Vigabatrin Induces Tonic Inhibition Via GABA Transporter Reversal Without Increasing Vesicular GABA Release

Yuanming Wu, Wengang Wang, and George B. Richerson

INTRODUCTION

It is widely recognized that there is normally a finite amount of GABA in the extracellular space (Lerma et al. 1987; Tossman et al. 1986) and that under some conditions this ambient GABA reaches levels sufficient to tonically activate GABA_A receptors (Brickley et al. 1996). The density of the tonic inhibitory current that is induced can be surprisingly large, exceeding the total charge transfer induced by fast GABAergic synaptic transmission (Brickley et al. 1996; Nusser and Mody 2002).

The GABA responsible for tonic inhibition comes in part from spillover of synaptic GABA that has escaped the synaptic cleft before it can be taken back up by GABA transporters (Brickley et al. 1996; Rossi and Hamann 1998). There is also a contribution from the continuous barrage of spontaneous miniature inhibitory postsynaptic potentials (mIPSPs) that occurs in the absence of action potential dependent GABA release (Otis et al. 1991). These mIPSPs depend in part on influx of calcium (Llano et al. 2000), but continue at a lower rate in the absence of extracellular calcium (Llano et al. 2000; Otis et al. 1991). Tonic inhibition is primarily due to activation of high-affinity extrasynaptic GABA receptors (Bai et al. 2001; Nusser and Mody 2002; Stell and Mody 2002).

We have recently shown that nonvesicular GABA release can also contribute to ambient GABA. Chronic treatment (2–5 days) with the anticonvulsant vigabatrin (0.05–100 μM), which blocks GABA transaminase, potently increases [GABA]^0 in primary hippocampal cultures (Wu et al. 2001). The increase in [GABA]^0 is not prevented by removal of extracellular Ca^{2+} and is blocked by the GABA transporter antagonist SKF-89976a. Therefore the nonvesicular GABA release induced by vigabatrin under these conditions is due to reversal of the GABA transporter on surrounding neurons and/or glia. Vigabatrin also increases ambient [GABA]^0 in rat hippocampal slices during subacute exposure (2–5 h) to vigabatrin (Overstreet and Westbrook 2001) and in cultured rat hippocampal slices during chronic exposure (Engel et al. 2001). In contrast to the increase in ambient [GABA], vigabatrin only induces a small decrease in mIPSC size during acute exposure in slices (Overstreet and Westbrook 2001) and a small increase in mIPSC size during chronic exposure in cultured slices (Engel et al. 2001).

Here it is shown that chronic treatment with vigabatrin markedly increases tonic GABAergic inhibition of cultured hippocampal neurons without increasing IPSCs induced by presynaptic stimulation. Furthermore, blockade of the GABA transporter causes an increase in tonic inhibition when whole-cell recordings are made without Na^+ and GABA in the electrode solution, but causes a decrease in inhibition during perforated patch recordings or when whole cell recording electrodes contain Na^+ and GABA. Thus whole cell recordings can lead to erroneous conclusions about the role of the GABA transporter in setting tonic [GABA]^0. Theoretical predictions suggest that the GABA transporter plays a major role in es-
establishing ambient [GABA]. We propose that the transporter will work in the forward or reverse direction to seek an equilibrium GABA gradient with a finite [GABA]e, which under some conditions is high enough to induce tonic activation of GABA receptors.

METHODS

Cell culture

Primary cell cultures of hippocampal neurons and glia were prepared from neonatal (P0–P2) Sprague-Dawley rats as described previously (Gaspar et al. 1998). Briefly, hippocampi were dissociated and plated at a density of 2.5–5 × 10^5 cells/ml on poly-L-ornithine and laminin-coated coverslips and incubated in culture medium (63% modified Eagle’s medium (MEM), 7% fetal bovine serum (FBS), 30% Neurobasal medium with B27 supplement, with the following additives: 1 ng/ml basic fibroblast growth factor (bFGF), 10 ng/ml FGF-5, 100 U/ml penicillin, and 100 μg/ml streptomycin) at 37°C with 5% CO₂ in room air. At 24 h, medium was switched to 100% Neurobasal/B27 with additives. Cytosine β-arabino-furanoside hydrochloride (Ara-C; 3 μM) was used to control glial growth after approximately 7 days. Cultures were fed with half-medium changes on day 7 and then every 1 to 4 weeks. In most cases, cultures were grown for 7 days. Cultures were fed with half-medium changes on day 7 and their physiological state, the number of synaptic connections between neurons, the surface area of each neuron in contact with glia, with respect to neuronal density, the number of synaptic connections touching each other.

Drug treatment of cultures

As described previously (Wu et al. 2001), changes in tonic GABAergic inhibition were measured with patch-clamp recordings while applying different solutions using the “multipuffer” technique (Greenfield and Macdonald 1996). In all cases, the multipuffer solution was identical to the bath solution at that time, except for the specific stimulus (high K⁺, bicuculline, SKF-89976a, or NO-711) being tested. Application of bath solution alone has previously been shown to have no effect (Wu et al. 2001).

Recordings were made from coverslips for less than about 3 h from the time they were removed from the incubator. Values expressed as n = x are the total number of neurons recorded. All experiments were performed at room temperature. QX-314, TTX, and tetanus toxin was purchased from Alomone Labs (Jerusalem, Israel). SK-89976a and CGP-55845 were purchased from Tocris Cookson (Ballwin, MO). AP-5, CNQX, Vigabatrin, NO-711, bicuculline, and all salts and chemicals not otherwise listed were purchased from Sigma Chemical.

Data analysis

For analysis of spontaneous mIPSCs, two patch-clamp amplifiers were used to simultaneously make whole cell patch-clamp recordings from two neurons. These neurons were selected in the same high power field (400×), but without the two somata directly touching each other.

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with a rapid onset and slow decay. This method was used instead of automatic detection using a fixed threshold, because this reduced the false positives resulting from the increase in baseline noise induced by vigabatrin treatment, reduced false negatives that would have resulted from using a fixed threshold and eliminated those events due to superimposition of more than one mIPSC. The lower limit of accurate detection of mIPSCs with this method was typically 15–20 pA for most recordings, which was likely to be too high to detect all small mIPSCs. Histograms were made using the following method. For each cell, a histogram was constructed of the amplitude distribution of all mIPSCs that had been selected for that neuron, with the number of mIPSCs in each bin expressed as the percentage of total mIPSCs. For each treatment group, an average histogram was then calculated from the histograms for all cells within that group. The histograms for each treatment group were then scaled, so that each bin was expressed as the mean number of mIPSCs per minute. Using this method, all cells contributed equally to the shape of each histogram, while information was preserved about differences in frequency of mIPSCs.

