Received: 12 February 2021 | Accepted: 16 February 2021

DOI: 10.1111/epi.16866

Epilepsia®

CRITICAL REVIEW – INVITED COMMENTARY

Reply to the commentary by Ben-Ari and Delpire: Bumetanide and neonatal seizures: Fiction versus reality

Wolfgang Löscher[1,2] | **Kai Kaila**[3,4]

[1]Department of Pharmacology, Toxicology, and Pharmacy, University of Veterinary Medicine, Hannover, Germany

[2]Center for Systems Neuroscience, Hannover, Germany

[3]Molecular and Integrative Biosciences, University of Helsinki, Helsinki, Finland

[4]Neuroscience Center (HiLIFE), University of Helsinki, Helsinki, Finland

Correspondence
Wolfgang Löscher, Department of Pharmacology, Toxicology, and Pharmacy, University of Veterinary Medicine, Hannover, Germany.
Email: wolfgang.loescher@tiho-hannover.de

Kai Kaila, Neuroscience Center, Helsinki Institute of Life Science, University of Helsinki, Helsinki, Finland.
Email: kai.kaila@helsinki.fi

Funding information
Deutsche Forschungsgemeinschaft; Academy of Finland; Sigrid Jusélius Foundation

Abstract

In this response to a commentary by Ben-Ari and Delpire on our recent study on the pharmacology of neonatal seizures in a novel, physiologically validated rat model of birth asphyxia, we wish to rectify their inaccurate descriptions of our model and data. Furthermore, because Ben-Ari and Delpire suggest that negative data on bumetanide from preclinical and clinical trials of neonatal seizures have few implications for (alleged) bumetanide actions on neurons in other brain disorders, we will discuss this topic as well. Based on the poor brain penetration of bumetanide, combined with the extremely wide cellular expression patterns of the target protein NKCC1, it is obvious that the numerous actions of systemically applied bumetanide described in the literature are not mediated by the drug's effects on central neurons.

KEYWORDS

asphyxia, blood–brain barrier, diuretics, GABA, KCC2, NKCC1, pharmacokinetics

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 was proposed to potentiate the antiseizure activity of phenobarbital, but we found that bumetanide is ineffective in a novel rat model of birth asphyxia
- In a commentary article, Drs. Ben-Ari and Delpire discussed our data, but incorrectly described our model and the outcome of our experiments
- Here we respond to their commentary and also briefly discuss the (alleged) bumetanide actions on neurons in other brain disorders

1 | RESPONSE TO THE COMMENTARY BY BEN-ARI AND DELPIRE

We thank Drs. Y. Ben-Ari and E. Delpire[1] for their interest in our study[2] on the actions of bumetanide, phenobarbital (PB), and midazolam on neonatal seizures carried out on a novel, physiologically validated model of birth asphyxia[3]; both papers appear in this issue of *Epilepsia*. In our reply to their commentary,[1] we wish to rectify the inaccurate descriptions of our model and data. Furthermore, because Ben-Ari and Delpire[1] suggest that negative data on bumetanide from

preclinical and clinical trials of neonatal seizures have few implications for (alleged) bumetanide actions on neurons in other brain disorders (see also Ben-Ari et al.[4]), we will discuss this topic as well.

2 | BIRTH ASPHYXIA IS NOT A HYPOXIA-ONLY CONDITION

The original model paper by us[3] (see also Pospelov et al.[5]) is focused on showing that exposure to pure hypoxia, as done in a large number of studies on rodents, does not mimic birth asphyxia (i.e., the combination of hypoxia and hypercapnia), and totally fails to reproduce its key physiological and pathophysiological manifestations within and outside the brain. Among the major differences in the physiology and pathophysiology between pure hypoxia and our asphyxia models is that, in the former, seizures are triggered during the insult, whereas in our "intermittent asphyxia" protocol (for details see Johne et al.[2] and Ala-Kurikka et al.[3]), the seizures commence after the insult, akin to the situation in human neonates. Thus, we were surprised to read in the commentary of Ben-Ari and Delpire[1] that our work on bumetanide, PB, and midazolam (with asphyxia) was done under "experimental conditions with very minor differences" when compared to the work of Cleary at al.,[6] which is based on hypoxia only.

