

## SELECTIVE CHANGES IN HIPPOCAMPAL GABA<sub>A</sub> RECEPTORS DURING STATUS EPILEPTICUS

**Subunit-Specific Trafficking of GABA<sub>A</sub> Receptors During Status Epilepticus.** Goodkin HP, Joshi S, Mtchedlishvili Z, Brar J, Kapur J. *J Neurosci* 2008 5;28(10):2527–2538. It is proposed that a reduced surface expression of GABA<sub>A</sub> receptors (GABARs) contributes to the pathogenesis of status epilepticus (SE), a condition characterized by prolonged seizures. This hypothesis was based on the finding that prolonged epileptiform bursting (repetitive bursts of prolonged depolarizations with superimposed action potentials) in cultures of dissociated hippocampal pyramidal neurons (dissociated cultures) results in the increased intracellular accumulation of GABARs. However, it is not known whether this rapid modification in the surface-expressed GABAR pool results from selective, subunit-dependent or nonselective, subunit-independent internalization of GABARs. In hippocampal slices obtained from animals undergoing prolonged SE (SE-treated slices), we found that the surface expression of the GABAR  $\beta$ 2/3 and  $\gamma$ 2 subunits was reduced, whereas that of the  $\delta$  subunit was not. Complementary electrophysiological recordings from dentate granule cells in SE-treated slices demonstrated a reduction in GABAR-mediated synaptic inhibition, but not tonic inhibition. A reduction in the surface expression of the  $\gamma$ 2 subunit, but not the  $\delta$  subunit was also observed in dissociated cultures and organotypic hippocampal slice cultures when incubated in an elevated KCl external medium or an elevated KCl external medium supplemented with NMDA, respectively. Additional studies demonstrated that the reduction in the surface expression of the  $\gamma$ 2 subunit was independent of direct ligand binding of the GABAR. These findings demonstrate that the regulation of surface-expressed GABAR pool during SE is subunit-specific and occurs independent of ligand binding. The differential modulation of the surface expression of GABARs during SE has potential implications for the treatment of this neurological emergency.

### COMMENTARY

Status epilepticus (SE) is a life-threatening condition that afflicts more than 150,000 people per year in the United States alone (1). Although the criteria for classifying a prolonged seizure or repetitive seizures as being definitively SE have been the subject of ongoing debate, it is clear that a longer duration of seizure, particularly the generalized convulsive type lasting 30 minutes or more, carries a much higher risk of neuronal injury and permanent neurological sequelae. Therefore, an understanding of the mechanisms that underlie the transition from typical brief, self-limited seizures to this dangerous form of seizure are important to understand as the knowledge may lead to more effective treatment or preventative strategies.

Neurons have a remarkable ability to acutely modify their excitability through alterations in the function or expression of ion channels and in receptors or other proteins. During prolonged seizures, some of these changes may be adaptive responses to high levels of activity, but others may be maladaptive and enhance seizure susceptibility. In the clinical setting, it is known that the longer a seizure lasts, the less likely it is to stop spontaneously and the more likely it is to require intensive medical intervention (2). In addition, longer seizures are more apt to be resistant to existing therapies, leading to even greater delays in successful treatment and poorer prognosis for recovery (3). These clinical findings have led to the hypothesis that pro-

longed epileptiform activity results in a collapse of the brain's inhibitory mechanisms, which is most likely due to changes in the GABA-mediated neurotransmission.

