

## Looking for GABA in All the Wrong Places: The Relevance of Extrasynaptic GABA<sub>A</sub> Receptors to Epilepsy

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Editor's note: Please use commentary by Mody pages 248–249 which highlights the same topic.

*It comes as no surprise that a high concentration of  $\gamma$ -aminobutyric acid (GABA)<sub>A</sub> receptors exists across the synapse from presynaptic terminals that contain GABA. Oddly, though, many GABA<sub>A</sub> receptors also are far away from synapses. These extrasynaptic GABA<sub>A</sub> receptors are tonically activated by the low levels of GABA normally present in the extracellular space. Many of these extrasynaptic GABA<sub>A</sub> receptors contain the  $\delta$  subunit. This subunit confers molecular properties on GABA<sub>A</sub> receptors that are well suited for a function in tonic inhibition, with a high affinity for GABA and little desensitization to continuous activation. Recent data linked a genetic variant of the  $\delta$  subunit to epilepsy, providing a missing link between tonic inhibition and control of brain excitability.*

We have all become comfortable with the model of neuronal communication in which neurotransmitters are released from presynaptic terminals and activate receptors on the postsynaptic membrane. Why then are many  $\gamma$ -aminobutyric acid (GABA)<sub>A</sub> receptors located immediately outside the synapse (perisynaptic) and far away from any synapses (extrasynaptic)? What are these misplaced GABA<sub>A</sub> receptors doing? One possibility is that neurons are not very good at targeting proteins where they should be located; in which case, they are simply in the wrong place at the wrong time. However, accumulating data has led to the emergence of an entirely different theory. Extrasynaptic GABA<sub>A</sub> receptors appear to be there for a very specific purpose of sampling the low but finite levels of ambient

GABA that are normally present in the extracellular space, leading to a continuous or “tonic” inhibitory current that controls the level of excitability of neurons.

### Tonic Inhibition: A Newly Discovered Form of GABAergic Inhibition

When an electrical recording is made from a neuron within a network, the electrical signature of GABAergic synaptic communication can easily be seen as inhibitory postsynaptic potentials (IPSPs). The onset of an IPSP occurs when GABA is released from a presynaptic terminal and binds to postsynaptic GABA<sub>A</sub> receptors (activating the associated chloride current) and ends when GABA diffuses out of the synaptic cleft or is removed by GABA transporters. This transient electrical response has been called “phasic” inhibition to differentiate it from a newly recognized “tonic” form of inhibition.

Tonic inhibition was first identified in the cerebellum, where it is particularly strong (1), but it is now known to be more widespread, including within the hippocampus (2–4). It is typically seen during electrophysiologic recordings as a continuous current (or offset in holding current), which is blocked by the GABA<sub>A</sub> receptor blocker bicuculline. This continuous current is due to tonic activation of GABA<sub>A</sub> receptors by the low levels of GABA that are always present in the extracellular space. The maximal amplitude of tonic inhibition is typically smaller than that of phasic inhibition, but the average current can often be far greater for tonic inhibition because it occurs continuously instead of transiently. It has been estimated that the total charge movement across the membrane of some neurons that is due to tonic current is 3 to 4 times greater than that from phasic current (2,5). It is a great surprise that a form of inhibition that presumably plays such a large role in control of neuron function was discovered so recently.

### The $\delta$ Subunit of the GABA<sub>A</sub> Receptor Mediates Tonic Inhibition

It is now known that the two types of inhibition are mediated by different isoforms of the GABA<sub>A</sub> receptor (4). Evidence for this conclusion includes the observation that tonic inhibition and phasic IPSCs can be pharmacologically separated. For example, NO-711 and midazolam selectively enhance tonic inhibition (2,7), whereas zolpidem selectively enhances phasic inhibition (2). A low concentration of gabazine blocks phasic inhibition without affecting tonic inhibition, whereas higher concentrations also block tonic inhibition (3,7). In addition, neurosteroids selectively enhance tonic inhibition (8).

