

Selective Modulation of Tonic and Phasic Inhibitions in Dentate Gyrus Granule Cells

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Nusser, Zoltan and Istvan Mody. Selective modulation of tonic and phasic inhibitions in dentate gyrus granule cells. *J Neurophysiol* 87: 2624–2628, 2002; 10.1152/jn.00866.2001. In some nerve cells, activation of GABA_A receptors by GABA results in phasic and tonic conductances. Transient activation of synaptic receptors generates phasic inhibition, whereas tonic inhibition originates from GABA acting on extrasynaptic receptors, like in cerebellar granule cells, where it is thought to result from the activation of extrasynaptic GABA_A receptors with a specific subunit composition ($\alpha_6\beta_x\delta$). Here we show that in adult rat hippocampal slices, extracellular GABA levels are sufficiently high to generate a powerful tonic inhibition in δ subunit-expressing dentate gyrus granule cells. In these cells, the mean tonic current is approximately four times larger than that produced by spontaneous synaptic currents occurring at a frequency of ~10 Hz. Antagonizing the GABA transporter GAT-1 with NO-711 (2.5 μ M) selectively enhanced tonic inhibition by 330% without affecting the phasic component. In contrast, by prolonging the decay of inhibitory postsynaptic currents (IPSCs), the benzodiazepine agonist zolpidem (0.5 μ M) augmented phasic inhibition by 66%, while leaving the mean tonic conductance unchanged. These results demonstrate that a tonic GABA_A receptor-mediated conductance can be recorded from dentate gyrus granule cells of adult rats in *in vitro* slice preparations. Furthermore, we have identified distinct pharmacological tools to selectively modify tonic and phasic inhibitions, allowing future studies to investigate their specific roles in neuronal function.

INTRODUCTION

Two types of GABA_A receptor-mediated inhibition have been described in cerebellar granule cells (Brickley et al. 1996, 2001; Rossi and Hamann 1998; Wall and Usowicz 1997). Synaptically released GABA acting on postsynaptic GABA_A receptors produces “phasic” inhibition, whereas “tonic” inhibition results from the persistent activation of extrasynaptic receptors by ambient GABA. If tonic inhibition is produced by low concentrations of ambient GABA thought to be in the low- or submicromolar range (Attwell et al. 1993), the extrasynaptic GABA_A receptors should have a high affinity and should not desensitize on the prolonged presence of agonist. The α_6 and δ subunit-containing receptors expressed by cerebellar granule cells have such properties (Haas and Macdonald 1999; Saxena and Macdonald 1994, 1996; Tia et al. 1996) and are exclusively present extrasynaptically (Nusser et al. 1998). Recent studies, using pharmacological tools demonstrated that these

receptors can be activated by an overspill of synaptically released GABA (Rossi and Hamann 1998), while experiments with knock-out animals (Brickley et al. 2001) have conclusively shown their role in the generation of tonic inhibition in cerebellar granule cells.

There is little known about the presence of tonic inhibition in other cell types of the mammalian CNS, although in some cells, differences between the properties of synaptic and extrasynaptic receptors have been reported (Bai et al. 2001; Banks and Pearce 2000). Of the two GABA_A receptor subunits involved in mediating tonic inhibition in cerebellar granule cells, the α_6 subunit has a very restricted distribution (granule cells of the cerebellum and dorsal cochlear nucleus), while the δ subunit is widespread in the CNS (Fritschy and Mohler 1995; Wisden et al. 1992). In the forebrain, the δ subunit mainly forms functional GABA_A receptors with α_4 and β subunits (Sieghart 1995; Sur et al. 1999). Such receptors may underlie tonic inhibition in many brain regions if their properties and subcellular distributions are comparable with those of cerebellar $\alpha_6\beta_x\delta$ receptors. In the present study, we investigated whether tonic inhibition could be detected in δ subunit-expressing dentate gyrus granule cells in acute *in vitro* brain slices obtained from adult rats. Provided tonic inhibition is found in this cell type, we also aimed to develop specific pharmacological tools to selectively modulate the two types of inhibition.

