Paradoxical Reduction of Synaptic Inhibition by Vigabatrin

LINDA S. OVERSTREET AND GARY L. WESTBROOK
Vollum Institute, Oregon Health Sciences University, Portland, Oregon 97201

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Overstreet, Linda S. and Gary L. Westbrook. Paradoxical reduction of synaptic inhibition by vigabatrin. J Neurophysiol 86: 596–603, 2001. GABAergic inhibition, a primary target for pharmacological modulation of excitability in the CNS, can be altered by multiple mechanisms including alteration of GABA metabolism. Gamma-vinyl GABA (vigabatrin, GVG) is an irreversible inhibitor of the GABA catabolic enzyme GABA transaminase, thus its anticonvulsant properties are thought to result from an elevation of brain GABA levels. We examined the effects of GVG on GABAergic synaptic transmission in hippocampal slices. GVG unexpectedly reduced miniature and evoked inhibitory postsynaptic currents (IPSCs) in dentate granule cells. The reduction in synaptic events was accompanied by an increase in tonic GABA receptor-mediated current. These effects developed slowly and persisted following wash out of GVG. The GVG pretreatment reduced sucrose-evoked GABA release as well as postsynaptic sensitivity to exogenous GABA, indicating that both pre- and postsynaptic mechanisms contributed to the reduction in synaptic currents. These results suggest that tonic rather than phasic increases in GABA underlie the anticonvulsant properties of GVG, and that mechanisms that elevate brain neurotransmitter levels do not necessarily correlate with enhanced synaptic release.

Introduction

The GABA synthetic enzyme glutamate decarboxylase (GAD) and the catabolic enzyme GABA transaminase affect CNS excitability by regulating transmitter levels in GABAergic neurons. Experimental manipulation of GAD activity suggests that the cytoplasmic GABA concentration in presynaptic terminals may influence vesicular GABA release. For example, genetic deletion of GAD65, the GAD isoform located in nerve terminals, reduces K+–induced GABA release (Hensch et al. 1998) as well as GABA that is released during sustained stimulation (Tian et al. 1999). The increased seizure susceptibility observed in GAD65 knockout mice may be a consequence of reduced GABA release (Asada et al. 1996; Kash et al. 1997). Pharmacological inhibition of GAD activity also reduces GABA release (Golan and Grossman 1996), and it has been proposed that estradiol suppresses GABAergic inhibition via inhibition of GAD (Murphy et al. 1998). Surprisingly, the reduction in synaptic GABA release in GAD65-deficient animals is due to fewer vesicles released rather than a decrease in the number of molecules per vesicle (Tian et al. 1999). Together these results suggest that the level of GABA in the presynaptic terminal influences the release of synaptic vesicles.

Presynaptic GABA levels may be elevated by interfering with its degradation. For example, gamma-vinyl GABA (vigabatrin, GVG), an irreversible inhibitor of GABA transaminase, increases GABA levels in synaptosomes (Lüscher et al. 1989). In addition to elevating the intracellular concentration of GABA (Jung et al. 1977), GVG increases the basal level of extracellular GABA and K+–stimulated GABA release (Abdul-Ghani et al. 1981; Neal and Shah 1989; Neal and Fowler 1997; Qume et al. 1995). The anticonvulsant properties of GVG in animal models of temporal lobe epilepsy and human partial epilepsy are thought to arise from an elevation of brain GABA levels. But it is not clear whether the anticonvulsant properties result from the elevation in basal extracellular GABA or from an increase in activity-dependent GABA release.

We investigated the action of GVG on GABAergic synaptic transmission in dentate granule cells of rat hippocampal slices. Contrary to the notion that elevated intracellular GABA enhances synaptic GABA release, our results indicate that GVG dramatically reduces evoked GABA release. This reduction was accompanied by an increase in ambient GABA as measured by tonic GABA receptor–mediated currents. Our results suggest that the anticonvulsant properties of GVG result from an elevation of basal extracellular GABA rather than enhanced synaptic release.