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GABA<sub>A</sub> receptors are composed of  $\alpha$  and  $\beta$  subunits that combine within the brain with  $\delta$ ,  $\gamma$ , or  $\varepsilon$  subunits. Different combinations of these subunits assemble into GABA<sub>A</sub> receptors with different properties. Early work, with heterologous expression of GABA<sub>A</sub>-receptor subunits in fibroblasts, showed that those receptors containing the  $\delta$  subunit have properties that are expected for mediation of tonic inhibition. For example, receptors assembled from the combination of  $\alpha_6\beta_2\delta$  or  $\alpha_6\beta_3\delta$  are very sensitive to low levels of GABA ( $EC_{50}$ , 0.19–0.27  $\mu M$ , compared with 1.9–13.6  $\mu M$  for receptors without the  $\delta$  subunit) and show little in the way of desensitization (a gradual reduction in chloride current despite continuous application of GABA) (9,10). Because ambient GABA has been measured at 0.2 to 0.8  $\mu M$  in the rat hippocampus (11,12), it is predicted that  $\delta$ -subunit-containing GABA<sub>A</sub> receptors would normally be activated and produce a nondesensitizing (i.e., sustained) current.

Good evidence exists that the  $\delta$  subunit mediates tonic inhibition in some neurons. For example, within the cerebellum, the  $\delta$  subunit preferentially combines with the  $\alpha_6$  subunit to form  $\alpha_6\beta_X\delta$  GABA<sub>A</sub> receptors. Genetic deletion of the  $\alpha_6$  subunit in knockout mice causes loss of expression of  $\delta$ -subunit-containing receptors in the cerebellum (13), and the loss of this subunit combination leads to a corresponding loss of tonic GABAergic inhibition in this region (14).

The  $\alpha_6$  subunit is found primarily within cerebellar granule cells (13), which means that  $\alpha_6\beta_X\delta$  receptors cannot be responsible for tonic inhibition in most of the rest of the brain. However, in many cases, the  $\delta$  subunit is probably still involved, but in combination with other  $\alpha$  subunits. The role of the  $\delta$  subunit in tonic inhibition was tested directly by genetic deletion of the  $\delta$  subunit itself in knockout mice (8). In these animals, greatly reduced tonic inhibition occurred in the cerebellum as well as in dentate gyrus granule cells of the hippocampus. Consistent with the selective effect of neurosteroids on tonic inhibition, reduced sensitivity of GABA<sub>A</sub> receptor currents to neurosteroids also was found (8).

The  $\delta$  subunit does not mediate all of the tonic GABAergic inhibition in the brain (4). For example, tonic inhibition is still present in hippocampal CA1 neurons after knockout of the  $\delta$  subunit (8). Thus other combinations of subunits must contribute to tonic inhibition that remain to be determined (6).

### GABA<sub>A</sub> Receptors That Mediate Tonic Inhibition Are Extrasynaptic

Many GABA<sub>A</sub> receptors are not localized to synapses, but are instead distributed across the extrasynaptic surface of neurons. Extrasynaptic GABA<sub>A</sub> receptors are not mistargeted to this location, because different subtypes of GABA<sub>A</sub> receptors appear

to be actively sorted to the two locations. For example, the single-channel properties of extrasynaptic GABA<sub>A</sub> receptors are distinct from those within the synapse (15). Likewise, the  $\delta$ -subunit-containing GABA<sub>A</sub> receptors that mediate tonic inhibition are not within synapses but, instead, are found only in extrasynaptic membrane (16).

### The GABA Transporter Regulates the Level of Tonic Inhibition

Extrasynaptic  $\delta$ -containing GABA<sub>A</sub> receptors must be activated by low levels of GABA that surround neurons in the brain, rather than large, transient spikes of GABA within synapses. Where does this ambient GABA come from? Some of it clearly is due to “spillover” of GABA out of synapses (1,17). Evidence exists for release of GABA from astrocytes (18). Under some conditions, the GABA transporter can operate in reverse, releasing GABA (19–21), and in other cases, different forms of non-vesicular GABA release also may be involved (22). The relative importance of each of these and other mechanisms in tonic inhibition may vary depending on the prevailing conditions and is the subject of ongoing studies.

