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Is It Safe to Use a Diuretic to Treat Seizures Early in Development ?

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There has been considerable interest in using bumetanide, a diuretic chloride importer NKCC1 antagonist, to reduce intracellular chloride ($[Cl^-]_i$) in epileptic neurons, thereby shifting the polarity of GABA from excitatory to inhibitory and ameliorating the actions of GABA-acting antiepileptic drugs. However, a recent study raises the important issue of potential deleterious actions of bumetanide on immature neurons, because reduction of $(Cl^-)_i$ also alleviates a major source of excitation in developing neurons, upon which GABA exerts a trophic action. This review considers the importance of separating intrauterine from postnatal effects of bumetanide in normal versus pathologic neurons.

Immature neurons have higher intracellular concentrations of chloride ($[Cl^-]_i$) than adult neurons, leading to depolarizing and excitatory actions of GABA, which exert trophic actions on the formation of cortical networks (1). These actions are mediated by many mechanisms, most notably an early expression of the chloride importer NKCC1and a somewhat delayed expression of the chloride exporter KCC2 (2–9). Blocking NKCC1 with the diuretic NKCC1 antagonist bumetanide reduces $(Cl^-)_i$ and the depolarizing actions of GABA (1). Immature neurons need excitatory GABA during development, because GABA exerts a clear trophic action on many major developmental processes, including migration; growth; synapse formation; and early network activities, notably giant depolarizing potentials that provide much of early synapse-driven activity (1, 10–13). However, recurrent seizures and epilepsies that also have a higher incidence in immature neurons also shift GABA polarity to excitation as $(Cl^-)_i$ is elevated in human and animal epileptic neurons (9, 14, 15). Therefore, immature neurons require "GABA to be excited but also to be hyperexcited" (16). These observations raise considerable interest in using agents that reduce $(Cl^-)_i$ to reinstate hyperpolarizing and inhibitory GABA in epileptic neurons, thereby also enabling the use of GABA-acting AEDs that often exert paradoxical aggravating actions on these neurons (9, 14, 17). In addition to epilepsies, elevated neuronal $(Cl^-)_i$ has been documented in autism spectrum disorders (ASDs), brain edema, spinal cord lesions, spasticity, and other disorders. Two clinical trials are presently being conducted—the CRA/INMED trial (18) on the use of bumetanide to treat children with ASD the NEMO project of the European Community (19) and the US trial gov/ct2/show/NCT00830531on the use of bumetanide to treat phenobarbital-refractory encephalopathy in 2-day-old babies.

Clearly, the excitatory actions of GABA have both positive and deleterious actions in physiologic and pathologic conditions, challenging its usefulness in the neonatal period. A recent elegant study reports that long-lasting administration of bumetanide at early developmental stages leads to cortical malformations and behavioral sequelae that are reminiscent of autism and schizophrenia (20). The authors suggest that the use of bumetanide may have teratogenic actions on the developing brain, challenging the promising therapeutic efficacy of this drug. Here, I discuss two issues: 1) the precise period during which long-term diuretic use is potentially deleterious, and 2) the major differences in GABA actions on normal versus pathologic neurons and the need to differentiate excitation (of normal neurons) and hyperexcitation (of pathologic neurons).

The Dangers of Diuretics During Pregnancy

Wang and Kriegstein (20) report a highly significant increase in glutamatergic EPSCs when bumetanide was continuously injected between E15 to E17 to P7 (Wang and Kriegstein, 2010). However, curiously, the diuretic had no effect when administered entirely during the prenatal (E15 to E19) or postnatal (P0 to P7 or P7 to P14) periods although GABA depolarizes neurons in utero (1Ben-Ari et al., 2007) and alters neuronal migration (21–23). Also, GABA depolarizes neurons during the first postnatal week in the hippocampus, the neocortex, and many other structures (1, 4, 7, 8, 11, 12, 24, 25). These different reported actions are due either to longer time periods (a week vs 11–13 days) or to the inclusion in the deleterious experiment of delivery; administration of bumetanide entirely before or after delivery appears not to have deleterious actions. Delivery is associated with a dramatic shift of the actions of GABA mediated by the hormone oxytocin, which also triggers labor (8). Oxytocin-mediated GABA exerts dramatic neuroprotective

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and analgesic actions (8, 26). It is conceivable that bumetanide reduces oxytocin release and ameliorates its actions, thereby impacting critical events that occur during delivery. Although the exact correspondence between human and rodents can be debated, there is little doubt that the vulnerable period reported in rodents (20) corresponds to the preterm period in humans and that the long-term use of bumetanide-like drugs duringgestation can have adverse actions on the developing brain. There have been no clinical trials aimed at determining the usefulness of bumetanide during pregnancy. If such a trial ever occurs, care will need to be exercised regarding the safety of this agent, especially during the second trimester of gestation.

