

THE DUAL ROLES OF GABA IN SEIZURES AND EPILEPSY GENERATE MORE EXCITEMENT

Anomalous Levels of Cl⁻ Transporters in the Hippocampal Subiculum from Temporal Lobe Epilepsy Patients Make GABA Excitatory. Palma E, Amici M, Sobrero F, Spinelli G, Di Angelantonio S, Ragozzino D, Mascia A, Scoppetta C, Esposito V, Miledi R, Eusebi F. *Proc Natl Acad Sci USA* 2006;103:8465–8468. Erratum in *Proc Natl Acad Sci USA* 2006;103:11814. The mRNA levels of NKCC1, an inwardly directed Na⁺, K⁺-2Cl⁻ cotransporter that facilitates the accumulation of intracellular Cl⁻, and of KCC2, an outwardly directed K⁺-Cl⁻ cotransporter that extrudes Cl⁻, were studied in surgically resected brain specimens from drug-resistant temporal lobe (TL) epilepsy (TLE) patients. Quantitative reverse transcription polymerase chain reaction (RT-PCR) analyses of the mRNAs extracted from the human TLE-associated brain regions revealed an upregulation of NKCC1 mRNA and a downregulation of KCC2 mRNA in the hippocampal subiculum, compared with the hippocampus proper or the TL neocortex, suggesting an abnormal transcription of Cl⁻ transporters in the TLE subiculum. In parallel experiments, cell membranes isolated from the same TLE-associated brain regions were injected into *Xenopus* oocytes that rapidly incorporated human GABA_A receptors into their surface membrane. The GABA currents elicited in oocytes injected with membranes from the subiculum had a more depolarized reversal potential (E_{GABA}) compared with the hippocampus proper or the neocortex. The NKCC1 blocker bumetanide or a temperature decrease of 10°C shifted the GABA-current E_{GABA} more negative in oocytes injected with membranes from TLE hippocampal subiculum, matching the E_{GABA} of TL neocortex-injected oocytes. We conclude that the anomalous expression of both Cl⁻ transporters, KCC1 and NKCC2, in TLE hippocampal subiculum probably causes altered Cl⁻ transport in the “epileptic” neurons, as revealed in the microtransplanted *Xenopus* oocytes, and renders GABA aberrantly “exciting,” a feature that may contribute to the precipitation of epileptic seizures. The authors note that the last sentence of the abstract should read: “We conclude that the anomalous expression of both Cl⁻ transporters, NKCC1 and KCC2, in TLE hippocampal subiculum probably causes altered Cl⁻ transport in the ‘epileptic’ neurons, as revealed in the microtransplanted *Xenopus* oocytes, and renders GABA aberrantly “exciting,” a feature that may contribute to the precipitation of epileptic seizures. This error does not affect the conclusions of the article.

Epileptogenic Actions of GABA and Fast Oscillations in the Developing Hippocampus. Khalilov I, Le Van Quyen M, Gozlan H, Ben-Ari Y. *Neuron* 2005;48:787–796. GABA excites immature neurons and inhibits adult ones, but whether this contributes to seizures in the developing brain is not known. We now report that in the developing, but not the adult, hippocampus, seizures beget seizures only if GABAergic synapses are functional. In the immature hippocampus, seizures generated with functional GABAergic synapses include fast oscillations that are required to transform a naive network to an epileptic one: blocking GABA receptors prevents the long-lasting sequels of seizures. In contrast, in adult neurons, full blockade of GABA(A) receptors generates epileptogenic high-frequency seizures. Therefore, purely glutamatergic seizures are not epileptogenic in the developing hippocampus. We suggest that the density of glutamatergic synapses is not sufficient for epileptogenesis in immature neurons; excitatory GABAergic synapses are required for that purpose. We suggest that the synergistic actions of GABA and NMDA receptors trigger the cascades involved in epileptogenesis in the developing hippocampus.

COMMENTARY

A dramatic rethinking of the role of GABA—traditionally regarded as the brain’s main inhibitory neurotransmitter—in seizures and epilepsy has been occurring in recent years. The shift in thinking began more than 10 years ago with the discoveries that during the early stages of brain development, GABA acts as an excitatory neurotransmitter and plays a key role in the shaping of synaptic connections. Because GABA exerts its primary (fast) effects through chloride currents associated with GABA_A receptors, it either depolarizes or hyperpolarizes the postsynaptic neuron, depending on chloride’s electrochemical gradient across the neuronal membrane. Early in development, expression of the Na⁺/K⁺/Cl⁻-cotransporter,

NKCC1, results in a high intracellular chloride concentration that makes GABA depolarizing; negatively charged chloride ions flow out of the cell upon GABA_A receptor activation. During the first two postnatal weeks in rats and likely during the late prenatal and early postnatal period in humans, the chloride concentration within neurons decreases simultaneously with a decrease in expression of NKCC1 and an increase in expression of the K⁺/Cl⁻-cotransporter, KCC2. KCC2 transports chloride out of the neuron, resulting in the low intracellular chloride concentration found in mature neurons that makes GABA hyperpolarizing.