The peak amplitudes of evoked IPSCs were analyzed using custom-written software. Peak amplitudes of IPSCs evoked by single presynaptic action potentials were measured as the difference between the maximum IPSC amplitude and the baseline holding current. In response to 10-s trains of spikes, the peak amplitudes were measured as the mean amplitudes of the first three evoked IPSCs, and the steady-state amplitudes were determined as the mean amplitudes of the last three evoked IPSCs.

For analysis of responses to multipuffer application of solutions, the peak amplitude and the area under the curve (AUC) of the current response were calculated as described previously (Gaspary et al. 1998). We used the peak amplitude of the response to bicuculline to estimate ambient [GABA]. This tonic GABA_A receptor-mediated current was calculated as the holding current during the peak of the response to 50 μM bicuculline minus the baseline holding current (both measured for 10 s). The AUC, or total charge transfer, was expressed in nanocoulombs (nC = nanoampere × seconds). Probability values were determined using a two-tailed t-test assuming unequal variances. All values expressed as x ± y are mean ± SD and all error bars are SE.

RESULTS

Vigabatrin did not affect the amplitude of miniature IPSCs

To determine whether vigabatrin induces an increase in mIPSC amplitude, we measured changes in the peak amplitude of GABAergic mIPSCs after 3–5 days of treatment with vigabatrin—a time during which there is a nearly maximal increase in [GABA], (Wu et al. 2001). The concentration we chose to use (100 μM) was 2,000 times greater than that required to produce a significant increase in ambient [GABA] (Wu et al. 2001), so that if an increase in quantal content is important for its mechanism of action, this concentration should be sufficient to produce a detectable effect. Whole cell voltage-clamp recordings were made at a holding potential of −60 mV, using cesium chloride electrode solution and supplemented Ringer in the bath (including CGP-55845 to block GABA_A-mediated presynaptic inhibition). In hippocampal cultures treated with 100 μM vigabatrin for 82 ± 13 h, the frequency of mIPSCs was lower [88 ± 67 mIPSCs/min; n = 33; age of cultures 33 ± 1.7 days in vitro (DIV)] than in control neurons from sister dishes (180 ± 87 mIPSCs/min; n = 31; P < 0.0001). However, the distribution of mIPSC amplitudes was not different in vigabatrin-treated neurons compared with control neurons (Fig. 1). It might have been predicted that the increase in ambient [GABA] induced by vigabatrin would reduce mIPSC amplitude due to desensitization of GABA_A receptors. However, the fractional availability of GABA_A receptors only decreases by approximately 15% in response to a [GABA] of 0.4 μM (Overstreet et al. 2000), an ambient [GABA] that is slightly less than that predicted to be induced by vigabatrin in these experiments (0.5 μM; see next paragraph). Thus the effect of desensitization on mIPSC amplitude may have been too small to be detected in the present experiments.

We examined whether the decrease in mIPSC frequency induced by vigabatrin might have been due to the increase in [GABA]. We compared mIPSC frequency and amplitude distribution in neurons treated with vigabatrin (100 μM) for 126 ± 12 h (n = 13; age of cultures 24.5 ± 0.5 days) with that of neurons from sister cultures that were not treated with vigabatrin, but instead were acutely exposed to GABA (only during the time that recordings were made; n = 16). Recordings were made as above, including the use of CGP-55845 (Fig. 1, A–D). The addition of 0.5 μM GABA to the bath solution induced a tonic GABA_A receptor-mediated current of 468 ± 145 pA, which was the same magnitude as that seen spontaneously in recordings from vigabatrin-treated sister neurons (440 ± 175 pA). The frequency of mIPSCs in neurons bath-exposed to GABA (70 ± 51 mIPSCs/min) was the same as in neurons pretreated with vigabatrin (69 ± 48 mIPSCs/min). The amplitude distribution of mIPSCs was also the same in the two groups (Fig. 1, E and F). Note that there was a significant increase in baseline noise when GABA was added to the bath solution and after pretreatment with vigabatrin, apparently due to tonic activation of GABA_A receptors. We cannot exclude the possibility that this increase in noise masked small mIPSCs, although the manual selection of mIPSCs should have reduced this error. If such an error existed, it would not have prevented detection of a significant shift to large-amplitude mIPSCs.
potential for chloride = −108 mV). Neurons were superfused with supplemented Ringer containing CNQX and AP-5, but not TTX or CGP-55845. Recordings were made in current-clamp mode from presynaptic GABAergic neurons and in voltage-clamp mode from postsynaptic neurons (holding potential = −50 mV). Under these conditions, when postsynaptic responses could be evoked by presynaptic stimulation, they were blocked by bicuculline (n = 6 of 6). The peak amplitudes of IPSCs were compared between control neurons (n = 21 pairs) and neurons treated with 100 μM vigabatrin for 73 ± 33 h prior to recording (n = 22 pairs). Recordings were included in the analysis only if every presynaptic stimulus induced an action potential in the soma and was followed by an IPSC in the postsynaptic neuron.

In response to a single presynaptic spike, the peak amplitude of the IPSC was 493 ± 254 pA in control neurons and 285 ± 230 pA in vigabatrin-treated neurons (P < 0.01). In response to trains of spikes (at 5, 10, and 20 Hz for 10 s each), the peak amplitude of the IPSCs in vigabatrin-treated neurons was 43–48% of that in control neurons, whereas the steady-state amplitude at the end of the 10-s train was 30–39% of that in control neurons (Fig. 2). Thus chronic treatment with vigabatrin decreased the amplitude of evoked IPSCs.

