Gabapentin Increases a Tonic Inhibitory Conductance in Hippocampal Pyramidal Neurons

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Background: The mechanisms underlying the therapeutic actions of gabapentin remain poorly understood. The chemical structure and behavioral properties of gabapentin strongly suggest actions on inhibitory neurotransmission mediated by γ-aminobutyric acid (GABA); however, gabapentin does not directly modulate GABA_A or GABA_B receptors. Two distinct forms of GABAergic inhibition occur in the brain: postsynaptic conductance and a persistent tonic inhibitory conductance primarily generated by extrasynaptic GABA_A receptors. The aim of this study was to determine whether gabapentin increased the tonic conductance in hippocampal neurons in vitro. As a positive control, the effects of vigabatrin, which irreversibly inhibits GABA transaminase, were also examined.

Methods: GABA_A receptors in hippocampal neurons from embryonic mice were studied using whole cell patch clamp recordings. Miniature inhibitory postsynaptic currents and the tonic current were recorded from cultured neurons that were treated for 36–48 h with gabapentin, vigabatrin, or gabapentin and vigabatrin. To determine whether gabapentin increased the expression of GABA_A receptors, Western blots were stained with antibodies selective for α_1, α_2, and α_3 subunits.

Results: GABA_A receptors were insensitive to the acute application of gabapentin, whereas chronic treatment increased the amplitude of the tonic current threefold (EC_50 = 209 μM) but did not influence miniature inhibitory postsynaptic currents. Vigabatrin increased the tonic conductance, and the maximally effective concentration did not occlude the actions of gabapentin, which suggests that these compounds act by different mechanisms. Neither gabapentin nor vigabatrin increased the expression of GABA_A receptors in the neurons.

Conclusions: Gabapentin increases a tonic inhibitory conductance in mammalian neurons. High-affinity GABA_A receptors that generate the tonic conductance may detect small increases in the ambient concentration of neurotransmitter caused by gabapentin.

GABAPENTIN (1-aminomethyl) cyclohexanecacetic acid) is widely used as an analgesic and anticonvulsant. The therapeutic indications have recently expanded to include bipolar disorders,† motor neuron degeneration,‡ hot flashes,§ and acute postoperative pain.¶ Optimum dose schedules and therapeutic plasma levels for many of these disorders, most notably acute postoperative pain, are only beginning to be investigated.¶ Further- more, the molecular mechanisms underlying the therapeutic actions of gabapentin remain uncertain.¶

A high-affinity gabapentin binding site has been unequivocally identified in animal brain membranes as the auxiliary α_δ subunit of voltage-activated calcium channels.¶ Inhibition of voltage-gated calcium channels by gabapentin is thought to reduce the release of an excitatory neurotransmitter associated with the central sensitization that occurs in neuropathic pain.¶ Several lines of evidence suggest that a high-affinity site may not fully account for the behavioral effects of gabapentin, particularly with doses that exceed 3,600 mg/day.¶ High concentrations may occur in the brain after large doses because gabapentin easily crosses the blood–brain barrier via a high-affinity L-amino acid transporter.¶ Also, the increased expression of the α_δ subunit of voltage-gated calcium channels that occurs over several days in animal models of hyperalgesia is not a prerequisite for the short-term analgesic actions of gabapentin.¶ A comparison of the antinociceptive properties of gabapentin and stereoselective analogs of gabapentin has indicated that binding to the α_δ subunit does not completely account for gabapentin’s effects.¶ Therefore, it is plausible that multiple molecular targets underlie the therapeutic properties of gabapentin in vitro, as has been observed with narcotics,¶ nonsteroidal antiinflammatory drugs,¶ and other analgesic compounds.