Models of brain injury in adult animals, including prolonged epileptiform activity, have demonstrated a downregulation of neuronal KCC2 expression and a transformation of GABA into a depolarizing, excitatory neurotransmitter in affected brain regions (1). Therefore, it is possible that acute and chronic changes in chloride transporter expression, by reducing

or even reversing the inhibitory influence of GABA, underlie the generation of seizures acutely or the process of epileptogenesis. If this were true, pharmacological agents that act at chloride transporters could provide novel anticonvulsant therapies. Indeed, the loop diuretic bumetanide, which potently and selectively blocks NKCC1, reduces chloride accumulation in neurons and shifts the reversal potential for GABA toward more hyperpolarized levels, thereby reducing or even terminating seizure activity in the immature mouse brain (2,3).

Another aspect of the changing perspective on GABA concerns the key role it plays in the development of neuronal circuits. It is a major excitatory neurotransmitter in the immature brain at a time when glutamatergic synaptic connections are beginning to mature (4). In the early postnatal period in rats, the pairing of depolarizing GABA-mediated responses with glutamate release at immature excitatory synapses that contain only *N*-methyl-D-aspartate (NMDA) receptors relieves their voltage-dependent block by magnesium ions. The resulting NMDA receptor-mediated calcium influx provides the signal required for insertion of AMPA-type glutamate receptors into the postsynaptic membrane and, therefore, leads to their maturation into active synapses. Given this crucial role in the development of excitatory synapses, it is likely that the depolarizing action of GABA contributes to the particular vulnerability of neonates to seizures and epilepsy. This developmental process appears to be analogous to the insertion of AMPA receptors that occurs during induction of long-term potentiation in more mature brain circuits, when glutamate provides the depolarizing signal. Considering the importance of long-term potentiation in learning and memory functions, disruption of these analogous signaling patterns by seizures may have long-lasting consequences in patients at a vulnerable age.

Several questions have been raised by these recent findings. First, do changes in chloride transporter expression that recapitulate early development occur in adult epilepsy, such that GABA has less inhibitory efficacy or perhaps is excitatory? Second, because GABA is a major excitatory neurotransmitter early in development, what role does it play in the generation of seizures and in the development of chronic epilepsy? Two new studies have begun to address these questions.

Although it is now clear that the high expression of NKCC1 in immature brain does play a role in the increased susceptibility of neonates to seizures, whether altered chloride transporter expression plays a role in adult human epilepsy remains unclear. To explore this possibility, Palma et al. measured the expression of NKCC1 and KCC2 in temporal lobe resected from four patients with hippocampal sclerosis who underwent surgery for the treatment of intractable epilepsy. Comparing mRNA levels in the temporal neocortex, hippocampus, and subiculum, they found that on average NKCC1 was upregulated in the subiculum approximately threefold compared with the cortex. In addition, KCC2 was downregulated by approximately 80% in

the subiculum. Although not part of the hippocampus proper, the subiculum is the major output region from the hippocampus, receiving inputs from CA1 pyramidal neurons and sending outputs to many other brain regions. Interestingly, one report of electrophysiological recordings from acute slices of resected human temporal lobe found the subiculum to be the origin of interictal-like epileptiform activity (5), suggesting that it may be an important locus of epileptogenesis. The mRNA changes, if they are reflected in altered protein levels, are expected to shift neuronal chloride gradients and make GABA less hyperpolarizing. The authors investigated the functional consequences of this expression pattern by injecting membranes prepared from the subiculum, hippocampus, and cortex into *Xenopus* oocytes. This technique effectively reconstituted in oocyte membranes the complement of GABA receptors and chloride transporter proteins expressed in those regions. When membranes from subiculum were used, the reversal potential for chloride, as determined by application of GABA, was shifted toward more depolarized potentials compared with those of hippocampus or cortex. In support of the hypothesis that an increased ratio of NKCC1 to KCC2 is responsible for this difference, application of bumetanide shifted the chloride reversal potential from subicular membranes toward the values in hippocampus and cortex. These findings suggest that chloride transporter expression in adult epileptic subiculum is similar to early stages of development. The results of Palma and colleagues suggest that in the epileptic subiculum, GABA has diminished efficacy as an inhibitory transmitter.

