Dynamic Equilibrium of Neurotransmitter Transporters: Not Just for Reuptake Anymore

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Richerson, George B. and Yuanming Wu. Dynamic equilibrium of neurotransmitter transporters: Not just for reuptake anymore. J Neurophysiol 90: 1363–1374, 2003; 10.1152/jn.00317.2003. Many electrophysiologists view neurotransmitter transporters as tiny vacuum cleaners, operating continuously to lower extracellular neurotransmitter concentration to zero. However, this is not consistent with their known behavior, instead only reducing extracellular neurotransmitter concentration to a finite, nonzero value at which an equilibrium is reached. In addition, transporters are equally able to go in either the forward or reverse direction, and when they reverse, they release their substrate in a calcium-independent manner. Transporter reversal has long been recognized to occur in response to pathological stimuli, but new data demonstrate that some transporters can also reverse in response to physiologically relevant stimuli. This is consistent with theoretical calculations that indicate that the reversal potentials of GABA and glycine transporters are close to the resting potential of neurons under normal conditions and that the extracellular concentration of GABA is sufficiently high when the GABA transporter is at equilibrium to tonically activate high-affinity extrasynaptic GABAA receptors. The equilibrium for the GABA transporter is not static but instead varies continuously as the driving force for the transporter changes. We propose that the GABA transporter plays a dynamic role in control of brain excitability by modulating the level of tonic inhibition in response to neuronal activity.

Traditional View of Neurotransmitter Transporters as “Vacuum Cleaners"

Synaptic communication relies on rapid de-activation of postsynaptic receptors to terminate synaptic responses. In many synapses, specific neurotransmitter transporters on neurons and glia contribute to this de-activation by clearing neurotransmitter from the synaptic cleft. These neurotransmitter transporters are members of a large superfAMILY with four major branches: Na+- and Cl−-dependent symporters (e.g., for GABA, monoamines and glycine), Na+- and K+-dependent antiporters (e.g., for glutamate), vesicular transporters, and amino acid transport systems. The Na+- and Cl−-dependent symporters can be divided into four subfamilies in mammals (Fig. 1A). In each case, these transporters utilize the energy available from the transmembrane ion gradients and membrane potential to provide the energy to drive their neurotransmitter substrate up a steep concentration gradient. Under normal conditions, a thermodynamic reaction cycle for GAT-1 involves coupled translocation of two Na+ ions, one Cl− ion and one uncharged GABA molecule (Fig. 1B) (Kanner and Schuldiner 1987). Thus GABA is carried up its concentration gradient using the energy from the inward Na+ gradient and the flow of one positive charge down the electrical gradient.

There is no doubt of the validity of the traditional view that transporters are important for neurotransmitter clearance and recycling after vesicular release. For example, their location is appropriate, with GABA transporters concentrated on presynaptic terminals and peri-synaptic glial membrane (Radian et al. 1990; Ribak et al. 1996). In addition, blocking GABA or glutamate transporters can prolong inhibitory or excitatory postsynaptic currents (IPSCs or EPSCs) (Fig. 2, A and B) (Dingledine and Korn 1985; Isaacson et al. 1993; Overstreet et al. 1999; Roepstorff and Lambert 1992; Thompson and Gutwiler 1992), and blockade of GABA transporters increases extracellular [GABA] as measured with a sniffer patch (Isaacson et al. 1993). Direct evidence for uptake after synaptic neurotransmitter release has also been obtained using measurement of GABA and glutamate transporter currents in glia (Fig. 2, C and D) (Bergles and Jahr 1997; Kinney and Spain 2002).

Despite clear support for this model of synaptic function, there are reasons to believe that it does not provide a complete picture of transporter function. For example, the effect of transporter antagonists on IPSCs and EPSCs can be small, especially in response to low-frequency stimulation (Isaacson et al. 1993; Nusser and Mody 2002; Overstreet and Westbrook 2003; Overstreet et al. 1999; Rossi and Hamann 1998). Glutamate transporter blockade has relatively little effect on AMPA receptor-mediated glutamatergic EPSCs induced by a single stimulus unless cells are first treated with cyclothiazide to reduce receptor desensitization (Overstreet et al. 1999) (Fig. 2B). Rather than simply controlling the duration of EPSCs, there is evidence that transporters may be more important for prevention of overspill onto extrasynaptic receptors and crosstalk to neighboring neurons (Overstreet and Westbrook 2003; Rossi and Hamann 1998). Transporters are also present in locations where they would not be involved in termination of synaptic transmission, such as on extrasynaptic membranes. For example, in the optic nerve, a white matter tract without synapses, GABA transporters are located on oligodendrocytes and GABA receptors are present on axons (Sakatani et al. 1996).
GABA transporters are members of the Na\(^+\) and Cl\(^-\) dependent transporter family. The GABA transporter subfamily includes transporters of GABA (GAT-1, GAT-2, GAT-3, and GAT-4), taurine (TAUT), betaine (BETAT), and creatine (CRET). The monoamine transporter subfamily includes transporters of serotonin (SEROT), norepinephrine (NORAT), and dopamine (DOPAT). The amino acid transporter subfamily includes transporters of glycine (GLYT1 and GLYT2) and proline (PROT). The “orphan” transporter subfamily (identified by sequence homology, substrate unknown) includes renal osmotic stress-induced transporter (ROSIT), neurotransmitter transporter rB21 and neurotransmitter transporter 4 (NTT4).

For ion channels, substrate transporters on the transmembrane ion gradients. Just as is the case for ion channels, substrate flux through transporters is determined by the transmembrane electrochemical potential ($\Delta \mu$), which is the sum of the electrical potential ($\Delta \Psi$) and chemical potential ($\Delta G$). For a single molecule X, the driving force is quantified as

$$\Delta \mu_X = \Delta \Psi_X + \Delta G_X = z_X \cdot F \cdot E_m + R \cdot T \cdot \ln \left( \frac{[X]_{in}}{[X]_{out}} \right)$$

where $z_X$ is the valence of X, $F$ is Faraday’s constant, $E_m$ is membrane potential, $R$ is universal gas constant, and $T$ is temperature. It should be noted that when $\Delta \mu = 0$ (i.e., X is at equilibrium), this equation reduces to the Nernst equation.