Changes in GABAergic neurotransmission have been explored in animal models of SE and of chronic epilepsy. Studies have revealed alterations in phasic (synaptic) inhibition as well as in tonic inhibition, mediated by ambient GABA acting at extrasynaptic receptors. Both types of inhibition have important but unique roles in limiting neuronal excitability, and they depend on distinct subsets of GABA<sub>A</sub> receptors composed of different subunit combinations within the pentameric GABA<sub>A</sub> receptor complex. The synaptic receptors comprise  $\alpha$  and  $\beta$  subunits along with  $\gamma$ 2 subunits, while extrasynaptic receptors contain  $\delta$  instead of  $\gamma$ 2 subunits and mostly utilize the  $\alpha$ 4-subunit. Earlier studies (4,5) had demonstrated that GABA<sub>A</sub> receptors are internalized and synaptic inhibition is reduced during SE, while tonic inhibition may be increased (5). Other animal studies have shown that prolonged seizures become resistant to the ability of benzodiazepines to stop them (6) and synaptic GABA responses become less sensitive to these drugs (7). Taken together, these studies have suggested that rather than nonspecific internalization of GABA<sub>A</sub> receptors, SE may induce specific alterations in the complement of GABA<sub>A</sub> receptors mediating the different types of inhibition. Thus, Goodkin and colleagues investigated the SE-induced changes in GABA<sub>A</sub> receptors and the physiological consequences to address this possibility.

In their study, Goodkin et al. induced SE in rats using the muscarinic agonist, pilocarpine, then rapidly prepared

hippocampal slices for analysis. They used biochemical techniques to quantify GABA<sub>A</sub> receptor subunits that were located on the surface of neurons and indeed, found subunit-specific changes. In particular, the surface expression of  $\beta 2/3$  and  $\gamma 2$  subunits were markedly reduced compared with control slices, but the  $\delta$  subunit was unchanged. The total cellular content of all of these subunits remained unchanged, indicating that receptors containing  $\beta 2/3$  and  $\gamma 2$  subunits had been translocated from the surface to the interior of the cells. To show that the changes were functionally significant, the investigators used electrophysiological techniques to record inhibitory synaptic responses and tonic GABA-mediated currents in dentate granule cells of hippocampal slices. After SE, the amplitude and frequency of miniature inhibitory synaptic currents (i.e., the responses to release of single vesicles of GABA) were reduced compared with those from control animals. In contrast, the authors found no difference between the groups in the tonic currents recorded in dentate granule cells, in agreement with the lack of change in the surface  $\delta$  subunit. However, when GABA uptake was blocked, the tonic current in the dentate granule cells from SE-treated rats was greater than that from control rats. There is no obvious explanation for this finding, except that there appears to be a change in the steady-state balance of GABA release and reuptake (by GABA transporters) during SE. Therefore, although there was no significant change in the surface expression of  $\delta$  subunits that mediate most of the tonic GABA-mediated current, other alterations, possibly in GABA release, reuptake, or surface expression of other receptor subunits, are likely to occur in SE.

To further explore the cellular mechanisms underlying these changes in GABA<sub>A</sub> receptors during SE, the authors used a model of hyperexcitability in cultured hippocampal neurons. Incubation of dissociated neuronal cultures with elevated extracellular potassium depolarizes neurons and increases network activity, and this treatment resulted in a decrease in surface  $\gamma 2$ , but not  $\delta$ , subunits within 15 minutes, much like the *in vivo* SE results. In organotypic hippocampal cultures, which more closely resemble the synaptic organization of intact hippocampus, the investigators were able to achieve similar results to those using dissociated cultures, but the addition of NMDA to the elevated potassium solution was required for the effect. Replication of the *in vivo* results *in vitro* allowed the investigators to analyze the mechanisms of subunit-specific trafficking in more detail.

Similar to the trafficking of many other receptor types, clathrin-dependent endocytosis appears to be the major route of GABA<sub>A</sub> receptor internalization and may thereby contribute to a dynamic regulation of inhibition through surface expression of receptors (8). Making the extracellular solution hyperosmolar by the addition of sucrose, which impairs clathrin-mediated

endocytosis, partially inhibited the high potassium-induced reduction in surface  $\gamma 2$ , suggesting that this mechanism was playing a role in SE-induced internalization of receptors. In addition, because a dependence on ligand binding for internalization is common among other receptor types, the authors considered whether activation of GABA<sub>A</sub> receptors was the stimulus for their internalization. Incubation of organotypic cultures with either GABA, GABA and an uptake inhibitor, or muscimol (a GABA<sub>A</sub> agonist) failed to reproduce the reduction in surface  $\gamma 2$  seen with SE. Although these results do not rule out the possibility that activation of GABA<sub>A</sub> receptors is required for internalization to occur, they indicate that nonspecific activation of receptors by bath application of GABA is not sufficient to explain the observed SE-induced changes.