The profound differences between the birth asphyxia and hypoxia-only models are likely to account for the finding that bumetanide did not potentiate PB's effect on seizures in our study,[2] whereas such an effect was reported by Cleary et al.[6] Importantly, our negative data on bumetanide are in line with those of a clinical trial (the NEMO trial) in human neonates.[7]

Ben-Ari and Delpire[1] argue that "the NEMO trial is irrelevant to the use of bumetanide to treat brain disorders," because "bumetanide was injected intra-venously at very high doses that might indeed be toxic and are certainly not used in other clinical situations." However, this is not true. The intravenous doses of bumetanide in the NEMO trial (.05, .1, .2, and .3 mg/kg) are in the dose range used (and approved) for induction of diuresis in neonates and are generally considered safe.[8] Similar doses were also used in the experiments on neonatal seizures in our study[2] and the study of Cleary et al.[6] As correctly noted by Ben-Ari and Delpire,[1] we have repeatedly shown by direct measurements from brain tissue samples that at such low doses of bumetanide, the maximum total and unbound brain levels of this drug are at least an order of magnitude below concentrations needed to inhibit the Na-K-2Cl cotransporter NKCC1 expressed in central neurons.[2,9–11] This is plain fact, and not a result of speculations on pharmacokinetic factors. We will discuss below some of the many additional reasons why bumetanide is not a promising drug candidate for targeting central nervous system (CNS) neurons.

3 | HYPOXIA, SEIZURES, AND INTEGRITY OF THE BLOOD–BRAIN BARRIER

Ben-Ari and Delpire[1] suggest that "brain levels are, as expected, higher after the asphyxia insult" and cite Cleary et al.[6] in this respect. However, in the hypoxia-only postnatal day 10 (P10) rat model used by Cleary et al.,[6] bumetanide brain levels were not significantly higher than determined by the same group in neonatal control rats or, in a previous study, in adult rats.[12] Thus, 30 minutes following administration of .3 mg/kg ip bumetanide, the average brain bumetanide level was 1.07 ng/g in P10 rats exposed to hypoxia compared to .94 ng/g in controls,[6] which is not a relevant (or statistically significant) difference. These values have to be corrected by the substantial (>80%) binding of bumetanide to lipids and proteins in the brain parenchyma.[11] Thus, both total and the functionally more relevant free bumetanide brain levels are far below those needed to inhibit NKCC1.[13] Similarly, the brain levels of bumetanide determined by us in postasphyxic P11 rats with seizures[2] were not higher than those determined in adult rats,[10] further refuting the assumption that brain insults by hypoxia or asphyxia lead to an impairment of blood–brain barrier (BBB) integrity, and to a consequent increase in the brain penetration of bumetanide in the studies discussed presently. It is a widely held misconception that BBB impairment due to brain insults "opens" the BBB to drugs.[14]

Ben-Ari and Delpire[1] note that "the main reason for this poor brain availability [of bumetanide] is the fact that ~97% of BUM binds to serum proteins, e.g., albumin." However, this is not a major factor in the present context. For instance, midazolam is ~95% bound to plasma proteins but, following intravenous administration, it has an almost immediate onset of anticonvulsant action.[15] As shown by Johne et al.[2] for rat neonates, midazolam's brain:plasma concentration ratio exceeds 2, which is 400-fold higher compared to the brain:plasma ratio of .005 reported at peak brain levels of bumetanide in hypoxic rat neonates.[6] Rather than plasma protein binding, the extremely high ionization rate (>99%) of bumetanide in plasma and active efflux transport at the BBB restrict brain entry of this drug.[13,16,17] It is of interest to note here that seizures may increase active drug efflux at the BBB and thus further reduce drug brain levels.[18]

4 | PB AND NEONATAL SEIZURES

In contrast to the comments of Ben-Ari and Delpire,[1] we certainly do not claim that the convulsive seizures in our novel model of birth asphyxia are—to any degree—resistant to PB.[2] A relatively high (30 mg/kg) dose applied before the asphyxia exposure was effective in fully blocking the convulsive postasphyxia seizures, whereas nonconvulsant

seizures were resistant to PB, which is in line with clinical experience.[19] The lack of effect of PB when administered after the asphyxia simply reflects the brief time window (~2–3 minutes) between the end of the asphyxia and onset of seizures in our model, and the slower tissue distribution and brain penetration of PB when compared to midazolam, as explained in detail in our paper.[2] Thus, one of the major points of the commentary[1] is based on a misunderstanding. Although PB is certainly not an ideal treatment for neonatal seizures, as stated by Ben-Ari and Delpire,[1] it is unfortunately still the best available option in clinical practice[20] (but see also Davidson et al.[21]). Notably, midazolam was very effective in preventing neonatal seizures in our experimental study.[2] Midazolam is the most effective benzodiazepine currently available and widely used as an emergency treatment for termination of seizures.[22] Contrary to the statement by Ben-Ari and Delpire,[1] midazolam is not related to zolpidem, which is a "Z drug" and not a benzodiazepine, but an imidazopyridine. Zolpidem differs from midazolam in its effects on γ-aminobutyric acid type A ($GABA_A$) receptors and other targets.[23,24]