We investigated whether this reduction in IPSCs was due to presynaptic inhibition via GABAB receptor activation. Dual recordings were made from pairs of neurons treated with vigabatrin (100 μM) for 90 ± 9 h. The same methods and solutions were used as above for evoking IPSCs. IPSCs were measured in supplemented Ringer containing 0.5 μM GABA. Note the similarity to the recording in B, F: amplitude distribution histograms from 14 vigabatrin-treated neurons and 16 control neurons bathed in 0.5 μM GABA (this is a different subset of neurons than those in B and D). There was no difference in mIPSC frequency or amplitude distribution.
Vigabatrin increased [GABA]o, after blockade of vesicular release with tetanus toxin

The previous results do not support the hypothesis that the anticonvulsant effect of vigabatrin is due to an increase in phasic synaptic inhibition. Instead, the anticonvulsant effect may be due to the substantial increase in ambient [GABA] that induces tonic inhibition. This effect occurs in the absence of extracellular Ca2+ (Wu et al. 2001), consistent with a nonvesicular release mechanism. However, since a small amount of vesicular GABA release may occur in the absence of extracellular calcium (Llano et al. 2000; Otis et al. 1991), we measured tonic GABA receptor activation in cultures pretreated with both vigabatrin and tetanus toxin to confirm that vesicular fusion is not required for vigabatrin to increase ambient [GABA]. Cultures were pretreated with vigabatrin for 44 ± 9 h prior to recording, and also with tetanus toxin (1 μg/ml) for 24–46 h prior to recording. Whole cell voltage-clamp recordings were made using cesium chloride electrode solution and supplemented Ringer. In culture dishes pretreated with vigabatrin and tetanus toxin, there was an increase in [GABA]o, that induced a tonic GABA receptor-mediated current of 745 ± 318 pA at a holding potential of −60 mV (n = 23) (Fig. 3). In paired culture dishes pretreated with tetanus toxin (1 μg/ml for 25–49 h) but not vigabatrin, the tonic GABA receptor-mediated current was only 99 ± 148 pA (n = 21; P < 0.0001). There was a difference in baseline leak current between the two groups of neurons that was consistent with superimposition of tonic GABAergic inhibition onto nonspecific leak (~1.17 ± 0.49 nA in vigabatrin-treated neurons vs. −0.44 ± 0.33 nA in control neurons; P < 0.0001). Thus the tonic increase in ambient [GABA] after vigabatrin treatment is not due to vesicular GABA release.

![Image](http://jn.physiology.org/)

**FIG. 2.** Vigabatrin decreased the size of IPSCs evoked by presynaptic stimulation during paired recordings. A: inhibitory postsynaptic current (IPSC; bottom) in response to a single spike evoked in a presynaptic GABAergic neuron (top, truncated). B: same experiment as in A, from 2 neurons that had been pretreated with vigabatrin (100 μM) for 88 h. Note the increase in outward holding current due to an increase in ambient [GABA] and the decrease in the size of the IPSC compared with the control neuron in A. C: IPSC in a postsynaptic neuron in response to presynaptic stimulation of a GABAergic neuron at 20 Hz for 10 s (bar). D: same experiment as in C, from 2 neurons pretreated with vigabatrin (100 μM) for 90 h. E: there was a statistically significant decrease in the size of IPSCs in vigabatrin-treated neurons (gray bars) compared with control neurons (black bars) in response to single presynaptic spikes and to trains of spikes at 5, 10, and 20 Hz. *P < 0.01; **P < 0.005; ***P < 0.001.

pA), or in response to a train of action potentials at 20 Hz (control: 697 ± 306 pA; CGP-55485: 788 ± 242 pA; wash: 734 ± 439 pA). Similar results were obtained when the area under the curve of IPSCs was quantified.

We also found that a decrease in evoked IPSCs could not be induced by bath application of GABA at a concentration sufficient to induce a similar amount of tonic inhibition as induced by treatment with 100 μM vigabatrin for 3–5 days. Dual recordings were made from pairs of neurons that had not been treated with vigabatrin. The same methods and solutions were used as above for evoking IPSCs. IPSCs were measured in supplemented Ringer containing CNQX and AP-5, but not CGP-55845 or TTX, and then from the same neurons in the same bath solution to which 0.5 μM GABA was added (n = 5 neurons, means obtained from 2 exposures for each neuron to GABA). Bath application of GABA did not affect the peak amplitude of IPSCs in response to single spikes (control: 856 ± 439 pA; GABA: 879 ± 514 pA; wash: 967 ± 448 pA), or in response to a train of action potentials at 20 Hz (control: 846 ± 421 pA; GABA: 865 ± 496 pA; wash: 896 ± 464 pA). Similar results were also obtained when the area under the curve of IPSCs was quantified.

![Image](http://jn.physiology.org/)

**FIG. 3.** The increase in ambient [GABA] induced by vigabatrin was not prevented by blocking vesicular GABA release by pretreatment with tetanus toxin. Voltage-clamp recording from a neuron treated with vigabatrin (100 μM) and tetanus toxin (1 μg/ml), both for 47 h prior to recording. Application of bicuculline (50 μM) for 20 s led to a decrease in tonic GABAergic inhibition, seen as a decrease in inward holding current at a holding potential of −60 mV.
GABA transporter blockade has opposite effects on ambient [GABA] during whole cell recording compared with perforated patch recording

Using perforated patch recordings, we have previously shown that SKF-89976a (a noncompetitive antagonist of GAT-1) blocks the increase in [GABA]o by vigabatrin (Wu et al. 2001). Since then, we used whole cell recordings and obtained the opposite result with NO-711 (also a noncompetitive antagonist of GAT-1). To determine whether these conflicting results occurred as a result of the recording configuration or the specific antagonist used, we first made perforated patch recordings and measured the response to NO-711. We then converted the recording to whole cell mode and measured the response to NO-711 again (n = 8 neurons) (Fig. 4). Neurons were pretreated with 100 μM vigabatrin for 74 ± 15 h and then placed into the recording chamber and superfused with zero calcium supplemented Ringer. Perforated patch recordings were made using perforated patch solution. The chloride concentration was chosen to minimize the change in reversal potential for the GABAα receptor on whole cell breakthrough (predicted Nernst potential for chloride = −83 mV). Recordings were made in voltage-clamp mode (holding potential = −50 mV) and were allowed to stabilize until the access resistance decreased to a stable level after 10–15 min (typically 20–40 MΩ). Bicuculline (50 μM) and NO-711 (100 μM) were applied with the multipuffer for 20 s each, and responses were measured as the area under the curve of the current trace for 20 s after the onset of the response. During these perforated patch recordings, application of bicuculline induced a response of −1,711 ± 485 pC (Fig. 4; first traces), consistent with a decrease in GABAα receptor activation. Application of NO-711 led to a response of −1,148 ± 664 pC (Fig. 4; second traces), consistent with a decrease in [GABA]o. The membrane patch was then broken using brief suction, converting the recording to whole cell mode. This led to a change in holding current from 188 ± 111 to 322 ± 203 pA. Three (±0) minutes after conversion to whole cell recording mode, the response to bicuculline increased to −3,681 ± 1,522 pC (Fig. 4, third traces). Eight (±1) minutes after whole cell breakthrough, the response to NO-711 was highly variable, but on average was opposite that of bicuculline (806 ± 2197 pC) (Fig. 4; fourth traces). This was often accompanied by a change from a monophasic response to a biphasic or more complex response. The reason for these complex responses is unclear, but may be due to spatial heterogeneity in our experiments. After stabilization for another 7 ± 3 min (15 ± 3 min after whole cell breakthrough), a second response to NO-711 was less variable, more consistently monophasic, and in all cases was opposite the response to bicuculline (1090 ± 1110 pC; range 136-3039 pC) (Fig. 4; last traces). The response to NO-711 during perforated patch recordings was significantly different from the first response to NO-711 during whole cell recordings (P < 0.05), as well as the second response to NO-711 during whole-cell recordings (P < 0.0005). Similar results were also obtained with SKF-89976a. Thus, if taken at face value, the results from whole cell recordings led to the conclusion that GABA transporters are constantly taking up GABA to reduce ambient [GABA], whereas the results from perforated patch recordings lead to the opposite conclusion that GABA transporters are constantly working in reverse to release GABA in a nonvesicular manner.