The findings in this study may help to explain two phenomena occurring in experimental, and possibly human, SE. First, the tendency of prolonged seizures to continue rather than spontaneously terminate, as most seizures do, may result, at least partially, from weakened inhibition that is due to internalization of GABA<sub>A</sub> receptors within inhibitory synapses. Second, the development of resistance to the therapeutic benefit of benzodiazepines may be related to the selective loss of surface  $\gamma 2$ -containing receptors within the synapses and the relative preservation of  $\delta$ -containing extrasynaptic receptors that are insensitive to benzodiazepines. Clearly, there are other adaptive and maladaptive changes that occur with the extreme stress incurred during SE. For instance, chloride homeostasis is altered, which also will influence GABA-mediated inhibition. The importance of the specific changes described in this study relative to the many molecular and cellular processes that accompany prolonged seizure activity is presently unknown. Moreover, many experimental animals and human patients who experience SE will subsequently develop epilepsy, and the role of these acute changes in GABA<sub>A</sub> receptors in the epileptogenic process is not clear. In conclusion, while Goodkin et al. have demonstrated that there is a complex dynamic regulation of receptor cell surface expression during SE that alters the balance between synaptic and tonic inhibition, many questions still remain about how and why the changes occur and about their ultimate consequences. The findings they report and future studies in this area will undoubtedly influence the way SE is understood and treated in humans.

by Gregory C. Mathews, MD, PhD

## References

1. DeLorenzo RJ, Hauser WA, Towne AR, Boggs JG, Pellock JM, Penberthy L, Garnett L, Fortner CA, Ko D. A prospective, population-based epidemiologic study of status epilepticus in Richmond, Virginia. *Neurology* 1996;46(4):1029–1035.

2. Shinnar S, Berg AT, Moshe SL, Shinnar R. How long do new-onset seizures in children last? *Ann Neurol* 2001;49(5):659–664.
3. Treiman DM, Meyers PD, Walton NY, Collins JF, Colling C, Rowan AJ, Handforth A, Faught E, Calabrese VP, Uthman BM, Ramsay RE, Mamdani MB; for the Veterans Affairs Status Epilepticus Cooperative Study Group. A comparison of four treatments for generalized convulsive status epilepticus. Veterans Affairs Status Epilepticus Cooperative Study Group. *N Engl J Med* 1998;339(12):792–798.
4. Goodkin HP, Yeh JL, Kapur J. Status epilepticus increases the intracellular accumulation of GABAA receptors. *J Neurosci* 2005;25(23):5511–5520.
5. Naylor DE, Liu H, Wasterlain CG. Trafficking of GABA(A) receptors, loss of inhibition, and a mechanism for pharmacoresistance in status epilepticus. *J Neurosci* 2005;25(34):7724–7733.
6. Walton NY, Treiman DM. Response of status epilepticus induced by lithium and pilocarpine to treatment with diazepam. *Exp Neurol* 1988;101(2):267–275.
7. Kapur J, Macdonald RL. Rapid seizure-induced reduction of benzodiazepine and Zn<sup>2+</sup> sensitivity of hippocampal dentate granule cell GABAA receptors. *J Neurosci* 1997;17(19):7532–7540.
8. Jacob TC, Moss SJ, Jurd R. GABA(A) receptor trafficking and its role in the dynamic modulation of neuronal inhibition. *Nat Rev Neurosci* 2008;9(5):331–343.