

Wrong-Way Chloride Transport: Is It a Treatable Cause of Some Intractable Seizures?

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Despite decades of research and a half dozen new anticonvulsant agents, some types of seizures are as untreatable now as they were in the days of bromides. These treatment-resistant seizures suggest that some of the assumptions about anticonvulsant mechanisms may need revision. This review will focus on one of the bedrock assumptions of epileptology that the neurotransmitter GABA inhibits neuronal activity, and therefore, agents that increase GABA activity should increase inhibition and consequently decrease the abnormal neuronal activity that occurs during a seizure.

Seizures occurring in the neonatal period, during intractable temporal lobe epilepsy, and after hypoxic-ischemic injury seem to have nothing in common other than a poor response to medication, but these three seizure syndromes have recently been linked to potentially treatable abnormalities in neuronal chloride transport. Chloride is the principal ion that flows through channels activated by the GABA_A receptor. Normally, neurons have very little chloride in their cytoplasm. When GABA opens the GABA_A chloride channel, negatively charged chloride flows from the extracellular space into the neuronal cytoplasm. The extra negative charge carried by the chloride ions drives the neuronal membrane potential to more negative values. The negative membrane potential, in turn, strongly reduces the likelihood that the neuron will fire an action potential; in effect, the neuron is put to sleep by GABA_A receptor activation.

A host of anticonvulsants, including the barbiturates, benzodiazepines, tiagabine, vigabatrin, and possibly valproate work by enhancing the opening of GABA_A channels (1,2). Conversely, pentylenetetrazol, one of the drugs most widely used in animal models to screen new compounds for anticonvulsant

activity, is a GABA_A antagonist (3). So, putting neurons to sleep by enhancing the effects of GABA seems to be an effective anticonvulsant strategy, albeit complicated by the predictable side effects of sedation and cognitive slowing engendered by sleepy neurons.

The foundation of GABA-based anticonvulsant strategies is a low intracellular chloride concentration that makes possible the inward flux of negatively charged chloride. However, recent discoveries demonstrate that neuronal chloride is not always maintained at a conveniently low level. The first situation in which neuronal chloride was found to be elevated was in the immature brain (4). Developing neurons express Na⁺,K⁺,2Cl⁻ cotransporter (NKCC1), a transporter that uses the energy stored in the transmembrane sodium gradient to import a potassium ion, along with one sodium ion and two chloride ions (5,6). NKCC1 activity results in an accumulation of cytoplasmic chloride such that opening of chloride-permeable GABA_A channels produces an efflux of negatively charged chloride, depolarizing the developing neuron's membrane. These events trigger action potentials and increase intracellular calcium (4). While such GABA-mediated excitation is necessary for proper circuit formation (7), it clearly hampers the effects of GABA-based anticonvulsants: opening the GABA_A channel more frequently will only increase the loss of negatively charged chloride, resulting in more membrane depolarization and more action potentials (8). As will be discussed, curiously, GABAergic anticonvulsants are by far the most frequently used agents in the treatment of neonatal seizures (9).

NKCC1 is not expressed in most adult neurons (10); a chloride-exporting transporter, KCC2, is expressed instead (11, 12). KCC2 uses the potassium gradient to energize the cotransport of one potassium and one chloride ion from the cytoplasm to the extracellular space (13). Like NKCC1, KCC2 ion transport is electroneutral (i.e., the positive charge on the potassium ion balances the negative charge on the chloride ion), so these transporters do not directly affect membrane potential. However, transport of potassium chloride out of the neuron by KCC2 depletes the cytoplasm of chloride. Opening the GABA-gated chloride channel allows only the negatively charged chloride back in, thus GABA currents are hyperpolarizing and inhibitory in mature neurons.