The effect of GABA transporter blockade on ambient [GABA] during whole cell recordings was dependent on the electrode [Na+] and [GABA]

The results described above are consistent with whole cell recordings causing an artifactual response to NO-711. We hypothesized that adding GABA and Na+ to the electrode solution during whole cell recording might restore the response to normal. To test this hypothesis, we pretreated neurons with

![Figure 4](http://jn.physiology.org/10.22038/jn.2003.2006) The effect of NO-711 on ambient [GABA] during perforated patch recordings was opposite of that during whole cell recordings. A: 5 traces all from the same recording obtained from a neuron pretreated with vigabatrin (100 μM) for 92 h. During the first 2 traces, a voltage-clamp recording was made in perforated patch mode. Bicuculline induced a decrease in outward (inhibitory) current (first trace; holding potential = −50 mV). NO-711 induced a decrease in ambient [GABA] leading to a decrease in tonic inhibition (second trace). The recording was then converted to whole cell mode by rupturing the patch, and the next 3 traces were recorded. In whole cell mode, bicuculline still induced a decrease in tonic inhibition (third trace; Nernst potential for chloride = −83 mV), but now NO-711 induced an increase in ambient [GABA] (fourth trace) that became more pronounced with time (fifth trace). B: same experiment in a second neuron pretreated with vigabatrin (100 μM) for 70 h.
vigabatrin and then measured the response to SKF-89976a while systematically changing [GABA] and [Na\(^+\)] in the recording electrode solution.

Neurons were pretreated with vigabatrin for 3–5 days. Whole cell patch-clamp recordings were made using cesium chloride electrode solution (with 0 mM NaCl and 0 mM GABA) and zero calcium supplemented Ringer bath solution. Application of 50 \(\mu\)M bicuculline for 20 s induced a decrease in inward holding current at a holding potential of −60 mV (Fig. 5A). In contrast, application of SKF-89976a (40 \(\mu\)M) induced an opposite response. This response to SKF-89976a was prevented when 50 \(\mu\)M bicuculline was added to the bath solution. Similar results were obtained in 15 neurons. It should be noted that transporter currents did not contribute to the responses to SKF-89976a (or NO-711), in part because these responses were blocked by bicuculline, and also for other reasons outlined previously (Gaspary et al. 1998). Thus the response to blockade of the GABA transporter under these conditions was due to an increase in ambient [GABA].

When this experiment was repeated under the same conditions except that 20 mM NaCl and 20 mM GABA were added to the electrode solution (reducing CsCl from 135 to 105 mM to maintain osmolarity), SKF-89976a induced a response that was in the same direction as bicuculline, although smaller in size (Fig. 5B). The response to SKF-89976a under these conditions was also prevented by bicuculline in the bath solution \((n = 10)\).

We then quantified the effect of Na\(^+\) and GABA in the electrode solution on the response to SKF-89976a. Whole cell recordings were made from a group of age- and treatment-matched neurons using experimental methods that were identical for each subgroup, except for the concentration of Na\(^+\) and GABA in the electrode solution. Neurons were pretreated with 100 \(\mu\)M vigabatrin for 3–4 days, and then recordings were made in zero calcium supplemented Ringer at a holding potential of −60 mV. Electrodes were filled with potassium chloride solution (with 1.5 mM Mg-ATP) containing different concentrations of NaCl and GABA (maintaining osmolarity and [Cl\(^-\)] constant by adjusting [KCl]). The higher the [Na\(^+\)] and [GABA] in the pipette, the more closely the response to SKF-89976a (40 \(\mu\)M for 60 s) approached the response to bicuculline (50 \(\mu\)M for 60 s) (Fig. 6). The first response to SKF-89976a was typically more negative (and variable) than when it was repeated an average of 8 min later (Table 1, Fig. 6). The response to bicuculline was not significantly different with changes in [Na\(^+\)] and [GABA] (Table 1), whereas the first and second response to SKF-89976a were dependent on the [Na\(^+\)] and [GABA] in the recording electrode (Table 1, Fig. 6). GABA alone was effective in preventing reversal of the response to SKF-89976a, and there was a trend toward an effect of Na\(^+\) alone. However, the two together were more effective than either one alone.

We hypothesized that Na\(^+\) and GABA in the cytosol of the recorded neuron altered the driving force for GABA transporters on the recorded neuron. If this were true, then it would be expected that ambient [GABA] (and tonic inhibition) would be lower during whole cell recordings without Na\(^+\) and GABA in the electrode, but we did not detect a difference in the response to bicuculline as [Na\(^+\)] and [GABA] were changed within the recording electrode (Table 1). However, there was significant variability of the bicuculline response within each subgroup of neurons in these experiments. Therefore, we designed a separate set of experiments to directly address the question of whether Na\(^+\) and GABA within the cytosol of the recorded neuron could induce an increase in [GABA] outside that same neuron. Whole cell recordings were made at a holding potential of −60 mV with electrode solution containing the following (in mM): 105 KCl, 30 potassium methanesulfonate, 10 HEPES, 1 EGTA. Application of bicuculline induced a response of 396 ± 247 pA. When the same experiment was repeated with an electrode solution containing the following (in mM): 60 NaCl, 60 GABA, 45 KCl, 10 HEPES, 1 EGTA, the response to bicuculline was 664 ± 266 pA \((P < 0.01)\). There was no difference in the nonspecific leak current \((-182 ± 419\) pA vs. \(-130 ± 319\) pA), indicating that this solution did not adversely affect cell viability. Thus ambient [GABA] can be influenced by the [Na\(^+\)] and [GABA] in the postsynaptic neuron.