Regardless of the source of GABA, the GABA transporter appears to play a major role in regulating the amount of tonic inhibition, because it helps determine the level of GABA in the extracellular space. Under normal conditions, the GABA level at which the GABA transporter is at equilibrium is relatively high—estimated to be from 0.1  $\mu M$  in the hippocampus to 0.26  $\mu M$  in the cerebellum (20). Thus when the transporter is at equilibrium, the GABA level would still be high enough to cause tonic activation of high-affinity, extrasynaptic GABA<sub>A</sub> receptors. If GABA levels increase above this equilibrium level (e.g., by synaptic GABA release or other mechanisms), the GABA transporter would try to reduce GABA levels, but only down to the equilibrium level (20). If GABA levels are lower than the equilibrium level, then the GABA transporter will reverse, releasing GABA until the GABA transporter is again at equilibrium. Thus the GABA transporter plays a major role in regulation of tonic inhibition, sometimes by reversing and at other times by maintaining a finite floor level of GABA.

### Functions of Tonic Inhibition

The functions of tonic inhibition are only now beginning to be understood. The existence of specialized receptors designed to produce tonic inhibition, and the large magnitude of this inhibition, imply that it must be important. However, the loss of GABAergic tonic inhibition in  $\alpha_6$  knockout mice did not result in any behavioral changes (14), suggesting that it was not important. If the authors of that study had stopped there, tonic inhibition might be viewed as just an interesting electrophysiologic observation with no functional relevance (23). However,

by looking deeper into this question, they made an even more interesting observation. In response to the loss of GABAergic tonic inhibition, a compensatory appearance of a different form of tonic inhibition was noted in these animals, mediated by a leak potassium channel (14). This corrective response implies that tonic inhibition is so important that neurons can recognize when it is not present, and they can devise ingenious ways to replace it, when it is absent.

The widespread distribution of the  $\delta$  subunit implies that tonic inhibition occurs in many neurons throughout the brain (24). How is this inhibition involved in normal brain function? A variety of proposals exist for the function of tonic inhibition (4,5,25). It may be a way to monitor the activity of many surrounding neurons, instead of just one (23). Because tonic inhibition is due to a chloride current, it would normally inhibit the firing of action potentials, causing an overall decrease in excitability in the brain. Rather than simply decreasing the number of action potentials a neuron generates, tonic inhibition also can alter the pattern of neuron firing, from a regular to an irregular pattern, by modifying the response to synaptic inputs (26). A change in pattern of firing implies a role for tonic inhibition in information processing and not just in control of overall brain excitability. This is consistent with the idea that background inputs can alter the gain of a neuronal response to excitatory drive (25). Defining the contribution of each of these mechanisms to overall brain function will likely require development of conditional knockout animals, or  $\delta$ -subunit specific drugs, because it is not possible to use embryonic knockout mice because of the compensatory changes in tonic inhibition that occur (14).

The preceding discussion may imply that neurons in the brain are always subjected to the same amount of tonic inhibition. However, this appears unlikely, because tonic inhibition may be increased or decreased in response to neuronal activity (20), drugs (21), modulatory inputs, or endogenous signaling pathways (22). These changes occur, in part, because the level of GABA in the extracellular space changes with alterations in the amount of GABA released, as well as with alterations in the equilibrium of the GABA transporter (which depends on intracellular  $\text{Na}^+$ , intracellular GABA, and membrane potential). In addition, modulation of  $\delta$ -subunit-containing GABA<sub>A</sub> receptors also would alter the amount of tonic inhibition. Thus although this form of inhibition is tonic, it is not static.

### Relevance to Epilepsy

One of the axioms of epileptology is that a decrease in GABAergic inhibition leads to an increase in seizure susceptibility. Thus a selective loss of tonic inhibition would be predicted to cause seizures. Although this conclusion seems obvious, it has been

difficult to obtain direct evidence for a link between tonic inhibition and seizures. For example, neither the  $\alpha_6$  nor the  $\delta$  knockout mice were reported to have spontaneous seizures. However, indirect evidence supports this conclusion. For example, the anticonvulsant vigabatrin (VGB) is a GABA-transaminase inhibitor that leads to an increase in brain GABA levels (27), which was presumed to lead to an increase in the size of IPSPs. Surprisingly, VGB does not increase IPSPs, but instead causes a large increase in tonic GABAergic inhibition (21). It is believed that the anticonvulsant effect of this drug is due to this selective enhancement of tonic inhibition, which would be expected to reduce seizures, for example, by acting on a subset of hippocampal interneurons to regulate network excitability (4).