Gabapentin is structurally related to the major inhibitory transmitter, γ-aminobutyric acid (GABA), as depicted in figure 1A. It is derived by addition of a cyclohexyl group to the carbon backbone of GABA and was originally designed as a GABA-mimetic compound that could readily cross the blood–brain barrier.¶ However, gabapentin does not bind to or directly modulate the function of ionotropic GABA_A receptors or metabotropic GABA_B receptors.¶ In humans, gabapentin causes a dose-dependent increase in the brain concentration of GABA that correlates with improved seizure control, as evidenced by functional magnetic resonance imaging studies.¶ In vitro studies show that gabapentin interacts with three cytosolic enzymes that regulate amino acid metabolism¶ and increases the synthesis of GABA in several brain regions, including the hippocampus.¶
aptic transmission.\textsuperscript{16} pentin reduced rather than increased inhibitory postsynaptic GABA compared with postsynaptic GAB\textsubscript{A} receptors, which suggests that the inhibitory charge generated by the tonic conductance is primarily generated by GABAergic inhibition in the behavioral effects of gabapentin, and thus prevents the breakdown of GABA\textsuperscript{28,29} and increases the ambient concentration of GABA.\textsuperscript{28,29} and gabapentin. (B) Effects of GABA type A (GAB\textsubscript{A}) receptor antagonists, positive allosteric modulators, and increased GABA on tonic conductance and miniature inhibitory postsynaptic current (mIPSC). The tonic current is revealed by an outward shift in the baseline ($I_{\text{baseline}}$) and a reduction in the holding current ($I_{\text{hold}}$) caused by GAB\textsubscript{A} receptor antagonists, which also inhibit mIPSCs, as evidenced by the loss of the transient inward currents. Positive allosteric modulators and increased ambient GABA increase $I_{\text{hold}}$ and shift the baseline inward.

The current study focuses on a novel form of tonic inhibition that is thought to be primarily generated by extrasynaptic GAB\textsubscript{A} receptors (fig. 1B). These receptors have a different subunit composition and different pharmacologic and biophysical properties than most postsynaptic receptors.\textsuperscript{22,25} The tonic conductance is activated by low ambient concentrations of GABA that have been released from neighboring synapses or from neurons and glia.\textsuperscript{22,24} In pyramidal neurons, the underlying GAB\textsubscript{A} receptors likely have a higher sensitivity to GABA compared with postsynaptic GAB\textsubscript{A} receptors and may be activated by low concentrations of extracellular GABA.\textsuperscript{25,25} Under certain experimental conditions, the inhibitory charge generated by the tonic conductance is several times greater than that generated by postsynaptic GAB\textsubscript{A} receptors, which suggests that tonic inhibition contributes to neuronal regulation.\textsuperscript{26} Indeed, a tonic inhibitory conductance recorded in cerebellar granule cells \textit{in vivo} was shown to regulate processing of somatosensory input.\textsuperscript{27} We tested the hypothesis that gabapentin increases a tonic inhibitory conductance in hippocampal neurons. For comparison and as a positive control, we also studied the effect of vigabatrin, a compound that inhibits GABA transaminase and thus prevents the breakdown of GABA\textsuperscript{28,29} and increases the ambient concentration of GABA and the tonic conductance.\textsuperscript{29–31}

**Materials and Methods**

**Cell Culture and Electrophysiologic Techniques**

Experiments were approved by the Animal Care Committee of the University of Toronto (Toronto, Ontario, Canada). Primary cultures of hippocampal neurons were prepared from embryonic Swiss White mice as previously described.\textsuperscript{32} Briefly, fetal pups (17 days \textit{in utero}) were removed from pregnant mice killed by cervical dislocation. The hippocampi were dissected from each fetus and placed in an ice-cooled culture dish. Neurons were then dissociated by mechanical trituration and plated on 35-mm culture dishes at a density of approximately $1 \times 10^6$ cells/ml. For the first 10 days \textit{in vitro}, the cells were maintained in minimum essential medium supplemented with 10% fetal bovine serum and 10% horse serum (Life Technologies, Grand Island, NY). The neurons were cultured at 36.5°C in an environment of 5% CO\textsubscript{2} and 95% air. After the background cells had grown to confluence, 0.1 ml of a mixture of 5-fluorodeoxyuridine (4 mg) and uridine (10 mg) in 20 ml minimum essential medium was added to the extracellular solution to reduce the number of dividing cells. Subsequently, the medium was supplemented with 10% horse serum and changed every 3 or 4 days. Cells were maintained in culture for 14–21 days, after which a tonic current could readily be detected. On the day of the experiments, the cultures were removed from the incubator and the medium was exchanged with an extracellular recording solution that contained a chemical buffer system designed for atmospheric conditions.