Before treating an infant with a GABA-based anticonvulsant, it would be important to know when the developing neuron's chloride-importing NKCC1 activity will transition to the adult neuron's KCC2-based chloride-exporting activity. A seminal paper by Stein et al. provided an unexpected answer that

offers compelling insights into neonatal seizures (14). In the rat pup, the KCC2 chloride exporter was expressed first in the spinal cord and brainstem, and only later in the cortex. This caudal–rostral expression of KCC2 is similar to the marked difference between the clinical response and the EEG response to anticonvulsant therapy seen with neonatal seizures. The terms *electroclinical dissociation* (15) and *uncoupling* (16) have been used to point out that up to 85% of neonates that have a clinical anticonvulsant response will not have an EEG response (17,18). If the caudal–rostral developmental progression of KCC2 expression is considered, this electroclinical dissociation makes sense. At some point in development, GABA should still be excitatory in the cortex because KCC2 is not yet expressed, and thus GABAergic anticonvulsants should not suppress EEG seizure activity. However, in the brainstem and spinal cord, KCC2 expression renders GABA inhibitory, so that GABAergic anticonvulsants will suppress the transmission of seizure activity through these structures, thereby blocking the clinical manifestations of seizures. Thus, one explanation for electroclinical dissociation of neonatal seizure activity after treatment with GABAergic anticonvulsants is the differential expression of inward versus outward chloride transporters in the cortex versus in the brainstem and spinal cord.

Dzhala et al. demonstrated that in the rat pup for up to 12 days after birth (P12), NKCC1 indeed continues to be expressed in the cortex, and GABA still is excitatory (19). Furthermore, they showed that the human term neonate also expresses NKCC1 in the cortex up to the age of 1–2 months. Conversely, KCC2 is not heavily expressed in the cortex until 1–2 months after birth. These data suggest that NKCC1-mediated chloride accumulation is the reason that electrographic neonatal seizures are so refractory to GABAergic anticonvulsants. NKCC1 is readily inhibited by bumetanide ($K_i = 0.28 \mu\text{M}$) (20). Dzhala et al. demonstrated that this agent, which has been extensively characterized in human term and preterm neonates, (21–23) prevented kainate-induced seizures in P9–P12 rat pups. It should be noted that blocking NKCC1 does not make GABA_A currents hyperpolarizing; rather, chloride becomes passively distributed across the neuronal membrane so that opening GABA_A channels short circuits any changes in the neuronal membrane potential. This phenomenon is referred to as shunting and is an effective means of inhibiting neurons, for example, by diminishing the impact of depolarizing synaptic activity mediated by glutamate (24).

The evidence for altered neuronal chloride transport in temporal lobe epilepsy is based on an intriguing finding reported by Cohen et al (25). In human temporal lobe tissue resected for intractable epilepsy, the investigators found that GABA excited rather than inhibited neurons in the subiculum. These data suggest that one component of medical intractability may be a reversion to the immature neuron's chloride transport pattern

(26). Why would such a reversion happen? Several other laboratories have demonstrated that specific neuronal experiences, including seizures, can produce long-term depolarizing shifts in the GABA_A reversal potential (27–29). Although it remains to be demonstrated that chloride transport has been altered in these neurons, the shifts in GABA reversal potential, together with reductions in KCC2 expression (30), strongly suggest that chloride transport can undergo long-term modification in such a manner as to reduce the efficacy of GABA-mediated inhibition and the efficacy of anticonvulsants, particularly those that modulate GABA_A receptor activity.

If changes in the direction of neuronal chloride transport contribute to intractability in temporal lobe epilepsy, should not blocking NKCC1 work as well for temporal lobe epilepsy as it does for neonatal seizures? Hesdorfer et al. recently published an epidemiological study demonstrating that diuretics are associated with a decreased risk of developing epilepsy (31). In patients undergoing temporal lobe resection, intravenous administration of furosemide, a loop diuretic that also blocks NKCC1, sharply reduced interictal spike frequency (32). These data support the idea that activation of NKCC1-mediated chloride import and the subsequent change in GABA currents are etiologically important in the development of temporal lobe epilepsy and provide an attractive potential mechanism for intractability.

