0026-895X/06/6902-564–575$20.00
MOLECULAR PHARMACOLOGY
Vol. 69, No. 2
16683/3080741
Mol Pharmacol 69:564–575, 2006
Printed in U.S.A.

High-Affinity, Slowly Desensitizing $GABA_A$ Receptors Mediate Tonic Inhibition in Hippocampal Dentate Granule Cells

Zakaria Mtchedlishvili and Jaideep Kapur

Department of Neurology, University of Virginia-Health Sciences Center, Charlottesville, Virginia

Received July 11, 2005; accepted November 10, 2005

ABSTRACT

The tonic form of GABA-mediated inhibition requires the presence of slowly desensitizing $GABA_A$ receptors with high affinity, which has not yet been directly demonstrated in hippocampal neurons. Low concentration of GABA (1 μM) persistently increased baseline noise, increased membrane slope conductance, but did not affect spontaneous inhibitory postsynaptic currents (sIPSCs) in dentate granule cells (DGCs). Higher concentrations of GABA (10–100 μM) desensitized synaptic currents quickly, and there was a large residual current. Saturating concentration of GABA (1 mM) completely desensitized synaptic currents and revealed a slowly desensitizing, persistent current. Penicillin (300 μM) inhibited baseline noise without affecting mean current and inhibited decay time of sIPSCs. $GABA_A$ receptors mediating baseline noise in DGCs were sensitive to allopregnanolone, furosemide, and loreclezole and insensitive to diazepam and zolpidem. These studies demonstrate persistently open $GABA_A$ receptors on DGCs with high affinity for GABA, slow desensitization rate, and pharmacological properties similar to those of recombinant receptors containing α_4, β_1, and the δ subunits.

Fast synaptic inhibition in the forebrain is mediated by transient activation of synaptic $GABA_A$ receptors by high concentrations of GABA released from the presynaptic terminals. GABA in the extracellular space mediates a slow inhibition of neurons by persistent activation of extrasynaptic receptors, which is commonly termed tonic inhibition. Tonic inhibition has been well characterized in cerebellar granule cells (Brickley et al., 1996; Hamann et al., 2002) and more recently in hippocampal dentate granule cells (DGCs) (Nusser and Mody, 2002; Stell et al., 2003). For $GABA_A$ receptors to be activated by the low concentrations of GABA present in the extracellular space, these receptors must have a high affinity for GABA. Because persistent activation of many types of $GABA_A$ receptors leads to their desensitization, specific subsets of receptors with slow rates of desensitization would be required for maintaining tonic inhibition. Recombinant $GABA_A$ receptors containing the δ subunit in combination with the α_4 or α_6 subunit form receptors that have a high affinity for GABA as well as a slow rate and limited degree of desensitization (Brown et al., 2002; Wohlfarth et al., 2002). The δ subunits are expressed perisomatically in hippocampal DGCs (Wei et al., 2003) and extrasynaptically in cerebellar granule cells, suggesting that these receptors are capable of mediating tonic inhibition.

In keeping with this hypothesis, previous studies on cerebellar and hippocampal granule cells demonstrated that tonic inhibition has pharmacological properties similar to those of recombinant receptors containing the δ subunit. For example, zolpidem does not affect holding (mean) current, whereas it enhances synaptic currents in DGCs in vivo (Nusser and Mody, 2002) and in cultured hippocampal neurons (Yeung et al., 2003). In cerebellar granule cells, receptors mediating tonic inhibition are sensitive to the neurosteroid THDOC (Stell et al., 2003) and furosemide (Hamann et al., 2002; Wall, 2002) but insensitive to diazepam (Hamann et al., 2002). These pharmacological properties are shared with recombinant receptors containing the α_6 and δ or α_4 and δ subunits. Postembedding electron microscopic studies further demonstrated that δ subunit-containing $GABA_A$ receptors are present on extrasynaptic membranes in cerebellar granule cells (Nusser et al., 1998). Based on these studies it has been proposed that δ subunit-containing receptors mediate tonic inhibition. However, earlier studies do not provide direct evidence of the presence of high-affinity, slowly desensitizing $GABA_A$ receptors in neurons that are necessary to mediate tonic inhibition.

This study was supported Grants R01-NS040337 and R01-NS044370 (to J.K.).
Article, publication date, and citation information can be found at http://molpharm.aspetjournals.org.
doi:10.1124/mol.105.016683.

ABBREVIATIONS: DGC, dentate granule cell; THDOC, tertrahydrodeoxycorticosterone; ACSF, artificial cerebrospinal fluid; sIPSC, spontaneous inhibitory postsynaptic current; DL-AP5, DL-2-amino-5-phosphonopentanoic acid; RMS, root mean square; KS, Kolmogorov-Smirnov; SR 95531, 2-(3-carboxyl)-3-amino-6-(4-methoxyphenyl)-pyridazinium bromide; CGP 55845, (2*S*)-3-[[(1*S*)-1-(3,4-dichlorophenyl)ethyl]amino-2-hydroxypropyl](phenylmethyl)phosphinic acid.

We directly demonstrate that DGCs express persistently open $GABA_A$ receptors with high GABA affinity. Furthermore, these cells express $GABA_A$ receptors with a slow rate and limited extent of desensitization, which remain open when synaptic receptors have desensitized. We also characterized pharmacological properties of tonic currents, which were similar to those of recombinant receptors containing α_4, β_1, and δ subunits.

Materials and Methods

Adult male Sprague-Dawley rats (150–200 g) were anesthetized with halothane before decapitation according to the University of Virginia Animal Care and Use Committee guidelines. Brains were dissected free and immersed in ice-cold (2–4°C) artificial cerebrospinal fluid (ACSF) saturated with 95% O_2, 5% CO_2. The ACSF consisted of 127 mM NaCl, 2 mM KCl, 1.5 mM $CaCl_2$, 1.5 mM $MgSO_4$, 25.7 mM $NaHCO_3$, 1.1 mM KH_2PO_4, and 10 mM glucose (osmolarity, 300–305 mOsM). After cooling, the brains were blocked and mounted on a vibratome stage (Camden Instruments Ltd., Leicester, UK), and 300-μm-thick horizontal sections containing the ventral hippocampus were cut. Slices were maintained in continuously oxygenated ASCF, at 32°C in a holding chamber for 30 to 45 min, and then at room temperature in a recording chamber mounted on the stage of an Olympus BX51 microscope equipped with a 40× water-immersion objective, infrared-differential interference contrast optics, and video. DGCs were identified in dentate granule layer as small- and medium-sized neurons with typical oval-shaped soma and single process. Patch electrodes (final resistances, 6–8 MΩ) were pulled from borosilicate glass (Sutter Instrument Company, Novato, CA) on a horizontal Flaming-Brown microelectrode puller (model P-97; Sutter Instrument Company), using a two-stage pull protocol. Electrode tips were filled with a filtered internal recording solution consisting of 153.3 mM CsCl, 1.0 mM $MgCl_2$, 10.0 mM HEPES, and 5.0 mM EGTA (pH adjusted to 7.2 with CsOH; osmolarity, 285–295 mOsM). The electrode shank contained 3 mM ATP Mg^{2+} salt, 0.1 mM GTP Na^+ salt. Neurons were voltage-clamped to −65 mV with an Axopatch 1D or Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA). Whole cell capacitance and series resistance were compensated by 70 to 75% at 10-μs lag. Recording was performed when series resistance after compensation was 20 MΩ or less. Access resistance was monitored with a 10-ms, −5-mV test pulse once every 2 min, and if the series resistance increased by 25% at any time during the experiment, the recording was terminated. Currents were filtered at 5 kHz and then digitized using a Digidata 1322 digitizer and acquired using Axoscope 8.0 software (Molecular Devices) on an IBM PC-compatible computer hard drive.

For recording of $GABA_A$ receptor-mediated spontaneous inhibitory postsynaptic currents (sIPSCs) and tonic GABAergic current, 50 μM DL-2-amino-5-phosphonopentanoic acid (DL-AP5) and 20 μM 6-cyano-7-nitroquinoxaline-2,3-dione (Tocris-Cookson Inc., Ellisville, MO) were included in the ACSF to block *N*-methyl-D-aspartate and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid/kainate receptor-mediated currents, respectively. All other reagents were obtained from Sigma-Aldrich (St. Louis, MO).

Acquisition and Analysis. The digitized current traces were analyzed with Mini Analysis software (Synaptosoft, Leonia, NJ). The software was used to detect and analyze sIPSCs. To detect sIPSCs, a detection threshold five times the root-mean-square (RMS) amplitude was used. RMS noise (I_{rms}) was defined as a square root of the average of the squares of the deviation from the I_{avg} (amplitude) over the chosen time interval using the equation

$$I_{rms} = \left[\frac{(I_{avg} - I_1)_2 + (I_{avg} - I_2)_2 + \ldots + (I_{avg} - I_n)_2}{n}\right]^{1/2}$$

where I_{avg} is an average amplitude, I_n is amplitude in an individual point, and n is the number of measurements in an epoch.

To study the tonic inhibition, transient events were manually removed from the current trace, so that it consisted only of holding current, the current required to voltage-clamp the cell. Two features of the holding current were studied: the mean current and the noise. Mean current was measured in 100-ms epochs with 1-s interval between epochs in 30 epochs. The measurements were taken 30 s before and 5 min after application of a drug. The segments of a current trace that contained synaptic event were omitted. Mean current (I_{avg}) was defined as an arithmetic mean of peak-to-peak amplitudes of individual points during that epoch. To assess the effect of a drug on I_{avg} in an individual neuron, the distribution of I_{avg} before application of the drug during the baseline period was compared with that after drug application by means of a Kolmogorov-Smirnov (KS) test. To compare the data obtained from a group of neurons, mean of I_{avg} from all neurons before and after drug application was compared.

A second measure of holding current, I_{rms}, was studied. The time interval (epoch) for each measurement was 50 ms and contained 250 amplitude measurements (5-kHz digitization rate). The time interval between two epochs was 500 ms. Sixty epochs were analyzed for each experimental condition (60 control and 60 after a drug application in each cell). To assess the effect of a drug on I_{rms} in an individual neuron, the distribution of I_{rms} in epochs before the application of a drug (during the baseline period) was compared with that after drug application by means of KS test. To compare data obtained from a group of neurons, I_{rms} values in individual epochs before and after drug application were averaged. Mean I_{rms}, mean I_{avg}, and mean sIPSC frequency and amplitude values were compared using *t* tests unless specified otherwise.