Methods

Whole cell voltage-clamp recordings of granule cells in transverse hippocampal slices were made from postnatal day 14–20 Sprague-Dawley rats. During recordings, slices were continuously perfused with an extracellular solution containing (in mM) 125 NaCl, 25 NaHCO3, 2.5 KCl, 1.25 NaH2PO4, 2 CaCl2, 1 MgCl2, and 25 d-glucose, bubbled with 95% O2–5% CO2. Patch pipettes were filled with (in mM) 140 KCl, 10 EGTA, and 2 Mg2+ATP, adjusted to pH 7.3 and 310 mOsm (1.5–4 M resistance). Visually identified granule cells were voltage clamped at −70 mV using an Axopatch 200B amplifier (Axon Instruments) and maintained at room temperature. Currents were filtered at 2 kHz and sampled at 10 kHz. Miniature inhibitory postsynaptic current events (mIPSCs) were isolated by adding TTX (0.5 μM) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 5 μM) to the extracellular solution. CGP55845 (1–2 μM) was used to block GABA.A receptors. GVG (100–400 μM) was either included in the incubation chamber (pretreatment), or bath applied in the recording chamber (acute treatment). mIPSCs were detected with the template-matching procedure of Axograph 4.0 (Axon Instruments), using the sum of one rising (τ = 400 μs) and one falling (τ = 20 ms) exponential as a template. To prevent overlapping events, the mIPSC decay time course was measured by averaging the subset of events separated by ≥100 ms. The decay was fitted with the

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sum of two exponential functions, and the weighted decay was calculated from the equation $A_1 \tau_1 + A_2 \tau_2$, where $A$ is the relative amplitude of each component and $\tau$ is its time constant. Tonic GABA$_A$ receptor–mediated currents were measured during a 1-min segment in the presence and absence of SR95531 (5 μM). All points were fitted with a Gaussian function. Because the amplitude of tonic GABAergic currents was small and occasionally obscured by gradual changes in the holding current, tonic currents were sometimes reported as the standard deviation of the current. IPSCs were evoked with a bipolar stimulating electrode (FHC, Bowdoinham, ME) placed in the granule cell layer (0.1 ms duration, 2–20 mV). For measurements of $E_{eq}$, CsCl replaced KCl, and QX314 (10 mM) was included in the intracellular solution to block voltage-gated $K^+$ and Na$^+$ currents, respectively. Junction potentials were not compensated. GABA and high osmolar solutions (extracellular solution increased to 650 mOsmol with sucrose) were applied via pressure compensation. GABA and high osmolar solutions (extracellular solution increased to 650 mOsmol with sucrose) were applied via pressure ejection (2–4 psi, Picospritzer, General Valve) from a recording pipette placed near the soma of the recorded cell. Series resistance was monitored throughout each experiment (4–12 MΩ before 70–90% compensation); data were discarded if substantial increases were observed. Data are expressed as means ± SE. Unless noted, statistical significance was determined by two-tailed t-tests or Mann-Whitney U tests at the $P < 0.05$ level.

All drugs were dissolved in water or DMSO (final concentration <0.3%) and added to the bath perfusion solution or incubation chamber. CNQX, 2-amino-5-phosphonopentanoic acid (AP5), and CGP55845 were purchased from Tocris Cookson (Ballwin, MO). 1-(2-(Diphenylmethylene)imino)oxyethyl)-1,2,5,6-tetrahydropyridine-carboxylic acid (NO-711) and SR95531 were purchased from Research Biochemicals (Natick, MA). GABA, gamma-vinyl GABA, strychnine, and TTX were purchased from Sigma (St. Louis, MO).

RESULTS

**Vigabatrin pretreatment reduces GABAergic IPSCs**

To examine the effect of elevated GABA on inhibitory synaptic transmission, hippocampal slices were incubated for 2–5 h in GVG (100–400 μM). This protocol resulted in an accumulation of extracellular GABA (see below) following irreversible inhibition of GABA transaminase. Recordings were made from pretreated slices following wash out of GVG (>20 min). GVG pretreatment resulted in a concentration-dependent reduction in mIPSCs recorded in 5 μM CNQX, 0.5 μM TTX, and the GABA$_A$ receptor antagonist CGP55845 (2 μM, Fig. 1). GVG (400 μM) reduced the median mIPSC amplitude by 57% (10.9 ± 1.0 pA, mean ± SE, $n = 11$, vs. 25.2 ± 2.5 pA in untreated controls, $n = 8$) and greatly reduced the mIPSC frequency (interevent interval 10 ± 4 s vs. 1.0 ± 0.2 s in controls). The decay of mIPSCs was unaffected by GVG (Fig. 1C, inset). Thus GVG pretreatment did not affect the gating of the underlying channels, but fewer channels were activated.