For a symporter like GAT-1, all of the co-transported molecules are coupled to each other as they cross the membrane (i.e., they are not independent). Therefore the total electrochemical driving force for GAT-1, $\Delta \mu_{GAT-1}$, is the sum of all the linked contributions from each co-transported molecule (Aronson et al. 2003). Because one thermodynamic reaction cycle for GAT-1 involves coupled translocation of two sodium ions, one chloride ion and one uncharged GABA molecule, this is quantified as

$$\Delta \mu_{GAT-1} = 2 \cdot (\Delta \Psi_{Na} + \Delta G_{Na}) + 1 \cdot (\Delta \Psi_{Cl} + \Delta G_{Cl}) + 1 \cdot (\Delta G_{GABA})$$

and when $\Delta \mu_{GAT-1} = 0$, this equation reduces to

$$E_m = -\frac{R \cdot T}{2 \cdot Z_{Na} + Z_{Cl}} \cdot \ln \left( \frac{[GABA]_{in}}{[GABA]_{out}} \right) \left( \frac{[Na^+]_{in}}{[Na^+]_{out}} \right)^2 \left( \frac{[Cl^-]_{in}}{[Cl^-]_{out}} \right)$$

which defines the reversal potential for the transporter. It should be noted that this latter equation has a very similar form as the Goldman-Hodgkin-Katz equation for an ion channel. However, there are two major differences. First, if an ion channel is permeable to an uncharged molecule like GABA, that molecule does not contribute to the G-H-K equation. Second, if an ion channel is permeable to more than one ion, each ion moves independently of the others (i.e., the movement of each one is dependent only on the driving force for that ion). In contrast, for a symporter like GAT-1, all co-transported molecules will cross the membrane together, requiring that each of their driving forces summate to give a total driving force for all of them together. When there are two molecules of one ion (e.g., Na\(^+\)) that move together, each one contributes to...
the driving force. Despite these quantitative differences in the form of theequations for the transmembrane electrochemical potential for ion channels and transporters, the calculated driving force for a transporter can be viewed in the same way as the driving force for an ion channel. In other words, there will be no net substrate flux when membrane potential is equal to the reversal potential, and when it is not there will be a driving force generated to move solute in the direction needed to return membrane potential back to the reversal potential.

The solution to the equation for $\Delta_{\phi_{\text{GAT-1}}}$ is shown in Fig. 3A, under three different conditions. Under control conditions, the concentrations of $Na^+$, $Cl^-$, and GABA were chosen to approximate those in the normal hippocampus. These values resulted in a calculated reversal potential of $-62\, \text{mV}$, which is close to the normal resting potential of neurons. This may seem surprising, but is actually what should be expected because the GABA transporter would be likely to reach its equilibrium under normal conditions. From this control curve, it can be seen that as long as the membrane potential is more negative than the reversal potential, the transporter will operate in the inward direction and cells will take up GABA (forward transport). However, if the membrane potential is above the reversal potential, then the transporter will reverse and release GABA (reverse transport). Just like an ion channel, the driving force is determined by the combined chemical and electrical potential difference for all of the co-transported substrates. For GAT-1, this total transmembrane electrochemical potential, $\Delta_{\phi_{\text{GAT-1}}}$, or driving force, is dependent on the membrane potential and the transmembrane gradients for sodium, chloride, and GABA. Based on the equations in the text, this driving force is plotted for 3 conditions, as illustrated at the top. Control conditions were chosen to approximate the normal concentrations of the substrates inside and outside of a hippocampal neuron. The calculated reversal potential under these conditions ($-62\, \text{mV}$) was close to the normal resting potential of neurons. As would be the case for an ion channel, there would be net influx of substrate (forward transport) when the membrane potential was more negative than the reversal potential, and there would be net efflux (reverse transport) when the membrane potential was more positive. An increase in intracellular $[Na^+]$ to 30 mM led to a negative shift in the reversal potential to $-99\, \text{mV}$, so that there would be reverse transport at normal resting potential. An increase in extracellular [GABA] to 10 $\mu$M led to a positive shift in the reversal potential to $+60\, \text{mV}$, so that there would be a large driving force for GABA uptake at all physiologically relevant membrane potentials. Vertical dotted line highlights the reversal potential for the control conditions, and the direction of the arrows in the 3 schematics at top represent the direction of substrate flux when the membrane potential is equal to $-62\, \text{mV}$.

**FIG. 2.** GABA and glutamate transporters terminate postsynaptic responses by removing neurotransmitter from the synaptic cleft. A: presynaptic stimulation of a hippocampal GABAergic neuron induces an inhibitory postsynaptic current (IPSC) in a pyramidal cell (top). The GABA transporter antagonist tiagabine causes prolongation of the IPSC (bottom). Adapted from Thompson and Gahwiler (1992). B: the glutamate transporter antagonist L-trans-pyrrolidine-2,4-dicarboxylate (L-trans-PDC) has no effect on an excitatory postsynaptic current (EPSC) induced in a cerebellar granule cell (top; control and L-trans-PDC responses are superimposed). Treatment with cyclothiazide decreases desensitization and prolongs the EPSC (bottom). After cyclothiazide, L-trans-PDC further prolongs the EPSC [from Overstreet et al. (1999)]. C: presynaptic stimulation in the neocortex induces a current in astrocytes that is partially blocked by the GAT-1 inhibitors NO-711 and SKF-89976a (top). The difference between these 2 curves (bottom) is the transporter current induced by uptake into glia of synaptically released GABA [from Kinney and Spain (2002)]. D: stimulation of Schaffer collateral fibers induces a current in glia in hippocampal slices. This is a transporter current induced by reuptake of synaptically released glutamate, because it is blocked by the glutamate transporter antagonist D,L-threo-3-hydroxyaspartate [THA; from Bergles and Jahr (1997)].
determined by the difference between the membrane potential and the reversal potential. It should be noted that the driving force is expressed in kilocalories per mole of substrate transported across the membrane. This driving force can be converted using appropriate constants to an equivalent electrical potential (in millivolts) as is the convention for the driving force for an ion channel.