Results

Tonic Inhibition Revealed by Competitive Antagonists. Several recent studies have used competitive antagonists to measure tonic inhibition. To measure tonic inhibition, sIPSCs interspersed with the mean current were recorded from DGCs in the hippocampal slices by blocking excitatory neurotransmission with 50 μM DL-AP5 and 20 μM 6-cyano-7-nitroquinoxaline-2,3-dione. The neurons were voltage-clamped to −65 mV, under nearly symmetrical Cl^- conditions, at a room temperature (20–22°C; see *Materials and Methods* for details). After the input resistance became stable, sIPSCs and mean current were recorded for 5 min, and then 100 μM bicuculline was bath-applied. A typical recording with 100 μM bicuculline application is shown in Fig. 1A. In response to bicuculline, a slow outward current occurred, accompanied by loss of sIPSCs and reduction in baseline noise.

For analysis of tonic currents, I_{avg} and I_{rms} were measured. I_{avg} was analyzed in 30 successive 100-ms epochs (2000 points), and at every second interval they were analyzed after manually excluding rapid transient, synaptic currents from each epoch. A typical experimental change in I_{avg} after application of bicuculline was 67 pA. The experiment was replicated on four DGCs and the mean reduction of I_{avg} was 72 ± 19 pA. I_{rms}, the deviation from the mean of 250 individual digitized current measurements in each epoch lasting 50 ms (RMS noise; Fig. 1C), was measured in 60 epochs during the baseline period and 60 epochs during application of bicuculline after manually removing rapid transient synaptic currents. Each epoch was separated from the subsequent epoch by 500-ms intervals. The I_{rms} during application of bicuculline was less than that during the baseline period in each of four cells (KS test; $p < 0.001$). Pooled values from all four cells were compared using a paired *t* test, and mean I_{rms}

during the baseline period was 7.082 ± 0.56 pA, and during 100 μM bicuculline application it was 5.997 ± 0.20 pA (p = 0.001).

Application of another competitive $GABA_A$ receptor antagonist, SR 95531 (100 μM), to 5 DGCs produced similar results on synaptic currents in each cell (Fig. 1B). Unlike bicuculline, SR 95531 did not have an effect on I_{avg} in three DGCs (82.8 ± 6.9 versus 80.1 ± 3.6 pA; p = 0.57; paired t test). The I_{rms} was significantly decreased (Fig. 1C). Data were pooled from all five cells, and during baseline period the mean I_{rms} was 6.671 ± 0.11 pA and during SR 95531 application it was 5.496 ± 0.07 pA (KS test; D = 0.24; $p < 0.001$).

High-Affinity $GABA_A$ Receptors. Studies with bicuculline and SR 95531 confirmed that tonic inhibition was present in DGCs; however, these drugs blocked synaptic inhibition along with tonic inhibition. It has been suggested that the $GABA_A$ receptors specifically mediating tonic inhibition possess the high affinity for GABA that makes them less sensitive to competitive $GABA_A$ receptor antagonists than those receptors mediating synaptic inhibition. However, this high affinity should render these receptors mediating tonic inhibition more sensitive to agonists than synaptic receptors. We determined whether a low concentration of GABA (1 μM), similar to that found in the extracellular space, could selectively activate $GABA_A$ receptors mediating tonic inhibition without altering synaptic currents.

After making a baseline recording for 5 min, a low concentration of GABA (1 μM) was bath-applied, and 5 min was allowed to elapse to allow the drug to equilibrate within the slice. The holding current and sIPSCs in the subsequent 5 min were compared with those during the 5-min baseline period before GABA application. A typical experiment is shown in Fig. 1. Note that there was no apparent change in I_{avg} on visual examination of the trace (Fig. 2A). A detailed quantitative examination of the I_{avg} confirmed the visual impression. The distribution of I_{avg} in 30 epochs before GABA application was compared with that in 30 epochs after application KS test (Fig. 2B), and the two distributions were not different (71.3 ± 11.2 versus 69.3 ± 6.7 pA; D = 0.04; p = 0.94). The experiment was repeated in four DGCs, and 1 μM GABA did not change $I_{avg.}$ in any of the cells.

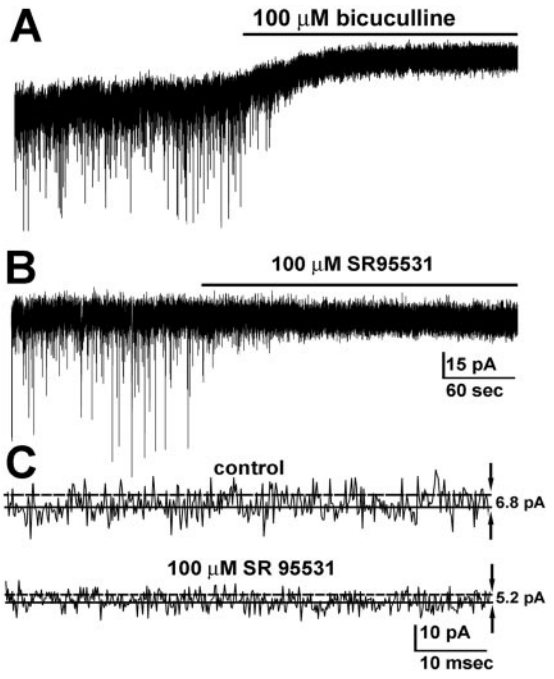

Fig. 1. Bicuculline (100 μM) inhibits sIPSCs mean baseline current (I_{avg}). A, current trace recorded from a dentate granule cell. Bicuculline (100 mM) (solid line) eliminated synaptic currents and decreased I_{avg}. Decrease of I_{avg} was accompanied by decrease of baseline noise (I_{rms}). Averaged 5-ms epochs of I_{avg} are scattered wider before application of bicuculline. B, competitive $GABA_A$ receptor antagonist SR 95531 (100 μM; solid line) inhibited synaptic currents with no effect on I_{avg} in a DGC. Note that unlike bicuculline, SR 95531 did not affect I_{avg}. C, SR 95531 inhibited I_{rms} in DGCs. The traces display baseline noise during a 50-ms epoch before application of SR 95531 (top trace) and in the presence of 100 μM SR 95531 (bottom trace). I_{rms} (shown by arrows between two solid lines) was defined as a square root of the average of squares of the deviation of individual points from the average value over 50-ms period. Note larger I_{rms} in the bottom trace compared with the top trace in B.

Visual inspection of the trace also suggested that the baseline trace was thicker after application of 1 μM GABA, and when expanded further it seemed to possess greater noise (Fig. 2C). The I_{rms} during baseline period was 5.30 pA (Fig. 2D, top trace), and 5 min after application of 1 μM GABA it was 6.0 pA. The distribution of I_{rms} measurements from each of 60 epochs before and after GABA application was compared using the KS test, indicating that they were significantly different (D = 0.68; p = 0.001; Fig. 2E). This finding was confirmed in four DGCs, and in each one the KS test revealed that I_{rms} was significantly increased. To further understand the impact of 1 μM GABA on I_{rms}, an I_{rms} amplitude frequency distribution histogram was constructed (Fig. 2F), which demonstrated that 1 μM GABA increased the frequency of large I_{rms} epochs. In four DGCs tested, the mean I_{rms} increased from 6.29 ± 0.02 to 6.66 ± 0.03 pA ($p < 0.0001$) in response to 1 μM GABA.

Using a different approach, we studied the slope of the current-voltage relationship (slope conductance) to confirm that 1 μM GABA indeed increased $GABA_A$ receptor conductance. Recordings were obtained in DGCs at baseline and after application of 1 μM GABA voltage-clamped to −65, −35, and −15 mV. I_{rms} in 60 epochs was obtained for each holding potential, and these values were plotted against the membrane holding potential (Fig. 2G). During the baseline period, the I_{rms} decreased proportionally to the driving force (r^2 = 1) and ordinate intercept was 2.9 pA. The regression line crossed the abscissa at close to 0 mV, the chloride reversal potential, suggesting that passage of chloride ions through the membrane contributed to I_{rms}. The slope of the RMS noise (current)-voltage relationship in the control condition was 18 pS. GABA (1 μM) increased I_{rms} at each holding potential, and the ordinate intercept was also close to 0 (2.9 mV). The slope of the RMS noise (current)-voltage relationship in the presence of 1 μM GABA in this neuron was 26.2 pS; therefore, 1 μM GABA increased the slope conductance. These findings were confirmed in four DGCs, and in each cell 1 μM GABA increased the slope conductance of I_{rms}.

We tested whether increased I_{rms} by 1 μM GABA was accompanied by inhibition of sIPSCs, because low concentrations of GABA are known to desensitize synaptic $GABA_A$ receptors and diminish synaptic currents in DGCs (Overstreet and Westbrook, 2001). The mean amplitude of sIPCSs remained unchanged after application of 1 μM GABA, 31.2 ± 5.3 pA in baseline and 33.7 ± 8.6 pA (approx. 500 events; paired t test; p = 0.82; n = 4). The mean decay time constant was also unchanged (9.9 ± 0.9 versus 10.12 ± 1.1 ms; p = 0.88). The frequency of sIPSCs was 1.38 ± 0.7 Hz at baseline

and 1.49 ± 0.7 Hz after application of 1 μM GABA ($p = 0.92$). Therefore, 1 μM GABA selectively enhanced baseline noise but did not have an effect on synaptic currents.

GABA Concentration-ΔI_{rms} Relationship. Theoretical studies on receptor-gated channel noise and subsequent recordings predict that the relationship between agonist concentration and I_{rms} is parabolic, with minimum noise occurring at low and high concentrations of the agonist and maximum noise occurring at intermediate concentrations (Traynelis and Jaramillo, 1998). As noted above, 1 μM GABA increased mean I_{rms} by 0.37 pA. In three DGCs, 10 μM GABA increased mean I_{rms} by 1.69 pA at the maximum change of I_{avg}. However, when the concentration of GABA was increased further to 30 μM, the increase in mean I_{rms} was smaller, 0.41 pA. These results indicated that the GABA concentration versus I_{rms} noise change relationship was parabolic, thus further confirming that $GABA_A$ receptor conductance contributes to I_{rms}.