5 | DEPOLARIZING GABA ACTIONS AND BIRTH ASPHYXIA

We are fully aware that "GABAergic inhibition is particularly activity-dependent and can even reverse polarity."[1] The widely used term "ionic plasticity" of GABAergic signaling was coined by one of us (K.K.; see Rivera et al.[25]; reviewed in Kaila et al.[26,27]). However, the detailed discussion on "depolarizing GABA actions" in the commentary of Ben-Ari and Delpire[1] has little to do with our work. We do not provide any evidence, nor do we argue that NKCC1-dependent depolarizing GABA actions would have any role in the postasphyxia seizures in the present model. We would also like to note here that we certainly do not postulate a "universal common underlying mechanism" (see Summary in Ben-Ari and Delpire[1]) for neonatal encephalopathic seizures.

The NKCC1 expressed in neurons was originally suggested as a promising therapeutic target of bumetanide by Dzhala et al.,[28] who used kainate (sic) in P9–10 rats as a model of neonatal seizures. In support of their hypothesis that neuronal NKCC1 facilitates neonatal seizures in human neonates, they erroneously reported that in the neonatal human cortex, neuronal expression of NKCC1 would be high and KCC2 would be low, leading to depolarizing and excitatory GABAergic signaling. Although depolarizing actions of GABA on pyramidal neurons have been observed in neonatal rats and mice,[29] it is important to note that these animals are at a much more immature stage of cortical development than the human term neonate.[30–32] Work on the expression patterns of cation-chloride cotransporters (CCCs) has shown that KCC2 mRNA and protein expression are high in human cortical neurons at term birth,[33–36] clearly indicating that GABAergic transmission is hyperpolarizing at this stage (for reviews, see Kaila et al.,[26,27,37]).

That neonatal seizures and neuronal damage in the postasphyxic human neocortex might lead to downregulation of KCC2 and decreased extrusion of neuronal Cl^-—as first shown by us in adult kindled adult mice[38]—is entirely possible. However, specifically targeting these neurons with systemically applied bumetanide is not feasible, as explained above.

Moreover, even with direct application of bumetanide or any other NKCC1 blocker into the brain, a very high number of off-target effects would be expected. This is because neuronal NKCC1 constitutes only a minor fraction of the total brain NKCC1, which is expressed in practically all cells in the CNS parenchyma, as well as in the BBB and vasculature, as described below.

Despite the advances in research on the developmental patterns of CCCs in the developing human neocortex (see above), the discussion section of a recent clinical study[39] on the possible mechanisms of bumetanide actions on neonatal seizures is still based on the false data and assumptions by Dzhala et al.[28] In this pilot randomized, controlled double-blind clinical trial, neonates with electroencephalographically (EEG)-confirmed seizures after ≥20 and <40 mg/kg PB were randomized to receive additional PB with either placebo (control) or .1, .2, or .3 mg/kg bumetanide.[39] Drug efficacy was not analyzed as percentage seizure reduction, which is the benchmark readout in antiseizure medication trials,[7,40,41] but as quantitative change in seizure burden. This was, strikingly, the case also in the original Dzhala et al. study,[28] where the conclusions of the "seizure-suppressing effect" of bumetanide were based on quantification of EEG power (not seizures as such) in six rats treated with kainate and six controls. 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.[42]

Similar to the study of Pressler et al.,[7] add-on treatment with bumetanide was associated with ototoxicity,[39] which might have been potentiated by the concurrent treatment with the aminoglycoside antibiotic gentamicin. As noted by Ben-Ari and Delpire,[1] ototoxic aminoglycosides should not be used together with bumetanide. Notably, however, in both clinical studies with bumetanide in neonates,[7,39] there was an increased risk of ototoxicity also in those neonates that did not receive aminoglycosides.

Soul et al.[39] concluded that definitive proof of bumetanide's efficacy awaits an appropriately powered Phase 3 trial, which we would emphatically advise against because of the many reasons explained in this commentary.