Consistent with the dramatic reduction in mIPSCs, GVG pretreatment also reduced the amplitude of evoked IPSCs. GVG pretreatment (200 μM) reduced the amplitude of IPSCs evoked by focal stimulation of the granule cell layer in CNQX (591 ± 167 pA following GVG pretreatment, $n = 11$, vs. 1,099 ± 156 pA in untreated controls, $n = 6$, not shown). Evoked IPSCs were reduced in GVG-pretreated slices even though greater stimulus intensities (10 ± 2 mV vs. 3.6 ± 0.5 mV in untreated controls) were required to evoke responses. However, GVG did not appear to effect the release probability or the GABA transient. Paired-pulse depression (200-ms interval) was not affected by GVG pretreatment (0.82 ± 0.03, $n = 7$ vs. 0.88 ± 0.06 in untreated controls, $n = 6$), suggesting that there was not a sizeable change in the probability of release. Displacement of a low-affinity antagonist by synaptically released transmitter can be used to estimate the concentration profile of the transmitter in the cleft (Clements et al. 1992). The low-affinity GABA$_A$ receptor antagonist SR95301 (5 μM) (Jones et al. 1998) reduced the IPSC by a similar degree in GVG-pretreated and control cells (by 62% vs. 61 ± 5% in untreated controls, $n = 3$), suggesting that GVG did not alter the GABA transient in the synaptic cleft.

**Vigabatrin pretreatment elevates tonic GABA**

GVG pretreatment increased baseline current noise by 177% ($n = 13$ control, $n = 17$ GVG). A saturating concentration of the high-affinity GABA$_A$ receptor competitive antagonist SR95531 reduced the baseline noise by 60 ± 5% in GVG-

![Image](http://jn.physiology.org/)}
treated slices \((n = 8)\), compared with \(30 \pm 12\%\) in control slices \((n = 7, \text{Fig. 2})\). This indicates that the reduction in mIPSC amplitude and frequency was accomplished by an elevation of ambient extracellular GABA. Tonic GABA\(_A\) receptor–mediated currents were quantified by making Gaussian fits to all-points histograms from stable 1-min current segments in the presence and absence of the antagonist. The contribution of synaptic events to the Gaussian fits was negligible. When GABA\(_A\) receptors were blocked with SR95531, there was no difference in the mean or standard deviation of the membrane noise between control and GVG-pretreated cells \((13.0 \pm 1.2\ pA, \ n = 8, \text{vs.} 15.6 \pm 1.8\ pA \text{in control, } n = 7)\).

We next compared the effects of acute applications of GVG or exogenous GABA to untreated slices in the presence of TTX, CNQX, and CGP55845. In each cell GVG increased tonic current fluctuations after several minutes of application (Fig. 3A, asterisk). After 20 min, GVG increased the current to \(221 \pm 11\% \ (n = 5)\), compared with \(105 \pm 7\%\) in untreated control cells \((n = 5)\). Acute treatment with GVG reduced the mIPSC amplitude \((69 \pm 4\% \text{ control})\), while the interevent interval was unaffected \((105 \pm 11\%\) \text{in control slices})\). In control slices the mIPSC amplitude and interevent interval were stable over the same time period \((n = 5)\). The tonic current and mIPSC amplitude reduction produced by GVG were similar to that produced by a low concentration of exogenous GABA (Fig. 3C), although the effects of GVG developed more slowly. GABA \((5\ \mu M, n = 4)\) increased tonic current fluctuations \((230 \pm 100\%\) \text{and decreased the mIPSC amplitude}) \((78 \pm 5\%\) \text{without changing the mIPSC interevent interval}) \((99 \pm 18\%\) \text{in control} \((n = 5)\). The increased tonic current and reduced synaptic current were not due to a direct action of GVG on GABA\(_A\) receptors because GVG \((400\ \mu M)\) did not significantly affect currents activated by GABA \((10\ \mu M, 400\ ms)\) in nucleated patches from untreated granule cells \((93 \pm 3\% \text{ of control, } n = 3, \text{not shown})\). GVG also did not activate a current in nucleated patches \((n = 3)\). Thus the acute effects of GVG are compatible with an accumulation of extracellular GABA in the micromolar range that desensitizes postsynaptic GABA\(_A\) receptors (Overstreet et al. 2000). However, the dramatic reduction of mIPSC frequency observed in the pretreatment protocol was not observed during acute applications of either GVG or GABA.

