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Letter to the Editor

Combined effect of bumetanide, bromide, and GABAergic agonists: An alternative treatment for intractable seizures

To the Editor:

The epilepsies constitute one of the most common serious brain disorders and know no geographic, social, or racial boundaries, occurring in men and women and affecting people of all ages, though more frequently affecting young people in the first two decades of life and people over the age of 60 [1,2]. Worldwide, there are at least 50 million people who have epilepsy, and many of these persons have seizures that are refractory to treatment with the currently available therapies [1,3,4]. The most common risk factors for epilepsy are cerebrovascular diseases, brain tumors, alcohol, traumatic head injuries, genetic inheritance, and malformations of cortical development [5,6]. In resource-poor countries, endemic infections such as malaria and neurocysticercosis seem to be major risk factors [7].

The paradoxical excitatory action of GABA observed in the immature brain plays a role in neonatal development [8,9], but also is responsible for the increased seizure propensity and lowered seizure threshold of neonates [10]. Differently from mature neurons of adults, the immature neurons of neonates exhibit a much higher $[Cl^-]_i$ induced by higher Na^+, K^+,$2Cl^-$ co-transporter type 1 (NKCC1) expression and lower K^+,Cl^- type 2 (KCC2) expression. In this circumstance, the activation of $GABA_A$ receptors is followed by an efflux of Cl^-, induced by the positivity of the Nernst potential for Cl^- with respect to the membrane potential, which accounts for the excitatory action of GABA.

Almost all neurological insults (hypoxia–ischemia, metabolic derangement, hemorrhage, and infection) sustained during the neonatal period can trigger the synchronous firing of hyperexcitable neurons that underlie epileptogenesis [11]. Investigations conducted in human epileptogenic tissue have shown that dysplastic tissue may retain immature properties, exhibiting mechanisms of seizure generation resembling that observed during development in the immature brain [12,13].

To unravel the mechanisms involved in seizure initiation, epileptogenesis, and spontaneous recurrent seizures and to search for new treatment options, several experimental models mimicking different aspects of the epileptic process have been developed. Along with the seizure induction process, which leads to spontaneous seizures, many studies conducted with experimental models have identified activation of an inflammatory state [14]. Concomitantly with the inflammation, changes in expression of NKCC1 and KCC2 co-transporters have been observed that resemble the immature brain [15]. An increasing number of investigations suggest an important role for cation chloride co-transporters in controlling neuronal functions [6]. Deregulation of their expression may contribute to the mechanisms of hyperexcitability, which, in combination with neuronal coupling, may lead to synchronization and, therefore, to seizures. The hyperexcitability is attributed mainly to the accumulation of chloride in the intracellular space [15], and the hypersynchronism, to the nonsynaptic coupling promoted by the decrease in extracellular space volume [16,17].

Neurophysical studies of the molecular mechanisms that underlie the hyperexcitation and synchronization of neurons have a profitable role in the search for targets for novel antiepileptic drugs to control seizures that affect mainly infants. The treatment of refractory seizures, normally associated with intracellular chloride accumulation, involves a complex interaction of the mechanisms responsible for controlling the intracellular chloride level. Combining multiple drugs, each one targeting a different mechanism, is a promising powerful strategy to be investigated. It can be conjectured that more efficient action would be coupled with fewer side effects, as each compound would be used at a low dose, presuming their synergistic effect on the system.

Initially, attention to cation chloride co-transporters (CCCs) focused on their physiological roles in the recovery of cell volume after swelling or shrinkage in hypotonic or hypertonic media [18]. Later, attention turned to the importance of CCCs in regulation of intracellular Cl^- in the central nervous system (CNS) [19]. In mature brain, the normal level of intracellular chloride must be low enough to establish inhibitory GABAergic neurotransmission. This condition is due to minimal NKCC1 expression but robust KCC2 expression.

