COMMENTARY

Blocking seizures with the diuretic bumetanide: Promises and pitfalls

As recently described by Maa et al. (2011), the diuretic bumetanide (BUMEX) blocks the chloride importer Na^+-k^+- $2Cl^-$ (NKCC1) chloride cotransporter and appears promising as an antiepileptic drug (AED), at least in experimental conditions. In some cases, epileptic neurons appear to have an excessively high intracellular chloride concentration ($[Cl^-]_i$), leading to excitatory actions of γ-aminobutyric acid (GABA) that perturb behaviorally relevant brain waves and contribute to seizure generation. BUMEX is thought to reduce $[Cl^-]_i$, shift the polarity of GABA-activated currents, and thus preserve the positive (i.e., hyperpolarizing) effects of GABA-acting AEDs such as diazepam and phenobarbital. But as usual, the devil is in the details—and things are far from simple.

Immature neurons have higher $[Cl^-]_i$ than adult neurons, leading to depolarizing and occasionally excitatory actions of GABA (Ben-Ari et al., 2007). The depolarization produced by GABA is sufficient to trigger action potentials—directly or by the additional activation of voltage-gated currents such as the noninactivating sodium current. Voltage- and transmitter-gated currents tend to be ''sloppier'' in immature neurons relative to adult neurons, and this feature must be taken into account when infantile seizures are considered. In addition, GABA hyperexcites both immature and adult epileptic neurons subsequent to chronic intracellular accumulation of chloride that perturbs the capacity of the neuron to control $[Cl^-]_i$ (e.g., during and after seizures, when there is a massive activation of GABAergic synapses).

Because $[Cl^-]_i$ and the chloride gradients are important in so many functions, it comes as no surprise that there is a plethora of control mechanisms, including chloride cotransporters, chloride channels, and GABA-receptor channels (Blaesse et al., 2009). Two cotransporters have attracted much interest, as they appear to play a major role in the developmental shifts of the polarity of GABA actions: the chloride importer NKCC1 that operates early in development, and the chloride exporter k^+- $2Cl^-$ (KCC2) chloride cotransporter that operates later (when the inhibitory shift takes place) (Rivera et al., 1999). The driving force of GABA (DF_{GABA}), as determined with single-channel recordings, is indeed more positive in embryonic than adult cells. In addition, the time required to wash out chloride that accumulates during synchronized giant depolarizing potentials (GDPs) and network events is much longer in immature than in adult neurons, consistent with the relative inefficiency of KCC2 in immature neurons.

What about epileptic neurons? We have developed a three-chamber in vitro preparation in which interactions between the convulsive drug and the postulated AED, and between acute and more chronic actions of seizures AEDs can be readily dissociated (Khalilov et al., 2005). Using this preparation, we observed that recurrent (but not single) propagated seizures beget seizures and that neurons have an elevated $[Cl^-]_i$ and excitatory GABA (Fig. 1). These results demonstrate an altered neuronal capacity to control $[Cl^-]_i$. Long-lasting application of the NKCC1 antagonist BUMEX to one chamber and concomitant application of a convulsive agent to another chamber did not prevent the seizure-induced GABA shifts (Fig. 1). Similarly, in hippocampi dissected from pups in which NKCC1 has been genetically disabled, $[Cl^-]_i$ was increased with recurrent seizures and GABA polarity shifts, indicating that NKCC1 is neither necessary nor sufficient for these events to occur (Nardou et al., 2011). However, the time course of the kinetics of chloride extrusion confirmed that these epileptic neurons (like immature neurons) are ''sloppy.'' Furthermore, light and electron microscopy showed that KCC2 is internalized in these cells, and thus unable to exert a cotransporter function (Nardou et al., 2011). Of interest, single seizures were shown to upregulate KCC2 (Khirug et al., 2010). Lee et al. (2010) have shown that a tyrosine phosphorylation site controls the internalization of KCC2, which is promoted by recurrent seizures, thereby leading to the following scenario: Recurrent seizures enhance a phosphatase action, leading to reduced KCC2 trafficking to the membrane, and loss of KCC2 activity. The dynamic regulation of KCC2 by enhanced activity may be a defining difference between single seizures and genuine epilepsies. Once KCC2 fails to operate, it becomes difficult to regulate DF_{GABA}, and small electrical events may shift to whole-blown seizures. Because GABA synapses are extremely active during seizures, and even single bursts can alter DF_{GABA}, it is reasonable to conclude that GABA itself contributes to the shift of DF_{GABA}. In summary, it is clear that KCC2 is labile, but NKCC1 is much less so; GABAergic signals readily alter polarity, and this plasticity is promoted by hyperactivity and alters on a shorter or longer time course, depending on the severity of seizures.