### Presynaptic mechanisms

The dramatic reduction in mIPSC frequency following GVG pretreatment potentially could be explained either by a presynaptic reduction in GABA release or a marked postsynaptic reduction in GABA sensitivity such that mIPSCs were below detection thresholds. Activation of presynaptic GABA\(_B\) receptors by elevated tonic GABA was an obvious possibility that was ruled out because mIPSCs were recorded in the presence of the GABA\(_B\) receptor antagonist CGP55845. Inclusion of CGP55845 \((10\ \mu M)\) in the incubation chamber also failed to block the reduction in mIPSC frequency caused by GVG.

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![GABA pretreatment increased the tonic GABA\(_A\) receptor–mediated current. Left panels: examples of the reduction in GABA\(_A\) receptor–mediated tonic current by SR95531 (5 \(\mu M\)) in control and GVG-pretreated slices. Right panels: all-points histograms were made from 1-min segments in control (\(\bullet\)) and SR95531 (\(\blacksquare\)). The mean current and standard deviation were determined by Gaussian fits to the histograms (solid lines). Tonic current fluctuations were quantified by normalizing the standard deviation in SR95531 to that in control. In GVG-pretreated slices, SR95531 reduced the standard deviation by 60\% (bottom panels), whereas in control it was reduced by only 30\% (top panels). In the presence of SR95531, there was no difference in tonic current fluctuations between control and GVG-pretreated cells.](Image)

**Fig. 2.** GVG pretreatment increased the tonic GABA\(_A\) receptor–mediated current. Left panels: examples of the reduction in GABA\(_A\) receptor–mediated tonic current by SR95531 (5 \(\mu M\)) in control and GVG-pretreated slices. Right panels: all-points histograms were made from 1-min segments in control (\(\bullet\)) and SR95531 (\(\blacksquare\)). The mean current and standard deviation were determined by Gaussian fits to the histograms (solid lines). Tonic current fluctuations were quantified by normalizing the standard deviation in SR95531 to that in control. In GVG-pretreated slices, SR95531 reduced the standard deviation by 60\% (bottom panels), whereas in control it was reduced by only 30\% (top panels). In the presence of SR95531, there was no difference in tonic current fluctuations between control and GVG-pretreated cells.

**Fig. 3.** Acute GVG or GABA reduced mIPSC amplitudes. A: GVG \((400\ \mu M)\) applied to an untreated slice produced an increase in tonic current that was apparent after a couple of minutes (asterisk) and increased gradually throughout the experiment. Recordings were made in CNQX, TTX, and CGP55845. B: cumulative amplitude and interevent interval probabilities were measured after 20 min of acute GVG application. GVG reduced the mIPSC amplitude in all cases (Mann-Whitney U tests, \(n = 5\)), without affecting the interevent interval. C: the tonic current, mIPSC amplitude and mIPSC frequency were unchanged during a 25-min recording period in control slices. The effects of GVG were mimicked by acute application of a low concentration of GABA \((5\ \mu M)\) in the presence of CGP55845.
pretreatment (median interevent interval 15.6 ± 5.7 s in GVG + CGP, n = 5; 10.6 ± 4.1 s in GVG, n = 11, Fig. 4, B and D). In the absence of CGP55845, acute treatment with GVG reduced the mIPSC frequency in three of five cells (median interevent interval increased by 223%, n = 3; Mann-Whitney U test, Fig. 4, A and C), whereas no frequency reduction was observed in the presence of CGP55845 (n = 5, Fig. 4C). Thus elevated ambient GABA can reduce the frequency of mIPSCs via presynaptic GABA_B receptors, but it does not account for the dramatic reduction of mIPSCs that occurs following prolonged pretreatment with GVG.