A recent article now provides direct evidence of a role for the  $\delta$  subunit in epilepsy, presumably via its contribution to tonic inhibition (28). The authors of the article identified a genetic variant in the  $\delta$  subunit of the GABA<sub>A</sub> receptor in a family with generalized epilepsy with febrile seizures plus. They cloned the  $\delta$ -subunit variant, expressed it in HEK293 cells, along with  $\alpha_1$  and  $\beta_2$  subunits, and showed that the variant leads to greatly reduced amplitudes of the response to GABA. Although it is conceivable that the mechanism of epilepsy in these patients is due to a role of the  $\delta$  subunit in some aspect of neurophysiology other than tonic inhibition, this conjecture seems unlikely. Thus the study provides a missing link between the strong evidence for a role of the  $\delta$  subunit in tonic inhibition and the role of tonic inhibition in controlling brain excitability.

The same study also identified a polymorphism in the  $\delta$  subunit in the general population (without epilepsy). The investigators further showed that the second genetic variant also leads to a decrease in GABA responses when heterologously expressed with the  $\alpha_1$  and  $\beta_2$  subunits (28). Because most inherited human epilepsies are not due to single gene mutations, but instead are polygenic, the conclusion is that the  $\delta$  subunit is a susceptibility locus for polygenic generalized epilepsy. Thus a defect in tonic inhibition, together with genetic variation in other signaling molecules, may lower seizure threshold.

Only a few short years ago, it was believed that tonic inhibition was a curiosity observed only in the cerebellum. It now seems likely that it exists in many (if not all) parts of the brain, and in some cases, may be the dominant mechanism of inhibition. The demonstration of a prominent role of tonic inhibition in the hippocampus and the link to human epilepsy makes tonic inhibition much more than a curiosity to neurologists. Defining the mechanisms and function of this newly discovered tonic form of inhibition and the role of the  $\delta$  subunit in its expression may be an important new avenue for treatment of a variety of epilepsies.