As expected, the reversal potential varies depending on the local prevailing conditions, and is sensitive to the sodium and GABA gradients. When intracellular [Na\(^+\)] was doubled to 30 mM, this caused a shift in the reversal potential to \(-99\) mV (Fig. 3). This example illustrates two important points: an increase in intracellular [Na\(^+\)] can lead to net efflux of GABA at resting potential and the GABA transporter is more sensitive than an ion channel to changes in the sodium gradient (the corresponding change in Nernst potential for Na\(^+\) would only be \(18\) mV). This is due to the coupled translocation of two sodium ions.

When extracellular [GABA] was increased 100-fold, there was a shift in reversal potential to \(+60\) mV (Fig. 3). Thus unlike an ion channel, a change in the gradient of an uncharged molecule leads to a shift in reversal potential, and this shift was \(-60\) mV with each 10-fold change in the gradient. This dependence of reversal potential on the GABA gradient is due to the fact that there is obligatory co-transport of one net positive charge with each GABA molecule. This example illustrates that a sufficiently large increase in extracellular [GABA] can lead to net influx of GABA at all physiologically relevant membrane potentials, so that as expected would be net uptake of extracellular GABA in response to synaptic GABA release. However, the transporter could still reverse given sufficiently strong depolarization.

There have been many experimental studies that have confirmed that the GABA transporter will reverse, in most cases using biochemical measures of GABA release. For example, after removal of extracellular Ca\(^{2+}\), GABA release still occurs from cortical slices in response to an increase in K\(^+\) to 50 mM, to exposure to 50 \(\mu\)M veratridine, or to high-frequency electrical stimulation (Szerb 1979). Similarly, GABA release still occurs in the absence of extracellular Ca\(^{2+}\) from cultured striatal neurons (Pin and Bockaert 1989) and from cultured cortical neurons (Belhage et al. 1993) in response to 55–56 mM K\(^+\) or 100 \(\mu\)M glutamate. In fact, GABA release can even occur after treatment with tetanus toxin combined with removal of extracellular Ca\(^{2+}\) (Fig. 4A). This GABA release is due to transporter reversal because it is blocked by GABA transporter antagonists (Belhage et al. 1993; Pin and Bockaert 1989).

GABA transporter reversal has also been measured directly using electrophysiological recordings from GABA transporters. For example, GAT-1 transporter currents reverse in response to depolarization (Fig. 4B) or an increase in intracellular Na\(^+\) (Lu and Hilgemann 1999). Similar experiments have revealed that the GABA transporter can sometimes operate in an “uncoupled” manner, whereby the stoichiometry is not fixed, instead diverging from the normal ratio of 2 Na\(^+\):1 Cl\(^-\):1 GABA (Cammack et al. 1994). This slippage is generally believed to only occur under artificial conditions and be an imperfection in the design of transporters; but it has been suggested that slippage may actually play an important role as a safety valve in dissipating excess driving force (Nelson et al. 2002). However, the majority of electrophysiological and biochemical assays have indicated that GABA transporters operate with a fixed stoichiometry under most physiological conditions, and their behavior, including passive reversal, is accurately predicted by the equations shown above. For example,
TABLE 1. **Differences between vesicular and transporter-mediated GABA release**

<table>
<thead>
<tr>
<th></th>
<th>Vesicular Release</th>
<th>Transporter Reversal</th>
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<tbody>
<tr>
<td>Cell depolarization</td>
<td>Enhances</td>
<td>Enhances</td>
</tr>
<tr>
<td>Low calcium</td>
<td>Blocks</td>
<td>No effect</td>
</tr>
<tr>
<td>Tetanus or botulinum toxin</td>
<td>No effect</td>
<td>Enhances</td>
</tr>
<tr>
<td>Energy depletion</td>
<td>Reduces</td>
<td>Enhances</td>
</tr>
<tr>
<td>Source of GABA</td>
<td>Neurons only</td>
<td>Neurons and glia</td>
</tr>
<tr>
<td>Location of pool</td>
<td>Synaptic vesicles</td>
<td>Cytosol</td>
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<tr>
<td>Nipecotic acid</td>
<td>Prolongs IPSPs</td>
<td>Induces release, then blocks</td>
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<tr>
<td>Noncompetitive transport blockers</td>
<td>Prolong IPSPs</td>
<td>Block</td>
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Energy depletion leads to a decrease in the ability to repackaging vesicles but enhances transporter-mediated GABA release due to breakdown of the Na\(^+\) gradient and membrane potential. Nipecotic acid induces transporter reversal via heteroexchange followed by blockage of release and reuptake. Noncompetitive transport blockers (e.g., NO-711 and SKF-89976a) only block release and reuptake.

The validity of these calculations has been experimentally verified for both the glutamate (Zerangeh and Kavavanaugh 1996) and GABA transporters (Lu and Hilgemann 1999).

GABA transporter reversal can also be induced pharmacologically by nipecotic acid. This compound is a GABA analogue that induces a phenomenon referred to as heteroexchange release (Honmou et al. 1995; Solis and Nicoll 1992; Szerb 1982a). Nipecotic acid does not directly activate GABA receptors. However, it indirectly induces GABA receptor-mediated currents when applied to cultures or brain slices by causing nonvesicular release of GABA from neighboring cells. This release is blocked by antagonists of GABA transporters. The molecular mechanism of heteroexchange GABA release has not been well defined but is widely believed to be due to the exchange of one intracellular GABA molecule for one extracellular nipecotic acid molecule (Fig. 4C). Continued application of nipecotic acid later causes GABA transporter blockage. These dual effects of this drug can sometimes make it difficult to interpret a response to its application.

In summary, there is a large body of experimental data showing that GABA transporters can reverse, and there is a solid theoretical framework for explaining why this can happen. Table 1 compares the conditions that induce vesicular release with those that induce transporter-mediated release.