**Nonvesicular GABA release can make up a large fraction of total depolarization-induced GABA release**

We have previously shown that the nonvesicular GABA release that occurs spontaneously after vigabatrin treatment also occurs in response to depolarization induced by an increase in [K\(^+\)]\(_o\) from 3 to 6 or 12 mM (Gaspary et al. 1998; Wu et al. 2001). When extracellular [K\(^+\)] increases, vesicular and nonvesicular GABA release are both induced. When measured biochemically, the nonvesicular component is a large percentage of total release (Belhage et al. 1993; Pin and Backaert 1989). Here, we recorded from neurons that had not been treated with vigabatrin and quantified the relative contributions to the electrophysiological response of the vesicular and nonvesicular components of total GABA release induced by 12 mM K\(^+\).

The response to elevated [K\(^+\)]\(_o\) was studied using methods described previously (Gaspary et al. 1998; Wu et al. 2001).
without TTX was 28.1/11006 lar release). The response of these same neurons in the absence of 1998; Wu et al. 2001) that K previously shown under these same conditions (Gaspary et al. 2001). In our culture system, this tonic increase in calcium (zero calcium supplemented Ringer without TTX) was 11.8 ± 6.9 nC (i.e., nonvesicular release alone), or 42% of the response in the presence of calcium. Thus in response to a rise in [K+]o to 12 mM, the nonvesicular contribution to the electrophysiological response was nearly as large as the vesicular contribution even without vigabatrin treatment.

**DISCUSSION**

Whole cell recordings can alter conclusions about the role of the GABA transporter in control of tonic inhibition

It has now been demonstrated using several different approaches that vigabatrin induces a large increase in ambient [GABA] (Engel et al. 2001; Overstreet and Westbrook 2001; Wu et al. 2001). In our culture system, this tonic increase in [GABA]o occurs after blockade of vesicular release either by

**TABLE 1. Effect of [Na+] and [GABA] in the recording pipette on the response to SKF-89976a**

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<th>[GABA], mM</th>
<th>[Na⁺], mM</th>
<th>Duration of Vigabatrin Treatment, h</th>
<th>Bicuculline Response, nC</th>
<th>First Response to SKF-89976a, nC</th>
<th>Time After Breakthrough, min</th>
<th>Second Response to SKF-89976a, nC</th>
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Values are mean ± SD; N is number of cells. Responses were measured as the area under the curve, in nanocoulombs (nC). "Time after Breakthrough" is the time since the membrane patch was broken to obtain the whole cell recording.
removal of \([Ca^{2+}]_o\) (Figs. 4–6) (Wu et al. 2001) or by pre-treatment with tetanus toxin (Fig. 3). Thus, under these conditions, vigabatrin increases tonic inhibition by inducing nonvesicular GABA release.

During perforated patch recordings, NO-711 (Fig. 4) and SKF-89976a (Wu et al. 2001) blocked the increase in ambient \([\text{GABA}]\) induced by vigabatrin, indicating that the nonvesicular GABA release was due to continuous, spontaneous reversal of the GABA transporter. However, during whole cell recordings, the opposite result was obtained if \(Na^+\) and GABA were not included in the recording solution. The effect of whole cell dialysis could be reversed by adding \(Na^+\) and GABA to the recording solution. These may have been the essential components lost, or alternatively they may restore the response to normal even though they are not what was lost. The latter seems likely in the case of recordings from pyramidal cells, which would not be expected to normally have a high \([\text{GABA}]\).

These results indicate that cytosolic \([Na^+]\) and \([\text{GABA}]\) in the recorded neuron can alter the dynamics of GABA transport. The most obvious explanation for this is that they change the driving force for GABA transporters in the plasma membrane of the recorded neuron. If GABA transporters were present on the recorded neuron, then this neuron would act as a sink for GABA uptake when cytosolic \([\text{GABA}]\) and \([Na^+]\) were zero. Under these conditions, blocking these GABA transporters would cause a rise in ambient \([\text{GABA}]\) surrounding the recorded neuron. At the same time, if neighboring cells (neurons and/or glia) are releasing GABA via reversal of GABA transporters, the opposite result was obtained if \(Na^+\) and GABA were not included in the recording solution. The effect of whole cell dialysis could be reversed by adding \(Na^+\) and GABA to the recording solution. These may have been the essential components lost, or alternatively they may restore the response to normal even though they are not what was lost. The latter seems likely in the case of recordings from pyramidal cells, which would not be expected to normally have a high \([\text{GABA}]\).

An alternative explanation is that the absence of \(Na^+\) and GABA in the recorded neuron led to an increase in the driving force for GABA uptake into neighboring cells (neurons and/or glia), causing GABA transporters on surrounding cells to operate primarily in uptake mode, rather than in reverse mode. There is no clear mechanism to explain how this might occur, although it is possible that whole cell recordings led to changes in the extracellular milieu (e.g., 0 mM \([Na^+]\), would inhibit the Na/K ATPase and increase the activity of other Na\(^{2+}\)-driven pumps), which could indirectly affect neighboring neurons. If this explanation were true, then it would still be necessary to explain the source of ambient GABA, since vesicular release had been blocked by removal of extracellular calcium, and there is no clearly defined mechanism of nonvesicular GABA release other than GABA transporter reversal.

The difficulty in defining a mechanism to explain why GAT-1 blockade produced opposite results with the two different recording methods was compounded by the complexity of the dynamics of GABA uptake during our experiments. Ambient \([\text{GABA}]\) is controlled by different GABA transporter isoforms on different types of neurons and on glia and is influenced by ionic homeostasis controlled by other types of transporters. Spatial heterogeneity in ambient \([\text{GABA}]\) would exist normally, and dialysis of the soma might induce additional gradients in ambient \([\text{GABA}]\) between dendrites and the soma. The extracellular space is not uniform, with some parts of the cell surface being exposed to flowing solution and other parts adjacent to a restricted extracellular space apposed to different types of cells. Therefore, it may not be possible to view GAT-1 antagonists as simply blocking uptake or transporter reversal uniformly at all points around a neuron.