Conventional whole cell currents were recorded under voltage clamp conditions (–60 mV) by means of an Axopatch 200 amplifier (Molecular Devices Corporation, Sunnyvale, CA) interfaced with a Digidata 1200 (Instrutech Corp., Elmont, NY). Records were filtered at 2 kHz and digitized at 10 kHz for off-line analysis with pCLAMP\textsuperscript{6} software (Molecular Devices Corporation). The extracellular fluid contained 120 mM NaCl, 1.3 mM CaCl\textsubscript{2}, 5.4 mM KCl, 30 mM HEPES, and 28 mM glucose; the pH was adjusted to 7.4 by adding 1 M NaOH. Tetrodotoxin (300 M) was added to the extracellular fluid to block voltage-sensitive sodium channels, and 6-cyano-2,3-dihydroxy-7-nitroquinoxaline (10 M) and 2-amino-5-phosphonovalerate (40 M) were added to inhibit ionotropic glutamate receptors. Recording electrodes were filled with a solution that contained 120 mM CsCl, 30 mM HEPES, 11 mM EGTA, 2 mM MgCl\textsubscript{2}, 1 mM CaCl\textsubscript{2}, 4 mM MgATP, and 2 mM tetraethylammonium chloride; the pH was adjusted to 7.3 by adding 2 M CsOH. Extracellular fluid and drug-containing solutions were delivered to the cultured neurons through glass perfusion barrels that were positioned close to the somata.

**Measurement of Tonic and Synaptic Inhibitory Conductance**

Whole cell recordings were obtained from the neurons cultured for 14–21 days. Two protocols for gabapentin...
application were selected. To determine whether gabapentin directly modified the function of the GABA$_A$ receptors that generated the tonic and synaptic conductances as well as current evoked by exogenous GABA, gabapentin was applied acutely to the neurons. To determine whether prolonged exposure to gabapentin would increase the tonic and synaptic conductances, neurons were preexposed to gabapentin for 36–48 h. The neurons were also treated with vigabatrin or a combination of gabapentin and vigabatrin for 36–48 h before the recordings. We previously observed that the maximum effect of vigabatrin on the tonic conductance occurred after 36–48 h of treatment. The same period was used before the recordings. We previously observed that the maximum effect of vigabatrin and gabapentin was observed after 36–48 h of treatment. The same period was used for the maximum effect of vigabatrin on the tonic conductance. The same period was used for the maximum effect of vigabatrin on the tonic conductance. The neurons were preexposed to gabapentin for 36–48 h. The same period was used for the maximum effect of vigabatrin on the tonic conductance. The neurons were preexposed to gabapentin for 36–48 h.

Measurement of GABA-evoked Current

For some experiments, GABA$_A$ receptor function was tested by application of exogenous GABA at either a subsaturating concentration (3 μm, EC$_{50}$) or a saturating concentration (600 μm) for 2 s, followed by a washout period of 1 or 2 min, respectively, to allow the GABA$_A$ receptors to recover from desensitization. Gabapentin was applied for at least 30 s before the application of a test pulse of GABA plus gabapentin.

The GABA sensitivity of GABA$_A$ receptor in neurons chronically treated with gabapentin (300 μm) was studied by constructing a concentration–response plot. GABA (0.1–600 μm) was applied for 10 s with a 2-min washout period between each application. The peak GABA-activated current was recorded at each concentration. Concentration–response curves were generated by fitting the data using nonlinear regression analysis and the sigmoidal equation described above. The amplitude of the maximum current generated by a saturating concentration of GABA (600 μm) was also measured.

Membrane Preparation and Receptor Solubilization

Membranes from cultured neurons were prepared as described elsewhere with all of the reagents stored on ice. Briefly, the neurons were scraped from the culture dishes and washed with phosphate-buffered saline (Invitrogen, Carlsbad, CA). The cells were then disrupted through a 22-gauge needle and lysed on ice for 40 min in 1 ml radioimmunoprecipitation assay lysis buffer (50 mM Tris-HCl, pH 8.0; 150 mM NaCl; 0.5% sodium deoxycholate; 0.1% sodium dodecyl sulfate; and 5 mM EDTA supplemented with protease inhibitors: 25 mM benzamidine (Sigma-Aldrich, St. Louis, MO), 0.1 mM sodium orthovanadate (Sigma-Aldrich), 5 μg/ml aprotinin (Sigma-Aldrich), 4 mM Pefabloc (Roche Applied Science, Laval, Quebec, Canada), 20 μg/ml leupeptin (Roche), and 1 μg/ml pepstatin (Roche)) per culture dish. The lysates were centrifuged for 20 min (10,000g at 4°C), and the supernatant was collected. The protein concentrations of the supernatant were determined with use of the bicinchoninic acid protein assay reagent (Pierce Biotechnology, Rockford, IL).