**The GABA transporter reverses surprisingly easily**

The stimuli used to induce GABA transporter reversal in many previous studies would not occur under physiological conditions (e.g., 55 mM K\(^+\) or 100 \(\mu\)M glutamate). The use of such strong stimuli has led many to assume that the GABA transporter won’t reverse without a pathologically large stimulus. However, this is not the case, and the use of strong stimuli was probably a result of the relative insensitivity of the biochemical assays used to measure GABA release. As suggested by the calculations in Fig. 3, the reversal potential for GAT-1 would be expected to be close to the normal resting potential of neurons, so that a small level of depolarization would cause reversal. This is supported by some early experiments that clearly demonstrated that GABA transporters can reverse under physiologically relevant conditions. For example, calcium-independent GABA release can be induced by electrical stimulation of slices from the rat cortex (Szerb 1979) and striatum (Bernath and Zigmond 1988). In the fish retina, depolarization of GABAergic horizontal cells, which are deficient in synaptic vesicles, induces a postsynaptic response in bipolar cells (Schwartz 1987). This response is due to GABA release via reversal of the GABA transporter because it is not dependent on calcium and is blocked by nipecotic acid.

We directly tested the hypothesis that a small depolarizing stimulus could induce reversal of GABA transporters from the mammalian hippocampus and that the resulting nonvesicular GABA release leads to neuronal inhibition (Gasparly et al. 1998; Wu et al. 2001). Depolarization was induced by an increase in [K\(^+\)]\(_i\) from 3 to 6–12 mM. This stimulus is physiologically relevant, since [K\(^+\)]\(_i\) increases during neuronal firing in vivo. For example, an increase in [K\(^+\)]\(_i\), to >12 mM occurs in the rat hippocampus in vivo in response to electrical stimulation of the angular bundle (Somjen and Giacchino 1985). The method chosen to assay GABA release was to use electrophysiological recordings from neurons because this is a very sensitive way of detecting the component of GABA release that is functionally relevant (Fig. 5A). Patch-clamp recordings were made from rat hippocampal neurons in culture under conditions designed to block all ion channels in the recorded neuron except GABA\(_A\) receptors (Gasparly et al. 1998; Wu et al. 2001). Recordings were made in the absence of extracellular Ca\(^{2+}\) or after pretreating neurons with tetanus toxin, both of which are widely used to block vesicular GABA release. An increase in [K\(^+\)]\(_i\) to 12 mM led to a large chloride current. This current was due to activation of GABA\(_A\) receptors because it was blocked by bicuculline (Fig. 5B) and picrotoxin. The GABA release that caused the activation of GABA\(_A\) receptors was due to transporter reversal because it was blocked by SKF-89976a and NO-711, two GAT-1 antagonists. An increase in [K\(^+\)]\(_i\) to as little as 6 mM was sufficient to induce transporter reversal (Wu et al. 2001). These results demonstrate that even a small increase in [K\(^+\)]\(_i\) induces GABA transporter reversal, increasing ambient [GABA] and inducing neuronal inhibition.

The experiments described here are consistent with the calculations of Fig. 3 predicting that the reversal potential of GAT-1 is close to its equilibrium under normal resting conditions. In addition, when neurons increase their firing rate, there is an increase in cytosolic [Na\(^+\)], which would cause a negative shift in the reversal potential (Fig. 3). When combined with the increase in membrane potential (due to firing as well as increased extracellular [K\(^+\)]) (Somjen and Giacchino 1985), this would lead to a large outward driving force for GABA efflux. For these reasons, reversal of GABA transporters would be greatest during high-frequency firing. These predictions should be placed in context with the normal role of the GABA transporter in reuptake of synaptic GABA. During an IPSP, [GABA] within the synaptic cleft rises to near 1 mM (Jones and Westbrook 1995; Mozrzymas et al. 1999). This would lead to a theoretical reversal potential of +182 mV, assuming all other values are the same as for the control example in Fig. 3. Thus the increase in synaptic [GABA] during an IPSP would lead to such a large driving force that reuptake would still...
Because it was blocked by bicuculline (middle). The GABA release was due to C4 heteroexchange GABA release induced by nipecotic acid (Fig. and Honmou 1994), it was found that gabapentin increases recordings from rat hippocampal slices (Honmou et al. 1995) nance spectroscopy (Petroff et al. 1996). Using patch-clamp levels in humans in vivo as measured using magnetic reso-

the GABA transporter. However, it does increase brain GABA

transporter reversal. Gabapentin was designed as a lipophilic convulsant drugs, gabapentin and vigabatrin, enhance GABA (e.g., tiagabine). Recently, we have shown that two new anti-

tors (e.g., benzodiazepines, barbiturates) or blocking reuptake GABAergic inhibition, such as by potentiating GABA recep-

FIG. 5. An increase in [K+]o causes depolarization, which induces an increase in ambient [GABA]. A: patch-clamp readings of GABA transporters mediated currents were used to assay GABA release. Experiments were performed in 0 calcium bath solution. When the GABA transporter reversed, GABA release was detected as a change in holding current in the recorded neuron. B: application of 12 mM K+ (bar) caused GABA release that induced an inward current (left). This current was due to GABA_A receptor activation because it was blocked by bicuculline (middle). The GABA release was due to GAT-1 reversal because it was blocked by SKF-89976a and NO-711 (not shown) [from Gaspary et al. (1998)].

occur at all physiologically relevant membrane potentials, consistent with a role of transporters in terminating IPSPs.

GABA TRANSPORTER REVERSAL IS ENHANCED BY TWO NEW ANTICONVULSANTS

Many anticonvulsant drugs prevent seizures by increasing GABAergic inhibition, such as by potentiating GABA receptors (e.g., benzodiazepines, barbiturates) or blocking reuptake (e.g., tiagabine). Recently, we have shown that two new anticonvulsant drugs, gabapentin and vigabatrin, enhance GABA transporter reversal. Gabapentin was designed as a lipophilic GABA agonist but has no direct effect on GABA receptors or the GABA transporter. However, it does increase brain GABA levels in humans in vivo as measured using magnetic resonance spectroscopy (Petroff et al. 1996). Using patch-clamp recordings from rat hippocampal slices (Honmou et al. 1995) as well as sucrose gap recordings from rat optic nerve (Kocsis and Honmou 1994), it was found that gabapentin increases heteroexchange GABA release induced by nipecotic acid (Fig. 4C). This observation provided the first electrophysiological evidence for an effect of gabapentin on GABAergic inhibition. Interestingly, gabapentin also leads to an increase in expression of GABA transporters on the cell surface (Whitworth and Quick 2001). It is unclear whether these two observations are related, or if one causes the other. However, it is conceivable that either an increase in number of functional GABA transporters increases the flux of GABA when transporter reversal occurs or an increase in ambient [GABA] due to enhanced transporter reversal leads to an increase in surface expression of transporters (Bernstein and Quick 1999). These findings clearly link the GABA transporter with gabapentin, a widely used medication whose mechanism has eluded definition.

