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COMMENTARY

Bumetanide for neonatal seizures: No light in the pharmacokinetic/dynamic tunnel

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Abstract

In his editorial, Kevin Staley criticizes our recent work demonstrating the lack of effect of bumetanide in a novel model of neonatal seizures. The main points in our response are that (1) our work is on an asphyxia model, not one on "hypercarbia only"; (2) clinically relevant parenteral doses of bumetanide applied in vivo lead to concentrations in the brain parenchyma that are at least an order of magnitude lower than what would be sufficient to exert any direct effect—even a transient one—on neuronal functions, including neonatal seizures; and (3) moreover, bumetanide's molecular target in the brain is the Na-K-2Cl cotransporter NKCC1, which has vital functions in neurons, astrocytes, and oligodendrocytes as well as microglia. This would make it impossible even for highly brain-permeant NKCC1 blockers to specifically target depolarizing and excitatory actions of γ-aminobutyric acid in principal neurons of the brain, which is postulated as the rationale of clinical trials on neonatal seizures.

KEYWORDS

animal models, bumetanide, hypoxic–ischemic encephalopathy, neonatal seizures, NKCC1

1 | INTRODUCTION

In his commentary[1] on our recent studies[2,3] and subsequent editorial in *Epilepsia*,[4] Kevin Staley addresses the potential use of bumetanide for the treatment of neonatal seizures and the possible mechanisms involved.

2 | BUMETANIDE ACTIONS ON γ-AMINOBUTYRIC ACID TYPE A RECEPTOR SIGNALING IN VITRO VERSUS IN VIVO

Dr Staley starts by explaining how seizures and neuronal injury can, and often do, lead to an increase in Cl^- uptake by enhancing the functional expression of NKCC1 in neurons, which promotes depolarizing γ-aminobutyric acid type A ($GABA_A$) receptor responses. Notably, all the available data on block by bumetanide of NKCC1-dependent depolarizing GABA actions in epileptic tissue are based on in vitro work,[5–7] with the drug typically applied at about 10 $\mu mol \cdot L^{-1}$. A dose–response study on immature hippocampal network events (giant depolarizing potentials) yielded a threshold concentration of ~.1 $\mu mol \cdot L^{-1}$, which is in line with data in other cells and tissues, and in ectopic expression models (see Fig 11 in Löscher and Kaila[8]).

Based on data by Cleary et al.[9] in neonatal rats, achieving concentrations of .1 $\mu mol \cdot L^{-1}$ within brain tissue would require extremely high doses (~9 mg/kg ip). In experiments by Staley and coworkers,[10] bumetanide is applied

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at clinically approved doses of .3 mg/kg ip or lower, which will produce a transient maximum concentration of only ~1–5 nmol·L^{-1} within brain tissue, as shown by direct chemical assays.[8,9,11,12] Thus, there is no "core challenge"[1] in explaining why bumetanide had no effect in our model of neonatal seizures.[2] Much higher doses (~2 mg/kg ip or higher) are needed for any central effect, even with a very brief duration.[8]

Staley argues that in our model of birth asphyxia (BA),[2] "a lack of effect of bumetanide... does not significantly challenge the demonstrated efficacy of bumetanide in several models of experimental neonatal seizures,[10,13–19] neonatal brain injury[20]..." The rodent studies cited by Staley in this context had the following observations: (1) Mazarati et al.[13] reported an effect on seizure propensity caused by bumetanide (.5 mg/kg ip) applied 20 min before kindling; (2) Edwards et al.[14] is a study on seizures after 3 h of anesthesia with sevoflurane, with age-specific effects of 5 μmol·L^{-1}/kg (=1.8 mg/kg ip) bumetanide; (3) Dhir and Chopra[15] showed that bumetanide (.15 mg/kg ip) enhanced the anticonvulsant potential of allopregnanolone against kainic acid convulsions in neonatal rats; (4) Willis et al.[16] is on propofol-induced seizures with intraperitoneal bumetanide at 1.8 mg/kg; (5) Nardou et al.[17] is an in vitro study [sic] with 10 μmol·L^{-1} bumetanide; (6) Marguet et al.[18] is not on bumetanide actions on seizures, but on twice-a-day application of the drug (.2 mg/kg sc) in neonatal mice, which prevented the establishment of subsequent seizures in a genetic epilepsy model via an unidentified mechanism; (7) Hu et al.[19] reported that twice-a-day application of bumetanide (.5 mg/kg ip) reduced post-hypoxic–ischemic encephalopathy (HIE) seizure susceptibility in neonatal rats via inhibition of aberrant neurogenesis; and (8) Liu et al.[20] had no data on seizures, but showed a positive effect of 10 mg/kg but not 2.5 mg/kg ip of bumetanide on sensorimotor outcome in postnatal day 7 (P7) rats 1–4 weeks after experimental HIE, when used in conjunction with hypothermia and 30 mg/kg of phenobarbital. We leave it up to the reader to judge whether the above studies support a "demonstrated efficacy of bumetanide in several models of experimental neonatal seizures."[1]

Importantly, the results of the original work by Dzhala et al.[10] do not show a convincing antiseizure action of bumetanide.[4,21] Here, we cite a commentary[21] on the above study by three leading clinician-researchers in *Epilepsia* in 2009: "The available *in vivo* evidence for the antiepileptic properties of bumetanide is based on data from six rats that were given bumetanide together with kainic acid ... [The] main finding was that electroencephalography (EEG) power was reduced during seizures in the bumetanide-treated animals. ... EEG power reduction is clearly not equal to an anticonvulsant effect, and may not even have anything to do with seizure suppression. Most importantly, changes in the spectral power of ictal EEG have little relevance to clinical seizure treatment, which aims at blocking—not modifying—the electrographic seizure activity."

