

CHRONIC CORTICAL INJURY AND EPILEPTOGENESIS: A POSSIBLE ROLE FOR INTRACELLULAR CHLORIDE HOMEOSTASIS

Impaired Cl^- Extrusion in Layer V Pyramidal Neurons of Chronically Injured Epileptogenic Neocortex

Jin X, Huguenard JR, Prince DA

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In the mature brain, the K^+/Cl^- cotransporter KCC2 is important in maintaining low $[\text{Cl}^-]_i$, resulting in hyperpolarizing GABA responses. Decreases in KCC2 after neuronal injuries result in increases in $[\text{Cl}^-]_i$ and enhanced neuronal excitability due to depolarizing GABA responses. We used the gramicidin perforated-patch technique to measure $E_{\text{Cl}}(\sim E_{\text{GABA}})$ in layer V pyramidal neurons in slices of partially isolated sensorimotor cortex of adult rats to explore the potential functional consequence of KCC2 downregulation in chronically injured cortex. E_{GABA} was measured by recording currents evoked with brief GABA puffs at various membrane potentials. No significant difference was found in E_{Cl} between neurons in control and undercut animals (-71.2 ± 2.6 and -71.8 ± 2.8 mV, respectively). However, when loaded with Cl^- by applying mus-

cimol puffs at 0.2 Hz for 60 seconds, neurons in the undercut cortex had a significantly shorter time constant for the positive shift in E_{Cl} during the Cl^- loading phase (4.3 ± 0.5 s for control and 2.2 ± 0.4 s for undercut; $p < 0.01$). The positive shift in E_{Cl} 3 seconds after the beginning of Cl^- loading was also significantly larger in the undercut group than in the control, indicating that neurons in undercut cortex were less effective in maintaining low $[\text{Cl}^-]_i$ during repetitive activation of GABA_A receptors. Application of furosemide eliminated the difference between the control and undercut groups for both of these measures of $[\text{Cl}^-]_i$ regulation. The results suggest an impairment in Cl^- extrusion resulting from decreased KCC2 expression that may reduce the strength of GABAergic inhibition and contribute to epileptogenesis.

COMMENTARY

Two classic concepts in experimental epilepsy research are that 1) hyperexcitability is the prelude to seizure generation and a signature for the development of chronic epileptogenesis, and 2) epilepsy arises from an imbalance of excitation and inhibition. Several studies have suggested that hyperexcitability does not simply equal a seizure and the occurrence of epileptogenesis and that the notion of an arithmetic sum of excitation and inhibition may be too simplistic to understand the actual complexity of the molecular and cellular mechanisms that contribute to acute seizure generation and chronic epileptogenesis. The present study by Jin and colleagues uses the *in vitro* brain-slice technique in conjunction with the chronic undercut or partially isolated sensorimotor cortex as an animal model for posttraumatic epilepsy and provides evidence that neurons in undercut cortex were less effective in maintaining a low level of chloride ions (Cl^-) during repetitive activation of GABA_A receptors. These data suggest that neuronal injury causes a subsequent depression in the function of the potassium–chloride

cotransporter KCC2, which leads to an alteration in GABA_A-mediated inhibition that is dependent on the level of synaptic activity. The net effect of this hypothetical change is that, after a cortical injury, the first GABA_A-mediated inhibitory postsynaptic potential (IPSP) in a series of IPSPs would be relatively normal (i.e., show a lack of detectable hyperexcitability, with an apparent balance of excitation/inhibition to a single stimulus); however, subsequent IPSPs, during repetitive activation, would progressively decrease in effectiveness. Thus, the presence of impaired Cl^- extrusion during repetitive activity may facilitate seizure spread into new areas and ultimately allow generalization. In injured cortex, high levels of normal, nonictal synaptic activity could shift the balance of inhibition and excitation by overwhelming the neuronal chloride-export capacity, thereby generating seizures.

The chloride equilibrium of a neuron must be reestablished after synaptic activation of the GABA_A receptor during an IPSP. Cl^- ions that have entered the neuron through the GABA_A-receptor channel are exported in an electroneutral fashion by the potassium–chloride cotransporter, KCC2. If Cl^- ions admitted by synaptic activation of the GABA_A receptor overwhelm KCC2, then Cl^- accumulates in the neuronal cytoplasm, shifting the chloride equilibrium potential (E_{Cl}) in a positive direction and potentially making subsequent

GABA_A-receptor activity excitatory. Thus, the maximal rate at which KCC2 can operate (relative to the maximal rate of synaptic activation) is an important consideration in the capacity of a neuron to maintain a proper level of inhibition in the face of high demand on GABAergic systems.

The homeostasis of intracellular chloride concentration $[Cl^-]_i$ has been most extensively studied in relation to the differences between immature and adult cortex. Cl^- ions are actively transported into immature neurons, so $[Cl^-]_i$ is higher and E_{Cl} is less negative than in adult neurons, which is considered to be an important cause of the enhanced seizure susceptibility in the immature brain. Therefore, the immature brain represents a naturally occurring case of increased seizure susceptibility based on dysregulation of $[Cl^-]_i$. By analogy, many recent studies of axotomized and chronically injured cultured neurons also report alterations in intracellular Cl^- homeostasis in which E_{Cl} is less negative than in the normal condition. Thus, under these conditions, GABA_A-mediated IPSPs are less effective and may become excitatory after injury. In a recent study on spinal cord neurons, the change in $[Cl^-]_i$ occurred in neurons that were postsynaptic to the injured cells but were, themselves, uninjured (1), suggesting that an area of impaired chloride transport may extend beyond the actual cortical injury. This suggestion is more speculation than a hypothesis about cortical injury and posttraumatic epilepsy, but it may explain why epileptic foci are not always located in the most obviously injured brain region.

Demonstration of the reduced chloride extrusion in this animal model of posttraumatic epilepsy suggests that increased GABA_A-receptor activity during repetitive IPSPs leads to an

activity dependence in the balance of excitation and inhibition. Activity dependence is one mechanism by which external or internal stimuli may sometimes trigger seizures when normally they do not. For example, because cortical activity becomes more synchronous on transitions to and from sleep, activity-dependent disinhibition, resulting from a reduced Cl^- gradient, could potentially contribute to the changes in seizure propensity that are related to states of arousal.

Studies in the undercut cortex and other models of acquired epilepsy—including models resulting from chemically or electrically induced status epilepticus—indicate that impaired Cl^- extrusion is just one of many changes that occur after a brain injury. The development of a chronic epileptic state depends on the overall constellation of the epileptogenic alterations. Thus, loss of inhibitory interneurons, axon sprouting with the formation of new recurrent excitatory circuits, changes in postsynaptic receptors (including *N*-methyl-D-aspartate [NMDA] receptors), and other mechanisms may ultimately combine to create a state of heightened seizure susceptibility and to determine whether spontaneous recurrent seizures occur in the injured brain.

by F. Edward Dudek, PhD, and Kevin J. Staley, MD

Reference

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