Vigabatrin is an anticonvulsant whose biochemical mechanism has been well defined, but whose electrophysiological mechanism has remained unclear. This drug works as a “suicide substrate” for GABA transaminase, binding irreversibly to inhibit the enzyme. Recovery requires synthesis of new protein. As would be predicted, blocking GABA transaminase induces an increase in brain GABA levels (Rothman et al. 1993; Schechter et al. 1977) and also enhances GABA release (Gram et al. 1988; Szerb 1982b). It has been widely assumed that the increase in GABA would enhance inhibitory synaptic transmission. However, there has been no direct evidence for an increase in inhibition, and in fact vigabatrin can cause a decrease in synaptic inhibition (Jackson et al. 1994; Overstreet and Westbrook 2001). To define the electrophysiological mechanism of this drug, we made patch-clamp recordings from neurons in rat hippocampal cultures pretreated with 100 μM vigabatrin. There was no effect of vigabatrin on the amplitude of spontaneous miniature IPSCs, and there was a small paradoxical decrease in the amplitude of evoked IPSCs during paired recordings (Wu et al. 2003). In contrast to the lack of an increase in this “phasic” inhibition, there was a marked enhancement of “tonic” GABAergic inhibition due to an increase in ambient [GABA] (Wu et al. 2001, 2003). The tonic inhibition was not prevented by calcium-free bath solution or tetanus toxin pretreatment (Fig. 6, A and B) (Wu et al. 2001, 2003). During perforated-patch recordings, the increase in ambient [GABA] was prevented with NO-711 and SKF-89976a, two GAT-1 specific antagonists (Wu et al. 2001, 2003). Thus the increase in ambient [GABA] was due to continuous GABA efflux via transporter reversal. These results, together with the equations shown above, suggest that the increase in cytosolic [GABA] induced by both gabapentin and vigabatrin causes the reversal potential for the GABA transporter to become more negative. This could in turn lead to GABA transporter reversal even at normal resting potential.

One of the most interesting results from these experiments was that when patch-clamp recordings were made in whole cell mode, GAT-1 blockade caused an increase in ambient [GABA] (Fig. 6C), which is opposite the response seen when using perforated-patch recordings (Wu et al. 2003). Thus whole cell recordings can lead to a conclusion about the role of the GABA transporter that is exactly opposite that reached when perforated-patch recordings are used. The reason for this artifact induced by whole cell recording is unclear, but it could be prevented simply by adding Na+ and GABA to the whole cell electrode solution (Fig. 6D). The simplest explanation is that there is a loss of Na+ and GABA from the recorded neuron during whole cell recordings. However, it is possible that some other factor is lost from cells, but Na+ and GABA in the pipette solution lead to normalization of the response. There are some reasons to believe that this latter possibility might be
served in vitro is likely to be related to the anticonvulsant effect of vigabatrin, and was concentration dependent. B: the tonic inhibition induced by vigabatrin, and was concentration dependent. B: the tonic inhibition induced by vigabatrin (100 \text{ \textmu M}) was very slow to develop, requiring 4–5 days to reach a maximum and was sustained for hours after washout of the drug (Fig. 7B). This slow onset and lingering effect is consistent with slow inactivation of GABA transaminase followed by a further delay in the increase in cytosolic [GABA]. This temporal profile reflects the anticonvulsant effect of the drug in vivo. After a single intraperitoneal injection of vigabatrin, the elevation in brain GABA levels and the depression of GABA transaminase activity both last for >4 days (Jung and Palfreyman 1995). Total GABA levels peak within 12 h, but the GABA pool associated with nerve terminals doesn’t peak until 60 h, and it is the latter that correlates with the peak anticonvulsant effect (Gale and Iadarola 1980). Thus both the potency of the drug and the temporal profile

FIG. 6. Vigabatrin causes tonic inhibition of cultured hippocampal neurons due to spontaneous GABA transporter reversal. Same methods as shown in Fig. 5A. A: after treatment with 100 \text{ \textmu M} vigabatrin for 4 days, a large tonic 

ambient [GABA] in culture indicates that much lower concentrations of the drug are effective than those suggested by the peak serum levels.

The time course of the effect of vigabatrin in vitro also suggests that the increase in ambient [GABA] is relevant to the anticonvulsant effect in vivo. Vigabatrin can directly inhibit the GABA transporter, but this effect is immediate and is reversible on washout (Jung and Palfreyman 1995). In contrast, tonic inhibition induced by vigabatrin in vitro required 4–5 days to reach a maximum and was sustained for hours after washout of the drug (Fig. 7B). This slow onset and lingering effect is consistent with slow inactivation of GABA transaminase followed by a further delay in the increase in cytosolic [GABA]. This temporal profile reflects the anticonvulsant effect of the drug in vivo. After a single intraperitoneal injection of vigabatrin, the elevation in brain GABA levels and the depression of GABA transaminase activity both last for >4 days (Jung and Palfreyman 1995). Total GABA levels peak within 12 h, but the GABA pool associated with nerve terminals doesn’t peak until 60 h, and it is the latter that correlates with the peak anticonvulsant effect (Gale and Iadarola 1980). Thus both the potency of the drug and the temporal profile

FIG. 7. Concentration and time dependence of the effect of vigabatrin on tonic inhibition. Summary of data from experiments like that in Fig. 6A. A: tonic inhibition was induced after 3–4 days of treatment with as little as 50 nM vigabatrin, and was concentration dependent. B: the tonic inhibition induced by vigabatrin (100 \text{ \textmu M}) was very slow to develop, requiring >4 days to reach a maximum. For both parts, all data points for vigabatrin-treated neurons are significantly greater than control data points [P < 0.001; from Wu et al. (2001)].
suggest that the anticonvulsant effect of the drug is due to the increase in tonic inhibition.