Penicillin Modulates Tonic and Synaptic Inhibition. Bicuculline and SR 95531 blocked synaptic inhibition along with tonic inhibition. When the $GABA_A$ receptor is going through open and desensitized states, the agonist remains bound to it (agonist trapping) and competitive antagonists cannot bind to the receptor or close it (Bianchi and Macdonald, 2001). Therefore, bicuculline cannot selectively inhibit tonic currents, which are mediated by bound open receptors. We reasoned that a drug that preferentially binds to $GABA_A$ receptors in the open state is likely to discriminate between receptors mediating tonic and synaptic inhibition. Penicillin G is a noncompetitive antagonist of the $GABA_A$ receptor (Macdonald and Barker, 1977) that inhibits $GABA_A$ receptors by blocking the chloride channel in all open states (Twyman and Macdonald, 1992). Penicillin at a concentration as low as 250 μM reduces total average current in a single channel by 38% (Twyman et al., 1992).

The effect 300 μM penicillin on tonic and synaptic currents

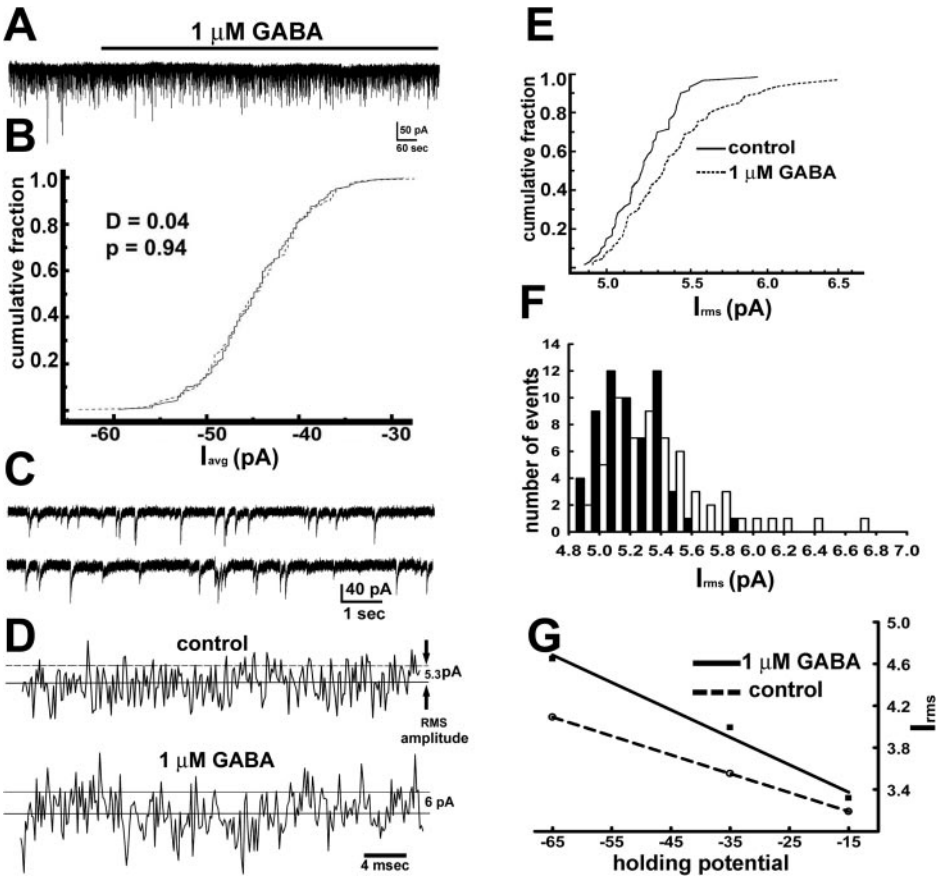

Fig. 2. GABA (1 μM) did not change I_{avg} but increased I_{rms}. A, current trace recorded from a dentate granule cell voltage-clamped to −65 mV in the presence of 50 μM DL-AP5 and 20 μM 6-cyano-7-nitroquinoxaline-2,3-dione, before (baseline recording) and during application of 1 μM GABA (solid line); the current trace did not seem to change. B, 1 μM GABA did not change I_{avg}. Measurements of I_{avg} in 30 epochs (1 epoch is 100 ms) at 1-s intervals during the baseline period (solid line) and in the presence of 1 μM GABA (dotted line). Note that the I_{avg} did not change after application of 1 μM GABA. C, current traces from the same DGCs display increased I_{rms} after application of 1 μM GABA. The top trace shows a 10-s recording before application of GABA, and the bottom trace shows a 10-s recording in the presence of 1 μM GABA. D, current traces display baseline noise during a 50-ms epoch before application of GABA (top trace) and in the presence of 1 μM GABA (bottom trace). I_{rms} (shown by arrows between two solid lines) was defined as a square root of the average of squares of the deviation of individual points from the average value over a 50-ms period. Note larger I_{rms} in the bottom trace compared with the top trace in B. E, KS test was used to compare I_{rms} before and after application of GABA in a neuron. I_{rms} in 60 epochs (1 epoch is 50 ms) at a 500-ms interval in the presence of 1 μM GABA (dotted line) was more than that during baseline period (solid line). F, distribution of RMS noise in cell during the baseline period (black columns) and in the presence of 1 μM GABA (white columns) were compared using a frequency distribution histogram with a bin size of 0.2 pA to demonstrate the rightward shift of I_{rms} in the presence of 1 μM GABA. G, membrane slope conductance before and after application of 1 μM GABA was compared. DGCs were voltage clamped to −65, −35, and −15 mV, and 60 epochs of I_{rms} were obtained at each holding potential during the baseline period and in the presence of 1 μM GABA, and I_{rms} values were plotted against holding potential. During the baseline period, the slope of the I_{rms} (current)-voltage relationship was 18.1 pS, and application of 1 μM GABA increased it to 26.2 pS.

was studied in seven DGCs. A typical experiment is shown on Fig. 3, where penicillin did not change the I_{avg} but it did diminish I_{rms} (Fig. 3, A and B). The decrease was significant for each cell, as confirmed by KS test with $p < 0.001$. The mean I_{rms} decreased from 4.364 ± 0.30 to 3.609 ± 0.21 pA ($p = 0.002$; $n = 7$). At the same time, penicillin effects on synaptic currents were subtle. It did not change peak amplitude and frequency of sIPSCs but significantly decreased decay time constant (Fig. 3C). The mean frequency of sIPSCs during the baseline period in seven neurons was 1.190 ± 0.19 Hz, and in the presence of 300 μM penicillin it was 1.25 ± 0.25 Hz ($p = 0.84$). The mean amplitude of sIPSCs was unchanged, 45.1 ± 13 pA during the baseline period and 43.7 ± 14 pA ($p = 0.95$) in the presence of penicillin. However, penicillin is an open channel blocker, and it is believed to inhibit channels after activation. We tested whether the decay of sIPSCs was accelerated by penicillin. The average decay time constant of sIPSCs decreased from 9.310 ± 0.9 to 5.240 ± 0.9 ms ($p = 0.002$; $n = 4$). The decrease of decay resulted in decreased charge transfer. It decreased from 512.1 ± 56.9 to 256.0 ± 33.4 pA/ms ($p = 0.006$; $n = 4$).

Desensitizing and Nondesensitizing $GABA_A$ Receptor Currents Elicited by 10 and 100 μM GABA. Several studies have shown that δ subunit-containing receptors desensitize less extensively and more slowly than γ2 subunit-containing receptors (Saxena and Macdonald, 1994; Haas and Macdonald, 1999; Bianchi et al., 2002). We tested whether higher concentrations of GABA (10 and 100 μM) could differentially desensitize lower affinity synaptic $GABA_A$ receptors while persistently activating high-affinity, slowly desensitizing receptors that are believed to mediate tonic inhibition.

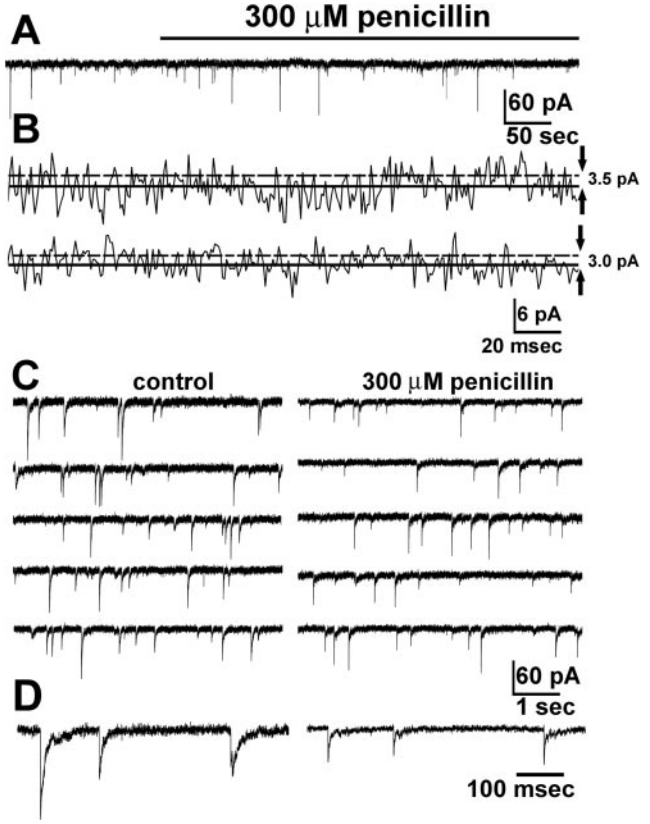

Fig. 3. The open channel blocker penicillin inhibits I_{rms}, synaptic currents but does not affect I_{avg}. A, current trace recorded from a DGC. Penicillin (300 μM; solid line) did not change I_{avg}. B, expanded current traces before application of penicillin (top trace) and 5 min after application of penicillin (bottom trace). I_{rms} (shown by arrows between solid and dashed lines) was smaller in the bottom trace compared with the top trace. C, penicillin (300 μM) did not change peak amplitude of sIPSCs but sharply decreased decay time constant. Current traces recorded from a DGC before application of penicillin (left) and 5 min after application of penicillin (right). Note faster decay in the presence of penicillin.