On the basis of theoretical arguments and a simplified model of the interactions between KCC2 and NKCC1, Staley and colleagues previously suggested that NKCC1 and KCC2 do not operate as push–pull devices (Brumback & Staley, 2008; Maa et al., 2011). Yet, this relationship is how pH is regulated (see Boron, 2004), suggesting that this type of regulation is not unprecedented. In addition, NKCC1 and KCC2 are not the only players in $[Cl_i]$ regulation; other chloride regulators—including KCC3, voltage gated, and anion-operated devices—cannot be ignored when calculating the import/export of chloride in neurons. Putting together a single ohmic-type formula to determine the kinetics of chloride regulation, where

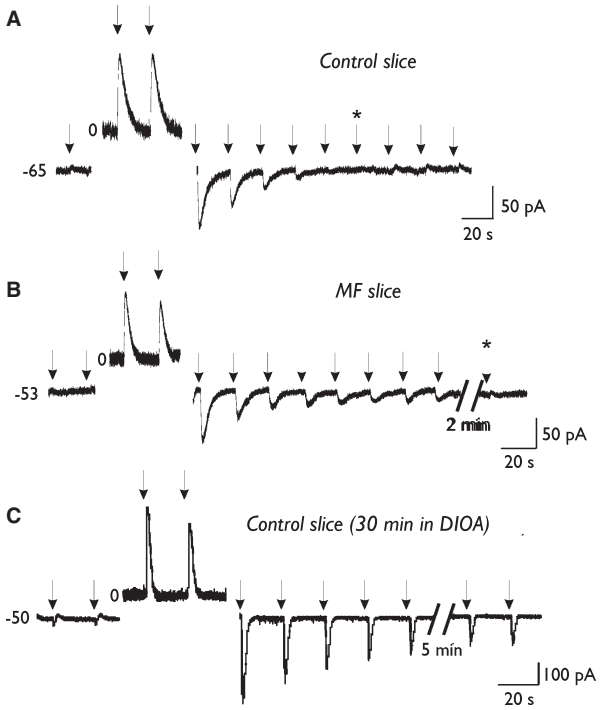

Figure 1.
Perforated patch recordings of currents evoked by puff applications of GABA on immature neurons. Holding membrane potential was adapted to the chloride equilibrium potential, and hence GABA initially evoked no current (left side). Then the neuron was strongly depolarized, leading to large GABA-evoked outward chloride currents. Upon return to the control holding membrane potential, GABA evoked a large inward current that returns to control values (GABA evoked no current) after a variable delay that is correlated with the efficacy with which chloride is washed out. Note that the kinetics of return was much faster in control neurons (**A**) than in epileptic neurons recorded in a mirror focus (MF) or (**C**) in the presence of KCC2 and NKCC1 antagonists. Similar results were obtained with a concentration of bumetanide that blocks NKCC1 AND KCC2.

NKCC1 is considered the sole importer and KCC2 and all other export mechanisms are lumped together (Brumback & Staley, 2008) is questionable considering the different mechanisms regulating these operations. We are clearly dealing here with an orchestra, not a dialog. In addition, this view reflects a static model of the regulation of $[Cl^-]_i$, and does not give sufficient weight to developmental issues, the dynamic alterations that occur during seizures (including the down regulation of KCC2), and the activity-dependent alteration of the kinetics of most other mechanisms.

The problem that we are facing is how to intervene in the transformation by seizures of an epileptic network and prevent the shift of GABA polarity and the internalization of KCC2. How can we prevent the failure of phenobarbital and diazepam and their paradoxic actions? In the triple chamber, bumetanide failed to prevent the formation by seizures of an epileptogenic mirror focus, but reduced the severity of ongoing seizures generated by a mirror focus, suggesting that BUMEX is more of an antiictal than an antiepileptogenic agent. This result also suggests that NKCC1, in contrast to KCC2, still operates in epileptic neurons and acts as a housekeeping device (Nardou et al., 2009, 2011). In addition, the history of seizures before the applications of the diuretic appears to be important (early, but not late, applications of pentobarbital reduced seizure severity, and BUMEX efficiently enhanced the antiepileptic actions of phenobarbital when provided early, but not late (Nardou et al., 2011)). Therefore, it seems appropriate to recommend aggressive strategies to block recurrent seizures at the earliest possible stage. Bumetanide will clearly not be the panacea but may offer interesting perspectives to facilitate the actions of other more conventional AEDs.