6 | CELLULAR TARGETS OF BUMETANIDE OUTSIDE THE BRAIN

It is obvious that the data presented by Soul et al.[39] (or any data from clinical trials using bumetanide) are not proof of concept that bumetanide acts by inhibiting NKCC1 in neurons or any other brain cells. Here, we would like to call attention to the finding that NKCC1 is expressed in practically all cells and organ systems in the body. Therefore, nonspecific manipulation of NKCC1 in the organism can lead to unexpected effects, and especially so if research is based on the outdated dogma referred to above. For instance, we found very recently that constitutive disruption of expression of the *Slc12a2* gene, which codes for NKCC1, leads to enhanced severity of seizures in the widely used kainate model of temporal lobe epilepsy.[43]

Interestingly, systemic administration of bumetanide has an anti-inflammatory action outside the brain, whereas direct application into the brain in vivo has the opposite effect.[44] There are also intriguing findings obtained using low systemic doses of bumetanide and other diuretics in rodents (e.g., Krystal et al.,[45] and Marguet et al.[46]) that point to beneficial actions, based on unidentified mechanisms outside the brain. However, effects of bumetanide may also be mediated by targets other than NKCC1. A recent example is the brain-permeant bumetanide derivative, bumepamine, which despite its structural similarity to bumetanide does not inhibit NKCC1 at all, but is a potent diuretic.[11,47,48] Strikingly, bumepamine is much more effective than bumetanide at potentiating the effect of PB on neonatal seizures in our birth-asphyxia seizure model,[49] but again, the underlying mechanisms remain to be identified.

7 | CONCLUSIONS

Although CCCs, such as NKCC1, are considered attractive CNS drug targets, bumetanide and other existing NKCC1 inhibitors are suboptimal because of pharmacokinetic constraints and lack of target specificity at the cellular level. Furthermore, with respect to the presumed role of neuronal NKCC1 in diverse neurological and psychiatric disorders, a major caveat here is that our understanding of the spatiotemporal expression patterns of NKCC1 in the brain is still in its infancy.[50] NKCC1 expression in certain glial subtypes is much higher than in neurons,[50] and glial NKCC1 seems to regulate neuronal signaling and plasticity in a robust manner (e,g., long-term potentiation[51]). We do not argue that bumetanide may not be beneficial in some brain disorders, such as autism spectrum disorders,[1,4] but such effects of bumetanide are obviously not related to inhibition of NKCC1 in CNS neurons. Effects of bumetanide on NKCC1 located in the BBB or in the hypothalamic–pituitary–adrenal axis, as well as secondary actions resulting from bumetanide's strong diuretic activity, may all be relevant here (see Puskarjov et al.[13]). Putative targets also include the choroid plexus, which has a uniquely high level of NKCC1.[50] This epithelium is directly exposed to blood-borne drugs and has been shown to regulate the neuronal microenvironment of immature cortical neurons during brain development.[52] Finally, chronic diuresis of offspring might influence parental attention and behavior in both mice and men, for the benefit of social and other aspects of brain development. We are convinced that open-minded research on the diverse effects of bumetanide will enhance our understanding of the multiple roles of NKCC1 in cells and organ systems, thus paving the way to rational design of NKCC1-modulatory drugs and to possible therapeutic applications thereof.

ACKNOWLEDGEMENTS

The original research work of the authors was supported by the German Research Foundation (W.L.), and by the Academy of Finland and the Sigrid Jusélius Foundation (K.K.).

CONFLICT OF INTEREST

Neither of the authors has any conflict of interest to disclose.

ORCID

Wolfgang Löscher https://orcid.org/0000-0002-9648-8973
Kai Kaila https://orcid.org/0000-0003-0668-5955

REFERENCES

1. Ben-Ari Y, Delpire E. Phenobarbital, midazolam, bumetanide, and neonatal seizures: The devil is in the details. Epilepsia. 2021;62:935–40.
2. Johne M, Römermann K, Hampel P, Gailus B, Theilmann W, Ala-Kurikka T, et al. Phenobarbital and midazolam suppress neonatal seizures in a noninvasive rat model of birth asphyxia, whereas bumetanide is ineffective. Epilepsia. 2021;62:908–22.
3. Ala-Kurikka T, Pospelov AS, Summanen M, Alafuzoff A, Kurki S, Voipio J, et al. A physiologically validated rat model of term birth asphyxia with seizure generation after, not during, brain hypoxia. Epilepsia. 2021;62:923–34.
4. Ben-Ari Y, Damier P, Lemonnier E. Failure of the Nemo trial: bumetanide is a promising agent to treat many brain disorders but not newborn seizures. Front Cell Neurosci. 2016;10:90.
5. Pospelov AS, Puskarjov M, Kaila K, Voipio J. Endogenous brain-sparing responses in brain pH and *P*O2 in a rodent model of birth asphyxia. Acta Physiol. 2020;229:e13467.
6. Cleary RT, Sun H, Huynh T, Manning SM, Li Y, Rotenberg A, et al. Bumetanide enhances phenobarbital efficacy in a rat model of hypoxic neonatal seizures. PLoS One. 2013;8:e57148.
7. Pressler RM, Boylan GB, Marlow N, Blennow M, Chiron C, Cross JH, et al. Bumetanide for the treatment of seizures in newborn babies with hypoxic ischaemic encephalopathy (NEMO): an open-label, dose finding, and feasibility phase 1/2 trial. Lancet Neurol. 2015;14:469–77.