Chloride accumulation, typical of immature brain, as well as some adult epilepsy syndromes, is promoted by the equilibrium established by the resulting changes in the expression of NKCC1 and KCC2. Investigation into the mechanisms involved revealed that co-transporter activities, driven by gradients established by Na/K pumps [20], have the net effect of controlling the intracellular chloride level. Studies conducted by our research group, based on computational simulation, have also shown the importance of the interplay between these mechanisms to determination of the excitability level [16]. The KCC co-transporter is responsible for Cl^- extrusion, counteracted by Cl^- influx, promoted by NKCC co-transporters. The simulations reproduce the Cl^- accumulation corresponding to an extracellular K^+ increase, typical of intense neuronal firing.

Granule cells of animals with epilepsy induced with pilocarpine exhibit a positive shift in the GABA reverse potential, E_{GABA} [15,21]. This shift in E_{GABA} alters synaptic integration, increasing granule cell excitability, and results in compromised "gate" function of the dentate gyrus.

Investigations have been carried out on the use of co-transporter inhibitors, such as furosemide and bumetanide, as antiepileptic agents [21,22]. These studies, conducted in hippocampal slices, suggest that blockage of NKCC is critical to the antiepileptic effects of chloride transport antagonism [23]. This fact was also confirmed by computational simulation [16]. According to these simulations, Cl^- accumulation in the intracellular space during the ictal state is mediated by the NKCC co-transporter, and occurs with almost the same flux as in the ictal state. Cl^- efflux is dominated by the KCC co-transporter and during the ictal state it is always smaller than Cl^- NKCC influx. Only at the transition between the ictal and interictal states does KCC efflux overcome NKCC influx and cause a decrease in intracellular Cl^-

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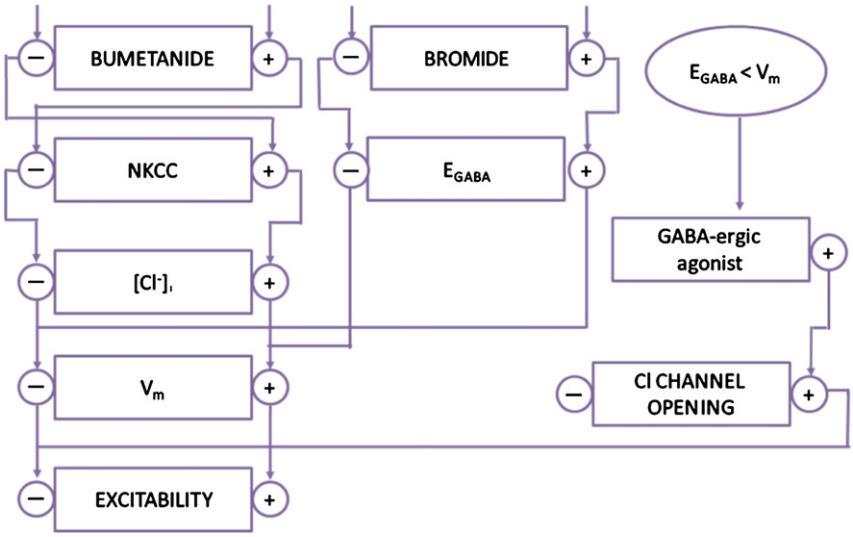

Fig. 1. Block diagram indicating the systemic action of the conjoint effect of bumetanide, bromide, and $GABA_A$ agonist. Bromide acts by blocking NKCC activity. This co-transporter is responsible for Cl^- influx, which induces the accumulation of this ion in the intracellular space, inducing an increase in excitability. An NKCC antagonist is, therefore, able to counteract the chloride accumulation, decreasing excitability. Bromide acts directly on E_{GABA}, improving its negativity and also decreasing excitability. The conjoint effects of an NKCC antagonist and bromide are therefore complementary. The clinical observation of seizure reduction could be indicative of induced E_{GABA} negativity with respect to V_m (intracellular potential), when the use of GABAergic drugs can be useful. NKCC = Na^+, K^+, $2Cl^-$ co-transporter; E_{GABA} = GABA reverse potential.