**AMBIENT GABA AND TONIC INHIBITION**

Until recently, GABAergic inhibition was believed to be mediated solely by phasic IPSPs due to vesicular GABA release. During the last decade, it has been recognized that there is also a tonic form of inhibition like that seen after vigabatrin treatment, but occurring spontaneously (i.e., without drug treatment). This tonic inhibition is due to an ambient [GABA] that is sufficiently high to continuously activate GABA<sub>A</sub> receptors on some neurons (Bai et al. 2001; Brickley et al. 1996, 2001; Liu et al. 2000; Nusser and Mody 2002; Otis et al. 1991; Rossi and Hamann 1998; Stell and Mody 2002; Vautrin et al. 2000; Wall and Usowicz 1997; Yeung et al. 2003). The magnitude of this tonic inhibition can be surprisingly high with the total charge transfer greatly exceeding that due to phasic GABA release (Brickley et al. 1996; Nusser and Mody 2002; Wall and Usowicz 1997). The specific role of tonic inhibition is unknown, but it would clearly have a large effect on brain excitability. The shunt current may also contribute to "gain modulation" or responsiveness to synaptic inputs (Chance et al. 2002).

Tonic GABAergic inhibition results from activation of extrasynaptic, high-affinity GABA<sub>A</sub> receptors that do not desensitize (reviewed in Mody 2001). The evidence that the two types of inhibition are mediated by different types of GABA receptor comes in part from the observation that tonic inhibition and phasic IPSCs can be pharmacologically separated. NO-711 and midazolam selectively enhance tonic inhibition (Nusser and Mody 2002; Yeung et al. 2003), whereas zolpidem selectively enhances phasic inhibition (Nusser and Mody 2002). A low concentration of gabazine blocks phasic inhibition without affecting tonic inhibition, whereas higher concentrations also block tonic inhibition (Stell and Mody 2002; Yeung et al. 2003).

Ambient [GABA] has been measured to be 0.2–0.8 μM in the rat hippocampus (Lerma et al. 1986; Tossman et al. 1986). This [GABA] is sufficient to activate extrasynaptic GABA<sub>A</sub> receptors but not GABA<sub>A</sub> receptors within the synaptic cleft. The specific subunit composition of high-affinity, extrasynaptic GABA<sub>A</sub> receptors is unclear, but δ subunits are only found in extrasynaptic membrane (Nusser et al. 1998). There is a preferential association of the δ subunit with the α<sub>6</sub> subunit, and GABA<sub>A</sub> receptors with the subunit composition of α<sub>6</sub>β<sub>2</sub>δ or α<sub>6</sub>β<sub>3</sub>δ have a high affinity for GABA (EC<sub>50</sub> = 0.19–0.27 μM) (Saxena and Macdonald 1996) and also have greatly reduced desensitization (Saxena and Macdonald 1994). These are precisely the properties expected of GABA<sub>A</sub> receptors involved in tonic inhibition, and in fact GABA<sub>A</sub> receptors containing the α<sub>6</sub> subunit appear to contribute to tonic inhibition in cerebellar neurons (Rossi and Hamann 1998). This is consistent with the finding that tonic GABAergic inhibition is absent in the cerebellum of mice lacking expression of extrasynaptic α<sub>6</sub>β<sub>3</sub>δ receptors due to targeted deletion of the α<sub>6</sub> gene (Brickley et al. 2001). Although the α<sub>6</sub> and δ subunits appear to be responsible for tonic inhibition in the cerebellum, the α<sub>6</sub> subunit is limited within the brain primarily to cerebellar granule cells (Jones et al. 1997). There is less known about the subunit composition of GABA<sub>A</sub> receptors that contribute to tonic inhibition in the hippocampus and elsewhere (Mody 2001).

A variety of other mechanisms may also be involved in producing tonic inhibition, including a continuous barrage of spontaneous miniature IPSCs that summate with each other (Otis et al. 1991), "spillover" of GABA out of synapses onto extrasynaptic membrane (Brickley et al. 1996; Rossi and Hamann 1998), detachment of GABA from binding sites on a surface matrix that becomes exposed during exocytosis (Vautrin et al. 2000), release of GABA from astrocytes (Liu et al. 2000), and constitutive activation of GABA<sub>A</sub> receptors in the absence of GABA (Neelands et al. 1999). The relative importance of each of these mechanisms in tonic inhibition is unclear, and may vary depending on the experimental conditions.

**ROLE OF THE GABA TRANSPORTER IN TONIC INHIBITION**

There has been relatively little focus on the role of the GABA transporter in regulating the level of tonic inhibition. The standard approach to determine whether GABA transporter reversal contributes to tonic inhibition is to apply GABA transporter antagonists and see whether tonic inhibition increases or decreases. However, as discussed in the preceding text, the existence of an artifact when using whole cell recordings was not previously recognized, so that some of the previous work using this approach may have reached an erroneous conclusion about the role of GABA transporter reversal in tonic inhibition. In addition, it is not necessary for GABA transporters to reverse to play an important role in regulating tonic inhibition. GABA transporters do not function like vacuum cleaners, working continually in the forward direction to reduce [GABA]<sub>i</sub> to zero. Instead, they try to maintain ambient [GABA] at their set point (i.e., the reversal potential). If the set point was sufficiently low, it would be likely that tonic inhibition would be low even if there was a large amount of vesicular GABA release. If the set point was high, then ambient [GABA] and tonic inhibition would be high even if there was only a small amount of vesicular GABA release. Under both conditions, the transporter may still operate in the forward direction to take up GABA released by vesicular fusion. However, an increase in the set point would result in a higher ambient [GABA] even if the amount of vesicular GABA release is small. Therefore, instead of focusing on the direction of GABA flux, an alternative way to view the role of the GABA transporter is to calculate the [GABA]<sub>i</sub> when the transporter is at equilibrium, and assume that the transporter is maintaining [GABA]<sub>i</sub> close to this equilibrium. This is a valid assumption, supported by the calculations in Fig. 3 for control conditions, which show that the reversal potential is near the normal resting potential.