After 5 min of baseline recording, 10 μM GABA was applied for 10 min. This resulted in an inward shift in I_{avg}, which reached a maximum (trough) and then decayed to persistent current that did not return to baseline I_{avg} for the duration of application of GABA (Fig. 4, A and B). In five DGCs tested, the peak I_{avg} evoked by 10 μM GABA was 134.7 ± 0.81 pA. The I_{avg} decayed to a persistent current (90.25 ± 0.57 pA), which was measured 5 min after application of 10 μM GABA was started, and the residual nondesensitizing component I_{avg} was 44.5 ± 1 pA ($n = 5$). Therefore, the extent of desensitization of I_{avg} was 33%. The channel noise can decrease because of opening or closing of channels; therefore, I_{rms} was not used to study desensitization of receptors.

We determined the extent of desensitization of synaptic currents by 10 μM GABA. GABA (10 μM) was applied after recording of sIPSCs at baseline from a DGC, and it reduced the amplitude and number of sIPSCs. The reduction of sIPSC amplitude was probably caused by desensitization of synaptic receptors; however, reduction of number of sIPSCs could be because of diminished presynaptic release of GABA (decrease of frequency) or could be because of disappearance of smaller amplitude events into increased baseline noise (Stell and Mody, 2002). To determine whether reduction of number of sIPSC was merely increase of failures to detect events, the Cl^- driving force was diminished. sIPCSs were recorded from three DGCs voltage-clamped to −65 mV for 5 min and then to −20 mV, thus reducing the driving force by 60%. The frequency of sIPSCs decreased from 2.05 to 1.23 Hz (by 40%), and the mean amplitudes decreased from 4.6 ± 18.6 to 40.6 ± 12.9 pA. Therefore, decrease of number of sIPSCs was proportional to decrease of driving force, which suggests that decrease of frequency with decrease of driving force reflects increase of failures to detect, rather than changes in presynaptic release of GABA.

In each of eight DGCs studied, 10 μM GABA inhibited sIPSC amplitude and frequency (Fig. 4, C and D). The mean amplitude of sIPSC decreased from 64.3 ± 6.6 to 47.1 ± 6.1 pA ($p < 0.05$) (Fig. 4E), and the frequency decreased from 1.26 ± 0.3 to 0.36 ± 0.04 Hz ($p = 0.01$). The total charge transfer mediated by sIPSCs before the application of GABA was 120.24 pA/s, and in the presence of GABA it was 42.39 pA/s. Therefore, prolonged application of 10 μM GABA desensitized 65% of synaptic currents, substantially more than desensitization of mean current, which was 33%, suggesting synaptic currents were more susceptible to desensitization than mean current.

We confirmed these findings using a higher concentration of GABA. In five DGCs, higher concentration of GABA (100 μM) produced a 1642 ± 164 pA inward shift of I_{avg} at the maximum (trough), which decayed to a persistent 676.5 ± 148.5 pA current. The effect of 100 μM GABA on sIPSCs was more dramatic, because they disappeared in all five cells. The mean frequency of sIPSCs before GABA application was

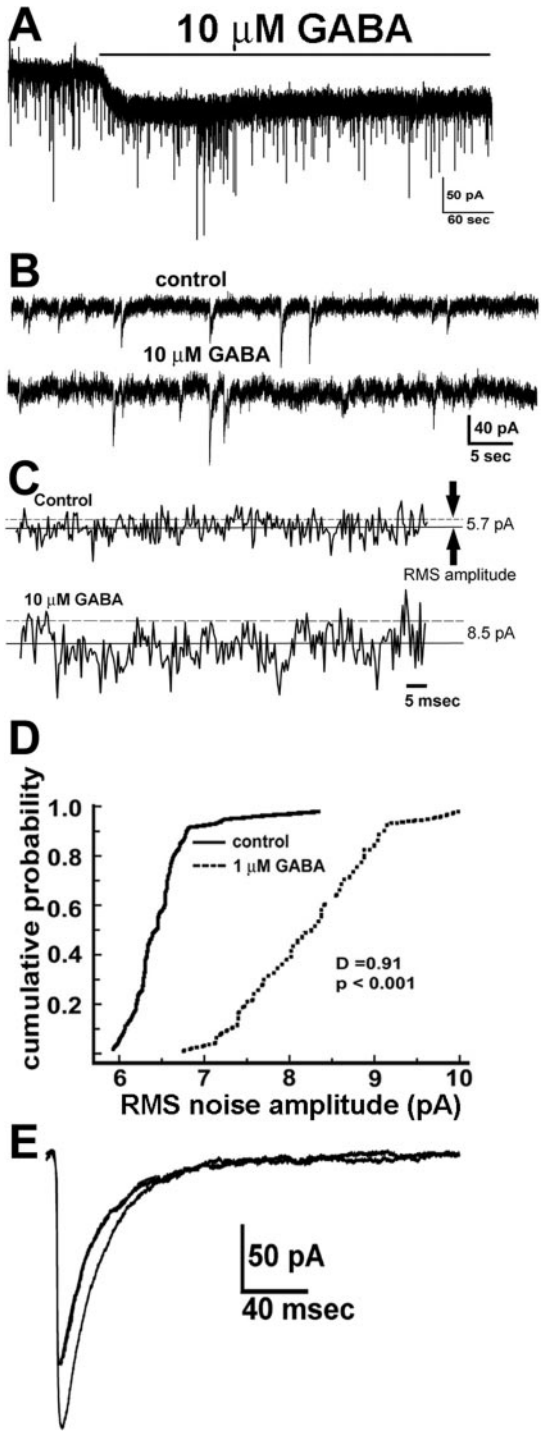

Fig. 4. GABA (10 μM) augmented baseline RMS noise and mean current. A, current trace recorded from DGC before (baseline period) and during application of 10 μM GABA (solid line). Note that 10 μM GABA resulted in an inward current that reached a maximum and then decayed to persistent current, which did not return to the baseline. B, expanded fragments from current trace in A; bottom trace recorded in the presence of 10 μM GABA demonstrated larger noise than the top trace recorded during baseline period. C, plot of KS test of I_{avg} in 30 epochs before and in 30 epochs after application of 10 μM GABA were compared with KS test. Note the significant increase of I_{avg} after application of 10 μM GABA (dotted line). D, normalized sIPSCs (average of approx. 500 sIPSCs) from the current trace recording shown in A. Thin line represents sIPSCs recorded in the baseline, and the thick line represents sIPSCs after application of 10 μM GABA. Note the reduction of peak amplitude after application of 10 μM GABA.

0.92 ± 0.12 Hz, and no transient event could be detected after the mean current reached the peak.

Activation of presynaptic $GABA_B$ receptors by GABA could have reduced applied to DGCs in the presence of CGP 55845 (10 μM) to block $GABA_B$ receptors. There was no change in frequency of sIPSCs when recordings without CGP 55845 were compared with recordings in the presence of CGP 55845 (0.92 ± 0.17 Hz, $n = 8$ and 1.22 ± 0.05 Hz, $n = 3$; $p = 0.36$). The sIPSCs were completely inhibited by 100 μM GABA in the presence of 10 μM CGP 55845. Therefore, desensitization of postsynaptic $GABA_A$ receptors alone without activation of $GABA_B$ receptors could explain the loss of synaptic currents.

Saturating Concentration of GABA to Measure Total Residual Conductance. Studies with 10 and 100 μM GABA suggested that GABA could be used to differentially desensitize two kinds of receptors that mediate tonic and phasic inhibition in DGCs. GABA activated both kinds of receptors, but a fraction of receptors desensitized rapidly, whereas others were persistently active in the presence of GABA. We determined the residual $GABA_A$ receptor conductance in the presence of a saturating concentration of GABA. GABA (1 mM) produced a large, whole cell inward I_{avg} with maximum amplitude of 1628 ± 194 pA ($n = 8$; see an example in Fig. 5A). Continuous application of 1 mM GABA (~10 min) produced desensitization of $GABA_A$ receptors. However, residual current remained regardless of duration of GABA application. This residual current had mean amplitude of 534 ± 126 pA. This residual current was probably mediated by a combination of slowly and rapidly desensitizing receptors.

Prolonged application of a saturating concentration of GABA could substantially affect the distribution of Cl^- ions, raising the possibility that a large persistent current in the presence of 1 mM GABA could result in the redistribution of Cl^- ions as opposed to increased conductance resulting from open $GABA_A$ receptor channels. To address this question, the DGCs were voltage-clamped to progressively more depolarizing potentials such that no synaptic activity could be detected, typically these potentials were +2 to +4 mV and conductance pulses (2 mV; 10 ms) at 1-Hz frequency were applied. After measuring membrane resistance by applying conductance pulses during 5 min of the baseline recording, 1 mM GABA was applied for 5 min. In response to 1 mM GABA, the I_{avg} changed very little (Fig. 5A), but the amplitude of the conductance pulse first increased to a peak and then decayed to a persistent value (Fig. 5, B and C). In all six DGCs, GABA caused a small inward current (42 ± 6 pA) and reduced the membrane resistance from 211 ± 9.3 MΩ to a minimum of 57.5 ± 0.7 MΩ. The resistance then increased to a persistent level, 95.6 ± 0.8 MΩ. Therefore, the persistent change in resistance was 115.4 MΩ or an 8.66-nS change in conductance, similar to that when the DGCs were voltage-clamped to −65 mV (8.21 nS).

Pharmacological Properties $GABA_A$ Receptors Mediating Tonic Currents. A metabolite of progesterone, 3a-OH-5a-pregnan-20-one or allopregnanolone, is a potent allosteric modulator of $GABA_A$ receptors. Allopregnanolone, in concentrations of the physiological range (10–30 nM), enhances whole cell $GABA_A$ receptor currents elicited from DGCs (Mtchedlishvili et al., 2001). Nanomolar concentrations of neurosteroid THDOC enhance $GABA_A$ receptor currents elicited from recombinant receptors containing the δ

subunit (Wohlfarth et al., 2002); thus, we tested whether low concentrations of allopregnanolone could modulate tonic inhibition in DGCs.

