STATUS EPILEPTICUS: *DANSE MACABRE* IN A BALLET OF SUBUNITS

Status Epilepticus Increases the Intracellular Accumulation of $GABA_A$ Receptors

Goodkin HP, Yeh JL, Kapur J

J Neurosci 2005;25:5511–5520

Status epilepticus is a neurological emergency that results in mortality and neurological morbidity. It has been postulated that the reduction of inhibitory transmission during status epilepticus results from a rapid modification of $GABA_A$ receptors. However, the mechanism(s) that contributes to this modification has not been elucidated. We report, using an *in vitro* model of status epilepticus combined with electrophysiological and cellular imaging techniques, that prolonged epileptiform bursting results in a reduction of GABA-mediated synaptic inhibition. Furthermore, we found that constitutive internalization of $GABA_A$ receptors is rapid and accelerated by the increased neuronal activity associated with seizures. Inhibition of neuronal activity reduced the rate of internalization. These findings suggest that the rate of $GABA_A$ receptor internalization is regulated by neuronal activity and its acceleration contributes to the reduction of inhibitory transmission observed during prolonged seizures.

Trafficking of $GABA_A$ Receptors, Loss of Inhibition, and a Mechanism for Pharmacoresistance in Status Epilepticus

Naylor DE, Liu H, Wasterlain CG

J Neurosci 2005;25:7724–7733

During status epilepticus (SE), GABAergic mechanisms fail and seizures become self-sustaining and pharmacoresistant. During lithiumpilocarpine-induced SE, our studies of postsynaptic $GABA_A$ receptors in dentate gyrus granule cells show a reduction in the amplitude of miniature IPSCs (mIPSCs). Anatomical studies show a reduction in the colocalization of the $\beta 2/\beta 3$ and $\gamma 2$ subunits of $GABA_A$ receptors with the presynaptic marker synaptophysin and an increase in the proportion of those subunits in the interior of dentate granule cells and other hippocampal neurons with SE. Unlike synaptic mIPSCs, the amplitude of extrasynaptic $GABA_A$ tonic currents is augmented during SE. Mathematical modeling suggests that the change of mIPSCs with SE reflects a decrease in the number of functional postsynaptic $GABA_A$ receptors. It also suggests that increases in extracellular [GABA] during SE can account for the tonic current changes and can affect postsynaptic receptor kinetics with a loss of paired-pulse inhibition. GABA exposure mimics the effects of SE on mIPSC and tonic $GABA_A$ current amplitudes in granule cells, consistent with the model predictions. These results provide a potential mechanism for the inhibitory loss that characterizes initiation of SE and for the pharmacoresistance to benzodiazepines, as a reduction of available functional $GABA_A$ postsynaptic receptors. Novel therapies for SE might be directed toward prevention or reversal of these losses.

Kainic Acid-Induced Status Epilepticus Alters GABA Receptor Subunit mRNA and Protein Expression in the Developing Rat Hippocampus

Lauren HB, Lopez-Picon FR, Korpi ER, Holopainen IE

J Neurochem 2005;94:1384–1394

Kainic acid-induced status epilepticus leads to structural and functional changes in inhibitory $GABA_A$ receptors in the adult rat hippocampus, but whether similar changes occur in the developing rat is not known. We have used in situ hybridization to study status epilepticus-induced changes in the $GABA_A\alpha 1$–$\alpha 5$, $\beta 1$–$\beta 3$, $\gamma 1$, and $\gamma 2$ subunit

Epilepsy Currents, Vol. 6, No. 3 (May/June) 2006 pp. 102–105
Blackwell Publishing, Inc.

mRNA expression in the hippocampus of 9-day-old rats during 1 week after the treatment. Immunocytochemistry was applied to detect the α1, α2, and β3 subunit proteins in the control and treated rats. In the saline-injected control rats, the α1 and α4 subunit mRNA expression significantly increased between the postnatal days 9 to 16, whereas those of α2, β3, and γ2 subunits decreased. The normal developmental changes in the expression of α1, α2, β3, and γ2 subunit mRNAs were altered after the treatment. The immunostainings with antibodies to α1, α2, and β3 subunits confirmed the in situ hybridization findings. No neuronal death was detected in any hippocampal subregion in the treated rats. Our results show that status epilepticus disturbs the normal developmental expression pattern of $GABA_A$ receptor subunit in the rat hippocampus during the sensitive postnatal period of brain development. These perturbations could result in altered functional and pharmacological properties of $GABA_A$ receptors.