METHODS

Slice preparation and in vitro electrophysiological recordings

Thirteen adult (>3 mo old) Wistar rats were anesthetized with Na-pentobarbital (70 mg/kg ip) in accordance with an animal protocol approved by the UCLA Chancellor's ARC. After decapitation, the brains were removed and placed into an ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM) 126 NaCl, 2.5 KCl, 2 CaCl₂, 2 MgCl₂, 1.25 NaH₂PO₄, 26 NaHCO₃, and 10 D-glucose, pH 7.3 when bubbled with 95% O₂-5% CO₂. Coronal slices (300–350 μ m in thickness) were cut with a vibratome (Leica VT1000S) and were stored at 32°C until they were transferred to the recording chamber. During recordings, the slices were continuously perfused with 34–36°C ACSF containing 3–5 mM kynurenic acid (Sigma). All recordings were made from the somata of visually identified neurons (Zeiss Axioscope and Leica DMS IR-DIC videomicroscopy, $\times 40$ water

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immersion objective) with an Axopatch 200B amplifier (Axon Instruments, Foster City, CA). Patch electrodes were filled with a solution containing (in mM) 140 CsCl, 4 NaCl, 1 MgCl₂, 10 HEPES, 0.05 EGTA, 2 Mg-ATP, and 0.4 Mg-GTP. The intracellular solution was titrated to a pH of 7.25 and to an osmolarity of 280–290 mosmol. The DC resistance of the electrodes was 4–8 M Ω when filled with the pipette solution. Series resistance (R_s) and whole cell capacitance were estimated by compensating for the fast current transients evoked at the onset and offset of 8-ms, 5-mV voltage-command steps. The R_s was either uncompensated or compensated by 75–80% (with 7- to 8- μ s lag values). Uncompensated R_s was 16.6 ± 7.2 M Ω (mean \pm SD).

To identify GABA_A receptor-mediated currents, 10–30 μ l of 20 mM aqueous solution of bicuculline methiodide (BMI, Sigma; for 16 cells) or SR95531 (Sigma; for 3 cells) was directly injected into the recording chamber where it rapidly mixed with the perfusate. Considering the volume of the chamber (~2 ml) and the flow rate (2.5 ml/min) of the ACSF, the final concentrations of BMI or of SR95531 were estimated to be between 100 and 150 μ M.

Data analysis

All recordings were low-pass filtered at 2 kHz and digitized on-line at 20 kHz. The inhibitory postsynaptic currents (IPSCs) were detected

and analyzed as described previously (Nusser et al. 2001). The mean phasic current was calculated by multiplying the charge of the averaged spontaneous, GABA_A receptor-mediated IPSC (sIPSC) with the sIPSC frequency. The frequency, charge per IPSC and the mean phasic current were compared between different experimental conditions using the unpaired *t*-test assuming unequal variances.

The baseline current was measured as follows. The mean of a 5-ms epoch taken every 100 ms served as one data point. By taking the mean of 100 baseline points over 5 ms (at 20-kHz sampling rate) allowed us to average out any high-frequency noise from the records. Baseline points falling on to the decay of IPSCs were discarded from the analysis. Such baseline points were usually identified from an increased SD of the 5-ms epoch. The mean and SD of the averaged baseline points were calculated for 10 s (~100 averaged baseline points) at three distinct times of the recordings (*periods a, b, and c*; see Fig. 1C). The time separation (30 s) between *periods a* and *b* was always the same as that between *b* and *c*. Another method was also developed to quantify the mean tonic current. In this method, the baseline was calculated by generating all-point histograms of 10-s epochs at *periods a, b, and c* (see Fig. 1D), and a Gaussian distribution was fitted to the histogram at *period c*, or single Gaussians were fitted to the positive side of the all-point histograms at *periods a* and *b*. The