A physiological concentration of allopregnanolone (10 nM) was applied to a DGC after a 5-min recording of sIPSCs, which did not alter I_{avg} but increased mean I_{rms} (Fig. 6, A and B). The effect of allopregnanolone was studied in five DGCs, and it increased I_{rms} in each of the five DGCs tested (KS test). When the data from individual cells were pooled, 10 nM allopregnanolone increased I_{rms} from 9.165 ± 0.5 to 9.612 ± 0.7 pA ($p < 0.001$; $n = 5$). When data from five cells were combined, allopregnanolone (10 nm) increased mean I_{avg} from 46.8 ± 3.1 to 48.3 ± 0.6 pA ($p = 0.4$). A higher concentration of allopregnanolone, 30 nM, also increased mean I_{rms} from 7.887 ± 0.47 to 8.121 ± 0.4335 pA (t test; $p = 0.003$; $n = 5$) without any effect on mean I_{avg} (69.1 ± 8 and 71.6 ± 3.2 pA; t test; $p = 0.38$).

Previous studies suggested that neurosteroids modulate mean current, so we tested whether higher concentration of allopregnanolone (300 nM) would modulate mean current. The mean current was enhanced by 300 nM allopregnanolone as shown in Fig. 6D. In three DGCs, 300 nM allopregnanolone increased mean I_{avg} by 98.2 ± 17 pA from 62.6 ± 4.9 to 160.8 pA ($p < 0.05$). It also increased I_{rms} from 7.258 ± 0.06 to 8.151 ± 0.1 pA ($p = 0.01$). These results suggest that the low nanomolar concentrations of allopregnanolone, which is likely to be found in the hippocampus in vivo, enhanced $GABA_A$ receptor-mediated tonic inhibition by increasing I_{rms}. The effect of 300 nM allopregnanolone on mean current could have been in part because of a direct effect of allopregnanolone on the chloride channel (Twyman and Macdonald, 1992).

Furosemide and Loreclezole. Furosemide is a noncompetitive antagonist, which inhibits $GABA_A$ receptor currents elicited from $\alpha 4$ subunit-containing recombinant receptors (Wafford et al., 1996) and does not discriminate between γ and δ subunit-containing receptors (Korpi and Luddens, 1997). In six DGCs, furosemide (300 μM) decreased I_{rms} from 7.611 ± 0.54 pA during baseline to 6.498 ± 0.35 pA ($p < 0.05$; Fig. 7A), but it did not change I_{avg}, which was 143.7 ± 23 pA during baseline and 145.6 ± 35 pA ($p = 0.38$) during drug application. The presence of tonic $GABA_A$ receptor-mediated current was confirmed by bath application of bicuculline (100 μM) in the presence of 300 μM furosemide. Bicuculline abolished all synaptic currents and decreased mean I_{avg} by 47 ± 8.31 pA ($n = 3$).

Loreclezole Decreased Tonic Current. Anticonvulsant loreclezole enhances β_2 or β_3, but it inhibits β_1 subunit-containing $GABA_A$ receptors (Fisher et al., 2000). Loreclezole (30 μM; Fig. 8) decreased I_{rms} from 7.573 ± 0.75 to 7.355 ± 0.63 pA ($p < 0.05$) in four DGCs. Loreclezole at 30 μM had the tendency to reduce mean I_{avg} from 174.0 ± 1.1 pA during baseline to 168 ± 0.57 pA during drug application ($p > 0.05$; $n = 4$).

Diazepam and Zolpidem Did Not Modulate Tonic Current. Diazepam (100 nM) did not alter I_{avg} and I_{rms} (Fig. 9, A–C). To confirm the presence of tonic currents, bicuculline was bath-applied to the cell, which caused a reduction in mean current. This result was confirmed, and in four cells diazepam did not change mean I_{avg}, which was 46.8 ± 3.1 pA during baseline and 48.3 ± 0.6 pA ($p = 0.4$) during drug application. The mean I_{rms} was slightly but not significantly diminished from 3.62 ± 0.05 pA during baseline to 3.39 ± 0.12 pA ($p = 0.15$; Fig. 9). Thus, tonic inhibition was not modulated by diazepam.

The imidazopiridine zolpidem acts on the benzodiazepine binding site and exerts maximal potentiation of $GABA_A$ receptor currents in the presence of α_1 subunits (Pritchett et

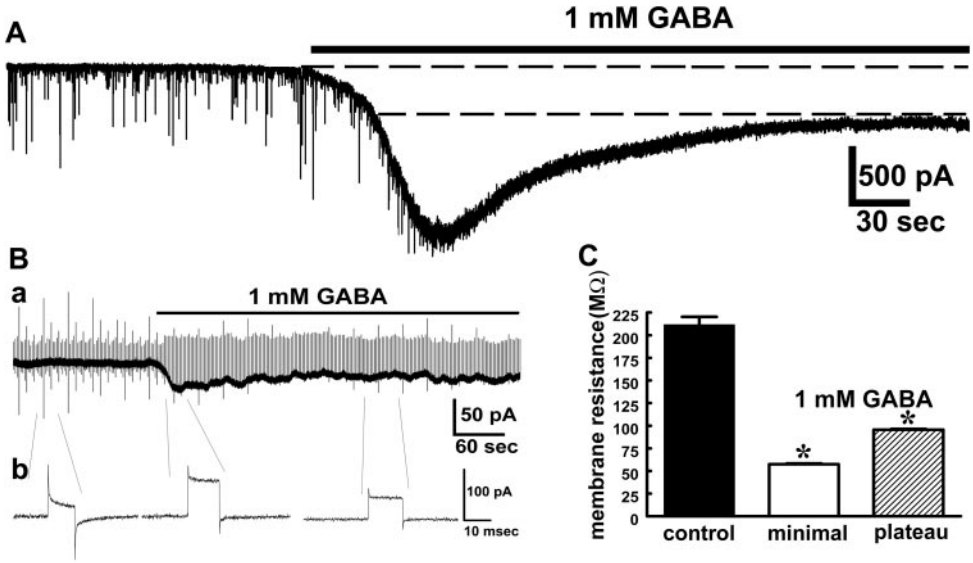

Fig. 5. Saturating concentration of GABA revealed a slowly desensitizing conductance. A, current trace from a DGC showing baseline recording followed by application of 1 mM GABA (solid line), which evoked a large whole cell current that decayed to a persistent, slowly desensitizing current. Note that sIPSCs gradually disappeared in during application of 1 mM GABA and were eliminated in the persistent slowly desensitizing component, suggesting that synaptic $GABA_A$ receptors were desensitized by 1 mM GABA. The difference between the baseline and the persistent, slowly desensitizing component (dashed lines) was measured. B, i, current trace from a DGC voltage-clamped to 4 mV (near reversal potential for Cl^-) with application of conductance pulses (5 mV; 2 ms; 1 Hz) before (baseline period) and during application of 1 mM GABA (solid line). ii, expanded potions of a current trace from i demonstrated a large increase in conductance in response to GABA by reduction of conductance (right trace), but the conductance remained increased compared with the baseline period. C, histograms demonstrating membrane resistance (in megaohms) at baseline (black) at maximally increased conductance evoked by 1 mM GABA (white) and in persistently increased conductance (dashed).

al., 1989) and least enhancement in the presence of α_5 subunits. Zolpidem (100 nM) was bath-applied to DGCs. Mean current and RMS noise was measured 30 s before and 5 min after the flow of diazepam was started. There was no change in mean current (56.1 ± 2.1 and 59.3 ± 0.6 pA; *t* test; $p = 0.48$; $n = 4$). The RMS noise also remained unchanged (3.62 ± 0.05 versus 3.39 ± 0.12 pA; *t* test; $p = 0.15$; $n = 4$; Fig. 10). To confirm that tonic currents were present in cells insensitive to zolpidem, bicuculline was applied, and it caused reduction of mean current (Fig. 10A).

Discussion

This study for the first time demonstrated high-affinity, slowly desensitizing $GABA_A$ receptors in hippocampal neurons. Furthermore, this study suggested that persistently open $GABA_A$ receptors contribute to membrane current fluctuations (I_{rms}) recorded from DGCs in baseline conditions. $GABA_A$ receptors contributing to tonic I_{rms} in DGCs were sensitive to allopregnanolone, furosemide, and loreclezole and insensitive to diazepam and zolpidem. These pharmacological properties were similar to those of recombinant receptors containing α_4, β_1, and the δ subunits.

Tonic $GABA_A$ Receptor Conductance Contributes to Membrane Noise. Membrane current fluctuations have long been used to study the properties of ion channels and receptors, but there are few studies demonstrating $GABA_A$ receptor contribution to membrane noise. Several experiments in the present study suggested that persistently open $GABA_A$ receptor channels contribute to the membrane noise (I_{rms}) in DGCs in hippocampal slices. The current fluctuations recorded from DGCs diminished in proportion to the chloride driving force, suggesting that passage of chloride ions through the membrane contributed to I_{rms}. Penicillin, which blocks open $GABA_A$ receptors, reduced I_{rms}. GABA enhanced I_{rms}, suggesting that activation $GABA_A$ receptors could contribute to I_{rms}. Pharmacological studies suggested that only a subset of $GABA_A$ receptors contribute to baseline membrane noise. I_{rms} was enhanced by neurosteroid allopregnanolone, whereas benzodiazepine site agonists diazepam and zolpidem, which enhance synaptic $GABA_A$ receptor currents in DGCs, did not enhance membrane noise.