We rearranged the equation for the reversal potential of GAT-1 (see preceding text) to solve for extrasomal [GABA] instead of membrane potential (Wu et al. 2003). These calculations predicted that ambient [GABA] would be high enough (>0.1 μM) under physiological conditions to activate high-affinity GABA<sub>A</sub> receptors. At a membrane potential of −60 mV, and a [Na<sup>+</sup>]<sub>i</sub> of 13 mM, [GABA]<sub>i</sub> at equilibrium would be 0.1 μM if cytosolic [GABA] is 2.5 mM (Fig. 8A). Although it is difficult to precisely measure free cytosolic [GABA], this value is consistent with experimentally measured [GABA] in
would have a $[\text{GABA}]_o$ of 0.26

an even greater increase in the equilibrium $[\text{GABA}]_o$ (Fig. 8).

Different levels of $[\text{GABA}]_i$. $[\text{Na}^+]$ to a large increase in the equilibrium $[\text{GABA}]_o$ from Wu et al. (2003). As long as there is vesicular GABA release, then either mechanism could lead to an increase in tonic inhibition. Thus tonic inhibition is not dependent on reversal of GABA transporters, and in fact GABA transporters would normally operate in the forward direction to prevent excessive tonic inhibition, by virtue of reuptake of vesicular release—precisely as predicted by the classical view of the GABA transporter. This dichotomy may seem counterintuitive, but these dual roles of the GABA transporter in reducing tonic inhibition by reuptake and regulating the level of tonic inhibition via changes in the equilibrium $[\text{GABA}]_i$, follow naturally from the biophysics of transporters. To accomplish the latter, the transporter will reverse whenever necessary, but reversal is not a prerequisite for an increase in $[\text{GABA}]_o$.

As shown in Fig. 3, the reversal potential for GAT-1 is sensitive to the GABA gradient, so the ambient $[\text{GABA}]_o$ is high enough to activate high-affinity, extrasynaptic GABA$_A$ receptors (see text). $B$: effect of membrane potential on the equilibrium $[\text{GABA}]_o$, at different levels of $[\text{Na}^+]$. $[\text{GABA}]_i$ was 5 mM. Note that depolarization combined with an increase in $[\text{Na}^+]$, such as would occur during a burst of firing, would lead to a large increase in the equilibrium $[\text{GABA}]_o$, [from Wu et al. (2003)].

Some cells (Otsuka et al. 1971). In fact, cytosolic [GABA] has been reported to be 6.6 mM in Purkinje cells in the cerebellum (Otsuka et al. 1971), a brain region in which tonic inhibition is particularly prominent (Brickley et al. 1996; Rossi et al. 2003). As shown in Fig. 3, the reversal potential for GAT-1 is sensitive to the GABA gradient, so the ambient [GABA] would be expected to be higher outside neurons with a relatively high cytosolic [GABA]. For example, cells with a cytosolic [GABA] of 6.6 mM and a membrane potential of $-60 \text{ mV}$ would have a $[\text{GABA}]_o$ of 0.26 $\mu\text{M}$ at equilibrium. These calculations suggest that the GABA transporter may regulate ambient GABA at a level high enough to cause spontaneous tonic inhibition in some parts of the brain. When neurons increase their firing rate, the resulting increase in membrane potential, combined with an increase in $[\text{Na}^+]$, would lead to an even greater increase in the equilibrium $[\text{GABA}]_o$ (Fig. 8B). This would be particularly important during stroke or seizures when there is a collapse of electrochemical gradients across membranes. However, as illustrated in Fig. 8, such extreme conditions would not be necessary for the equilibrium $[\text{GABA}]_o$ to reach a high level. The theoretical importance of the equilibrium value is that the GABA transporter would be incapable of lowering ambient [GABA] below it and would in fact reverse if $[\text{GABA}]_o$ dropped below this value.

In hippocampal cultures treated with vigabatrin, the increase in tonic inhibition is due to GABA transporter reversal (Wu et al. 2001, 2003). However, this does not necessarily mean that vigabatrin always causes transporter reversal. An alternative way of viewing this finding is that vigabatrin simply increases the equilibrium $[\text{GABA}]_o$, for the transporter (by increasing $[\text{GABA}]_i$). After treatment with vigabatrin in vivo, there are two ways that tonic inhibition could increase without reversal of the GABA transporter: by increasing the equilibrium $[\text{GABA}]_i$; or by decreasing the ability of the GABA transporter to reach this equilibrium by reducing the number or efficiency of functional GABA transporters in the cell membrane (Bernstein and Quick 1999; Deken et al. 2003). As long as there is vesicular GABA release, then either mechanism could lead to an increase in tonic inhibition. Thus tonic inhibition is not dependent on reversal of GABA transporters, and in fact GABA transporters would normally operate in the forward direction to prevent excessive tonic inhibition, by virtue of reuptake of vesicular release—precisely as predicted by the classical view of the GABA transporter. This dichotomy may seem counterintuitive, but these dual roles of the GABA transporter in reducing tonic inhibition by reuptake and regulating the level of tonic inhibition via changes in the equilibrium $[\text{GABA}]_i$, follow naturally from the biophysics of transporters. To accomplish the latter, the transporter will reverse whenever necessary, but reversal is not a prerequisite for an increase in $[\text{GABA}]_o$.