COMMENTARY

Status epilepticus (SE) has unique features that set it apart from other epilepsies. Most seizures in epilepsy are brief, even when frequent. Seizure arrest is not a passive process, but involves various endogenous anticonvulsant mechanisms, which are often triggered by the seizure itself. In contrast, SE is characterized by a progressive, either sequential or simultaneous, failure of anticonvulsant barriers. Therefore, SE does not cease without a therapeutic intervention. In some cases, even antiepileptic drug (AED) therapy fails to abort SE, which then is referred to as refractory SE. From a broader perspective, SE is a refractory state by virtue of its own mechanisms, because it is characterized both by the inability of endogenous anticonvulsant systems to control seizure activity and by the self-reinforcement of the seizure process.

The maladaptive nature and the refractory component of SE raise a number of questions. What is its primary cause—augmentation of the excitation or failure of the inhibition? What are the endogenous anticonvulsant mechanisms that fail? What are the proconvulsant mechanisms that are perpetuated? Is there a single mechanism that ultimately is responsible for the self-perpetuating character of SE; or, is there a combination of barriers that fail progressively? Are the events that underlie the self-sustaining nature of SE also responsible for the failure of the AED therapy; or, does the intractable feature of SE have a discrete mechanism?

GABA is a major inhibitory neurotransmitter and a target of many AEDs, particularly the benzodiazepines, which are commonly used to treat SE. It is, therefore, not surprising that a large number of studies have explored the hypothesis that both the progression and the refractory nature of SE occur as a result of GABAergic neurotransmission failure. It is a highly plausible scenario that the loss of postsynaptic $GABA_A$ receptors is a key mechanism responsible for the compromised GABAergic transmission. On the one hand, neurotransmitter receptors exhibit a high degree of plasticity: their synthesis, assembly, incorporation into the neuronal membrane, desensitization, and recycling occur under normal conditions; and, their actions and efficacy are dependent on the amount of the neurotransmitter available and on the level of neuronal activity (1). On the other hand, SE is characterized by an excessive neuronal activity as well as by increased GABA release (2).

The papers by Naylor et al. and Goodkin et al. explore the hypothesis that seizure activity leads to a relatively rapid translocation of $GABA_A$ receptors, from the cell surface into the cytoplasm. If SE indeed facilitates $GABA_A$ receptor internalization, this action would abolish the target, both for the inhibitory action of endogenous GABA and for the anticonvulsant effects of benzodiazepines. To test the hypothesis, the two groups applied similar methodologies to different systems. Goodkin et al. used an in vitro surrogate model of SE in which continuous seizure activity was induced in rat hippocampal neuronal cultures, either by removing Mg^{2+} from the medium or by increasing the extracellular K^+ concentration. Naylor et al. examined hippocampi of rats that had experienced 1 hour of SE, induced by lithium chloride and pilocarpine. Both groups used two experimental approaches: (i) studies of miniature inhibitory postsynaptic currents (mIPSCs) that represent an electrophysiological correlate of the number of $GABA_A$ receptors available for the effects of the neurotransmitter, and (ii) immunofluorescence examination of cellular distribution (membrane versus intracellular fractions) of $GABA_A$ receptor subunits $\beta2/\beta3$ that contribute to the binding site of GABA and $GABA_A$ receptor subunit $\gamma2$, which is responsible for the effects of benzodiazepines.

In both studies, seizure activity altered mIPSC properties in a way indicative of the deterioration in function of $GABA_A$ receptors and of the decline of GABAergic inhibition. Furthermore, in hippocampal slices from SE rats, diazepam failed to fully restore seizure-induced loss of GABAergic inhibition. Electrophysiological changes correlated with the modifications in cellular distribution of $GABA_A$ receptor subunits. Using different techniques to discriminate membrane and intracellular fractions, both Naylor et al. and Goodkin et al. found that seizures led to the rapid decline in the number of $\beta2/\beta3$ subunits on

the cell surface and their concurrent increase in the cytoplasm. In addition, Naylor et al. reported a similar redistribution of $\gamma 2$ subunits. Thus, the loss of GABAergic inhibition observed in electrophysiology experiments might be attributed to the internalization of $GABA_A$ receptor subunits responsible for the action of both GABA and of benzodiazepines.