Western Blot Detection of Subunit-containing GABA$_A$ Receptors

Protein samples were resolved on 10% sodium dodecyl sulfate–polyacrylamide gels and transferred to nitrocellulose membranes. Blots were incubated in blocking buffer containing 5% nonfat milk in Tween-Tris–buffered saline (500 mM NaCl, 20 mM Tris Base, and 0.1% Tween-20, pH 7.5) for 1 h at room temperature with α$_1$, α$_2$, or α$_6$ antibody rabbit polyclonal primary antibodies at dilutions of 1:1,000, 1:200, and 1:1,000, respectively. The α$_1$ antibody was purchased from Upstate Biotechnology Inc. (Waltham, MA), and the α$_6$ antibody was purchased from Alomone Labs (Jerusalem, Israel). The α$_6$ antibody,
which was previously described, was donated by Werner Sieghart, Ph.D. (Professor, Division of Biochemistry and Molecular Biology, Vienna University Medical School, Vienna, Austria). The blots were then washed for 10 min four times with Tween-Tris-buffered saline and incubated for 1 h at room temperature in horse radish peroxidase-conjugated goat anti-rabbit antibody (Amer- sham Biosciences, Little Chalfont, Buckinghamshire, England) at a dilution of 1:10,000 in blocking buffer. The blots were washed again for 10 min four times in Tween-Tris-buffered saline, developed with SuperSignal West Pico for detection, stripped with Restore Western Blot Stripping Buffer, and reprobed with 1:1,000 anti-α-actinin antibody (Sigma-Aldrich, Oakville, Ontario, Canada) and horseradish peroxidase-conjugated donkey anti- mouse antibody (Amersham). The immunoblots were scanned, and the optical density of the positive bands was measured with a calibrated gray scale. The density of α-subunit staining was normalized to that of α-actinin. The experiments were repeated three times for each experimental condition with the use of homogenates from three animals. The values for protein from the drug-treated cultures were normalized to those for vehicle controls.

Materials

Bicuculline methiodide, picrotoxin, and gabapentin were purchased from Sigma-Aldrich. Vigabatrin, baclofen, and CGP55845 were obtained from Tocris Cook- son (Ellisville, MO). All stock solutions were prepared by dissolving the appropriate amount of drug in distilled water, except for picrotoxin and CGP55845, which were dissolved in ethanol and dimethyl sulfoxide, respectively. The solutions were then filtered and stored at 4°C until used. The vehicle solutions did not influence the tonic conductance at the concentrations used.

Statistical Analysis

Results are presented as mean ± SEM. Data were scrutinized for deviations from normality and appropriately transformed. Statistical significance was determined with a paired or unpaired Student t test, as appropriate. When multiple groups were compared, an analysis of variance and Dunnett multiple comparison post hoc test were used. Differences between groups were considered significant at P < 0.05.

Results

The effects of gabapentin were assessed with the use of several electrophysiologic protocols. First, to examine direct effects of gabapentin on the function of GABA receptors, current was evoked by applying exogenous GABA at a sub saturating or a saturating concentration.

The GABA concentrations, 3 and 600 μM, were selected to mimic those that activate tonic and synaptic conductances, respectively. As shown in figure 2, gabapentin (300 μM) had no effect on the amplitude or time course of the current evoked by 3 μM GABA (control, 754.8 ± 54.1 pA; gabapentin, 732.6 ± 66.1 pA; n = 6) or 600 μM GABA (8.39 ± 0.61 and 8.12 ± 0.52 nA, respectively; n = 4). Similarly, gabapentin (1 mM) did not influence the current evoked by 3 μM GABA (265.3 ± 69.6 and 256.0 ± 67.4 pA, respectively; n = 3). These results confirm that gabapentin does not directly increase or reduce the function of GABA_A receptors in pyramidal neurons.

Next, the short-term effect of gabapentin on a tonic inhibitory conductance generated by endogenous neurotransmitter was studied. In keeping with our previous findings, pyramidal neurons grown in primary cultures generated a tonic inhibitory conductance that was revealed by applications of the GABA_A receptor antagonists bicuculline and picrotoxin. The antagonists decreased I_hold and reduced the baseline noise, consistent with a reduction in the number of open receptors. Brief application of gabapentin, at concentrations up to 1 mM, did not influence I_hold even when cells were patched and observed for up to 30 min (ΔI_hold with 300 μM gabapentin, 0.1 ± 0.9 pA; n = 5). Therefore, gabapentin had no direct effect on the GABA_A receptors that generated the tonic inhibitory conductance.