We found that the membrane noise was more sensitive to GABA, penicillin, and allosteric modulators of $GABA_A$ receptors than mean current, I_{avg}. Previous studies using competitive antagonists such as bicuculline found that these drugs reduce I_{avg} as well as membrane noise, I_{rms} (Stell and Mody, 2002). However, competitive antagonists block synaptic currents before inhibiting tonic currents. Penicillin altered I_{rms}

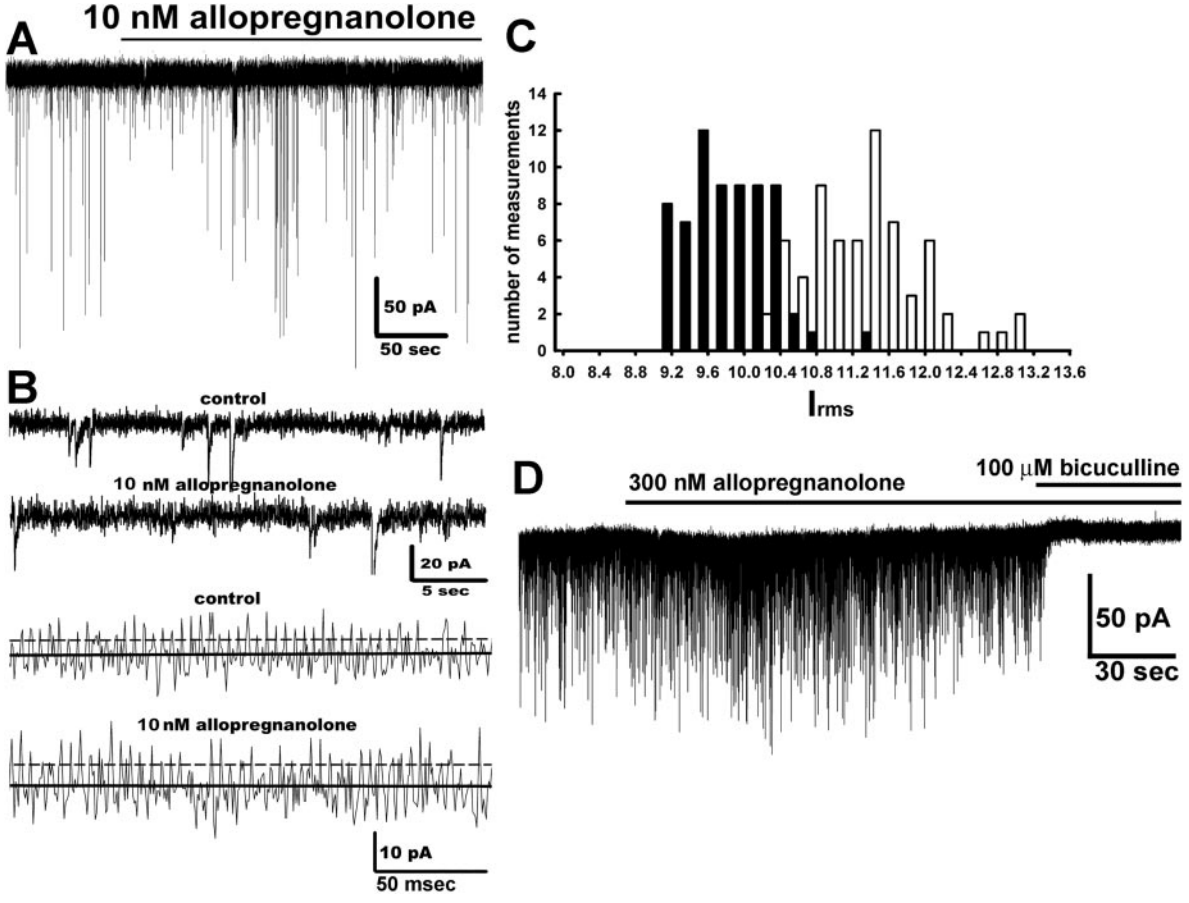

Fig. 6. Effect of allopregnanolone on $GABA_A$ receptor-mediated tonic current. A, typical recording of current trace before (baseline) and during bath application of 10 nM allopregnanolone (solid line) to a DGC demonstrating no change in I_{avg}. B, expanded fragments from current trace in A; bottom trace recorded in the presence of 10 nM allopregnanolone demonstrated larger noise than the top trace recorded during baseline period. C, frequency distribution histogram of I_{rms} in 60 epochs during the application of 10 nM allopregnanolone (white columns; bin size, 0.2 pA) demonstrated rightward shift compared with epochs in the baseline period (black columns). D, typical recording of current traces from a DGC before and after application of 300 nM allopregnanolone. The presence of tonic $GABA_A$ receptor-mediated current was confirmed by application of 100 μM bicuculline. Note the downward deflection of the holding current.

without any change in I_{avg}. Penicillin had a minimal effect on synaptic currents; it did not decrease peak amplitude or frequency of IPSCs, but it hastened IPSCs decay, which resulted in a 2-fold decrease in total charge transfer. In contrast, bicuculline completely eliminated synaptic currents, and this could have contributed to changes in I_{avg}. Likewise, furosemide, another noncompetitive $GABA_A$ receptor antagonist, reduced I_{rms} but not I_{avg}.

High-Affinity $GABA_A$ Receptors. Experiments with low concentrations of GABA (1 μM and 300 nM) revealed a high-affinity $GABA_A$ receptor on DGCs. Low concentrations of GABA increased membrane noise, but this could have resulted from closure of channels because of desensitization. Increased I_{rms} is not simply because of $GABA_A$ receptor channel openings because the relationship between the noise variance (RMS noise) and mean current amplitude is parabolic, described by the binomial theorem [$\sigma^2 = i^2 Np(1 - p)$, where is σ^2 is I_{rms}, N is number of channels, i is single channel currents, and p is probability of single channel opening. That is, the current fluctuations at lowest and highest concentrations of a ligand are minimal because most channels are in a closed or open state, but the fluctuations are maximal at intermediate concentrations of a ligand (for review, see Traynelis and Jaramillo, 1998). Several lines of evidence suggested that the augmentation of I_{rms} by 1 μM GABA was because of an opening of receptor channels and not because of closure of open channels. Comparison of membrane slope conductance in the presence and absence of GABA demonstrated increased conductance, suggesting that GABA opened more channels than it had shut. Furthermore, 1 μM GABA did not alter the frequency or amplitude of sIPSCs, suggesting that this concentration did not desensitize synaptic $GABA_A$ receptors. Finally, increasing the concentration of

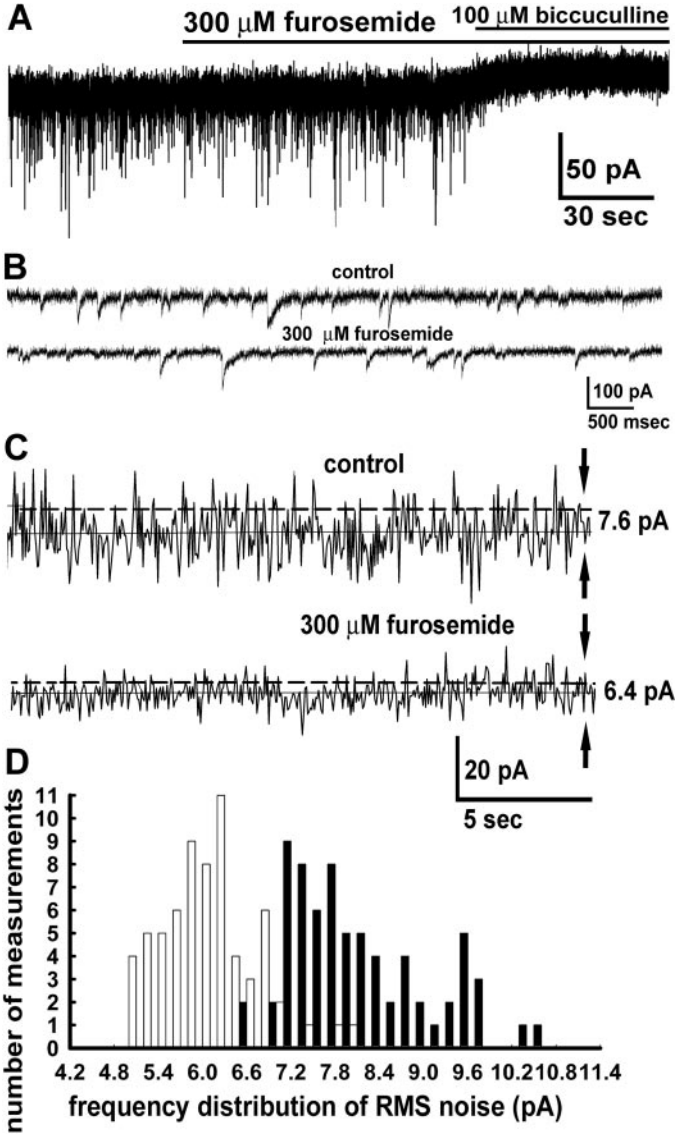

Fig. 7. Furosemide (300 μM) decreased $GABA_A$ receptor-mediated I_{rms} in DGCs. A, typical recording of a current traces from a DGC before (baseline) and during application of 300 μM furosemide indicated by a solid line. Note that 300 μM furosemide did not change I_{avg}, and bicuculline (100 μM) applied at the end of the experiment demonstrated the presence of tonic inhibition. B and C, expanded fragments from the trace shown in A show diminished I_{rms} during the furosemide application (bottom traces) than in the baseline period (top trace). Note decreased I_{rms} in the bottom trace compared with the top trace in C (distance between solid and dashed lines). D, frequency distribution histogram of I_{rms} during the furosemide application (white columns; bin size, 0.2 pA) was shifted to the left compared with that in the baseline period (black columns). Loreclezole (30 μM) decreased $GABA_A$ receptor-mediated I_{rms} in DGCs.

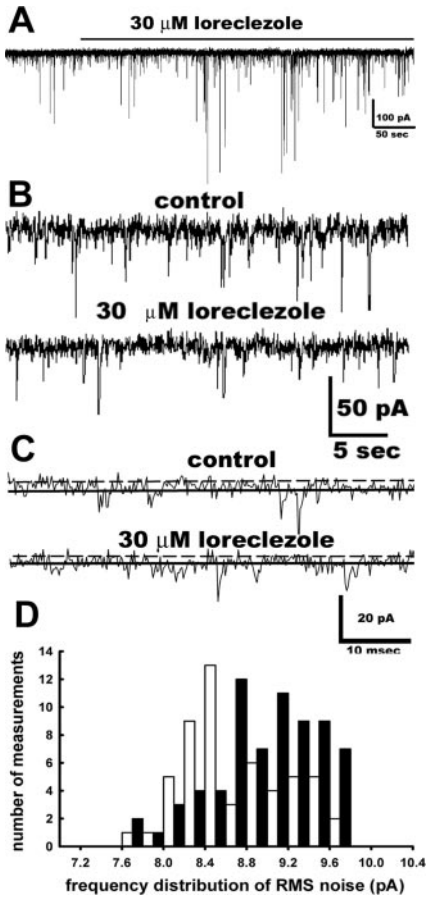

Fig. 8. A, typical recording of current trace from a DGC before (baseline) and during application of 30 μM loreclezole (solid line). Note that 30 μM loreclezole did not change I_{avg}. B and C, expanded fragments from the trace in A show diminished I_{rms} during the loreclezole application (bottom trace) compared with that in the baseline period (top trace). Note decreased I_{rms} in bottom trace compared with top trace in C (distance between solid and dashed lines). D, frequency distribution histograms of I_{rms} during loreclezole application (white columns; bin size, 0.2 pA) was shifted to the left compared with that in the baseline period (black columns).