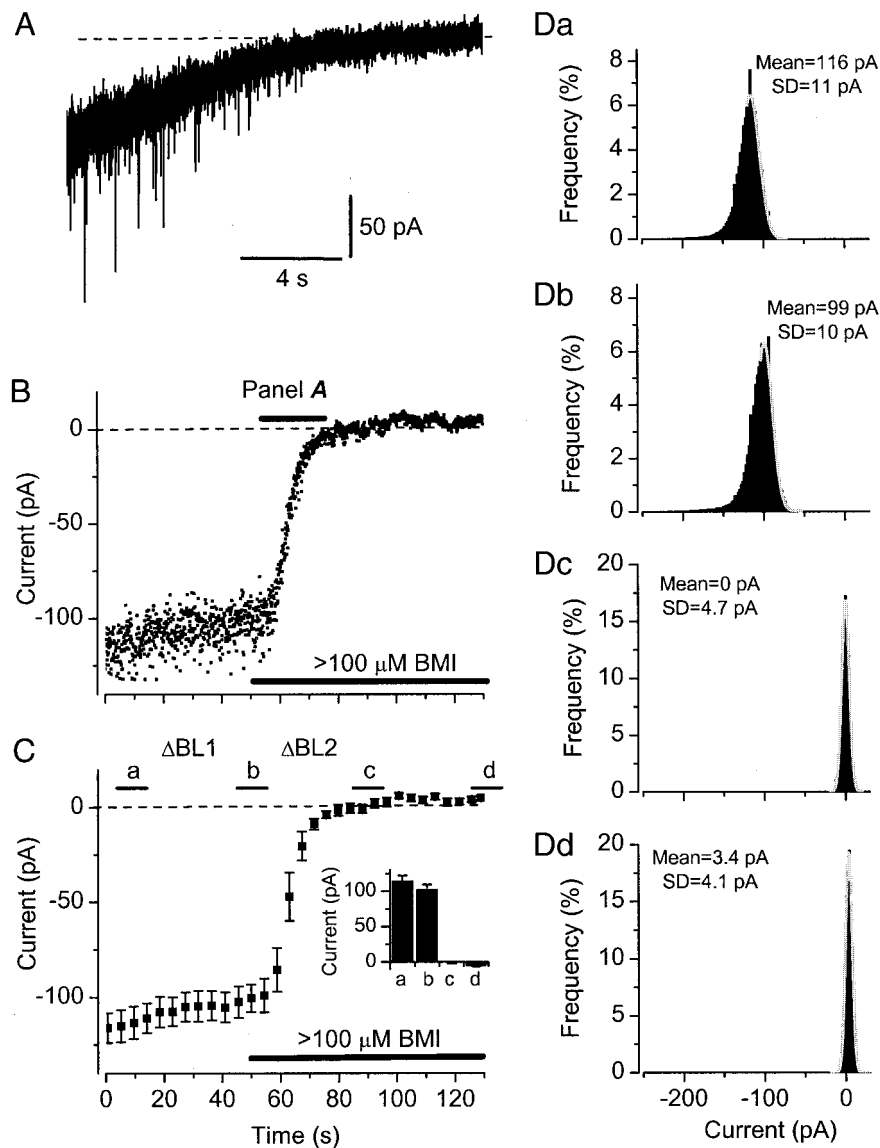


FIG. 1. Measurements of a GABA_A receptor-mediated tonic current in the presence of 2.5 μ M NO711. *A*: a whole cell voltage-clamp record showing the effect of ~100 μ M bicuculline methiodide (BMI) on the baseline current and on the sIPSCs. *B*: baseline values measured every 100 ms demonstrate a large, persistent GABA_A receptor-mediated inward current before the application of BMI. *C*: the mean \pm SD of baseline values (taken every 5 s) are shown. The inset illustrates the average baseline values between 0 and 10 s (*a*), 40 and 50 s (*b*), 80 and 90 s (*c*), and 120 and 130 s (*d*). The baseline level was set to zero during the recording period labeled *c*. *Da–Dd*: all-point histograms of 10-s traces at time points *a, b, c, and d* as indicated in *C*. Following BMI application (*periods c* and *d*), the histograms were fitted with single Gaussians, and the means and SD were determined. Before the application of the antagonist (at time points *a* and *b*), single Gaussian distributions were fitted only to the positive side of the all-point histograms, i.e., the side of the all-point histograms not contaminated by “negative-going” inhibitory postsynaptic currents (IPSCs).