Key Points

- Seizures are the most common neurological emergency in the neonatal period and only poorly respond to antiseizure drugs
- Birth asphyxia is a frequent cause of neonatal seizures, mortality, and poor neurodevelopmental outcome
- Bumetanide has been proposed to potentiate the antiseizure activity of phenobarbital by blocking NKCC1-dependent depolarization mediated by GABA, but this has not been demonstrated in vivo
- In his commentary in *Epilepsia* (this issue), Dr Staley incorrectly describes our noninvasive model of birth asphyxia
- Here, we will explain why bumetanide is not suitable for seizure suppression in neonates

3 | HELSINKI BA MODEL: SIMULATING THE CLINICAL SITUATION

BA is characterized by severely impaired respiratory gas exchange, which leads to progressive hypoxia, hypercarbia, and acidosis.[22] Based on this, Kai Kaila's group has developed a rat model of moderate BA in P11 rat pups (termed the "Helsinki model" in the following), in which the animals are exposed for 30 min to intermittent hypoxia (step changes between 9% and 5% ambient O_2) and maintained hypercapnia (20% CO_2).[3,23] We have demonstrated that this model mimics BA in human neonates in several important aspects.[2,3,23–25] Our model recapitulates the most salient physiological responses to BA in human neonates, including systemic acid–base changes caused by anaerobic energy metabolism (acidosis, accumulation of lactic acid, and fall in base excess) and used routinely in diagnosis of BA.[3] The model also shows the characteristic stress-hormone surge as monitored by blood copeptin,[3,25,26] a widely used clinical biomarker.[27]

In the Helsinki model, seizures are never observed during asphyxia,.[2,3,23,24] The 20% CO_2 used in this model suppresses the hypoxia-induced increase in neuronal excitability,[3] which reflects an important endogenous protective role for CO_2 in mammalian birth.[23] Importantly, and in contrast to commonly used hypoxia-only models

in which seizures are triggered already during the insult,[28] the pups develop seizures after the termination of asphyxia, that is, after full recovery from hypoxia, which is analogous to the clinical situation.[29]

Staley[1] argues that our model[3] "is closely based on the hypercarbia withdrawal model of acute seizures developed by Dixon Woodbury."[30–32] This is an erroneous conclusion. Woodbury et al. found that ambient CO_2 has immediate anticonvulsant effects at 5%–20% (see also Tolner et al.[33]), becomes proconvulsant at 25%–40%, and induces anesthesia at very high levels (>40%).[32] Animals exposed to CO_2 levels of >30% displayed seizures upon withdrawal. Hypercarbia-only is a condition that obviously had to be examined in the piloting phase of our work, and 20% CO_2 never led to postexposure seizures. Interestingly, we found that intermittent strongly hypoxic episodes (from 9% to 5% O_2) during the asphyxia exposure resulted in postasphyxia seizures, whereas asphyxia with continuous 5% O_2 hypoxia did not promote seizures at all, even when the total hypoxic load was equal in the two paradigms.

Staley's further criticism is that the time course of seizures in the Helsinki model is different from those in asphyxiated human newborns, in which seizures begin hours after delivery[29] and continue for hours to days, not minutes.[29] Here, we wish to emphasize that in the widely used rodent models of BA/HIE based on carotid ligation and/or hypoxia, the seizures start already during the insult.[28] Moreover, our model provides the possibility of testing fast-acting antiseizure medications such as midazolam and acetazolamide with drug application after the insult.[2,34] Thus, our model satisfies numerous criteria for translational validity better than the other current rat- and mouse-based approaches. A relevant question is obviously whether evoking "neonatal seizures" by exposing a neonatal animal to a proconvulsant agent (such as kainate)[10] has any translational validity at all.

4 | ION REGULATION AND SEIZURES

Staley makes the surprising statement that "The editorial[4] cited other studies finding higher levels of KCC2 in the human neonatal brain as evidence that the $GABA_A$ reversal potential was already sufficiently hyperpolarizing in human neonates, such that inhibition of NKCC1 by bumetanide would not be an effective anticonvulsant therapy." This is not what we state. We have worked on NKCC1-dependent excitatory GABA actions observed in damaged neurons in epileptic adult rodent and human tissue,[5,35] in which neurons that remain healthy have a high level of KCC2. Neither does our editorial claim anything about the relative expression of KCC2 versus NKCC1 at any developmental time point. As explained before,[8,36,37] KCC2 is a neuron-specific molecule, whereas NKCC1 is widely expressed in nearly all cell types of the brain, making the ratio (quantitative or qualitative) between KCC2 and NKCC1 expression at the tissue level (whether mRNA or protein) a meaningless parameter.