concentration. These findings indicate that NKCC is an important target for antiepileptic drugs, and bumetanide has been highlighted for its adequacy. Bumetanide has an approximately 500-fold greater affinity for NKCC1 (K_i = ~0.1 μM) than for KCC2 (K_i = ~25–50 μM); in contrast, furosemide inhibits NKCC1 and KCC2 with equal potency (K_i = ~25–50 μM). Therefore, at low doses (2–10 μM), bumetanide is a relatively specific inhibitor of NKCC1 [24].

Bromides were the first effective treatment for epilepsy. Introduced in 1853 by Sir Charles Locock, bromides remained useful as a therapeutic option for nearly 60 years. With the advent of less toxic and more effective treatments, the use of bromides became less common [25]. However, bromides are still a valuable tool in the treatment of epilepsies refractory to current antiepileptic drugs, such as medically refractory tonic–clonic seizures and severe pediatric myoclonic epilepsies [26–28]; the pharmacological activity of bromides remains incompletely elucidated.

The most acceptable hypothesis explaining how bromide blocks epileptic activity is through an increase in neuronal hyperpolarization mediated by inhibitory postsynaptic potentials [29]. The antiepileptic effect of bromide is believed to be due to the potentiation of inhibitory postsynaptic potentials by GABA, as Br^- ions cross cellular membranes more quickly than Cl^-, enhancing GABA-activated currents and leading to large hyperpolarization [30]. But not only are GABA-activated chloride channels more permeable to bromide, voltage-dependent channels are also more permeable (I>Br>Cl>F, 1.98:1.46:1:0.44) [31]. This fact justifies why bromide also has effects on nonsynaptic epileptiform activity (NEA). Meierkord et al. [32] observed consistent blockage of NEA when hippocampal slices bathed with low-Ca^{2+} solution were perfused with 11 mM NaBr. If bromide would also substitute for chloride in the CCCs, no objection would be considered to the assumption that bromide also accumulates intracellularly. Because this is not the case, bromide may exert antiepileptic activity not only because of its reinforcement of the Cl^- hyperpolarizing Nernst potential, but also because of its low affinity for the NKCC enzyme in comparison with Cl^- [33,34]. In summary, bromide's antiepileptic effect may be divided into three parts: (1) compensation of Cl^- accumulation by means of its hyperpolarizing effect on chloride channels; (2) antagonism of chloride flow through the channels because of its competition with chloride; (3) low affinity for the NKCC enzyme.

We propose the therapeutic use of bumetanide and bromide combined for the treatment of patients with refractory epilepsy. Bumetanide would reduce intracellular chloride concentration and bromide would reinforce E_{GABA} negativity, according to Fig. 1, where the systemic effects of bumetanide and bromide are depicted. Although acting by different mechanisms, both drugs reduce neuronal excitability. Once the effects are complementary, the doses of bumetanide and bromide could be reduced and, consequently, their side effects as well. In fact, in a recent case report on the use of bumetanide for treatment of autism, the authors did not observe, at the dosage investigated, any side effect [31]. To overcome the severe side effects of bromide described [32], it is paramount that bromide be administered with care, that follow-up testing be carried out, and that serum bromide levels be monitored periodically. We also propose that effort should be directed to find anions or anionic compounds that can replace bromide with the aim of avoiding its side effects. These proposals reflect a new perspective on the use of bromide: valuing its clinical efficacy associated with a reduction of its side effects. Furthermore, we also hypothesize that clinical observation of seizure reduction would be indicative of E_{GABA} negativity (Fig. 1). Under such clinical conditions, after the effect of bumetanide and bromide has already been established, shifting GABA from excitation to inhibition, the administration of GABA agonists should be considered in the treatment of uncontrollable seizures. With the action of these agonists, it is assumed that the doses of bumetanide and bromide could be reduced further.

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