Despite these complexities, the results presented here provide a direct demonstration that changes in \([Na^+]\) and \([\text{GABA}]\) in the cytosol of the recorded neuron can drastically alter the response to GABA transporter blockade. If the response to GABA transporter blockade during whole cell recordings was taken at face value, then it must be concluded that the increase in ambient \([\text{GABA}]\) induced by vigabatrin treatment in zero calcium solution or after tetanus toxin pretreatment is due to GABA release via some mechanism other than vesicular fusion or GABA transporter reversal. However, there is no reason to believe that the method of recording would lead to a change in the mechanism of GABA release from GABA transporter reversal to an undefined third mechanism of release. Instead, it is more likely that whole cell recordings lead to local changes in transporter dynamics, while the source of ambient GABA in both cases is transporter reversal, presumably from neighboring neurons and/or glia.

The most important conclusion from these results is that any experiments using whole cell recordings to measure the response to GABA transporter blockers should be interpreted cautiously, because there is the potential that this recording method can lead to an erroneous conclusion about the role of the GABA transporter in control of ambient GABA.

**Theoretical predictions of ambient \([\text{GABA}]\) based on GABA transporter stoichiometry**

The GABA transporter is not simply a GABA vacuum operating continually in the inward direction to suck all GABA out of the extracellular space. Instead, as with all ion-coupled...
transporters, the GABA transporter will seek an equilibrium GABA gradient determined by the stoichiometry of the transporter, the concentration gradients of the substrates, and the membrane potential. At equilibrium, there will be a finite, nonzero [GABA]o, and if [GABA]i drops below this equilibrium value, the transporter will operate in reverse. If this equilibrium [GABA]o is sufficiently high, then tonic GABAergic inhibition would not require continuous vesicular efflux of GABA with diffusion away from the synaptic cleft. Instead, a steady state could exist with no vesicular GABA efflux and no net flux of GABA through the GABA transporter, but with a sufficiently high-ambient [GABA]o to cause tonic activation of GABA receptors.

Under normal physiological conditions, a thermodynamic reaction cycle for the GABA transporter involves coupled translocation of two Na+ ions, one Cl− ion, and one uncharged GABA molecule (Kanner and Schuldiner 1987). Based on this stoichiometry, we calculated [GABA]o at equilibrium as a function of [GABA]i, the [Na+] and [Cl−] gradients, and membrane potential (E m) using the following equation for the reversal potential of the GABA transporter

\[ E_m = \frac{RT}{F(\Delta n_{Na} - \Delta n_{Cl})} \ln \left( \frac{[Na^+]_{o}}{[Na^+]_{i}} \frac{[Cl^-]_{i}}{[Cl^-]_{o}} [GABA]_{i}^{\text{calc}} \frac{[GABA]_{o}}{[GABA]_{i}} \right) \]

where \( n = 2 \) for Na+, 1 for Cl−, and 1 for GABA.

This equation has previously been validated using experimental recordings from GAT-1 and accurately predicts the steady-state equilibrium conditions (Lu and Hilgemann 1999). It is of the same form as an equation for extracellular glutamate at equilibrium, which has also been validated with experimental recordings (Zerangue and Kavanaugh 1996).

We assumed the baseline conditions of E m = −60 mV; [Na+]i = 13 mM (Rose and Ransom 1996, 1997); [Na+]o = 150 mM; [Cl−]i = 7 mM; and [Cl−]o = 135 mM. The [GABA] in the cytosol of GABAergic hippocampal neurons is unknown, but the mean [GABA] of the human occipital lobe is approximately 1 mM (Rothman et al. 1993), and [GABA] in the cell bodies of various neurons ranges from 0.2 to 6.6 mM, with the latter value measured in cerebellar Purkinje cells (Otsuka et al. 1971).

The predicted equilibrium values for [GABA]o were calculated as a function of E m, at different values of [GABA]i (Fig. 7A). These curves define the values of [GABA]o at which there would be no net flux through the GABA transporter (i.e., E m would be at the reversal potential for the transporter). If [GABA]o was greater than (or less than) the equilibrium value, then GABA would be carried into (or out of) the cell by the GABA transporter until [GABA]o reached the equilibrium value.

The following examples illustrate the sensitivity of the GABA transporter to physiologically relevant changes in E m, Na+, and GABA. When [GABA]i is 1 mM, depolarization to +6 mV would result in an increase in equilibrium [GABA]o to 0.5 μM. As seen experimentally (Fig. 1), this [GABA]o would induce a tonic GABA A receptor-mediated current of 450 pA in neighboring neurons at resting potential. High-affinity GABA A receptors are activated at a [GABA]o of <0.1 μM (Levitan et al. 1988; Saxena and Macdonald 1996), a [GABA]o that would be reached outside of neurons at a resting potential of −60 mV when [GABA]i is 2.5 mM, a value that is well below that measured in cerebellar Purkinje cells (Otsuka et al. 1971). Thus the equilibrium [GABA]o for the GABA transporter would likely be high enough to induce a small but measurable tonic GABA A receptor-mediated current in some neurons, even in the absence of vigabatrin treatment. These theoretical predictions are consistent with previously published experimental data demonstrating that tonic GABAergic inhibition occurs in some neurons spontaneously (Bai et al. 2001; Brickley et al. 2003).

**Fig. 7.** Theoretical predictions of the equilibrium [GABA]o for the GABA transporter. Values were calculated using the equation in the text. A: dependence of equilibrium [GABA]o on membrane potential at different [GABA]i; B: dependence of [GABA]o on [Na+]i at different [GABA]i; C: dependence of [GABA]o on membrane potential at different [Na+]i, when [GABA]i = 1 mM; D: dependence of [GABA]o on membrane potential at different [Na+]i, when [GABA]i = 5 mM.
It is not clear how high cytosolic [GABA] rises in neurons after treatment with vigabatrin. However, GABA rises 3.4-fold in human occipital lobe in vivo and fourfold in rat hippocampal slices after treatment with vigabatrin (Engel et al. 2001; Rothman et al. 1993). In addition, we needed to increase the [GABA] in whole cell electrodes to 20 mM to maintain the same size response to SKF-89976a as during perforated patch recordings (Table 1). Thus it is reasonable to assume that [GABA] might reach 10–20 mM in some GABAergic neurons after treatment with vigabatrin. This is interesting in light of the theoretical predictions that the equilibrium [GABA]o would be 0.5 $\mu$M at a membrane potential of $-55$ mV when [GABA]i is 10 mM (Fig. 7A), which correlates with the ambient [GABA] induced experimentally in neurons treated with 100 $\mu$M vigabatrin for 4–5 days (see preceding text and Wu et al. 2001).