To determine whether prolonged exposure to gaba- pentin modified the tonic conductance, neurons were incubated for 36–48 h with various concentrations of gabapentin or a vehicle control. Gabapentin caused a concentration-dependent increase in the amplitude of the tonic conductance, as shown in figure 3A. The EC_{50} of gabapentin, determined by fitting the concentration-response plot, was 209 μM (95% confidence interval, 172–254 μM; figs. 3B and C). The maximum increase in
1mM did not further increase the tonic conductance variance). Increasing the concentration of gabapentin to applying bicuculline and measuring the change in I hold.

GBP 300 μM

BAC 100 μM

GBP 300 μM

CGP 1 μM

4 s

50 pA

4 s

50 pA

Fig. 4. Application of the γ-aminobutyric acid type B receptor modulators CGP 55845 (CGP; 1 μM) and baclofen (BAC; 100 μM) did not modify the tonic conductance in neurons treated with gabapentin (GBP; 300 μM) for 36–48 h. The superimposed all-points histograms for current recorded before and during drug application indicate no change in the holding current.

was washed away, and picrotoxin (1 mM) was applied. Picrotoxin and bicuculline caused a similar reduction in \( I_{\text{hold}} \), which confirmed that GABA\(_A\) receptors generated the tonic conductance (data not shown). Furthermore, ion channels regulated by GABA\(_B\) receptors did not contribute to the tonic conductance, because application of a GABA\(_B\) receptor antagonist (CPG 55845; 1 μM) and a GABA\(_B\) receptor agonist (baclofen 100 μM) did not influence \( I_{\text{hold}} \) \( \Delta I_{\text{hold}} \equiv -0.4 \pm 0.5 \) pA, \( n = 11 \) and 0.0 ± 0.6 pA, \( n = 12 \), respectively; fig. 4).

In contrast to the nearly threefold increase in tonic conductance, prolonged exposure to gabapentin (300 μM) did not affect the frequency, amplitude, rise time, or time course of mIPSCs (table 1).

Gabapentin is reported to be a weak inhibitor of GABA transaminase, whereas vigabatrin is a potent inhibitor with irreversible effects. Several laboratories, including ours, have reported that vigabatrin increases the tonic conductance in hippocampal pyramidal neurons, presumably by increasing the concentration of GABA in the extracellular fluid. Vigabatrin caused a concentration-dependent increase in the tonic current, as shown in figures 5A and B. It was then postulated that if vigabatrin and gabapentin act through identical mechanisms, a maximally effective concentration of vigabatrin should occlude the actions of gabapentin. Neurons were pretreated with vigabatrin (100 μM or 1 mM) or a combination of gabapentin (300 μM) and vigabatrin (100 μM) for 36–48 h. The concentrations selected for coapplication caused a maximal increase in the tonic current when applied alone. As shown in figure 5C, coapplication had an additive effect: The increase was greater than that caused by gabapentin or vigabatrin alone (\( P < 0.05 \) by analysis of variance). Therefore, vigabatrin and gabapentin seem to act, at least in part, through different mechanisms.

An increase in tonic conductance results from an increase in either the expression of GABA\(_A\) receptors or the probability of channel opening. The most plausible explanation is an increase in the ambient concentration tonic current, 2.7-fold, was observed at a gabapentin concentration of 300 μM (control, 7.5 ± 1.1 pA; gabapentin, 23.6 ± 4.0 pA; n = 29, \( P < 0.05 \) by analysis of variance). Increasing the concentration of gabapentin to 1 mM did not further increase the tonic conductance (control, 8.9 ± 1.8 pA; gabapentin, 21.5 ± 4.6 pA; n = 10; \( P < 0.05 \)).