Disclosures

I have no conflicts of interest to disclose. I confirm that I have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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References

Ben-Ari Y, Gaiarsa JL, Tyzio R, Khazipov R. (2007) GABA: a pioneer transmitter that excites immature neurons and generates primitive oscillations. *Physiol Rev* 87:1215–1284.
Blaesse P, Airaksinen MS, Rivera C, Kaila K. (2009) Cation-chloride cotransporters and neuronal function. *Neuron* 61:820–838.
Boron WF. (2004) Regulation of intracellular pH. *Adv Physiol Educ* 28:160–179.
Brumback AC, Staley KJ. (2008) Thermodynamic regulation of NKCC1-mediated Cl- cotransport underlies plasticity of GABA(A) signaling in neonatal neurons. *J Neurosci* 28:1301–1312.
Khalilov I, Le Van QM, Gozlan H, Ben Ari Y. (2005) Epileptogenic Actions of GABA and fast oscillations in the developing hippocampus. *Neuron* 48:787–796.
Khirug S, Ahmad F, Puskarjov M, Afzalov R, Kaila K, Blaesse P. (2010) A single seizure episode leads to rapid functional activation of KCC2 in the neonatal rat hippocampus. *J Neurosci* 30:12028–12035.
Lee HH, Jurd R, Moss SJ (2010) Tyrosine phosphorylation regulates the membrane trafficking of the potassium chloride co-transporter KCC2. *Mol Cell Neurosci* 45:173–179.
Maa EH, Kahle KT, Walcott BP, Spitz MC, Staley KJ. (2011) Diuretics and epilepsy: will the past and present meet? *Epilepsia* 52:1559–1569.
Nardou R, Ben-Ari Y, Khalilov I. (2009) Bumetanide, an NKCC1 antagonist, does not prevent formation of epileptogenic focus but blocks epileptic focus seizures in immature rat hippocampus. *J Neurophysiol* 101:2878–2888.

Nardou R, Yamamoto S, Chazal G, Bhar A, Ferrand N, Dulac O, Ben-Ari Y, Khalilov I. (2011) Neuronal chloride accumulation and excitatory GABA underlie aggravation of neonatal epileptiform activities by phenobarbital. *Brain* 134:987–1002.

Rivera C, Voipio J, Payne JA, Ruusuvuori E, Lahtinen H, Lamsa K, Pirvola U, Saarma M, Kaila K. (1999) The K+/Cl- co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. *Nature* 397:251–255.

ANNOUNCEMENTS

14th Annual Meeting of the International Symposium on Surgery for Catastrophic Epilepsy in Infants

February 18–29, 2012 in Tokyo, Japan. http://www.iss-jpn.info

6th Latin American Summer School on Epilepsy

February 24–March 2, 2012 in Sao Paulo, Brazil. http://www.lasse.med.br

6th World Congress on Controversies in Neurology (CONy)

March 8–11, 2012 in Vienna, Austria. http://comtecmed.com/cony/2012

9th Asian and Oceanian Epilepsy Congress

March 21–25, 2012 in Manila, Philippines. http://www.epilepsymanila2012.org

2nd International Congress on Epilepsy, Brain and Mind

March 28–31, 2012 in Prague, Czech Republic. http://www.epilepsy-brain-mind2012.eu/en/welcome

11th Eilat Conference on New Antiepileptic Drugs (Eilat XI)

May 6–10, 2012 in Eilat, Israel. http://www.eilat-aeds.com

12th International Child Neurology Congress and 11th Asian and Oceanian Congress of Child Neurology

May 27–June 2, 2012 in Brisbane, Australia. http://www.icnc2012.com

22nd Meeting of the European Neurological Society

June 9–12, 2012 in Prague, Czech Republic. http://www.congrex.ch/ens2012

1st African Epilepsy Congress

June 21–23, 2012 in Nairobi, Kenya. http://www.epilepsynairobi2012.org

6th Baltic Sea Summer School on Epilepsy

July 8–13, 2012 in Rostock, Germany. The BSSSE is primarily addressed to medical postgraduates and junior researchers (to age 40), and is focused on the comprehensive aspects of diagnosis and treatment of epilepsy. The interactive course format involves combinations of lectures and group work, with case-oriented studies. Applications due by April 15, 2012. For more information, contact petra.novotny@wolfstiftung.org or see BSSSE6 at http://www.epilepsiestiftung-wolf.de/7.html

10th European Congress on Epileptology

September 30–October 4, 2012 in London, U.K. http://www.epilepsylondon2012.org

7th Latin American Congress on Epilepsy

November 14–17, 2012 in Quito, Ecuador. http://www.epilepsiaquito2012.org