GABA to 10 μM resulted in a greater increased membrane noise than that observed with 1 μM GABA.

A number of studies report the extracellular GABA concentration to be in the range from tens of nanomoles to a few micromoles. Extracellular GABA concentration was reported to be 30 nM in neocortex, measured by high-performance liquid chromatography (Zhang et al., 2005). Based on microdialysis studies, it has been suggested that the extracellular concentration of GABA in the hippocampus varies in the 2.1 to 3.8 μM range (Shin et al., 2002). It seems that extracellular GABA concentration in vivo varies in the 0.3 to 3 μM range (Timmerman and Westerink, 1997). Studies in the past used blockade of GABA uptake to modulate tonic current in DGCs (Nusser and Mody, 2002), but the exact change in extracellular concentration of GABA in these studies is unknown. The current study demonstrated that a low concentration of exogenously applied GABA could activate chloride conductance.

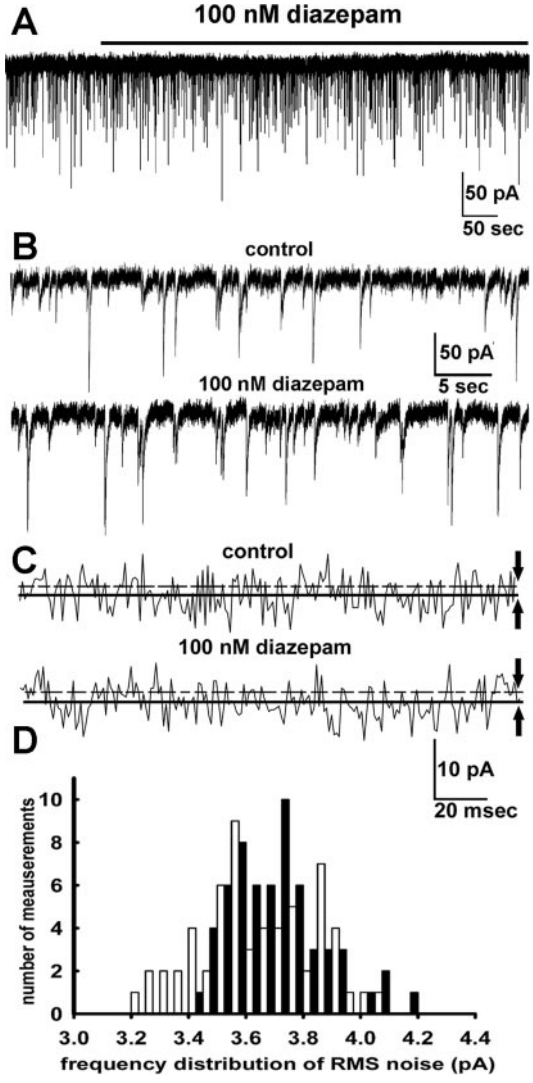

Fig. 9. Diazepam (100 nM) did not affect $GABA_A$ receptor-mediated tonic current in DGCs. A, current trace from a DGC before (baseline) and during application of 100 nM diazepam indicated by solid line. B and C, expanded fragments from the trace in A show unchanged I_{rms} during diazepam application (bottom trace) compared with the baseline period (top trace). D, frequency distribution histograms of I_{rms} during diazepam application (white columns; bin size, 0.2 pA) was unchanged compared with that in the baseline period (black columns).

This study also suggested that DGCs express $GABA_A$ receptors with a high affinity to GABA. A likely explanation for high GABA affinity of receptors mediating tonic inhibition is that they contain the α_4 subunit. Recombinant $GABA_A$ receptors containing the α_4 have a higher affinity for GABA than those containing the α_1 subunit (Wafford et al., 1996). The mRNA coding for the α_4 and polypeptide has been localized to DGCs in the past. Inhibition of tonic $GABA_A$ receptor-mediated currents by furosemide suggests that α_4 subunit-containing $GABA_A$ receptors participate in mediating tonic currents. Furosemide is known to inhibit α_4 subunit-containing receptors and was demonstrated to inhibit $GABA_A$ recep-

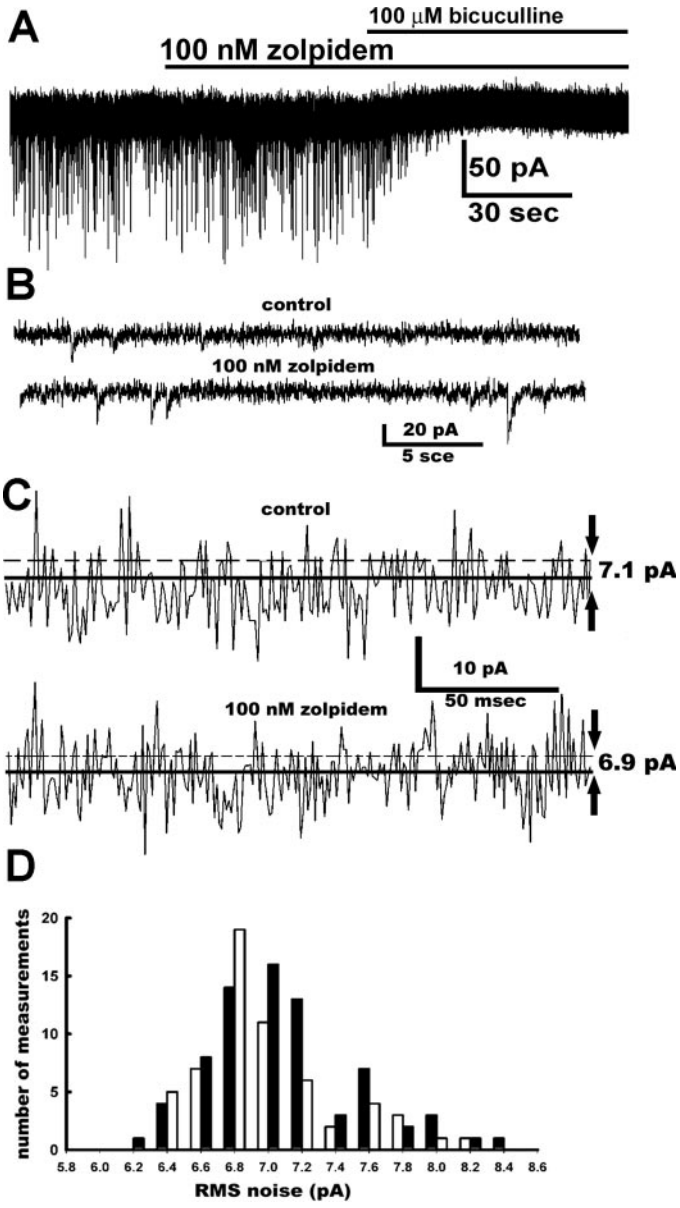

Fig. 10. Zolpidem (100 nM) did not affect $GABA_A$ receptor-mediated tonic current in DGCs. A, current trace from a DGC before (baseline) and during application of 100 nM zolpidem indicated by a solid line. Bicuculline (100 μM) was applied in the presence of zolpidem (solid line) to confirm presence of tonic inhibition. B and C, expanded fragments from the trace in A show unchanged I_{rms} during zolpidem application (bottom trace) compared with the baseline period (top trace). D, frequency distribution histograms of I_{rms} during zolpidem application (white columns; bin size, 0.2 pA) was unchanged compared with that in the baseline period (black columns).

tor currents in dentate granule cells, which express the α_4 subunit (Wafford et al., 1996; Kapur and Macdonald, 1999). In addition to furosemide sensitivity, recombinant $GABA_A$ receptors containing the α_4 subunit are insensitive to diazepam and zolpidem, which was also the case in our experiments with tonic currents recorded from DGCs.

Slowly Desensitizing Receptors on DGCs. A large residual $GABA_A$ receptor conductance was found in the equilibrium state after prolonged application of 100 μM and 1 mM GABA. This residual current was probably mediated by both synaptic and extrasynaptic receptors. Increasing the extracellular concentration of GABA inhibits synaptic $GABA_A$ receptors (Overstreet and Westbrook, 2001) by slow desensitization (Overstreet et al., 2000). Furthermore, the disappearance of synaptic currents was temporally correlated with reduction of the whole cell conductance, suggesting these receptors were being desensitized. Finally, previous studies have suggested that α_1 and γ_2 subunit-containing receptors are expressed in GABAergic synapses on DGCs. Recombinant receptors containing these subunits desensitize rapidly and to a substantial extent (90%) (Bianchi et al., 2002). In contrast, the δ subunit-containing receptors desensitize very slowly (Saxena and Macdonald, 1996; Haas and Macdonald, 1999) and to a lesser extent (35%) (Bianchi and Macdonald, 2002). However, studies with recombinant receptors have shown desensitization of γ2 subunit-containing receptors is never complete. Because γ subunit-containing receptors produce larger currents compared with δ subunit-containing receptors, even after 90% decrement of the current, the residual current corresponding to slow and ultraslow desensitization rates of γ subunit-containing receptors can be of similar amplitude as residual current mediated by δ subunit-containing receptors, which loose only 35% of current in response to 28-s application of 1 mM GABA (Bianchi and Macdonald, 2002). Therefore, it is likely that in our preparation, residual current after prolonged application of 1 mM GABA is generated by mixed population of γ and δ subunit-containing receptors. However, the current study does not permit to estimate the extent of participation of each subunit type-containing receptor because the ratio of expression of each subunit-containing receptor is unknown. Separate study will be needed to answer this question.

Pharmacological Properties of $GABA_A$ Receptors Mediating Tonic Currents. Pharmacological properties of persistently open $GABA_A$ receptors in DGCs were similar to those of recombinant receptors containing the δ subunits. Recombinant receptors containing the δ subunit are highly sensitive to neurosteroid enhancement. Enhancement of RMS noise by 10 nM allopregnanolone suggested that persistently open $GABA_A$ receptors on DGCs are sensitive to a physiological concentration of allopregnanolone. This high sensitivity to allopregnanolone is likely to be caused by the presence of δ subunit-containing $GABA_A$ receptors (Wohlfarth et al., 2002). Lack of diazepam and zolpidem sensitivity of receptors also suggested absence of γ_2 subunit and presence of δ subunit in these receptors.