The amplitude of the tonic current was studied by applying bicuculline and measuring the change in \( I_{\text{hold}} \). However, bicuculline also inhibits several subtypes of potassium channels that might be influenced by gabapentin. To ensure that the bicuculline-sensitive conductance was generated by GABA\(_A\) receptors, bicuculline...
of a ligand that activates GABA<sub>δ</sub> receptors, which in turn increases the probability of channel opening. Nevertheless, gabapentin could increase the total number of high-affinity receptor subtypes expressed on the surface of neurons. In hippocampal pyramidal neurons, tonic conductance is primarily generated by α<sub>δ</sub> subunit-containing GABA<sub>δ</sub> receptors, whereas lower-affinity synaptic GABA<sub>δ</sub> receptors in hippocampal pyramidal neurons likely contain α<sub>1</sub> or α<sub>2</sub> subunits. To determine whether gabapentin increased the number of synaptic or extrasynaptic GABA<sub>δ</sub> receptors, we performed semiquantitative Western blot analyses using subunit-selective antibodies. Homogenates were prepared from cultured neurons treated with placebo control, gabapentin (300 μM), or vigabatrin (100 μM) for 36–48 h. Experiments were performed three times with three sets of cultures, and the results were averaged. As shown in figures 6A and B, no differences were detected in the expression of subunit proteins after chronic treatment with gabapentin or vigabatrin. Therefore, the increase in tonic conductance by gabapentin or vigabatrin could not be attributed to increased expression of GABA<sub>δ</sub> receptors that generate the tonic conductance in hippocampal neurons.

We next determined whether the sensitivity of GABA<sub>δ</sub> receptors to gabapentin was changed by chronic treatment with gabapentin (300 μM). Concentration–response plots for current evoked by exogenous GABA revealed no difference in the EC<sub>50</sub> values for gabapentin-treated and control neurons (12.5 ± 0.9 μM, n = 6 vs. 11.7 ± 1.0 μM, n = 6, respectively; P > 0.05; fig. 6C). Also, the maximum amplitude of the current evoked by a saturating concentration of GABA did not change after chronic treatment with gabapentin (300 μM) compared with control (6.8 ± 1.0 nA, n = 6 vs. 7.6 nA ± 1.5 pA, n = 6, respectively; P > 0.05). Therefore, the increase in tonic current by gabapentin was not due to an increase in the number of expression of high-affinity GABA<sub>δ</sub> receptors on the cell surface.

### Discussion

This study has provided the first evidence that gabapentin has different actions on extrasynaptic and synaptic GABA<sub>δ</sub> receptors in hippocampal pyramidal neurons. Gabapentin increased tonic inhibitory conductance but did not change the frequency, amplitude, or time course of mIPSCs. The increase in tonic conductance could not be attributed to direct positive allosteric modulation of GABA<sub>δ</sub> receptors or to an increase in receptor expression. The most plausible explanation is that gabapentin indirectly increased tonic conductance by increasing the ambient level of neurotransmitter. Extrasynaptic GABA<sub>δ</sub> receptors have a higher affinity for GABA than do postsynaptic receptors. Hence, extrasynaptic receptors may act as biosensors, detecting small increases in neurotransmitter that do not modulate postsynaptic receptors. Alternatively, gabapentin may have increased the release of transmitter at sites that reside in close proximity to extrasynaptic but not synaptic GABA<sub>δ</sub> receptors. Our results also suggest that neuronal cultures can be used for future studies of the functional and biochemical consequences of long-term exposure to gabapentin.

These in vitro results certainly do not preclude actions on high-affinity sites such as voltage-gated calcium channels containing the α<sub>δ</sub> subunit in vivo. Nevertheless, they do suggest that gabapentin (as with most analgesics) may exert multiple effects, particularly at higher doses. The enhancement of the tonic current that we observed was consistent with the previous observation from electrophysiologic recordings in rat optic nerves that gabapentin (100 μM) increased responses caused by the nonvesicular release of GABA. Another study showed that gabapentin (100 μM) increased a GABAergic conductance activated by nipecotic acid, a drug that inhibited the reuptake of GABA in rat hippocampal slices. Gabapentin increased the release of [3H]GABA in slices from rat neostriatum incubated in vitro. The gabapentin concentration–response plot was bell-shaped, with 0.1 μM nearly doubling the release of [3H]GABA, whereas 1 and 10 μM were without effect. In studies of the human brain using ex vivo magnetic resonance spectroscopy analysis of necortical slices, treatment with gabapentin (100 μM) for 1 h increased the concentration of GABA. In vivo imaging of human occipital cortex with [1H] nuclear magnetic resonance spectroscopy also showed a modest increase in GABA levels.