The authors also addressed the question of why SE led to $GABA_A$ receptor internalization. Goodkin et al. found that the inhibition of seizure-like activity by agents that block *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid (AMPA) receptors as well as the inhibition of neuronal activity by tetrodotoxin alleviated the translocation of $\beta 2/\beta 3$ subunits into the cytoplasm, thus suggesting that the changes in $GABA_A$ receptors were secondary to the enhanced glutamatergic transmission and neuronal activity. Naylor et al. established that the application of GABA on control hippocampal-slice–induced changes in mIPSC properties similar to those found in SE, possibly indicating that a seizure-induced increase in GABA release was sufficient to cause the failure of $GABA_A$ receptors (i.e., ligand-dependent internalization) [2].

Can the decrease in the membrane fraction of $GABA_A$ receptors be explained solely by their enhanced trafficking? During the course of SE, the normally observed dynamic equilibrium between $GABA_A$ receptor internalization and their insertion into the plasma membrane evidently shifts in favor of the former (1). This effect may reflect the breakdown either in the $GABA_A$ receptor recycling, in $GABA_A$ receptor de novo synthesis, or in the externalization. If only $GABA_A$ receptor internalization was compromised in SE, then the increase of intracellular $GABA_A$ receptor would not be accompanied the decrease of surface subunits.

An alternative mechanism for failure of GABAergic transmission in SE was studied by Lauren et al. The authors examined the expression of $GABA_A$ receptor subunits in the hippocampus of immature rats after kainic-acid–induced SE. During the subacute period of seizures (6 hours after SE onset), Lauren et al. found a substantial decrease in the expression of $\gamma 2$ subunits in all hippocampal areas, along with several other, less profound and area-specific changes. A similar observation had been reported earlier in adult animals (3). In addition to a possible role for $\gamma 2$-subunit downregulation in the loss of benzodiazepines efficacy, such a change might have an important long-term implication. $\gamma 2$ subunits are critical for $GABA_A$ receptor clustering (i.e., proper spatial distribution on the cell membrane), especially in the immature brain (4). For instance, while $\gamma 2$-subunit knockout mice display a normal number of $GABA_A$ receptors, they are severely dysfunctional, and the mutation is often lethal at an early age. Other changes reported by Lauren et al., such as downregulation of $\alpha 4$, $\beta 3$, and other subunits (3), might contribute both to the loss of inhibition during SE and to long-term consequences, including impaired trafficking and assembly, and, hence, these changes might contribute to the impaired function of $GABA_A$ receptors.

$\alpha\beta\gamma 2$ subunits are responsible for synaptic effects of $GABA_A$ and of benzodiazepines. Other $GABA_A$ receptors have an extra or perisynaptic location. The nonsynaptic $GABA_A$ receptor subunits are different from synaptic ones, not only in location, but also in function. For example, the electrophysiological correlate of their activation differs from that of synaptic $GABA_A$ subunits. In particular, $\alpha\beta\delta$-receptors are responsible for what are known as tonic currents; tonic currents contribute to an ambient GABAergic inhibition that is dependent on extrasynaptic, "spillover" GABA concentration (5). Naylor et al. found that hippocampal slices from animals that had undergone SE exhibited a significant increase in tonic $GABA_A$ currents. Such an increase is not surprising considering two important facts: $GABA_A$ receptors that contain δ subunits (i) do not inactivate and (ii) have a higher affinity to GABA than synaptic receptors (5). Thus, the increased pool of GABA released during seizures would result in the enhanced tonic currents, when acting at $\alpha\beta\delta$-receptors.

Can the failure of $GABA_A$ receptors alone explain the self-sustaining and refractory nature of SE? Although the time between the onset of SE and the internalization of $GABA_A$ receptors has not been clearly identified, it is significantly longer than the duration of a single epileptic seizure. The decline of benzodiazepine efficiency occurs after 30 minutes of seizures, or later (6). Assuming that the internalization of $\alpha\beta\gamma 2$-receptors results in the failure of benzodiazepines, it is likely that other mechanisms are required to sustain seizure activity during the early stage of SE. Enhanced glutamatergic transmission, particularly an excessive activation of NMDA receptors, is a highly possible mechanism. Interestingly, Naylor et al. mention that the changes in $GABA_A$ receptors occur concurrently with NMDA receptors moving in the opposite direction, that is, from the cytoplasm into the cell membrane. Although the authors do not elaborate on possible mechanisms of this finding, such a phenomenon, if proven true, might contribute to the strengthening of excitatory transmission. The activation of NMDA receptors itself may lead to the loss of GABAergic inhibition (7). Furthermore, in several experimental models of SE, NMDA-receptor blockers were proven to be highly effective for inhibiting the seizures, even when administered at late time points for which GABAergic drugs failed (8). Finally, while internalization of $\alpha\beta\gamma 2$-receptors might explain the failure of benzodiazepines to arrest SE, it certainly does not explain the loss of anticonvulsant efficacy that is frequently observed with phenytoin, another AED commonly used to manage SE. The failure of a presynaptic process, which underlies the loss of phenytoin efficacy, might be attributed to translocation of calmodulin kinase from the