The equilibrium [GABA]o would be predicted to increase by the square of [Na+] (Fig. 7B). This is consistent with experimental data showing that depolarization induced by glutamate or veratridine leads to greater nonvesicular GABA release than depolarization induced by elevated [K+] (Belhage et al. 1993; Pin and Bockaert 1989), since the former cause more influx of Na+ than the latter.

These theoretical predictions were supported qualitatively by experimental data, in that raising intracellular [Na+] and [GABA] in the recorded neuron led to an increase in ambient [GABA]. In addition, depolarization also leads to an increase in ambient [GABA] (Gaspar et al. 1998; Wu et al. 2001). However, the model predicts the steady-state, equilibrium conditions, and the cells in culture in our experiments were not at steady state or at equilibrium. In addition, we could not control all the parameters of the model during our experiments. For example, the intracellular [Na+] and [GABA] within the recorded neuron would not be perfectly clamped by the recording pipette solution, especially in the dendrites. In addition, we could not control the contents of neighboring cells, and those cells would influence extracellular [Na+] and [GABA] in regions of cell apposition. Thus it would be necessary to use a simpler experimental system to test these predictions quantitatively, although there is no theoretical reason to assume that the behavior of the transporter under these physiological conditions would diverge from the predicted behavior already validated experimentally using nonphysiological conditions (Lu and Hilgemann 1999).

Normally, $E_m$ and [Na+] do not change independently, both increasing when neurons increase their firing rate. The equilibrium [GABA], would be predicted to be highly sensitive to a combination of depolarization and increased [Na+]. In neurons with an [GABA], of 1 mM, the combination of depolarization to $-30$ mV and an increase in [Na+], to 25 mM would lead to an increase in equilibrium [GABA],, to 0.46 $\mu$M (Fig. 7C). These calculations support the hypothesis that high-frequency neuronal activity, such as during a seizure, would induce nonvesicular GABA release via reversal of the GABA transporter, even without treatment with vigabatrin (Gaspar et al. 1998; Wu et al. 2001). Furthermore, even a modest increase in [GABA], would result in a large increase in equilibrium [GABA],, when combined with depolarization and increased [Na+] (Fig. 7D). The resulting tonic inhibition could explain the anticonvulsant effect of vigabatrin, without requiring that there be any increase in phasic synaptic inhibition.

The GABA transporter is a major determinant of the level of tonic GABAergic inhibition

We propose that tonic inhibition is an expected result of the fact that the GABA transporter is not capable of reducing [GABA]o to an infinitely low level. In a dynamic system, spillover of synaptic vesicular release (Brickley et al. 1996; Rossi and Hamann 1998) and spontaneous mIPSCs (Otis et al. 1991) would both contribute to ambient GABA. However, a high ambient [GABA] would not require a continuous source of GABA release. Even under physiologically relevant conditions, the equilibrium [GABA], for the GABA transporter may be sufficiently high to induce activation of GABA_A receptors even without vesicular release or net efflux through the GABA transporter.

These theoretical considerations for the GABA transporter contrast with predictions for the glutamate transporter (Zerangue and Kavanaugh 1996), which co-transport three Na+ ions and one proton equivalent with each glutamate molecule, while counter-transporting one K+ ion. In contrast to the 25,000-fold gradient that is the maximum theoretical limit for the GABA transporter under physiological conditions, the greater dependence of the glutamate transporter on Na+, and the additional driving force from the K+ gradient, leads to a theoretical transmembrane glutamate gradient exceeding 1,000,000-fold under physiological conditions, with a theoretical lower limit for extracellular glutamate at normal resting potential of 4.6 nM (Zerangue and Kavanaugh 1996). Thus there is a fundamental difference between these two systems, with glutamate being maintained at a level far below that required to activate its receptors, whereas the equilibrium [GABA]o for the GABA transporter is near the threshold for activation of high-affinity GABA_A receptors. In the hippocampus, this may be an advantage, since a very low extracellular glutamate concentration would reduce the chance of excitotoxicity, while a high tonic [GABA] could help raise seizure threshold. These predictions are also consistent with the hypothesis that reversal of the glutamate transporter is a phenomenon that occurs during pathological conditions (Attwell et al. 1993; Levi and Raier 1993), whereas reversal of the GABA transporter occurs under physiological conditions (Gaspar et al. 1998; Wu et al. 2001).

Nonvesicular GABA release can be modified independently from vesicular GABA release

A rise in cytosolic [GABA] might be assumed to lead to an increase in vesicular GABA release, because of an increase in GABA content in each vesicle. However, at a vigabatrin concentration and duration of exposure that produce a large increase in ambient [GABA], we found no effect on mIPSC amplitude, and a decrease in the amplitude of evoked IPSCs during paired recordings.

The decrease in amplitude of evoked IPSCs was not due to inhibition of spikes in the soma of the presynaptic neuron, because neurons were included in the analysis only if each stimulus induced a presynaptic spike and a postsynaptic response. We found no evidence for GABA_B receptor-mediated...
presynaptic inhibition. This effect was also not reproduced by acute bath application of GABA at a concentration sufficient to induce the same amount of tonic GABA_A receptor activation.

We interpret this last result as indicating one of the following: 1) acute application of GABA for 5–10 min does not have the same effect as chronic exposure to GABA for 5 days (e.g., the latter could lead to remodeling of synapses, reducing phasic inhibitory inputs to compensate for the large tonic inhibition); 2) vigabatrin has other effects in addition to an increase in extracellular [GABA]; or 3) the elevation of ambient [GABA] induced by vigabatrin is different from an increase in GABA in the superfused bath solution (e.g., the former may be primarily localized to the extracellular space between cells, whereas the latter would primarily be on the exposed surfaces of neurons). Finally, long periods of exposure to vigabatrin at high concentrations reduce the response to exogenous GABA (Wu et al. 2001), consistent with GABA_A receptor desensitization. However, our data did not support the conclusion that desensitization was the cause of the decrease in evoked IPSCs, because vigabatrin did not lead to a decrease in the size of mIPSCs, and because bath application of GABA did not lead to a decrease in the size of evoked IPSCs or mIPSCs. In addition, the degree of desensitization induced by 0.5 μM GABA (the amount required to produce an equivalent tonic inhibition) should be relatively small (Overstreet et al. 2000). However, it is possible that the elevation in ambient [GABA] is not spatially uniform. Ambient [GABA] may be higher within the synaptic space where it might selectively induce desensitization of synaptic GABA_A receptors. Therefore our data cannot rule out the possibility that desensitization contributed to the decrease in evoked IPSCs by vigabatrin. Such a role for desensitization in inhibition of evoked IPSCs by vigabatrin would be consistent with previously published results (Overstreet and Westbrook 2001).