Seizures associated with hypoxic-ischemic encephalopathy in adults also respond poorly to anticonvulsants, including barbiturate anesthesia (33). One puzzling aspect of these seizures is that they generally do not occur during the hypoxic event but rather hours later, after the brain has been reperfused. Pond and colleagues recently published an interesting *in vitro* study that may help explain both the intractability and the time course of the seizures (34). Using mouse neurons that expressed a chloride-sensitive dye, they demonstrated that neuronal chloride concentrations increase acutely during hypoxia, but recover when oxygen and glucose are resupplied. However, a secondary increase in neuronal chloride concentrations occurred 60 minutes after oxygen and glucose had been resupplied. This secondary increase in chloride was sufficient to bring the GABA_A reversal potential very close to action potential threshold and was temporally correlated with an increase in the neuronal expression of NKCC1. Furthermore, the late increase in neuronal chloride concentration was prevented by bumetanide. Of clinical interest was the finding that the bumetanide could be applied well after completion of the oxygen–glucose deprivation and still effectively block the increase in neuronal chloride concentration.

Should we begin using bumetanide to treat neonatal seizures, intractable temporal lobe epilepsy, and seizures in the setting of hypoxic-ischemic encephalopathy? There are caveats. First, bumetanide is thought to work as a diuretic after

being concentrated in the urine, so there is a question as to whether bumetanide concentration in the brain could reach levels that inhibit NKCC1. At physiological pH, bumetanide has a lipid:water partition coefficient of about 4:1 (Chris Lytle, PhD, personal communication, February 2006), supporting the idea that this agent is capable of crossing the blood–brain barrier. NKCC1 is exquisitely sensitive to bumetanide (19). The robust effects of diuretics on seizure activity in animal and human studies (19,32) also support the idea that systemically administered diuretics, such as bumetanide, reach neurons at sufficiently high concentrations to block NKCC1.

Physicians, with the commitment to “first, do no harm,” should be aware that inhibiting NKCC1 is not without deleterious effects in the developing nervous system. After all, if GABA-mediated excitation is important in neuronal circuit formation (7,35) then blocking GABA-mediated excitation should be expected to produce some alterations in circuit formation. Using retroviruses to label recently born dentate gyrus granule cells, Ge et al. demonstrated that when NKCC1 expression was suppressed with RNA interference (RNAi) that was coexpressed by the retrovirus, dendritic arborization was reduced (36). Not surprisingly, bumetanide suppresses the physiological activity, called giant depolarizing potentials (19), that is dependent on depolarizing GABA responses and thought to be important in the formation of a variety of different neuronal circuits (7). NKCC1 knockout mice should be the ideal model to assess the effect of blocking GABA-mediated depolarization on neuronal development. However, these animals, while viable, are small, weak, deaf, and have renal disease, so assessments of learning are not easily accomplished (37).

It may be that the abnormal synaptic signaling that occurs during a seizure is at least as detrimental to the developing nervous system as transiently blocking GABA-mediated excitation with a diuretic. However, the effects of status epilepticus in neonatal animals have been surprisingly subtle (38). The retroviral study of Ge et al. knocked out GABA-mediated excitation for many days in animals whose brains mature in weeks, and thus it may not be fair to extrapolate that result to the effects of pharmacologically blocking NKCC1 for a few hours in a human, whose brain matures over years. However, at a minimum, these data suggest that bumetanide should not be used for prolonged periods in the developing nervous system. In contrast, most of the other anticonvulsants exert far more profound effects on the developing nervous system, (39) with substantial increases in neuronal apoptosis after a single (large) dose of barbiturate, benzodiazepine, or phenytoin. In addition, the pharmacokinetic studies of bumetanide in term and preterm human neonates did not reveal any acute problems, although alterations in cortical wiring might be subtle and take years to manifest. There are no studies suggesting that diuretics should be detrimental in the adult nervous system, and these agents

have been used for years in adults as diuretics without adverse cognitive effects.

What is next? A variety of in vitro, in vivo, and clinical studies are on the horizon. An assay of the effect of transient bumetanide administration on dendritic arborization in the rat pup would help pave the way to a human trial of bumetanide for neonatal seizures. The delayed chloride accumulation after oxygen-glucose deprivation is an intriguing in vitro finding that would be wonderful to establish in vivo. There are no compelling models of posthypoxic seizures, and development of such a model would permit testing the efficacy of NKCC1 inhibitors for seizures that occur with hypoxic-ischemic encephalopathy. If a human trial of diuretics as anticonvulsants were contemplated, intractable and surgically nonresectable temporal lobe epilepsy in adult patients would be a safe place to start. A clinical trial of neonatal seizure treatment might carry higher risk, but the benefit may be great.

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