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**Table 1. Spontaneous mIPSCs Recorded in Pyramidal Neurons Showed No Change in Frequency, Amplitude, or Time Course after Treatment with Gabapentin or Vigabatrin for 36–48 Hours**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Amp, pA</th>
<th>Rise, ms</th>
<th>Q, pA · ms</th>
<th>Decay, ms</th>
<th>RMS, pA</th>
<th>Freq, Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7</td>
<td>35.7 ± 6.0</td>
<td>2.3 ± 0.3</td>
<td>784 ± 139</td>
<td>23.4 ± 3.4</td>
<td>3.5 ± 0.3</td>
<td>0.85 ± 0.2</td>
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<tr>
<td>VIG (100 μM)</td>
<td>7</td>
<td>48.0 ± 5.4</td>
<td>1.7 ± 0.3</td>
<td>1,089 ± 120</td>
<td>24.6 ± 2.0</td>
<td>4.9 ± 0.7</td>
<td>0.62 ± 0.2</td>
</tr>
<tr>
<td>GBP (150 μM)</td>
<td>8</td>
<td>34.2 ± 5.0</td>
<td>2.2 ± 0.2</td>
<td>812 ± 150</td>
<td>22.6 ± 1.1</td>
<td>3.7 ± 0.5</td>
<td>0.87 ± 0.3</td>
</tr>
<tr>
<td>GBP (300 μM)</td>
<td>6</td>
<td>39.8 ± 5.1</td>
<td>2.5 ± 0.3</td>
<td>875 ± 89</td>
<td>18.8 ± 1.2</td>
<td>5.5 ± 0.2</td>
<td>1.19 ± 0.4</td>
</tr>
<tr>
<td>GBP (600 μM)</td>
<td>6</td>
<td>34.9 ± 3.5</td>
<td>2.4 ± 0.2</td>
<td>915 ± 112</td>
<td>24.7 ± 2.6</td>
<td>4.4 ± 1.2</td>
<td>1.01 ± 0.4</td>
</tr>
<tr>
<td>GBP (300 μM) + VIG (100 μM)</td>
<td>5</td>
<td>43.2 ± 5.9</td>
<td>1.9 ± 0.1</td>
<td>1,038 ± 154</td>
<td>20.8 ± 3.0</td>
<td>6.0 ± 0.8</td>
<td>0.78 ± 0.4</td>
</tr>
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</table>

Amp = mIPSC amplitude; Decay = 10–90% decay time; Freq = frequency of mIPSCs; GBP = gabapentin; mIPSC = miniature inhibitory postsynaptic current; Q = charge transfer; Rise = 10–90% rise time; RMS = root mean square deviation of current noise; VIG = vigabatrin.
Gabapentin did not increase GABA concentrations in mouse brains. Compared with the previous in vitro studies, our results suggest that high concentrations of gabapentin (EC$_{50}$ 209 µM) are required to increase the level of ambient transmitter. The reasons underlying the apparent low sensitivity of the tonic conductance to enhancement by gabapentin are unknown. Gabapentin likely indirectly activates the tonic conductance by increasing the concentration of agonist at the GABA$_A$ receptor. The

![Fig. 6. Gabapentin (GBP) and vigabatrin (VIG) did not alter the expression of α$_1$, α$_2$, or α$_5$ subunits in hippocampal neurons. (A) Western blots were probed separately with antibodies against the various subunits. Molecular mass (in kilodaltons) is indicated to the left, and subunit antibody is indicated to the right. (B) Quantitative densitometry showed that neither GBP nor VIG altered the expression of the α$_1$, α$_2$, and α$_5$ subunits, as summarized in the bar chart (n = 3 for each condition). The results after drug treatment were normalized to placebo control. (C) The concentration–response plot for γ-aminobutyric acid (GABA)–evoked current recorded from GBP-treated and vehicle control (CONT)–treated neurons. The amplitude of the current ($I_{MAX}$) was normalized to the maximal response ($I_{MAX}$). The GABA EC$_{50}$ values for GBP-treated and CONT neurons, estimated from the fitted curves, were 12.5 ± 0.9 µM, n = 6 versus 11.7 ± 1.0 µM, n = 6, respectively (P > 0.05).]
low density of neurons and glia in neuronal cultures compared with brain slices may release lower amounts of transmitter, and therefore, a higher gabapentin concentration is required to detect an effect. Clearly, in vitro models, such as cultured neurons, do not reflect the complexity of GABA transport and metabolism or GABA<sub>Α</sub> receptor activation in vivo. Nevertheless, such models have been used by numerous laboratories to gain fundamental insights into the pharmacologic features of GABA<sub>Α</sub> receptors.