Is Bumetanide Teratogenic on Normal Neurons in Utero?

In the report of Wang and Kriegstein (20), an extensive loss of dendrites, dendritic spines, glutamatergic synapses, and EPSCs after in utero bumetanide was accompanied by interesting behavioral consequences. Most motor signs were either completely unaffected or exhibited a slight (i.e., 1-day) delay, which is barely significant and transient; such effects are not observed in adults. Only the startle response was significantly augmented, raising the possibility of altered maturation of hearing or sensory processes. Yet, the authors suggested that bumetanide reproduces an animal model of autism and schizophrenia, although the primary features of ASD—repetitive stereotyped movements and lack of social interaction (27–29) —were unaltered.

Epidemiologic studies provide interesting information about the use of diuretics during pregnancy. Diuretics are only prescribed when there is a clinical need, so the concern should be focused on their effects in pathologic situations. The most frequent use of diuretics during pregnancy is to treat hypertension, which can have severe teratogenic consequences. The EuroMap group has analyzed the effects of diuretics in large cohorts (9,125 individuals),on measures of fetal growth (30). They reported that diuretics during pregnancy are associated with larger birth weight and a higher incidence of preterm delivery (30). Children of mothers with hypertension during pregnancy who were also exposed to diuretic treatment in the third trimester were at significantly enhanced risk of developing schizophrenia (31). However, neither hypertension alone nor diuretic treatment alone for other reasons than hypertension increased the likelihood of developing schizophrenia. Diuretics may be teratogenic, but one must also take into account the disease for which the treatment is prescribed as the diuretics are not used during pregnancy unless there is a medical need. Therefore, the effects of diuretics on naïve brains are not relevant in a clinical perspective. A similar situation exists for epilepsies, in which several AEDs have teratogenic actions on neuronal migration, yet seizures during pregnancy also have many potentially dangerous sequelae; these benefit/risk considerations must be carefully weighed in the clinical setting.

Experimental Issues on Chloride CoTransporters

Wang and Kriegstein (20) conclude that blockade of NKCC1 and GABA-mediated excitation by bumetanide leads to a chain of reactions that culminates in brain malformations and adverse neurologic sequelae. However, several issues must be considered in this context.

First, because the serum or brain concentration of bumetanide has not been measured, it is difficult to determine whether its actions are direct and specific. At higher concentrations, bumetanide also blocks the chloride exporter KCC2, which also alters migration and developmental processes (6, 21, 32, 33), and exerts an important neuroprotective role (34). Compensatory alterations of KCC2 after bumetanide are likely a result of cross talk between these cotransporters (35, 36). In addition, bumetanide is metabolised within a few hours raising some concerns on the interpretation of single injections (36,B). In the clinical autism trial, we have used long lasting (3 months) bumetanide administration at a dose of 1 mg daily in children weighing approximately 20 kg. 0.1 mg/kg intravenously will be used to stop seizures in neonatal encephalopathy (19). Therefore, the effects of long-lasting administration of bumetanide may not necessarily be due only to NKCC1 blockade. This is particularly relevant in utero when the blood brain barrier is underdeveloped and the mother's serum levels will predominate in the embryos.