Very briefly, we would also like to point out that the concept of fixed charges participating in the generation of the Cl^- driving force across neuronal membranes[38] was not "initially controversial."[1] This concept violates the basic laws of thermodynamics,[39] and it is therefore not merely controversial but simply wrong.

5 | BUMETANIDE IS A POTENT OTOTOXIC DRUG

In striking contrast to Staley's statement that "bumetanide has not been shown to be ototoxic experimentally,"[1] this drug has been reported to be a potent ototoxic drug when administered alone in adult cats, dogs, and guinea pigs.[40–44] Significant ototoxic effects were observed at intravenous doses of ~2 mg/kg in cats[40–42] and .5 mg/kg in dogs.[43] In contrast, mice are strikingly less sensitive to the ototoxicity of bumetanide.[45] However, only the latter mouse study was cited by Staley to support his argument that bumetanide is not ototoxic when administered alone.[1]

In the two clinical trials on bumetanide in newborns with neonatal seizures, the combined total incidence of permanent hearing loss was ~12%, which might have been due to coincident risk factors, in particular, administration of aminoglycoside antibiotics.[46,47] Loop diuretics applied together with aminoglycoside antibiotics, such as gentamycin or kanamycin are known to have a synergistic ototoxic action, leading to irreversible hearing loss in doses that would not be expected to cause ototoxicity if either drug was used alone.[41,48] One of 13 neonates in the NEMO trial developed hearing loss after treatment with bumetanide in the absence of an aminoglycoside.[46] Staley[1] pointed out that neonates with HIE are at an increased risk of hearing loss, which is true and may increase the sensitivity to the ototoxic effect of bumetanide. Thus, based on the risk of ototoxicity alone, one may question further clinical trials on bumetanide in newborns with neonatal seizures.

6 | DO WE REALLY NEED MORE CLINICAL TRIALS WITH BUMETANIDE ON NEONATAL SEIZURES?

At the end of his commentary,[1] Staley states that "the next step is a randomized, controlled, multicenter phase II–III

trial of bumetanide for neonatal seizures that do not respond to phenobarbital, excluding neonates treated with aminoglycosides." Taken together, all available evidence speaks against another trial:

1. The pharmacokinetic properties of bumetanide as a central nervous system (CNS) drug are extremely poor. The physicochemical properties of bumetanide are consistent with its very low permeability across the blood–brain barrier (BBB).[8,49,50] In addition, active efflux transport at the BBB further restricts brain levels of bumetanide.[51] Lack of CNS access with clinically relevant doses has been convincingly shown by direct chemical measurements from cerebrospinal fluid[52] and from brain tissue.[2,9,11,12,53]
2. The original idea of targeting NKCC1 in the brain to specifically reduce the intracellular chloride concentration in damaged principal neurons with depolarizing or excitatory $GABA_A$ receptor responses[10] has turned out to be impossible. Even if a brain-permeable NKCC1 blocker were available, it would obviously act on NKCC1 in all kinds of cells, including astrocytes and oligodendrocytes, as well as microglia.[8,54] NKCC1 expressed in astrocytes and oligodendrocytes has robust effects on neuronal plasticity, and on the development of neuronal connectivity and axonal functions.[55–58] These insights call for a re-evaluation of the mechanistic basis of practically all in vivo data obtained by bumetanide doses that are high enough to lead to relevant drug levels in brain tissue, including our own work on sharp waves.[59] Bumetanide has also effects on cells and tissues other than the kidney outside the brain, which is, again, consistent with the ubiquitous expression patterns of NKCC1.[8,37,60]

Finally, on a more personal note, the present authors have invested a great deal of time and resources in basic research on the roles of NKCC1 in neuronal signaling and network functions, including seizures.[5,8,36,37,49,50,53,59,61–63] Not long ago, we were enthusiastic about the possibility of developing BBB-permeant NKCC1 blockers and prodrugs to generate a novel type of anticonvulsant medication.[49,64–66] However, in light of the steeply accumulating data on the vital roles of NKCC1 in various types of non-neuronal cells within brain tissue as described above,[8] this goal had to be abandoned.

It is obvious that much of the previous work on NKCC1 needs a reality check based on the steeply evolving data within this field. There are many novel and interesting observations on the in vivo effects of bumetanide and other NKCC1 blockers, which do not require brain access of the drug.[8] The putative targets include NKCC1-expressing cells in the autonomic nervous system, in endocrine glands, and in the immune system. Interestingly, bumetanide administered parenterally ameliorates inflammation in the brain, whereas an opposite effect is seen in response to intracerebral application of the drug.[54] The cellular and physiological mechanisms underlying the potential therapeutic actions of bumetanide deserve open-minded investigations based on scientific curiosity, not on outdated concepts.[1,10]

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CONFLICT OF INTEREST

Neither of the authors has any conflict of interest to disclose. We confirm that we 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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