

NEONATAL SEIZURES: IS A NOVEL, MECHANISM-BASED TREATMENT FINALLY ON THE HORIZON?

NKCC1 Transporter Facilitates Seizures in the Developing Brain

Dzhala VI, Talos DM, Sdrulla DA, Brumback AC, Mathews GC, Benke TA, Delpire E, Jensen FE, Staley KJ

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During development, activation of Cl⁻-permeable GABA_A receptors (GABA_A-R) excites neurons as a result of elevated intracellular Cl⁻ levels and a depolarized Cl⁻ equilibrium potential (E_{Cl}). GABA becomes inhibitory as net outward neuronal transport of Cl⁻ develops in a caudal-rostral progression. In line with this caudal-rostral developmental pattern, GABAergic anticonvulsant compounds inhibit motor manifestations of neonatal seizures but not cortical seizure activity. The Na⁺-K⁺-2Cl⁻ cotransporter (NKCC1) facilitates the accumulation of Cl⁻ in neurons. The NKCC1 blocker bumetanide shifted E_{Cl} negative in immature neurons, suppressed epileptiform activity in hip-

pocampal slices *in vitro* and attenuated electrographic seizures in neonatal rats *in vivo*. Bumetanide had no effect in the presence of the GABA_A-R antagonist bicuculline, nor in brain slices from NKCC1-knockout mice. NKCC1 expression level versus expression of the Cl⁻-extruding transporter (KCC2) in human and rat cortex showed that Cl⁻ transport in perinatal human cortex is as immature as in the rat. Our results provide evidence that NKCC1 facilitates seizures in the developing brain and indicate that bumetanide should be useful in the treatment of neonatal seizures.

Depolarizing GABA Acts on Intrinsically Bursting Pyramidal Neurons to Drive Giant Depolarizing Potentials in the Immature Hippocampus

Sipilä ST, Huttu K, Soltesz I, Voipio J, Kaila K

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Spontaneous periodic network events are a characteristic feature of developing neuronal networks, and they are thought to play a crucial role in the maturation of neuronal circuits. In the immature hippocampus, these types of events are seen in intracellular recordings as giant depolarizing potentials (GDPs) during the stage of neuronal development when GABA_A-mediated transmission is depolarizing. However, the precise mechanism how GABAergic transmission promotes GDP occurrence is not known. Using whole-cell, cell-attached, perforated-patch, and field-potential recordings in hippocampal slices, we demonstrate here that CA3 pyramidal neurons in the newborn

rat generate intrinsic bursts when depolarized. Furthermore, the characteristic rhythmicity of GDP generation is not based on a temporally patterned output of the GABAergic interneuronal network. However, GABAergic depolarization plays a key role in promoting voltage-dependent, intrinsic pyramidal bursting activity. The present data indicate that glutamatergic CA3 neurons have an instructive, pacemaker role in the generation of GDPs, whereas both synaptic and tonic depolarizing GABAergic mechanisms exert a temporally nonpatterned, facilitatory action in the generation of these network events.

COMMENTARY

Neonates are very prone to seizures. The neonatal period represents the time across the lifespan when seizure threshold is lowest. Nearly any insult to the brain (e.g., hypoxia-

ischemia, hemorrhage, infection, metabolic derangement) during this vulnerable time span can induce the hypersynchronous neuronal firing that underlies a clinical seizure. The mechanisms of seizure generation, propagation, and termination may be somewhat different early in development compared to more mature ages, and these age-related mechanisms have not yet been characterized fully (1). This issue is relevant clinically, as the existing antiseizure medications to treat neonatal seizures are

woefully inadequate. Phenobarbital and phenytoin have been the mainstays of treatment, along with more recent use of benzodiazepines (2). It has been estimated that fewer than half of neonatal seizures respond to these agents, even at high doses (3,4). Furthermore, in neonates, electrographic seizures often occur without a clinical correlate (i.e., electroclinical dissociation) and are considered to be particularly harmful to the developing brain (5). The hope is that understanding the developmental mechanisms of seizure generation will lead to improved therapeutics. The two papers discussed in this commentary offer some optimism that the mechanisms of neonatal seizures may yet be unraveled, with a mechanism-based therapy on the horizon.

A prevalent notion regarding the enhanced seizure susceptibility of the developing brain is that GABA, the primary inhibitory neurotransmitter in the mature brain, instead exerts depolarizing actions in subjects who are young. This idea has received extensive experimental support, as summarized in the accompanying review by Dr. Kevin Staley. In brief, the paradoxical action of GABA early in development is due, at least in part, to age-related differences in chloride homeostasis. Chloride transport is a function of two membrane pumps with different time courses of expression. Early in development, (roughly P3–P15 in the rat), the $\text{Na}^+, \text{K}^+, 2\text{Cl}^-$ cotransporter (NKCC1) imports large amounts of Cl^- into the neuron (along with Na^+ and K^+ to maintain electroneutrality). This pump sets the chloride equilibrium potential (E_{Cl}) positive to the resting potential, so that when the GABA_A receptor is activated, Cl^- flows out of the neuron, depolarizing it. Over time, NKCC1 expression diminishes, and another Cl^- transporter, KCC2, is expressed. KCC2 has the opposite effect—it extrudes Cl^- out of the neuron, placing the E_{Cl} more negative than the resting potential so that GABA_A receptor activation allows extracellular Cl^- to flow into the neuron, hyperpolarizing it and endowing GABA with an inhibitory action.