**SEEKING A CONSTANTLY CHANGING EQUILIBRIUM**

The solutions to the reversal potential equation shown in Fig. 8 are based on steady-state values of membrane potential and substrate concentrations. However, neurons in vivo are rarely at steady state, so the equilibrium $[\text{GABA}]_o$, is constantly changing. Therefore even though the net direction of GABA flux through the transporter is likely to be inward, the direction and magnitude of GABA flux would constantly vary. We envision the following scenario. During resting conditions, there would be only a small amount of inward GABA flux to recover basal vesicular release. When a burst of firing occurs, there would first be increased GABA uptake due to increased $[\text{GABA}]_i$. As $[\text{Na}^+]$ rises, GABA uptake would slow and then begin to reverse. This reversal would occur in a dynamic fashion with pulses of GABA release with each depolarization. Finally, at the end of the burst of activity there would be a large surge of GABA reuptake to recover the GABA that was released during the burst. Not only would transporter activity respond dynamically to the changing conditions, but it would also be spatially inhomogeneous, with differences in flux of GABA among the synapse, the perisynaptic regions, and distant extrasynaptic locations. It is also now recognized that GABAergic neurons cannot be viewed as homogeneous, with different classes of GABAergic neurons present even within a single brain region (Poncer et al. 2000). It is likely that the function of GABA transporters varies among these different types of GABAergic neurons as indicated by the recent finding that the density of GAT-1 varies among different types of synapses (Chiu et al. 2002).

In addition to the complexity of the system described in the preceding text, the properties of GABA transporters themselves are also dynamic. For example, the GABA transporter can be shuttled back and forth into the plasma membrane from...
intracellular sites of sequestration in response to an increase in extracellular [GABA] (Bernstein and Quick 1999). Interestingly, GABA transporters are also rapidly inserted into the plasma membrane of synaptic terminals in response to presynaptic depolarization (Deken et al. 2003). These transporters are sequestered in presynaptic vesicles that are a different subset than those that contain GABA and are rapidly inserted into the plasma membrane in response to a burst of activity, possibly to maximize the efficiency of reuptake after release. Thus not only are transporters constantly seeking an equilibrium that is a moving target, but the transporters themselves are constantly moving.

**ALL NEUROTRANSMITTER TRANSPORTERS CAN REVERSE**

It is not clear how many of the properties of GABA transporters described here are shared by other neurotransmitter transporters, but it is likely that there are many common features. Reversal is a predictable consequence of the biophysics of all membrane cotransporters and exchangers. For example, reversal of the Na\(^+\)-Ca\(^2+\) exchanger plays an important role in axonal injury during white matter ischemia (Stys et al. 1992). Reversal under pathological conditions is a well accepted property of many neurotransmitter transporters (Attwell et al. 1993; Levi and Raiteri 1993). For example, glutamate transporter reversal is the primary source of glutamate release during brain ischemia (Rossi et al. 2000). However, the conditions necessary for reversal of the glutamate transporter may only occur during pathological states. The glutamate transporter is a Na\(^+\)- and K\(^+\)-dependent antiporter. During each forward translocation cycle, the glutamate transporter carries three Na\(^+\) ions into the cell down its concentration gradient along with one proton and one glutamate, while also carrying one K\(^+\) ion out of the cell down its concentration gradient. This greater dependence of the glutamate transporter on the Na\(^+\) gradient, the additional energy from the K\(^+\) gradient, and the transport of one additional positive charge with each translocation cycle, together results in a much steeper glutamate gradient at equilibrium compared with GABA. This glutamate gradient has been calculated to be 1:1,000,000, with a lower limit for ambient [glutamate] of 4.6 nM at normal resting gradient has been calculated to be 1:1,000,000, with a lower limit for ambient [glutamate] of 4.6 nM at normal resting gradient, the additional energy from the K\(^+\)/H\(^+\) antiporter plays an important role in axonal injury during white matter ischemia (Stys et al. 1992). Reversal under pathological conditions is a well accepted property of many neurotransmitter transporters (Attwell et al. 1993; Levi and Raiteri 1993). For example, glutamate transporter reversal is the primary source of glutamate release during brain ischemia (Rossi et al. 2000). However, the conditions necessary for reversal of the glutamate transporter may only occur during pathological states. The glutamate transporter is a Na\(^+\)- and K\(^+\)-dependent antiporter. During each forward translocation cycle, the glutamate transporter carries three Na\(^+\) ions into the cell down its concentration gradient along with one proton and one glutamate, while also carrying one K\(^+\) ion out of the cell down its concentration gradient. This greater dependence of the glutamate transporter on the Na\(^+\) gradient, the additional energy from the K\(^+\) gradient, and the transport of one additional positive charge with each translocation cycle, together results in a much steeper glutamate gradient at equilibrium compared with GABA. This glutamate gradient has been calculated to be 1:1,000,000, with a lower limit for ambient [glutamate] of 4.6 nM at normal resting gradient has been calculated to be 1:1,000,000, with a lower limit for ambient [glutamate] of 4.6 nM at normal resting gradient.

**SUMMARY**

The emerging view of neurotransmitter transporters is more complex than the traditional view. The classical model, although accurate in a global and static sense, is too simplistic to describe subcellular and dynamic events. On average, transporters do function to terminate synaptic transmission and scavenge transmitter for reuse. However, transporters can also reverse, and there is strong evidence that some of them do so in response to physiological changes in substrate gradients and membrane potential. Thus transporters can no longer be viewed as tiny vacuum cleaners whose sole purpose is to continuously suck neurotransmitter completely out of synaptic clefts.

Understanding the role of transporters in synaptic function and in control of tonic inhibition is important because these molecules contribute to a variety of pathological conditions and are targets of many drugs. For example, reversal of the GABA transporter is likely to occur during seizures, and regulation of tonic inhibition by GABA transporters may modulate seizure threshold. There are many questions that still need to be answered about the complexities of transporter function. Does tiagabine have an anticonvulsant effect by blocking GABA reuptake (Thompson and Gahwiler 1992) as well as a proconvulsant effect by blocking transporter reversal? What is the relative role of GABA transporters on neurons versus glia? Is GABA transporter reversal always a good thing, given that the conditions for transporter reversal also favor the generation of depolarizing GABA responses (Staley et al. 1995)? Are there unrecognized mechanisms for modulation of GABA transporters by second-messenger systems or pharmacological agents? How many other neurotransmitter transporters share these properties described for GABA transporters? A more thorough understanding of the dynamic equilibrium of transporters will help to define their role in normal brain function and pathological states.

**REFERENCES**