membrane to cytosol, rendering the release of neurotransmitter independent of Na^+ entry (9).

A final question is whether the increased $GABA_A$ receptor internalization can be managed pharmacologically and whether a pharmacological intervention could improve therapeutic outcomes? Goodkin et al. showed that hyperosmolar extracellular medium inhibited $GABA_A$ receptor internalization. Therefore, using a compound such as mannitol might be useful in increasing the efficacy of GABAergic therapy. Augmenting tonic $GABA_A$ receptor currents, for example, with neurosteroids, which act at the δ subunit, might have an anticonvulsant effect during SE—although it currently is not known whether enhanced tonic inhibition would be sufficient to compensate for the increased excitatory transmission and compromised synaptic GABAergic inhibition. Furthermore, enhancing tonic $GABA_A$ receptor currents may augment the synchronization mechanisms in generalized epileptic discharges, such as the corticothalamic oscillator (10). However, the strategy certainly may limit SE of limbic origin. Indeed, tiagabine (a GABA-transporter blocker) is effective for partial epilepsies but can precipitate nonconvulsive SE in clinical practice. Exploring other targets, such as NMDA receptors or interference with glutamate release, might be beneficial in refractory cases of SE.

In conclusion, the complexity of the pathophysiology and of the refractory nature of SE suggests that both phenomena result from a variety of maladaptive changes rather than from a single mechanism. It is conceivable that while the initial self-reinforcement of the glutamatergic system supports the transition to SE, the secondary failure of synaptic GABAergic inhibition consolidates SE and results in treatment failure. The relative augmentation of GABAergic tonic inhibition probably limits sustained focal discharges, although it may enhance the exuberance of other epileptiform mechanisms, particularly those derived from the corticothalamic oscillator.

by Andrey Mazarati, MD, PhD, and Raman Sankar, MD, PhD

References

1. Kneussel M. Dynamic regulation of $GABA_A$ receptors at synaptic sites. *Brain Res Rev* 2002;39:74–84.
2. Wasterlain CG, Baxter CF, Baldwin RA. GABA metabolism in the substantia nigra, cortex and hippocampus during status epilepticus. *Neurochem Res* 1993;18:527–532.
3. Tsunashima K, Schwarzer C, Kirchmair E, Sieghart W, Sperk G. GABA(A) receptor subunits in the rat hippocampus III: altered messenger RNA expression in kainic acid-induced epilepsy. *Neuroscience* 1997;80:1019–1032.
4. Essrich C, Lorez M, Benson JA, Fritschy JM, Luscher B. Postsynaptic clustering of major GABAA receptor subtypes requires the gamma 2 subunit and gephyrin. *Nat Neurosci* 1998;1:563–571.
5. 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.
6. Mazarati AM, Baldwin RA, Sankar R, Wasterlain CG. Time-dependent decrease in the effectiveness of antiepileptic drugs during the course of self-sustaining status epilepticus. *Brain Res* 1998;814:179–185.
7. Kapur J, Lothman EW. NMDA receptor activation mediates the loss of GABAergic inhibition induced by recurrent seizures. *Epilepsy Res* 1990;5:103–111.
8. Mazarati AM, Wasterlain CG. N-methyl-D-asparate receptor antagonists abolish the maintenance phase of self-sustaining status epilepticus in rat. *Neurosci Lett* 1999;265:187–190.
9. Wasterlain CG, Bronstein JM, Morin AM, Dwyer BE, Sankar R. Translocation and autophosphorylation of brain calmodulin kinase II in status epilepticus. *Epilepsy Res Suppl* 1992;9:231–238.
10. Cope DW, Hughes SW, Crunelli V. GABAA receptor-mediated tonic inhibition in thalamic neurons. *J Neurosci* 2005;25:11553–11563.