differences between the means of the fitted Gaussians at *periods a* and *b* and *periods b* and *c* were then calculated. As both methods of baseline calculation provided practically identical results, we decided to employ the first method in this study. Two changes in the baseline current were calculated between the three periods. The first (ΔBL1), reflecting the time-dependent fluctuations in the baseline, was the *absolute* (i.e., ignoring the sign) value of the baseline difference during recording *periods a* and *b* (see black column in Fig. 3D). The second (ΔBL2), reflecting the effect of the GABA_A receptor antagonist, was obtained from the *absolute* (i.e., ignoring the sign) value of the baseline difference during recording *periods c* and *b*. The two baseline changes (ΔBL1 and ΔBL2) were then statistically compared (paired *t*-test) in each cell.

RESULTS

We tested whether tonic inhibition could be detected in $\alpha 4/\delta$ subunit-expressing dentate gyrus granule cells by obtaining whole cell voltage-clamp recordings in *in vitro* acute slices from adult rats. sIPSCs were readily observed. By measuring the charge carried by the averaged sIPSCs and the frequency of the sIPSCs, the mean phasic current could be calculated and compared between different experimental conditions. To obtain an objective measure of the mean tonic current, we used a method based on measuring the change in baseline current following the application of a GABA_A receptor antagonist (see Fig. 1 and METHODS).

The application of $\sim 100 \mu\text{M}$ SR95531 or BMI resulted in the complete disappearance of sIPSCs (Fig. 2B) and an outward shift in the baseline current (Figs. 1 and 2A). The value of ΔBL2 ($12.8 \pm 3.0 \text{ pA}$, mean \pm SE; $n = 5$) was significantly larger ($P < 0.05$, paired *t*-test) than ΔBL1 ($3.9 \pm 1.3 \text{ pA}$, $n = 5$),

showing that the change in baseline was indeed due to the effect of the antagonist (Figs. 2 and 3). The mean phasic current ($2.4 \pm 0.6 \text{ pA}$, $n = 9$) calculated from the sIPSC frequency ($10.1 \pm 2.1 \text{ Hz}$, $n = 9$) and from the charge carried by the average sIPSCs ($0.23 \pm 0.04 \text{ pC}$, $n = 9$) was around 25% of the mean tonic current under control conditions. These results demonstrate that $\alpha 4/\delta$ subunit-expressing adult rat dentate granule cells display a significant amount of tonic inhibition that is almost four times larger than that mediated by sIPSCs occurring at $\sim 10 \text{ Hz}$.

Next we sought to increase the concentration of ambient GABA by antagonizing GABA uptake using $2.5 \mu\text{M}$ of the nonsubstrate GAT-1 inhibitor NO711 (Borden et al. 1994), and to investigate its effect on phasic and tonic GABA_A receptor-mediated currents. The drug produced no significant change in the charge ($0.21 \pm 0.01 \text{ pC}$, $n = 5$ in NO711) and frequency ($11.0 \pm 3.7 \text{ Hz}$, $n = 5$ in NO711) of sIPSCs (Figs. 2 and 3). Consequently, the mean phasic current ($2.3 \pm 0.8 \text{ pA}$, $n = 5$ in NO711) was unaffected by NO711. Concomitantly, however, there was an almost fourfold increase in the mean tonic current ($47.6 \pm 10.1 \text{ pA}$, $n = 7$, $P < 0.01$ unpaired *t*-test) compared with control.

Following the specific increase in the tonic current by NO711, we aimed to augment the phasic current independently of the tonic conductance. If the tonic current is mediated by $\alpha 4\beta\delta$ or $\alpha 4\beta\gamma 2$ receptors in granule cells, this current should be insensitive to benzodiazepine agonists (Sieghart 1995), whereas synaptic currents are known to be sensitive to zolpidem, a benzodiazepine agonist (Hajos et al. 2000). In agreement with our prediction, $0.5 \mu\text{M}$ zolpidem did not alter the

