

## GABA RECEPTORS GONE BAD: THE WRONG PLACE AT THE WRONG TIME

### The GABA<sub>A</sub>-receptor $\gamma 2$ Subunit R43Q Mutation Linked to Childhood Absence Epilepsy and Febrile Seizures Causes Retention of $\alpha 1\beta 2\gamma 2S$ Receptors in the Endoplasmic Reticulum

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The GABA<sub>A</sub>-receptor  $\gamma 2$  subunit mutation R43Q is an autosomal dominant mutation associated with childhood absence epilepsy and febrile seizures. Previously, we demonstrated that homozygous  $\alpha 1\beta 3\gamma 2L(R43Q)$ -receptor whole-cell currents had reduced amplitude with unaltered time course, suggesting reduced cell-surface expression of functional receptors. In human embryonic kidney 293-T cells, we demonstrate that both heterozygous and homozygous  $\alpha 1\beta 2\gamma 2S(R43Q)$  GABA<sub>A</sub>-receptor current amplitudes were reduced when receptors were assembled from coexpressed  $\alpha 1$ ,  $\beta 2$ , and  $\gamma 2S$  subunits and from  $\beta 2$ - $\alpha 1$  tandem subunits coexpressed with the  $\gamma 2L$

subunit. By using fluorescence confocal microscopy, we demonstrated that mutant receptors containing enhanced yellow fluorescent protein-tagged  $\gamma 2S$  subunits had reduced surface expression and were retained in the endoplasmic reticulum. In addition, by using biotinylation of surface receptors and immunoblotting, we confirmed that  $\alpha 1\beta 2\gamma 2S(R43Q)$ -receptors had reduced surface expression. These results provide evidence that the  $\gamma 2S(R43Q)$  mutation impaired GABA<sub>A</sub>-receptor function by compromising receptor trafficking and reducing surface expression.

### Altered Expression of the $\delta$ Subunit of the GABA<sub>A</sub> Receptor in a Mouse Model of Temporal Lobe Epilepsy

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$\delta$  Subunit-containing GABA<sub>A</sub> receptors are located predominantly at nonsynaptic sites in the dentate gyrus, where they may play important roles in controlling neuronal excitability through tonic inhibition and responses to GABA spillover. Immunohistochemical methods were used to determine whether  $\delta$  subunit expression was altered after pilocarpine-induced status epilepticus in C57BL/6 mice in ways that could increase excitability of the dentate gyrus. In pilocarpine-treated animals, the normal diffuse labeling of the  $\delta$  subunit in the dentate molecular layer was decreased by 4 days after status epilepticus (latent period) and remained low throughout the period of chronic seizures. In contrast, diffuse labeling of  $\alpha 4$  and  $\gamma 2$  subunits, potentially interrelated GABA<sub>A</sub>-receptor sub-

units, was increased during the chronic period. Interestingly,  $\delta$  subunit labeling of many interneurons progressively increased after pilocarpine treatment. Consistent with the observed changes in  $\delta$  subunit labeling, physiological studies revealed increased excitability in the dentate gyrus of slices obtained from the pilocarpine-treated mice and demonstrated that physiologic concentrations of the neurosteroid tetrahydrodeoxycorticosterone were less effective in reducing excitability in the pilocarpine-treated animals than in controls. The findings support the idea that alterations in nonsynaptic  $\delta$  subunit-containing GABA<sub>A</sub> receptors in both principal cells and interneurons could contribute to increased seizure susceptibility in the hippocampal formation in a temporal lobe epilepsy model.

## COMMENTARY

In neurotransmission, as in real estate, location is (almost) everything. Even though neurotransmitter receptors are commonly referred to as “inhibitory” or “excitatory,” their real ef-

fect on overall excitability critically depends on where they are located and when they are activated. This issue is particularly well studied with GABA receptors. Putting aside instances when GABA<sub>A</sub> receptors are depolarizing, several instances are known in which GABA acts to increase the overall excitability of neural

systems. Particularly good examples include the GABA-induced hypersynchrony seen in models of absence epilepsy and the ability of fast interneuron activity to synchronize local excitatory cortical neurons during an interictal spike. In a web of excitatory and inhibitory synapses, GABA either may suppress an inhibitory component or may help synchronize the excitatory portions, thereby increasing the overall excitability of the system. The final effect of GABAergic neurotransmission depends on both the target cell type and position of the receptors on those cells. The two articles presented here investigate how pathologic misplacement of GABA<sub>A</sub> receptors may result in hyperexcitability, expressed in one instance as idiopathic generalized epilepsy and in the other as temporal lobe epilepsy.