Despite an increase in tonic conductance, gabapentin did not alter spontaneous postsynaptic inhibitory transmission. The later finding contrasts with a 37% reduction of evoked inhibitory postsynaptic current by gabapentin (300 μM) recorded in CA1 neurons in brain slices. The reduction in evoked synaptic currents may be attributed to inhibition of presynaptic voltage-gated calcium channels. A reduction in the concentration of cytosolic calcium in the presynaptic terminal may reduce the synchronized release of vesicular GABA during evoked stimulation. Unlike evoked inhibitory postsynaptic current, tonic conductance is likely generated by GABA that is released by calcium- and soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE)-independent mechanisms. Tonic conductance is insensitive to calcium channel blockers and botulinum toxin and occurs in neurons from Munc18-1–deficient mice, in which vesicular release is abolished. Our results are consistent with the notion that gabapentin preferentially increases the nonvesicular release of GABA.

Our results showed that a maximally effective concentration of vigabatrin did not occlude the action of gabapentin on tonic conductance. These results can be interpreted in several ways. Vigabatrin and gabapentin may act by different mechanisms. Biochemical studies have shown that gabapentin increases the activity of glutamic acid decarboxylase (GAD), the principal enzyme for GABA synthesis, and and causes sevenfold and twofold increases in the activity of the two isoforms of GAD that occur in neurons, GAD<sub>65</sub> and GAD<sub>67</sub>, respectively. Vigabatrin irreversibly inhibits GABA transaminase, the principal enzyme that degrades cytosolic GABA to succinic acid. Gabapentin’s inhibition of GABA transaminase is partially reversible. Gabapentin may therefore act at a different regulatory site on the enzyme than vigabatrin. Alternatively, gabapentin may exert actions independent of GABA transaminase and GAD activity. For example, gabapentin may increase the levels of other amino acids, such as β-alanine and taurine, which activate GABA<sub>Α</sub> receptors. Future studies will investigate these possibilities.

We observed that chronic treatment with vigabatrin did not influence the amplitude or frequency of mIPSCs in cultured hippocampal pyramidal neurons as previously reported. This finding contrasts with a dramatic reduction in frequency of mIPSCs by vigabatrin (100–400 μM) also reduced the frequency of mIPSCs without changing the time course of current decay. The authors attributed the reduction in frequency by vigabatrin, in part, to the accumulation of extracellular GABA and desensitization of GABA<sub>Α</sub> receptors. The reduction in frequency of mIPSCs frequency by gabapentin was attributed to GABA activation of presynaptic GABA<sub>Β</sub> receptors because the decrease in frequency was not observed in the presence of the GABA<sub>Β</sub> receptor antagonist CGP55845. Reasons underlying differences in vigabatrin sensitivity of postsynaptic currents in hippocampal pyramidal neurons compared with dentate granule cells are unknown but may relate to the duration of drug exposure, differences in the subunit composition of the underlying GABA<sub>Α</sub> receptors, or factors that regulate GABA metabolism in different populations of neurons.

Our results showed that the gabapentin EC<sub>50</sub> value for enhancement of the tonic inhibition was approximately 200 μM. The concentration of gabapentin that increased tonic inhibitory conductance in vitro is considerably higher than the therapeutic concentrations reported for the treatment of pain or seizure control. The high concentrations of gabapentin required to affect the tonic current may be relevant to the toxic effects such as memory impairment. The tonic conductance in hippocampal pyramidal neurons is primarily generated by GABA<sub>Α</sub> receptors containing the α<sub>3</sub> subunit. This subpopulation of GABA receptors has been strongly implicated in learning and memory processes. An increase in tonic inhibition is predicted to impair memory performance, as observed with general anesthetics. Therefore, we speculate that increased tonic inhibition in the hippocampus might contribute to the memory impairment reported with gabapentin treatment. Consistent with this notion, mild cognitive deficits were reported in healthy patients after gabapentin treatment compared with drug-free conditions. Any relevance of gabapentin action on the tonic current to pain control is highly speculative. Therefore, it will be of interest to determine in future studies whether lower concentrations of gabapentin increase the tonic conductance in experimental models where the architecture of neuronal networks remains intact.

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