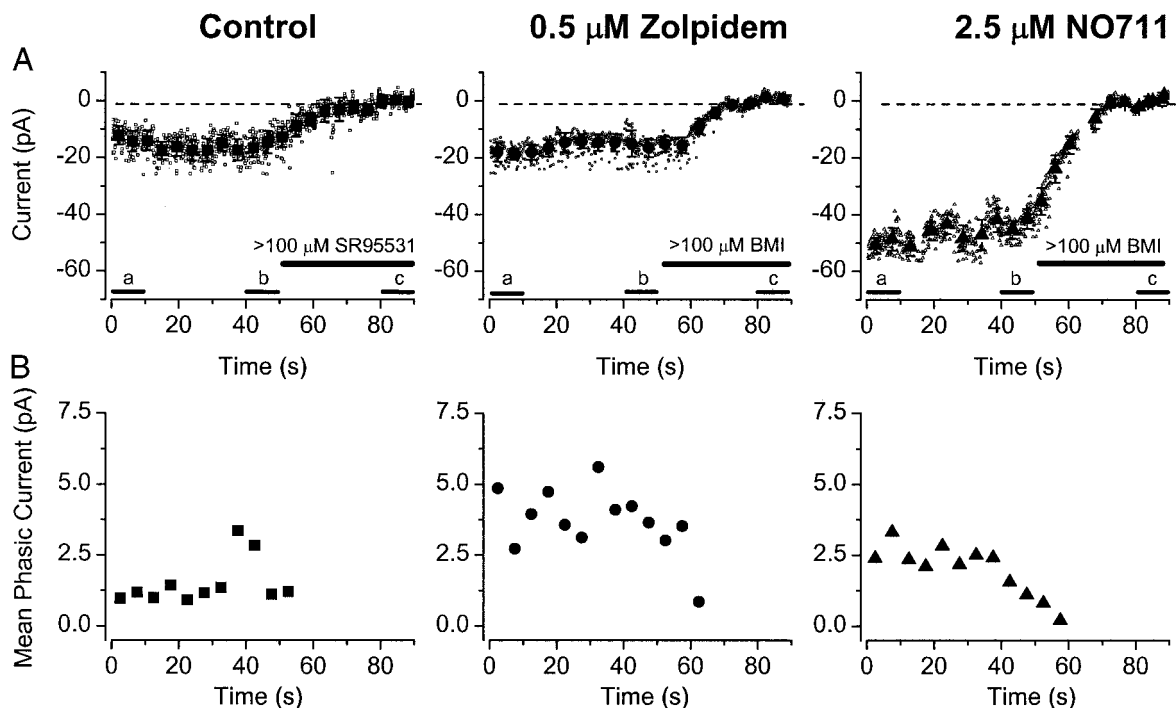


FIG. 2. Effect of a GABA uptake inhibitor ($2.5 \mu\text{M}$ NO711) and a benzodiazepine agonist ($0.5 \mu\text{M}$ zolpidem) on tonic and phasic inhibitions. A: the change in the holding current following antagonist application is similar in the presence of zolpidem (*middle panel*) to that in control (*left panel*). The GABA uptake inhibitor NO711 ($2.5 \mu\text{M}$) dramatically increased the persistent inward current in dentate granule cells as seen by the $\sim 50\text{-pA}$ shift in the baseline following BMI injection. B: the mean phasic current was calculated every 5 s by multiplying the frequency of spontaneous IPSCs (sIPSCs) with the charge carried by the averaged sIPSC. The mean phasic current is similar in $2.5 \mu\text{M}$ NO711 (*right panel*) to that in control (*left panel*), but it is larger in $0.5 \mu\text{M}$ zolpidem (*middle panel*).