A second explanation for the dramatic reduction in mIPSCs could be a decrease in the availability or filling of synaptic vesicles. This would be manifested as a decrease in the readily releasable vesicle pool evoked by hyperosmotic sucrose (Rosenmund and Stevens 1996; Stevens and Tsujimoto 1995). Indeed, the response elicited by hypertonic extracellular solution (650 mM) was greatly attenuated in GVG-pretreated slices. In control cells, sucrose elicited a barrage of mIPSCs (11 ± 0.7 Hz measured for 5 s following application, n = 5) compared with 2.3 ± 0.4 Hz in GVG-pretreated slices (n = 6, Fig. 5A). Thus GVG appears to reduce the readily releasable pool of GABAergic vesicles.

**Postsynaptic mechanisms**

Because GVG reduced mIPSC amplitudes as well as frequency, we also tested the possibility that there was a reduction in postsynaptic receptor sensitivity. Responses evoked by a brief local application of GABA (10 mM) were reduced in GVG-pretreated slices. In control slices, the GABA-evoked current was 8.6 ± 0.5 nA (n = 8), whereas following GVG pretreatment the GABA-evoked current was 4.5 ± 0.8 nA (n = 10, Fig. 5C). However, a concentration of SR95531 expected to produce at least a 90% reduction in postsynaptic responsiveness did not significantly alter the measured increase in sucrose-evoked mIPSC frequency in untreated slices (7.8 ± 1.6 Hz, n = 4, Fig. 5B). This suggests that the reduction in postsynaptic sensitivity following GVG pretreatment did not
reduce sucrose-evoked mIPSCs below detectable amplitudes, thereby causing an artifactual reduction in mIPSC frequency. Thus both pre- and postsynaptic mechanisms contribute to the reduction in synaptic currents.

It is unlikely that the reduction in sensitivity was due to a direct action of GVG on postsynaptic GABA<sub>A</sub> receptors because GVG was washed from the slice before experiments began, and GVG had no effect on GABA-activated currents in nucleated patches. The elevation in tonic ambient GABA could reduce postsynaptic GABAergic responses by several mechanisms, including agonist-dependent receptor internalization (Tehrani and Barnes 1991). To block agonist-dependent internalization, we pretreated slices with GVG and an antagonist cocktail of bicuculline (10 µM), CNQX (5 µM), and CGP55845 (2 µM). This treatment did not block the effects of GVG (n = 6, not shown), as the median mIPSC interevent interval (12.5 ± 3 s after GVG vs. 1.4 ± 0.3 s in control) and tonic GABA<sub>A</sub> receptor–mediated current (13 ± 2 pA, mean ± SD, after GVG vs. 4 ± 2 pA in control) were increased compared with slices pretreated only with the antagonist cocktail (n = 5). Furthermore, if agonist-induced internalization accounted for the reduction in mIPSCs, we would expect that prolonged GABA pretreatment would mimic the effects of GVG pretreatment. However, slice pretreatment with 10 µM GABA (>2 h) did not alter the mIPSC amplitude (24 ± 2 pA, n = 6) or frequency (interstimulus interval 2.5 ± 0.7 s, P = 0.06).

Two other possible explanations for the GVG-induced reduction in postsynaptic sensitivity are indirect changes in membrane properties (Frerking et al. 1999), and a shift in the IPSC reversal potential (Thompson and Gahwiler 1989). A tonic current could reduce the membrane resistance, causing a reduction in IPSC amplitude as a result of space-clamp error (Frerking et al. 1999; Spruston et al. 1993). A space-clamp error would be expected to reduce the amplitude of EPSCs as well as IPSCs. However, in GVG-pretreated slices, mEPSCs were not larger when GABAergic currents were blocked by SR95531 (2 µM, 15.8 ± 1.0 pA in the absence of SR95531 vs. 13.1 ± 1.5 pA in SR95531, n = 3). Finally, we examined the possibility that GVG reduced GABAergic inhibition by a shift in the Cl<sup>-</sup> reversal potential. Using an intracellular solution containing CsCl and QX314, we found that the reversal potential for the evoked IPSC was unaffected by GVG pretreatment (10 vs. 7 mV), but the synaptic conductance was reduced approximately fivefold (n = 4 control and GVG-pretreated cells, not shown). Thus GABA<sub>A</sub> receptor internalization, a change in membrane resistance, and a shift in the Cl<sup>-</sup> reversal potential do not appear to account for the reduction in postsynaptic sensitivity caused by GVG pretreatment.