## References

1. Brickley SG, Cull-Candy SG, Farrant M. Development of a tonic form of synaptic inhibition in rat cerebellar granule cells resulting from persistent activation of GABAA receptors. *J Physiol (Lond)* 1996;497:753–759.
2. Nusser Z, Mody I. Selective modulation of tonic and phasic inhibitions in dentate gyrus granule cells. *J Neurophysiol* 2002;87, 2624–2628.
3. Stell BM, Mody I. Receptors with different affinities mediate phasic and tonic GABAA conductances in hippocampal neurons. *J Neurosci* 2002;22:RC223.
4. Semyanov A, Walker MC, Kullmann DM, Silver RA. Tonic active GABA A receptors: modulating gain and maintaining the tone. *Trends Neurosci* 2004;27:262–269.
5. Hamann M, Rossi DJ, Attwell D. Tonic and spillover inhibition of granule cells control information flow through cerebellar cortex. *Neuron* 2002;33:625–633.
6. Mody I. Distinguishing between GABAA receptors responsible for tonic and phasic conductances. *Neurochem Res* 2001;26:907–913.
7. Yeung JYT, Canning KJ, Zhu GY, Pennefather P, MacDonald JF, Orser BA. Tonic activated GABAA receptors in hippocampal neurons are high-affinity, low-conductance sensors for extracellular GABA. *Mol Pharmacol* 2003;63:2–8.
8. Stell BM, Brickley SG, Tang CY, Farrant M, Mody I. Neuroactive steroids reduce neuronal excitability by selectively enhancing tonic inhibition mediated by  $\delta$  subunit-containing GABAA receptors. *Proc Natl Acad Sci U S A* 2003;100:14439–14444.
9. Saxena NC, Macdonald RL. Properties of putative cerebellar gamma-aminobutyric acid A receptor isoforms. *Mol Pharmacol* 1996;49:567–579.
10. Saxena NC, Macdonald RL. Assembly of GABAA receptor subunits - Role of the delta- subunit. *J Neurosci* 1994;14:7077–7086.
11. Lerma J, Herranz AS, Herreras O, Abaira V, Martin DR. In vivo determination of extracellular concentration of amino acids in the rat hippocampus: a method based on brain dialysis and computerized analysis. *Brain Res* 1986;384:145–155.
12. Tossman U, Jonsson G, Ungerstedt U. Regional distribution and extracellular levels of amino acids in rat central nervous system. *Acta Physiol Scand* 1986;127:533–545.
13. Jones A, Korpi ER, McKernan RM, Pelz R, Nusser Z, Makela R, Mellor JR, Pollard S, Bahn S, Stephenson FA, Randall AD, Sieghart W, Somogyi P, Smith AJ, Wisden W. Ligand-gated ion channel subunit partnerships: GABAA receptor  $\alpha 6$  subunit gene inactivation inhibits  $\delta$  subunit expression. *J Neurosci* 1997;17:1350–1362.
14. Brickley SG, Revilla V, Cull-Candy SG, Wisden W, Farrant M. Adaptive regulation of neuronal excitability by a voltage-independent potassium conductance. *Nature* 2001;409:88–92.
15. Brickley SG, Cull-Candy SG, Farrant M. Single-channel properties of synaptic and extrasynaptic GABAA receptors suggest differential targeting of receptor subtypes. *J Neurosci* 1999;19:2960–2973.
16. Nusser Z, Sieghart W, Somogyi P. Segregation of different GABAA receptors to synaptic and extrasynaptic membranes of cerebellar granule cells. *J Neurosci* 1998;18:1693–1703.
17. Rossi DJ, Hamann M. Spillover-mediated transmission at inhibitory synapses promoted by high affinity  $\alpha 6$  subunit GABA<sub>A</sub> receptors and glomerular geometry. *Neuron* 1998;20:783–795.
18. Liu QY, Schaffner AE, Chang YH, Maric D, Barker JL. Persistent activation of GABAA receptor Cl<sup>-</sup> channels by astrocyte-derived GABA in cultured embryonic rat hippocampal neurons. *J Neurophysiol* 2000;84:1392–1403.
19. Gaspary HL, Wang W, Richerson GB. Carrier-mediated GABA release activates GABA receptors on hippocampal neurons. *J Neurophysiol* 1998;80:270–281.
20. Richerson GB, Wu Y. The dynamic equilibrium of neurotransmitter transporters: Not just for reuptake anymore. *J Neurophysiol* 2003 (in press).
21. Wu Y, Wang W, Richerson GB. GABA transaminase inhibition induces spontaneous and enhances depolarization-evoked GABA efflux via reversal of the GABA transporter. *J Neurosci* 2001;21:2630–2639.
22. Rossi DJ, Hamann M, Attwell D. Multiple modes of GABAergic inhibition of rat cerebellar granule cells. *J Physiol (Lond)* 2003;548:97–110.
23. Soltesz I, Nusser Z. Neurobiology: Background inhibition to the fore. *Nature* 2001;409:24–27.
24. Pirker S, Schwarzer C, Wieselthaler A, Sieghart W, Sperk G. GABA(A) receptors: immunocytochemical distribution of 13 subunits in the adult rat brain. *J Neurosci* 2000;101:815–850.
25. Chance FS, Abbott LF, Reyes AD. Gain modulation from background synaptic input. *Neuron* 2002;35:773–782.
26. Hausser M, Clark BA. Tonic synaptic inhibition modulates neuronal output pattern and spatiotemporal synaptic integration. *Neuron* 1997;19:665–678.
27. Petroff OA, Behar KL, Mattson, RH, Rothman DL. Human brain gamma-aminobutyric acid levels and seizure control following initiation of vigabatrin therapy. *J Neurochem* 1996;67:2399–2404.
28. Dibbens LM, Feng HJ, Richards MC, Harkin LA, Hodgson BL, Scott D, Jenkins M, Petrou S, Sutherland GR, Scheffer IE, et al. GABRD encoding a protein for extra- or peri-synaptic GABAA receptors is a susceptibility locus for generalized epilepsies. *Hum Mol Genet* 2004;13:1315–1319.