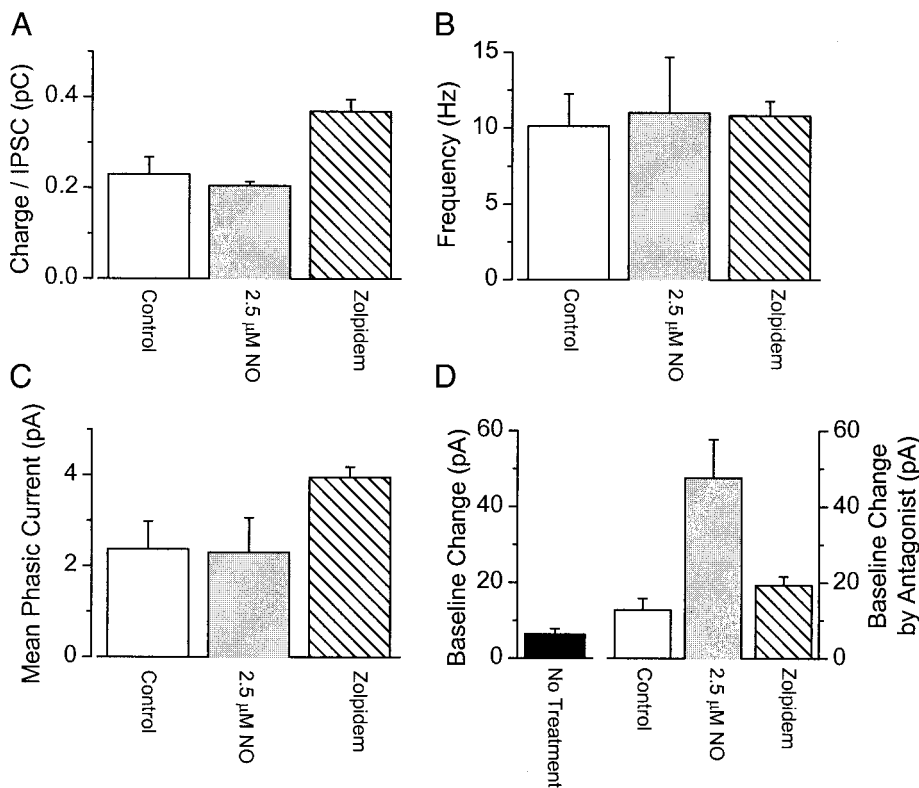


FIG. 3. Summary of the effects of 0.5 μ M zolpidem and 2.5 μ M NO711 on sIPSCs and on tonic inhibition. A–C: the GABA uptake inhibitor NO711 had no effect on the charge (A) and the frequency (B) of sIPSCs and therefore had no effect on the mean phasic current (C). In contrast, 0.5 μ M zolpidem increased the charge transfer (A) by 60% without any significant effect on the frequency (B), resulting in a 66% increase in the mean phasic current (C). D: the black column indicates the average change in the baseline (Δ BL1) prior to antagonist injection. Changes in baseline following the application of the antagonists (Δ BL2) in all 3 conditions were significantly ($P < 0.05$, paired t -test) larger than changes prior to the antagonist application. The mean tonic current did not significantly differ in zolpidem and control ($P > 0.05$, unpaired t -test), but was larger in NO711 than in control ($P < 0.01$, unpaired t -test). Data are expressed as means \pm SE.

mean tonic current ($P > 0.05$ compared with control, Figs. 2 and 3), but increased the mean phasic current (3.9 ± 0.2 pA, $n = 3$) by 66% through the prolongation of sIPSC decays without affecting their frequency (10.8 ± 1.0 Hz, $n = 3$). These results demonstrate that tonic and phasic inhibitions in adult rat dentate gyrus granule cells can be selectively increased by a GABA uptake inhibitor and by a benzodiazepine agonist, respectively.

DISCUSSION

A considerable amount of tonic current was present in the $\alpha 4/\delta$ subunit-expressing adult rat dentate gyrus granule cells, which was approximately four times larger than the total synaptic (phasic) current even when the frequency of spontaneous IPSCs was ~ 10 Hz. The differential sensitivities of the two types of inhibition to the benzodiazepine agonist zolpidem in these cells is consistent with different GABA_A receptor subtypes mediating the two conductances. Furthermore, the selective fourfold increase of the tonic inhibition on blocking the GABA transporter GAT-1 indicates that the tonic conductance is under the control of a powerful GABA uptake.