GABA<sub>A</sub> receptors are pentameric complexes, usually composed of two  $\alpha$  subunits, two  $\beta$  subunits, and either a  $\gamma 2$  subunit or a low-abundance subunit, such as  $\delta$ . Receptors containing  $\delta$  or  $\gamma$  subunits have different kinetic and pharmacologic properties. For instance, receptors with a  $\delta$  subunit are usually more sensitive to GABA, do not desensitize much, and some receptors are more sensitive to ethanol and neurosteroids. In contrast,  $\gamma 2$ -containing receptors activate very quickly and then desensitize extensively. They are less sensitive to neurosteroids but are modulated by benzodiazepines (unless they contain  $\alpha 4$  or  $\alpha 6$ ). In the cerebellum,  $\delta$ -containing GABA<sub>A</sub> receptors are located outside the synapse. Their high sensitivity to GABA and little desensitization makes them well suited for responding to ambient low levels of GABA. As is discussed later, they may play a role in tonic, extrasynaptic GABA currents. Again, in contrast,  $\gamma$ -containing GABA<sub>A</sub> receptors often are found localized to synapses, where they may be exposed to very brief (around 1 msec) pulses of GABA at high concentrations (around 1 millimolar). The rapid activation of these receptors allows them to respond to the brief pulses of synaptically released GABA. Furthermore, GABA<sub>A</sub>-receptor desensitization appears to involve a conformation in which the receptor has bound GABA but cannot conduct ions. This state has a very high apparent affinity for GABA and is sometimes referred to as agonist trapping. A key feature of GABA<sub>A</sub> receptors is that, even after ambient GABA is gone, desensitizing receptors open and close many times before releasing the bound GABA. Therefore, paradoxically, rapidly desensitizing currents actually have a longer-duration response to brief GABA applications than to non-desensitizing currents. These kinetic properties allow more long-lived postsynaptic currents, as well as summation, during a barrage of multiple postsynaptic currents. Although  $\delta$ -containing receptors are very sensitive to GABA, they tend to deactivate very quickly after ambient GABA is gone, which would result in very brief inhibitory postsynaptic currents (IPSCs). Therefore, it is important to synaptic and extrasynaptic signaling that the different GABA<sub>A</sub>-receptor subunit combinations are correctly

targeted within the neuron; how this process occurs is currently a very active field of research.

The article by Kang and Macdonald explores abnormal intracellular trafficking of the mutant GABA<sub>A</sub> receptor  $\gamma 2$  subunit that has been found in heterozygous patients with familial childhood absence epilepsy and febrile seizures. Initially some controversy occurred regarding the functional alteration associated with this mutation. One group showed a reduced maximal current but normal kinetics and pharmacology (1). Another group found the opposite results, with faster desensitization, slower deactivation, and reduced benzodiazepine (BZD) sensitivity (2,3). The discrepant findings of the second group may be explained by the inability of the mutant subunit to become incorporated into GABA<sub>A</sub> receptors, resulting in receptors containing only  $\alpha$  and  $\beta$  subunits, which would be BZD insensitive (4).

Kang and Macdonald used fluorescent-tagged  $\gamma 2$  subunits receptors to visualize the location of the receptors directly. The  $\gamma 2$  subunit was coexpressed with a modified protein containing an  $\alpha$  and a  $\beta$  subunit linked together by a peptide sequence (an  $\alpha/\beta$  tandem protein). Because no free  $\alpha$  or  $\beta$  subunits are found in these cells, the only way for a functional pentamer to form is to incorporate two  $\alpha/\beta$  tandem proteins and a single  $\gamma 2$  subunit. This manipulation produced a mutant GABA<sub>A</sub> receptor current with normal kinetics and BZD sensitivity but severely reduced current size, compared with cells expressing  $\alpha/\beta$  tandem protein with the normal  $\gamma 2$  subunit. Confocal microscopy and immunoprecipitation showed that this reduced current was associated with apparent trapping of the receptors within the endoplasmic reticulum.

If so little receptor makes it to the surface, why are patients with this form of epilepsy otherwise normal? In contrast to the artificial experimental situation in which only mutant receptors are expressed in heterologous cells, neurons in this autosomal dominant condition presumably express both mutant and wild-type receptors. Perhaps compensatory upregulation of the wild-type  $\gamma 2$  subunit occurs. Alternatively, given the important role of the  $\gamma 2$  subunit to alter subcellular localization and surface stability of GABA<sub>A</sub> receptors (5), it is reasonable to think that a combination of wild-type and mutant  $\gamma 2$  subunits may interact in unusual ways. Either the wild type may rescue the mutant from aberrant trafficking or the mutant may alter the normal trafficking of the wild type. A similar interaction has been suggested between full-length and alternatively spliced  $\alpha 4$  subunits (6). To address this problem, these studies included a mixture of wild-type and mutant  $2\gamma$  subunits to mimic the heterozygous state in patients. These cells had maximal currents and cell-surface expression that were midway between wild-type and pure mutant-containing cells, suggesting that for this particular mutation, no evidence exists for interaction between wild-type

and mutant proteins. These studies raise the possibility that a diffuse reduction in  $\gamma 2$ -subunit expression may be associated with some forms of idiopathic generalized epilepsy. However, caution is advisable in interpretation of these findings. Although the experiments were conducted in human and primate cells, they were not conducted in neurons. Because specific mechanisms in neurons target GABA<sub>A</sub> receptors to postsynaptic densities, it is possible that the trafficking of GABA<sub>A</sub> receptor may be different in actual neurons. Future studies should include neuron-like cells as well.

