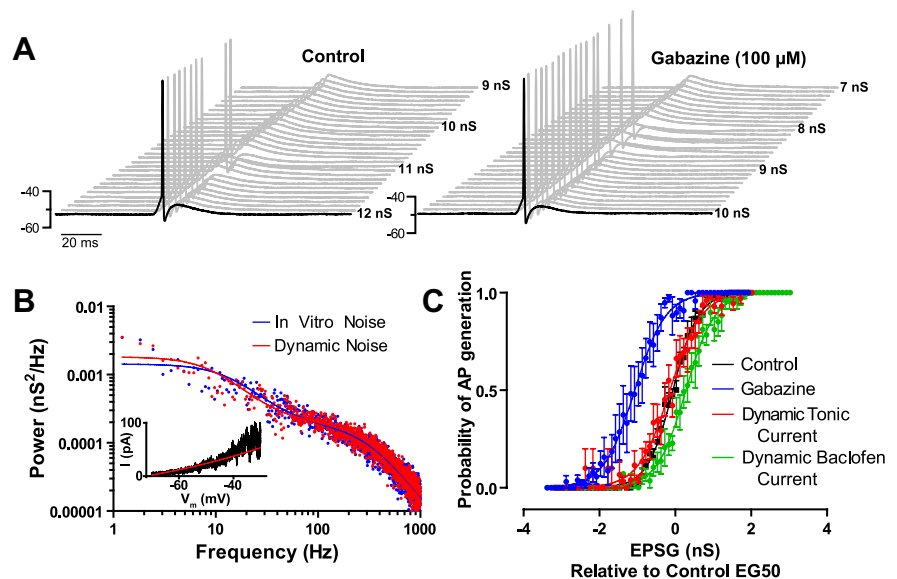


Figure 4. Mimicking the baclofen-induced enhancement of tonic GABA_A current using dynamic clamp reduces TC neuron excitability. **A**, A representative trace showing one repetition of the I/O protocol before (left) and after (right) the application of gabazine. **B**, The power spectrum of the gabazine-sensitive noise (*in vitro* noise) and the noise model used during dynamic clamp (dynamic noise). Inset, A representative I/V relationship of the gabazine-sensitive current, with the best fit shown in red, from which the reversal potential was calculated. **C**, The I/O curves showing the enhancement of excitability caused by blocking the tonic current (blue), the reinstatement of normal integrative properties by the injection of a model of the tonic current with dynamic clamp (red), and the reduction in excitability caused by injecting a model of the baclofen-enhanced tonic current with dynamic clamp (green).

GABA_A receptors. This may mean that PKA could act to integrate the signaling of many G protein-coupled receptors and modulate cellular excitability in neurons expressing a tonic GABA_A current. Indeed, it has been shown previously that dopamine can modulate the excitability of medium spiny neurons via a PKA-dependent modulation of the tonic GABA_A current (Janssen et al., 2009). However, it is also possible that there are other regulatory proteins downstream of PKA that mediated the GABA_B receptor-mediated enhancement of tonic GABA_A currents, rather than PKA phosphorylating extrasynaptic GABA_A receptors directly.

In conclusion, our results show for the first time, a functional postsynaptic crosstalk between GABA_B and GABA_A receptors. They also raise the intriguing possibility that several of baclofen's clinical actions could be, at least in part, due to the GABA_B-mediated enhancement of tonic GABA_A currents throughout the brain. The most obvious of these is baclofen's use in ethanol dependence and withdrawal because of ethanol's ability to enhance the tonic GABA_A current (Wei et al., 2004; Addolorato et al., 2007).

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