**Role of GABA transport**

In GVG-pretreated slices, application of the GABA uptake blocker NO711 (100 µM) produced a robust inward current and increase in membrane noise, whereas only a small current was detected in control slices (average current 56 ± 15 pA following GVG vs. 12 ± 5 pA in control; n = 6). This current was blocked by SR95531, consistent with activation of GABA<sub>A</sub> receptors by endogenous GABA (Fig. 6). A lower concentration of NO711 (20 µM) also increased the tonic GABA<sub>A</sub> receptor–mediated current fluctuation in GVG-pretreated slices by 430 ± 212% (n = 3) compared with control slices (160%, n = 2). These results suggest that the role of GABA transporters in maintaining low extracellular levels of GABA was enhanced following inhibition of GABA transaminase.

Interestingly, NO711 (20 µM) applied before and during the acute GVG protocol blocked the reduction in mIPSC amplitude (98% of control) and attenuated the increase in tonic GABA current (122% of control, n = 2, not shown). To further investigate this phenomenon, mIPSCs were recorded in slices after dual pretreatment with NO711 (20 µM) and GVG (400 µM, >3 h). In this protocol NO711 blocked the effect of GVG (Fig. 7, n = 6). mIPSC amplitudes were 27 ± 4 pA versus 25 ± 2 pA in untreated controls, compared with 11 ± 1 pA after GVG alone. The interevent interval was only slightly reduced, 2.1 ± 0.2 s versus 1.0 ± 0.2 s in control, compared with 10 ± 4 s after GVG alone. Tonic current fluctuations measured after dual pretreatment (following wash out of NO711 and GVG, 61 ± 17 pA, mean ± SD, n = 3) were not different from pretreatment with NO711 alone (69 ± 32 pA, n = 3). Following dual pretreatment the tonic current fluctu-
Mechanisms of action of GVG

The dramatic reduction in synaptic currents following GVG pretreatment is consistent with the reduced Ca\textsuperscript{2+} dependence of K\textsuperscript{+}-stimulated GABA release following GVG treatment (Quine and Fowler 1997). Our finding that GVG attenuated GABA release in response to a sucrose challenge strongly suggests a reduction in the readily releasable vesicle pool. This is consistent with our observation that the paired-pulse ratio, a common measure of release probability, was unaffected, whereas the mIPSC frequency was severely reduced. Although there may be a correlation between the size of readily releasable pool and release probability (Rosenmund and Stevens 1996), this relationship can be masked by a subset of unaffected terminals that have normal release characteristics (Augustin et al. 1999). The GVG-induced reduction of mIPSC frequency is similar to the actions of bafilomycin, an inhibitor of vesicular transport (Zhou et al. 2000). High concentrations of GVG can also inhibit the vesicular GABA transporter (IC\textsubscript{50} = 7.5 mM) (McIntire et al. 1997), suggesting that GVG may reduce the readily releasable pool by inhibition of vesicle filling. This mechanism could account for the reduction in mIPSC frequency if GVG accumulates in the presynaptic terminal during prolonged incubation, and is not readily cleared following GVG wash out.

GVG pretreatment also had postsynaptic effects that persisted after prolonged wash out of GVG. Most obvious was the elevation of ambient GABA that resulted in tonic GABA\textsubscript{A} receptor–mediated current fluctuations. The rate of glutamate transport is reduced following elevation of intracellular transporter substrates (Takahashi et al. 1996). In addition, GVG increased tonic GABAAergic current fluctuations more gradually than NO711 (Figs. 3A and 6A), presumably reflecting the gradual accumulation of intracellular GABA. The increase in extracellular GABA was accompanied by a reduction in postsynaptic receptor sensitivity that was not due a shift in the IPSC reversal potential, passive shunting, or agonist-induced receptor internalization. These results suggest that the increase in ambient GABA desensitized postsynaptic receptors (Overstreet et al. 2000), although the lack of an agent that blocks GABA\textsubscript{A} receptor desensitization prevented us from testing this possibility directly.