The effects of chronic vigabatrin on vesicular release described here are similar to those described previously after subacute exposure (100–400 μM for 2–5 h) (Overstreet and Westbrook 2001). In both cases, vigabatrin induced a decrease in evoked IPSC amplitude and a decrease in mIPSC frequency. However, subacute exposure induced a decrease in mIPSC amplitude (Overstreet and Westbrook 2001), whereas we found no effect of chronic exposure on mIPSC amplitude. In a third study (Engel et al. 2001), chronic exposure (4 days) to 40 μM vigabatrin induced a small increase (35%) in mIPSC amplitude. The reason for the different results from these three studies is not clear, although they could be explained by a variety of differences in methodology. The differences between subacute and chronic exposure also suggest that the effect of the drug at 2–5 h may be qualitatively different from the effect at 3–4 days, which could explain why vigabatrin has a proconvulsant effect at 4 h (Loscher et al. 1989), but an anticonvulsant effect after 1–3 days (Gale and Iadarola 1980; Loscher et al. 1989).

Perhaps the most interesting result from these experiments is that a concentration of vigabatrin 2000-fold greater than needed to induce a large amount of nonvesicular GABA release (Wu et al. 2001) does not induce an increase in vesicular GABA release. A vigabatrin concentration of 100–500 μM has been estimated to be a clinically relevant CNS concentration (Abdul-Ghani et al. 1981). However, this estimate was based on data obtained after a single acute dose of the drug. Vigabatrin has unusual pharmacology, as a suicide inhibitor of GABA transaminase (Gale and Iadarola 1980). Therefore the instantaneous concentration is a less important determinant of its effect than the time integral of the concentration. Thus chronic exposure to a low concentration would have the same effect as short exposure to a high concentration. As a result, the clinically relevant concentration during chronic use is likely to be much lower than 100 μM. The possibility should be considered that the effects of 40–400 μM vigabatrin on evoked IPSCs and mIPSCs reported here and by others (Engel et al. 2001; Overstreet and Westbrook 2001) may be irrelevant to the anticonvulsant mechanism. Instead, the relevant effect is more likely to be the large increase in tonic inhibition induced by a concentration as low as 50 nM.

An important conclusion from these results is that it is possible for tonic and phasic GABAergic inhibition to be independently controlled by pharmacological intervention. It is possible that intrinsic regulatory mechanisms also exist to take advantage of this independent control for modifying GABAergic inhibition in vivo.

Functional role of the GABA transporter in vivo

The equation for the reversal potential of the GABA transporter is entirely dependent on the stoichiometry, so that it would accurately define the steady-state, equilibrium conditions when the transporter is expressed in tissue culture, artificial expression systems, or in vivo. However, the dynamics of GABA transport would be strongly dependent on a variety of factors that may be different in culture compared with in vivo. These include the influence of surrounding glia, differences in size of the extracellular space, the ability of GABA to diffuse away from release sites, and regulation of extracellular [K+] and [Na+]. In our culture system, neurons are sitting on top of a glial monolayer superfused with flowing solution. In this situation, extracellular GABA would continuously diffuse away from cells and be carried away by the bath solution. Therefore the GABA transporter would never be at equilibrium and instead would be continuously operating in reverse, releasing GABA to try to raise [GABA]o to the equilibrium value. Under these conditions, blocking the GABA transporter would decrease [GABA]o, as we observed. In contrast, the same situation would not exist in vivo. GABA would still diffuse down its concentration gradient away from points of release. However, it would diffuse toward points of reuptake rather than continuously diffusing away from the brain. An increase in cytosolic [GABA] induced by vigabatrin in vivo would increase the equilibrium [GABA]o. However, as long as the membrane potential and the transmembrane gradients of Na+ and Cl– stay constant, the GABA transporter would not be expected to operate in reverse mode. Instead, there would be net uptake of GABA by the transporter to recover GABA released by vesicular fusion, albeit with a higher steady-state ambient [GABA] and greater tonic GABAergic inhibition. The situation in brain slices would likely be intermediate to that in culture and in vivo. Thus the effect of GABA transporter antagonists on ambient [GABA] would vary depending on the dynamics of the system, so that knowing the direction of GABA flux through the transporter may be less important than defining the equilibrium value for [GABA]o.

The data presented here demonstrate that ambient [GABA]
is highly sensitive to perturbations of the driving force for the GABA transporter and that the transporter normally operates closer to equilibrium than is generally believed. When there is a sustained rise in cytosolic [GABA] induced by drugs such as vigabatrin and gabapentin (Hommoou et al. 1995; Petroff et al. 1996a,b; Wu et al. 2001), there would be an increase in tonic inhibition. During high-frequency firing, depolarization and increased [Na+] would induce transient GABA efflux via the GABA transporter. This nonvesicular GABA release makes up 42% of total K+-induced GABA release measured by blocking vesicular GABA release with zero calcium solution (see RESULTS) and also makes up 42% of total K+-induced GABA release measured by blocking vesicular GABA release with tetanus toxin (Gaspary et al. 1998). Thus, the GABA transporter may make a large contribution to phasic GABAergic inhibition during high-frequency firing, in addition to setting the level of tonic inhibition. The GABA transporter would reverse during high-frequency firing and then return to the “normal” forward direction at the end of the burst of neuronal activity as the cell hyperpolarizes and Na+ is pumped out of the cell. When seizures occur, there is a large elevation in extracellular [K+] (Fisher et al. 1976) that can be worsened by a breakdown in glial homeostasis (Janigro 1999). The large increase in [K+] would in turn lead to depolarization that would cause an increase in ambient [GABA]. This could either help to reduce excitability or else contribute to excitability if depolarizing GABA receptor-mediated responses were to occur (Staley et al. 1995).

Thus rather than acting as a constant vacuum, the GABA transporter may be highly dynamic, with the direction and rate of GABA flux changing continuously with the prevailing conditions. We propose that the GABA transporter is a major determinant of the level of tonic GABAergic inhibition and also plays an important role as a source of nonvesicular GABA release during high-frequency firing.

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