Second, in contrast to bumetanide, genetic invalidation of NKCC1 exerts contradictory effects on glutamatergic currents and brain patterns as either an acceleration or a delay of their maturation (37, 38). Wang and Kriegstein (20) argue correctly that these differences may be due to compensatory mechanisms that occur in knockouts. However, similar compensatory mechanisms may occur when NKCC1 is blocked for long periods by bumetanide. Indeed, because bumetanide is metabolized within a few hours (39), transient shifts of GABA polarity with stop-and-go type of signals may complicate the interpretation of the results. Because the chloride gradient and the polarity of GABA actions E_{GABA} was determined solely at P0 but not later, when the behavioral effects were measured, it is unknown whether the GABA shift is permanent (20). Also, the use of hydrochlorothiazide at high concentrations to control for the possible deleterious actions of diuresis is problematic, because this agent is an efficient inhibitor of AMPA/kainate receptor desensitization (40) and would be expected to alter immature brain patterns.

Third, during delivery, the hormone oxytocin that triggers labor also produces a dramatic loss of $(Cl^-)_i$ and a hyperpolarizing shift of GABA actions (8) that occludes the actions of bumetanide and exerts neuroprotective and analgesic effects (8, 26, 34). The failure of Wang and Kriegstein (20) to observe this shift may be due to the invasive recordings (11) and/or the use of high concentrations of potassium (ie, 5 mM) in the artificial cerebrospinal fluid. Additional factors include the exquisite heterogeneity of transmitter-gated and voltage-gated currents in immature neurons that determine the actions of bumetanide. Extracellular recording techniques are required to better unravel the net actions of GABA.

Bumetanide in Pathologic Conditions

The fundamental assumption of Wang and Kriegstein (20) is that bumetanide exerts similar adverse effects in normal and pathological conditions and that the effects they report hamper the use of the diuretic in both conditions. However, neurons have different features in neurologic disorders, sug-

gesting that what applies to normal neurons may not apply to pathologic ones. Several factors underlie the excitatory actions of GABA on normal neurons. The early operation of the chloride importer NKCC1 and delayed operation of the chloride exporter KCC2 is one of these factors. In addition, the slow decay kinetic of GABA currents activates a slow sodium voltage-dependent current (I_{NaP}) that enables the voltage shift to reach spike threshold, and this is not attained by the chloride gradient solely (41). Additional factors include the large input resistance of immature neurons and their intrinsic tendency to oscillate, which are not genuine features of epileptic neurons or neurons in other pathologic disorders. The developmental gradient is yet another important consideration; even in utero, some neurons already have a hyperpolarizing GABA response, particularly in humans, who have prolonged gestation and heterogeneity of cortical neurons that are born during the second trimester, which preclude a homogeneous widespread general excitatory GABA action. This heterogeneity is illustrated by the different actions of GABA on rodent immature neocortical neurons; GABA excites pyramidal neurons of the earlier-operative deep cortical layers but only depolarizes superficial layer pyramidal neurons (24), stressing the dynamic role of GABA in seizure propagation (42).

These differences are also evidenced by investigations on the excitatory actions of GABA in experimental models of epilepsies and in human epileptic neurons (43, 44). Bumetanide reduces the enhanced $(Cl^-)_i$ in slices with a variable efficacy that depends on the convulsive agent used (9, 45, 46In the triple chamber, in which seizures generated in one hippocampus propagate to the other hippocampus and form an epileptogenic mirror focus (44), epileptic neurons accumulate chloride primarily because they fail to export it because of down regulation and internalization of KCC2 (47). NKCC1 contributes but is neither necessary nor sufficient to produce the GABA polarity shift, as it is also observed in NKCC1 knockouts (14).

In experimental models, bumetanide has shown promising therapeutic effects in pain, (48), spasticity after spinal cord lesions (49), and brain edema (50, 51). All these effects have been shown in acute protocols, in which the agents are administered during short time periods. Several clinical trials are presently being conducted. Relying on the paradoxical effects of GABA-acting agents in ASD and the alterations of benzodiazepine receptors (52), we have recently obtained very promising effects of bumetanide in the treatment of ASD. Bumetanide, given over 3 months at 1 mg daily to 3- to 11-year-old children with ASD, reduced the severity of the syndrome, with few adverse effects related to the diuresis, and these adverse effects could be readily compensated (18). A large, double-blind trial of bumetanide in ASD is being performed now.

In conclusion, there is an excellent theoretical case for additional testing of bumetanide in autism, epilepsy, and probably other neurodevelopmental disorders. The study of Wang and Kriegstein (20) is a good reminder that things are never simple but that there are ways to take into account the possible adverse actions of a therapy and exploit its dramatic beneficial effects.

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