This developmental “switch” in Cl^- homeostasis might influence the seizure susceptibility of the neonatal brain. The additional depolarization that is due to GABA_A receptor activation would add to the excitation initiated by glutamate neurotransmission and shift the excitation/inhibition balance toward excessive excitation and thus toward seizure activity. The paper by Dzhala et al. makes several major contributions to this emerging story. First, it describes the developmental profile of NKCC1 in the human neonate. In the early postnatal period (weeks in rats, months in humans), NKCC1 rises to a peak then declines to adult levels; while over the same time period, KCC2 expression gradually rises to adult levels. Second, blocking NKCC1 function with a commonly used diuretic, bumetanide, prevents the accumulation of intracellular Cl^- , thereby averting the depolarizing action of GABA_A receptor activation. Bumetanide reduces kainic acid–induced seizures in neonatal (but not adult) rats and

burst firing in hippocampal slices. Furthermore, in genetically engineered mice lacking NKCC1, bumetanide is not effective in ameliorating seizures, supporting its role as a specific inhibitor of NKCC1. Bumetanide is, therefore, a promising antiepileptic drug with a novel target—the immature chloride cotransporter NKCC1. This loop diuretic has been safely used clinically, including in neonates. Its long-term safety profile needs to be established more fully, but it offers a mechanistically sensible alternative to GABA_A receptor agonists for the treatment of neonatal seizures.

A well-established characteristic of some neonatal neurons is their tendency to fire in bursts of rhythmic activity. Such immature cortical rhythms are hypothesized to underlie a number of critical developmental processes, such as the establishment of synaptic connections (6). However, intrinsically generated rhythms can also be pathological and might contribute to the heightened seizure propensity of the developing brain. In neonatal hippocampal CA3 neurons, spontaneous network events termed giant depolarizing potentials (GDPs) can be recorded intracellularly (7). These GDPs are facilitated by GABA, which is depolarizing at this stage, though it is uncertain whether GDPs are driven by interneurons (constituting a pacemaker-like mechanism) or whether GABA leads to a generalized increase in network excitability.

The present paper by Sipilä et al. analyzes the synaptic basis of GDPs and roles of glutamatergic and GABAergic input that generate GDPs. The authors show that neonatal CA3 pyramidal neurons burst rhythmically when depolarized, that is, produce GDPs, and that the rhythmicity is produced by intrinsic voltage-dependent properties of CA3 neurons and is not dependent on patterned GABAergic input from interneurons. This conclusion is based on several lines of evidence, using field recordings and patch clamp techniques. Field GDPs (if monitored in the extracellular field, they are called fGDPs) are present when both glutamatergic and GABAergic synaptic transmission are blocked and can be generated when CA3 neurons are depolarized to the voltage range that activates intrinsic conductances. The authors identify a 0.3-Hz fGDP rhythm and argue that interneurons are entrained to fire at 0.3 Hz by rhythmic input from CA3 during fGDPs. GDP frequency is modulated by both synaptic and tonic extrasynaptic GABAergic input in the sense that GABA depolarizes CA3 neurons to the voltage range at which they utilize intrinsic mechanisms to generate rhythmic bursts. The authors claim that tonic GABA action, even in the absence of interneuron input, is sufficient to enhance fGDPs. It is important to differentiate spontaneous bursts in single CA3 neurons from synchronous population bursts from fGDPs. Furthermore, interictal bursts (population events in the presence of GABA antagonists) may be separable from GDPs. Whether CA3 neurons, as opposed to fGDPs, have an intrinsic burst rate remains to be verified.

Agents that specifically diminish bursting behavior in neonatal neurons could add another dimension to seizure treatment. In particular, the demonstration by Dzhala et al. that bumetanide rapidly suppresses synchronous bursts of network activity in P4–P8 hippocampal slices supports use of the agent as a potential anticonvulsant drug in this age range. Clinical trials would need to demonstrate that bumetanide, or other diuretics that inhibit seizure activity (8), reach the brain in appropriate concentrations and lack both short- and long-term adverse effects.

A crucial but unproven issue is whether human neonates exhibit GABA-mediated excitation and thus would be amenable to therapies based on this proposed mechanism. If the GABA-mediated mechanism is conserved evolutionarily, optimism is warranted. However, it is unclear why some GABAergic agents (e.g., phenobarbital, benzodiazepines) do not routinely make human neonatal seizures worse. While the efficacy of these agents is limited, they do stop seizures in a definite percentage of newborns. It is encouraging that experimental efforts are now addressing these critical clinical issues, so it is reasonable to remain optimistic that the realm of age-dependent epilepsy therapies is near at hand.

by Carl E. Stafstrom, MD, PhD

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