Powerful GABA uptake regulates tonic inhibition

It has been known for some time that GABA uptake can shape the decay of synaptic currents (Dingledine and Korn 1985; Korn and Dingledine 1986; Roepstorff and Lambert 1992; Thompson and Gahwiler 1992) and that it can regulate GABAergic signaling at both GABA_A and GABA_B receptors relatively distant from the release site (Isaacson et al. 1993). Our study demonstrated that, most likely through the GAT-1 GABA transporter, the GABA uptake system has a much more

profound influence on tonic than on phasic inhibition in adult dentate gyrus granule cells. Similar results were obtained recently in *postnatal day 6–18* (P6–P18) rat CA1 neurons, where ramp depolarizations exposed merely a leak conductance unless the GABA uptake inhibitor tiagabine was added to the perfusate (Frahm et al. 2001). It appears that at near physiological temperatures in acute brain slice preparations, GABA uptake may keep the ambient GABA concentration sufficiently low to prevent the activation of GABA_A receptors or to allow that of only a specific subsets of receptors (high affinity, nondesensitizing). Clearly, a slice preparation continuously washed with ACSF may not necessarily reflect the conditions for GABA uptake in the intact brain or that in other biochemical preparations (Wood and Sidhu 1986). Nevertheless, our finding that GABA uptake predominantly regulates the amount of tonic GABA_A receptor-mediated current may have significant consequences on the regulation of cellular and integrative properties of nerve cells.

In general, functional consequences of the tonic inhibition are not well understood. One of its possible roles could be to set the membrane potential close to the reversal potential (E_{GABA}) of GABA-induced currents, allowing an ambient GABA concentration-dependent regulation of the membrane potential. In dentate gyrus granule cells this results in a steady depolarization as E_{GABA} is about 15 mV positive to the resting membrane potential (Soltesz and Mody 1994). The amount of this depolarization would be a function of the ambient GABA, reflecting the overall network activity, particularly that of the GABA releasing neurons. In contrast to its action on the tonic current under our experimental conditions, inhibition of GABA uptake had no significant effect on the mean phasic current. The reason for this may be a dual consequence of the uptake inhibition. The resulting elevated ambient GABA concentra-

tion may have induced a small steady-state desensitization of the synaptic receptors (Overstreet et al. 2000), thus reducing the amplitude and the charge of the IPSCs. At the same time, the slowed GABA clearance may have prolonged the decay of the IPSCs (Williams et al. 1998), consequently increasing the charge transfer during synaptic events. These two effects of GABA uptake inhibition, decreasing and increasing the total charge per IPSC, may have canceled each other out, leaving the mean phasic current unaffected.

Tonic and phasic inhibitions are mediated by distinct GABA_A receptor subtypes

In cerebellar granule cells, where tonic inhibition was first described, distinct GABA_A receptor subtypes mediate tonic and phasic inhibitions. Our results with zolpidem also indicate that in dentate gyrus granule cells, $\alpha 4$ subunit-containing receptors could be primarily responsible for the tonic inhibition. In this cell type, $\alpha 4\beta_x\delta$ and $\alpha 4\beta_x\gamma_2$ subunit-containing receptors (Bencsits et al. 1999) may be exclusively present extrasynaptically, and they may have a high affinity for GABA and may not desensitize on the prolonged presence of agonist. Phasic inhibition is likely to be mediated by $\alpha 1\beta_x\gamma_2$ or $\alpha 2\beta_x\gamma_2$ receptors, as the synaptic currents are known to be benzodiazepine sensitive (De Koninck and Mody 1994; Hajos et al. 2000; Otis and Mody 1992). Although high-resolution anatomical studies are presently lacking in dentate granule cells, the $\alpha 4$ subunit-containing receptors may be confined to extrasynaptic sites just like the $\alpha 6\beta_x\delta$ receptors of cerebellar granule cells (Nusser et al. 1998). Another pharmacological difference between the sensitivities of tonic and phasic inhibitions to two competitive GABA_A receptor antagonists has been recently reported by Bai et al. (2001) in CA1 pyramidal cells. In contrast to this study, we did not find any difference in dentate gyrus granule cells between the two antagonists, albeit at higher concentrations, in their ability to block tonic and phasic inhibitions. The discovery of specific pharmacological tools allowing the modulation of one type of inhibition independently of the other will be extremely useful for elucidating their roles in the control of neuronal excitability.

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