Upregulation of NKCC1, and consequently reduced inhibition, in chronic epilepsy may be one determinant of the intractability of seizures to standard drug treatments. Of course, this study only examined tissue from four patients with intractable epilepsy, so it remains to be seen whether the finding can be generalized to all intractable patients. Moreover, because no nonepileptic brains were examined and the authors used the same patients' temporal neocortex as a "control," it is not clear whether this pattern of transporter expression is truly abnormal. If these expression patterns are related to epilepsy, when did the changes occur? It is possible that they occurred before the onset of clinical epilepsy and were part of the process of epileptogenesis or that they were the result of years of seizures that were ineffectively controlled. It also is possible that the changes were present early in the disease and were part of the reason that medications were not effective. Studies involving tissue from human patients present many challenges, but the findings in this study and the questions they raise should stimulate further research using animal models to address the relationship between chloride transporter expression, epileptogenesis, and the response of seizures to treatment.

Another recent study examined the role of GABA in the generation of seizures and chronic epilepsy in immature and mature hippocampus. Khalilov et al. used a preparation composed

of both hippocampi from an immature rat and the commissural fibers that connect them, which enabled the investigators to perfuse each hippocampus separately. They had demonstrated earlier (6) that seizures induced in one hippocampus propagate to the contralateral side acutely and after repeated seizures, produce lasting epileptogenic changes in the contralateral hippocampus (i.e., a “mirror focus”). The current study extended those findings to explore the mechanisms involved in the transition from acute, induced seizures to chronic, spontaneous seizures, mimicking the development of epilepsy. When inhibition was blocked (with GABA antagonists) or excitation enhanced (with kainic acid) in the ipsilateral hippocampus, acute seizure-like discharges were generated both in the ipsilateral and contralateral hippocampi. Repeated induction of ipsilateral seizures with either treatment was epileptogenic to the contralateral side, resulting in spontaneously generated seizures—even after the commissural connection was cut. But only repeated kainate treatment was epileptogenic on the ipsilateral side. Blocking GABA receptors appeared to protect the ipsilateral hippocampus from undergoing the long-lasting changes associated with chronic epilepsy, supporting the view that the action of GABA itself is required to produce the synaptic changes that underlie epilepsy in the immature brain.

Khalilov and colleagues also examined characteristics of the seizure patterns induced by both GABA antagonists and kainate; they found that during the ipsilateral kainate-induced seizures as well as during the propagated, contralateral seizures, fast oscillations were observed during the ictal discharges. Fast oscillations are rapid network-driven discharges (40–140 Hz) that depend in part on GABAergic interneurons, so seizures induced by GABA antagonists do not exhibit these discharges. The authors found that these largely interneuron-driven patterns of activity, while not required to generate seizures, are required to produce the changes associated with epilepsy. Both the fast oscillations and the epileptogenesis were prevented by an NMDA antagonist, suggesting that both GABA and NMDA receptors act together to promote these network patterns of activity and the lasting changes that result from them. Therefore, it appears that there is a critical developmental period during which GABAergic networks are mature enough to produce fast oscillations and, when driven in conjunction with NMDA receptor-containing synapses, can produce persistent epileptic changes.

Using adult rat hippocampi (i.e., after GABA becomes inhibitory and the critical developmental periods are over), Khalilov et al. assessed whether requirements for GABAergic synaptic activity and fast oscillations for epileptogenesis were similar to those of the immature brain. Unable to use the same intact preparation they used in immature animals, they placed adult and immature hippocampi in the same chamber. In contrast to the immature brain in which only kainate produced

lasting epileptic changes, both GABA antagonists and kainate were epileptogenic in adult hippocampus. These results indicate that GABAergic synapses are only required for epileptogenesis in immature brain. Their results point to a special role of GABA throughout a critical period of development, corresponding to the neonatal period in humans. During this time, GABAergic synapses are capable of driving ictal discharges as well as producing, in conjunction with NMDA-containing synapses, the chronic changes associated with epilepsy.

GABA's actions in the developing and mature brain are much more complex than suggested by earlier attempts to classify it simply as an inhibitory neurotransmitter. Because of the effects of GABA on developing synaptic connections, it is likely that seizures in the immature brain disrupt normal developmental processes and possibly produce long-lasting alterations in brain circuitry that either predispose to future seizures or to other neurological problems. Accordingly, the use of GABAergic modulators, such as benzodiazepines and barbiturates, to prevent seizures may not only lack the efficacy in neonates that is evidenced in adults, but also may interfere with normal developmental processes—at least theoretically. The impact of these considerations on clinical decision making will not be clear until more studies are available that examine the effects of both seizures and their treatment on developmental processes. Finally, the role of GABA in adult epilepsy is being reexamined in light of increasing evidence that altered chloride transporter expression may underlie the aberrant excitability within the epileptic tissue or may be a determinant of drug treatment response. These findings may lead to the development of novel therapeutics aimed at normalizing chloride distribution in brain regions with abnormal chloride transporter expression.

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References

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