The article by Peng et al. explores changes in the distribution of  $\delta$ -containing GABA<sub>A</sub> receptors in a mouse model of temporal lobe epilepsy. Previous work in rats has shown a diffuse reduction in  $\delta$ -receptor immunostaining after kainate-induced seizures (6). In contrast, other studies used single-cell polymerase chain reaction (PCR) to show an age-dependent increased expression of  $\delta$ -subunit mRNA in dentate granule cells in epileptic rats (7,8). Peng et al. used immunohistochemistry in mice that had become spontaneously epileptic after pilocarpine-induced status epilepticus.  $\delta$  Receptors are normally diffusely expressed in the molecular layer of the dentate gyrus, presumably representing, in large part, the dendritic tree of dentate granule cells. The  $\delta$  receptor also was expressed, albeit to a lesser extent, on the dentate granule cell somas. In epileptic mice, a redistribution of the  $\delta$  receptor staining occurred, with a large reduction in  $\delta$  receptors in the molecular layer of the dentate gyrus but an increase in both the number of interneurons expressing  $\delta$ -subunit protein and the intensity of that immunostaining. Similar to previous studies, a diffuse increase in  $\alpha 4$  staining was found in the dentate molecular layer as well as a smaller, more variable increase in  $\gamma 2$ -subunit expression. Field-potential responses to electrical stimulation showed that slices from chronically epileptic mice were more excitable than were those from controls. Furthermore, neurosteroids, which are known to enhance  $\delta$ -containing receptors, reduced the excitability of slices from control animals. However, slices from epileptic mice were insensitive to neurosteroids, similar to what has been seen in isolated dentate granule cells from epileptic rats (9). Although previous studies found increased  $\delta$ -subunit mRNA in epileptic animals, the actual protein expression appears to be reduced. Furthermore, a redistribution of these subunits away from presumed granule cell dendrites to interneuron cell bodies is associated with increased excitability in dentate gyrus.

Although much work remains to be done, these articles help to further the understanding of the importance of GABA<sub>A</sub>-receptor localization. GABAergic IPSCs are crucial to normal brain function, but how GABA<sub>A</sub> receptors are actually trafficked to the membrane and then to the synaptic cleft is just beginning to be understood. Moreover, as noted here and reviewed in more detail in a recent issue of *Epilepsy Currents* (10),

it is now clear that another system of GABA<sub>A</sub> receptors is constantly activated by low (about 1 micromolar) ambient levels of GABA. Although this is a small current, it carries significant charge across the cell membrane because of its long duration and probably plays an important role in regulating neuronal excitability. These receptors are likely extrasynaptic, but the exact subunits making up this population is not entirely clear. Based on the findings from pharmacologic, electron microscopy, and knockout-mice experiments, at least two types of tonic currents appear to exist: those mediated by  $\delta$ -containing receptors and those mediated by another set containing  $\alpha 5$  and  $\gamma$  subunits. The articles presented here suggest that regulation of the amount and distribution of specific GABA<sub>A</sub>-receptor subtypes may have profound effects on excitability. However, the actual role of tonic versus synaptic currents in normal physiology and disease must be studied further.

The exact mechanism whereby altered  $\delta$ -subunit distribution increases dentate gyrus excitability remains unknown. It is possible that the increased expression of  $\delta$  subunits results in hypoactive interneurons, whereas the reduction in  $\delta$ -subunit expression in presumptive dentate granule cells might increase their excitability. It has been notoriously difficult to find reproducible suppression of inhibitory synaptic activity in epileptic hippocampi. The authors note that tonic current in the dentate gyrus appears to be at least partly mediated by  $\delta$  subunit-containing receptors. Perhaps part of the hyperexcitability of epileptic hippocampi is related to alterations in the tonic GABA<sub>A</sub> receptor currents, which would not necessarily be evident when studying IPSCs. The authors did not actually perform the intracellular recordings necessary to address this issue. Therefore, future studies will have to distinguish the roles of tonic versus synaptic currents as well as to determine the GABA<sub>A</sub> receptor subunits involved in these functions. More globally, understanding the regulation of GABA<sub>A</sub> receptor localization is crucial to understanding normal brain function. By exploring how different receptors find their place, much more may be learned about normal and pathologic physiology, thus providing important avenues for selective pharmacologic intervention in specific disease states.

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