Our results are consistent with previous findings that acute GVG reduced GABAergic inhibition of evoked population spikes in CA1 (Jackson et al. 1994). GVG is also reported to reduce activity-dependent suppression of IPSCs that occurs during a train of stimuli by reducing the function of presynaptic GABA\textsubscript{B} autoreceptors (Jackson et al. 2000). The accumulation of ambient GABA may be expected to tonically activate GABA\textsubscript{B} receptors, thereby occluding further activation by synthetically released GABA. GABA\textsubscript{B} receptor activation has also been implicated in GVG’s inhibition of cocaine-induced increases in nucleus accumbens dopamine (Ashby et al. 1999). Our finding that acute GVG application reduced the mIPSC frequency only in the absence of GABA\textsubscript{B} antagonists confirms the ability of GVG to promote GABA\textsubscript{B} receptor–mediated modulation, even though this mechanism cannot account for the dramatic reduction in synaptic inhibition following prolonged pretreatment with GVG.

Inhibition of GABA transporters following GVG pretreatment revealed an increase in transporter activity. This may reflect an increase in tonic GABA transport to counteract the increase in ambient GABA, although an increase in the number of functional transporters in response to elevated extracellular...
GABA could also contribute (Bernstein and Quick 1999). This result also suggests that reversal of transport does not underlie the elevated ambient GABA, in which case NO711 would be expected to reduce tonic GABA\textsubscript{A} receptor–mediated currents. The simplest explanation for our finding that concurrent treatment with NO711 blocked the effect of GVG is that NO711 blocked uptake of GVG into neurons and glia (Eckstein-Ludwig et al. 1999; see also Schousboe et al. 1986), thereby preventing intracellular accumulation required for inhibition of GABA transaminase.

**Relevance to the use of GVG as an anticonvulsant**

The concentration of GVG used here is within the range estimated to occur in the rat CNS following a single anticonvulsant dose of 1500 mg/kg (Abdul-Ghani et al. 1981). A similar dose has time-dependent effects on seizure susceptibility. At 4 h following injection, GVG has proconvulsant effects, whereas anticonvulsant effects appear 24 h later (Lösch et al. 1989). The dramatic reduction in synaptic inhibitory currents observed here may correspond to the proconvulsant period following a single high-dose injection. However, the role of synaptic GABAergic inhibition in models of temporal lobe epilepsy is unclear. Depending on the region and parameter measured, synaptic inhibition following seizure activity can be enhanced, reduced, or unchanged (reviewed by Ben-Ari and Cossart 2000). For example, somatic inhibition of CA1 pyramidal cells is increased by hyperactivity of inhibitory interneurons, whereas degeneration of a subpopulation of interneurons reduces synaptic inhibition in the dentrites (Cossart et al. 2001). Experimental epileptogenesis is also associated with an increase in synaptic inhibition in dentate granule cells (Otis et al. 1994) and cortical pyramidal cells (Prince et al. 1997). Regardless of whether these increases in synaptic inhibition are compensatory or promote seizure activity by enhanced synchronization (Cobb et al. 1995), GVG would be expected to counteract enhanced synaptic inhibition by reducing synaptic GABA release as well as postsynaptic responsiveness. But the contribution of synaptic inhibition to cellular excitability may be overwhelmed by the elevation of ambient GABA produced by GVG. Tonic GABAergic inhibition arising from a persistent elevation of extracellular GABA would be expected to reduce excitability of both interneurons and principal cells. An elevation of tonic inhibition may also contribute to the clinical properties of the benzodiazepine midazolam and the anesthetic propofol (Bai et al. 2001). Thus tonic inhibition produced by ambient GABA may represent an important mechanism for modulating cellular excitability and network behavior.

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