We did not observe changes in mean current when low concentrations (10 and 30 nM) of allopregnanolone were applied; however, the RMS noise was increased. The mean current was increased by a 300 nM concentration of allopregnanolone (see *Results*). In contrast, low concentrations of the neurosteroid THDOC increased mean current in DGCs (Stell et al., 2003), which may suggest differential sensitivity of $GABA_A$ receptors to THDOC and allopregnanolone. We have previously reported that 10 nM allopregnanolone strongly enhanced whole cell currents in DGCs (Mtchedlishvili et al., 2001), but it was not clear whether the enhancement was because of effect of allopregnanolone on synaptic currents, tonic currents, or both. The current study demonstrated that the enhancement of whole cell currents at least in part was because of enhancement of tonic currents.

The loreclezole inhibition of tonic currents suggested the expression of β_1 subunit in receptors mediating tonic currents. Loreclezole is an anticonvulsant that enhances peak $GABA_A$ receptor currents elicited by subsaturating concentrations of GABA by acting at an allosteric regulatory site on β_2 and β_3 subunits (Wafford et al., 1994; Fisher and Macdonald, 1997). Peak currents elicited from receptors containing the β_1 subunit were not enhanced by loreclezole because the receptors lack the positive modulatory site present on the β_2 and β_3 subunits. In addition to potentiation of peak currents, loreclezole inhibited steady-state $GABA_A$ receptor currents by acting at a site distinct from the positive modulatory site on β_2 and β_3 subunits and increased the apparent desensitization. This inhibitory action of loreclezole occurred regardless of the β subunit. Thus, $GABA_A$ receptors containing the β_1 subunit may be inhibited by loreclezole or stay unaffected by it. Spontaneously opening $GABA_A$ receptors are present in hippocampal neurons (Macdonald et al., 1989; Birnir et al., 2000). It is possible that spontaneously opening $GABA_A$ receptors participate in the tonic current described in this article.

Conclusions. Ligand-gated ion channels can mediate at least three kinds of tonic currents: currents mediated by spontaneously open channels in the absence of the ligand, slowly desensitizing currents mediated by ligand-bound channels, and residual equilibrium currents recorded after desensitization of channels. These three forms of "tonic" currents may be mediated by overlapping pools of receptors as demonstrated in the current study. The current study demonstrated that on DGCs, some $GABA_A$ receptors are persistently open. These channels have high affinity for GABA; therefore, they may be ligand-bound open channels. Properties of persistently open $GABA_A$ receptors on DGCs were similar to recombinant receptors containing α_4, β_1, and δ subunits. A persistent current could be recorded after desensitization of $GABA_A$ receptors on DGCs.

References

Bianchi MT, Haas KF, and Macdonald RL (2002) alpha 1 and alpha 6 Subunits specify distinct desensitization, deactivation and neurosteroid modulation of GABA(A) receptors containing the delta subunit. *Neuropharmacology* **43:**492–502.

Bianchi MT and Macdonald RL (2001) Agonist trapping by GABA(A) receptor channels. *J Neurosci* **21:**9083–9091.

Bianchi MT and Macdonald RL (2002) Slow phases of GABA(A) receptor desensitization: structural determinants and possible relevance for synaptic function. *J Physiol (Lond)* **544:**3–18.

Birnir B, Everitt AB, Lim MSF, and Gage PW (2000) Spontaneously opening $GABA_A$ channels in CA1 pyramidal neurones of rat hippocampus. *J Membr Biol* **174:**21–29.

Brickley SG, Cull-Candy SG, and Farrant M (1996) Development of a tonic form of synaptic inhibition in rat cerebellar granule cells resulting from persistent activation of GABA(A) receptors. *J Physiol (Lond)* **497:**753–759.

Brown N, Kerby J, Bonnert TP, Whiting PJ, and Wafford KA (2002) Pharmacological characterization of a novel cell line expressing human alpha(4)beta(3)delta GABA(A) receptors. *Br J Pharmacol* **136:**965–974.

Fisher JL, Hinkle DJ, and Macdonald RL (2000) Loreclezole inhibition of recombinant $\alpha1\beta1\gamma2L$ $GABA_A$ receptor single channel currents. *Neuropharmacology* **39:**2235–2245.

Fisher JL and Macdonald RL (1997) Functional properties of recombinant GABA(A)

receptors composed of single or multiple beta subunit subtypes. *Neuropharmacology* **36:**1601–1610.
Haas KF and Macdonald RL (1999) GABA(A) Receptor subunit gamma 2 and delta subtypes confer unique kinetic properties on recombinant GABA(A) receptor currents in mouse fibroblasts. *J Physiol (Lond)* **514:**27–45.
Hamann M, Rossi DJ, and Attwell D (2002) Tonic and spillover inhibition of granule cells control information flow through cerebellar cortex. *Neuron* **33:**625–633.
Kapur J and Macdonald RL (1999) Postnatal development of hippocampal dentate granule cell γ-aminobutyric $acid_A$ receptor pharmacological properties. *Mol Pharmacol* **55:**444–452.
Korpi ER and Luddens H (1997) Furosemide interactions with brain GABA(A) receptors. *Br J Pharmacol* **120:**741–748.
Mtchedlishvili Z, Bertram EH, and Kapur J (2001) Diminished allopregnanolone enhancement of GABA(A) receptor currents in a rat model of chronic temporal lobe epilepsy. *J Physiol (Lond)* **537:**453–465.
Macdonald RL and Barker JL (1977) Pentylenetetrazol and penicillin are selective antagonists of GABA-mediated post-synaptic inhibition in cultured mammalian neurones. *Nature (Lond)* **5613:**720–721.
Macdonald RL, Rogers CJ, and Twyman RE (1989) Kinetic properties of the $GABA_A$ receptor main conductance state of mouse spinal cord neurons in culture. *J Physiol (Lond)* **410:**479–499.
Nusser Z, Hajos N, Somogyi P, and Mody I (1998) Increased number of synaptic GABA(A) receptors underlies potentiation at hippocampal inhibitory synapses. *Nature (Lond)* **395:**172–177.
Nusser Z and Mody I (2002) Selective modulation of tonic and phasic inhibitions in dentate gyrus granule cells. *J Neurophysiol* **87:**2624–2628.
Overstreet LS, Jones MV, and Westbrook GL (2000) Slow desensitization regulates the availability of synaptic GABA(A) receptors. *J Neurosci* **20:**7914–7921.
Overstreet LS and Westbrook GL (2001) Paradoxical reduction of synaptic inhibition by vigabatrin. *J Neurophysiol* **86:**596–603.
Pritchett DB, Sontheimer H, Shivers BD, Ymer S, Kettenmann H, Schofield PR, and Seeburg PH (1989) Importance of a novel GABA(A) receptor subunit for benzodiazepine pharmacology. *Nature (Lond)* **338:**582–585.
Saxena NC and Macdonald RL (1994) Assembly of GABAA receptor subunits—role of the delta-subunit. *J Neurosci* **14:**7077–7086.
Saxena NC and Macdonald RL (1996) Properties of putative cerebellar γ-aminobutyric acid(a) receptor isoforms. *Mol Pharmacol* **49:**567–579.
Shin RS, Anisman H, Merali Z, and McIntyre DC (2002) Changes in extracellular levels of amygdala amino acids in genetically fast and slow kindling rat strains. *Brain Res* **946:**31–42.
Stell BM, Brickley SG, Tang CY, Farrant M, and Mody I (2003) Neuroactive steroids reduce neuronal excitability by selectively enhancing tonic inhibition mediated by delta subunit-containing GABA(A) receptors. *Proc Natl Acad Sci USA* **100:**14439–14444.
Stell BM and Mody I (2002) Receptors with different affinities mediate phasic and tonic GABA(A) conductances in hippocampal neurons. *J Neurosci* **22:**1–5.
Timmerman W and Westerink BHC (1997) Brain microdialysis of GABA and glutamate: what does it signify? *Synapse* **27:**242–261.
Traynelis SF and Jaramillo F (1998) Getting the most out of noise in the central nervous system. *Trends Neurosci* **21:**137–145.
Twyman RE and Macdonald RL (1992) Neurosteroid regulation of GABAA receptor single-channel kinetic-properties of mouse spinal-cord neurons in culture. *J Physiol (Lond)* **456:**215–245.
Wafford KA, Bain CJ, Quirk K, McKernan RM, Wingrove PB, Whiting PJ, and Kemp JA (1994) A novel allosteric modulatory site on the GABAA receptor-beta subunit. *Neuron* **12:**775–782.
Wafford KA, Thompson SA, Thomas D, Sikela J, Wilcox AS, and Whiting PJ (1996) Functional characterization of human γ-aminobutyric $acid_A$ receptors containing the α4 subunit. *Mol Pharmacol* **50:**670–678.
Wall MJ (2002) Furosemide reveals heterogeneous GABA(A) receptor expression at adult rat Golgi cell to granule cell synapses. *Neuropharmacology* **43:**737–749.
Wei WZ, Zhang NH, Peng ZC, Houser CR, and Mody I (2003) Perisynaptic localization of delta subunit-containing GABA(A) receptors and their activation by GABA spillover in the mouse dentate gyrus. *J Neurosci* **23:**10650–10661.
Wohlfarth KM, Bianchi MT, and Macdonald RL (2002) Enhanced neurosteroid potentiation of ternary GABA(A) receptors containing the delta subunit. *J Neurosci* **22:**1541–1549.
Yeung JYT, Canning KJ, Zhu GY, Pennefather P, Macdonald JF, and Orser BA (2003) Tonically activated $GABA_A$ receptors in hippocampal neurons are high-affinity, low-conductance sensors for extracellular GABA. *Mol Pharmacol* **63:**2–8.
Zhang S, Takeda Y, Hagioka S, Takata K, Aoe H, Nakatsuka H, Yokoyama M, and Morita K (2005) Measurement of GABA and glutamate in vivo levels with high sensitivity and frequency. *Brain Res Brain Res Protoc* **14:**61–66.

Address correspondence to: Dr. Zakaria Mtchedlishvili, Department of Neurology, Box 800394, University of Virginia-Health Sciences Center, Charlottesville, VA 22908. E-mail: zm5e@virginia.edu