8. Pacifici GM. Clinical pharmacology of the loop diuretics furosemide and bumetanide in neonates and infants. Paediatr Drugs. 2012;14:233–46.
9. Brandt C, Nozadze M, Heuchert N, Rattka M, Löscher W. Disease-modifying effects of phenobarbital and the NKCC1 inhibitor bumetanide in the pilocarpine model of temporal lobe epilepsy. J Neurosci. 2010;30:8602–12.
10. Töllner K, Brandt C, Töpfer M, Brunhofer G, Erker T, Gabriel M, et al. A novel prodrug-based strategy to increase effects of bumetanide in epilepsy. Ann Neurol. 2014;75:550–62.
11. Brandt C, Seja P, Töllner K, Römermann K, Hampel P, Kalesse M, et al. Bumepamine, a brain-permeant benzylamine derivative of bumetanide, does not inhibit NKCC1 but is more potent to enhance phenobarbital's anti-seizure efficacy. Neuropharmacology. 2018;143:186–204.
12. Li Y, Cleary R, Kellogg M, Soul JS, Berry GT, Jensen FE. Sensitive isotope dilution liquid chromatography/tandem mass spectrometry method for quantitative analysis of bumetanide in serum and brain tissue. J Chromatogr B Analyt Technol Biomed Life Sci. 2011;879:998–1002.
13. Puskarjov M, Kahle KT, Ruusuvuori E, Kaila K. Pharmacotherapeutic targeting of cation-chloride cotransporters in neonatal seizures. Epilepsia. 2014;55:806–18.
14. Löscher W, Friedman A. Structural, molecular and functional alterations of the blood-brain barrier during epileptogenesis and epilepsy: a cause, consequence or both? Int J Mol Sci. 2020;21:591.
15. Pieri L. Preclinical pharmacology of midazolam. Br J Clin Pharmacol. 1983;16(Suppl 1):17S–27S.
16. Löscher W, Puskarjov M, Kaila K. Cation-chloride cotransporters NKCC1 and KCC2 as potential targets for novel antiepileptic and antiepileptogenic treatments. Neuropharmacology. 2013;69:62–74.
17. Römermann K, Fedrowitz M, Hampel P, Kaczmarek E, Töllner K, Erker T, et al. Multiple blood-brain barrier transport mechanisms limit bumetanide accumulation, and therapeutic potential, in the mammalian brain. Neuropharmacology. 2017;117:182–94.
18. Löscher W, Potschka H, Sisodiya SM, Vezzani A. Drug resistance in epilepsy: clinical impact, potential mechanisms, and new innovative treatment options. Pharmacol Rev. 2020;72:606–38.
19. Donovan MD, Griffin BT, Kharoshankaya L, Cryan JF, Boylan GB. Pharmacotherapy for neonatal seizures: current knowledge and future perspectives. Drugs. 2016;76:647–61.
20. Soul JS, Pressler R, Allen M, Boylan G, Rabe H, Portman R, et al. Recommendations for the design of therapeutic trials for neonatal seizures. Pediatr. Res. 2019;85:943–54.
21. Davidson JO, Bennet L, Gunn AJ. Evaluating anti-epileptic drugs in the era of therapeutic hypothermia. Pediatr Res. 2019;85:931–3.
22. Lingamchetty TN, Hosseini SA, Saadabadi A. Midazolam. StatPearls. Treasure Island, FL: StatPearls Publishing; 2020.
23. Sanger DJ, Depoortere H. The pharmacology and mechanism of action of zolpidem. CNS Drug Rev. 1998;4:323–40.
24. Rowlett JK, Platt DM, Lelas S, Atack JR, Dawson GR. Different GABAA receptor subtypes mediate the anxiolytic, abuse-related, and motor effects of benzodiazepine-like drugs in primates. Proc Natl Acad Sci U S A. 2005;102:915–20.
25. Rivera C, Voipio J, Kaila K. Two developmental switches in GABAergic signalling: the K+-Cl- cotransporter KCC2 and carbonic anhydrase CAVII. J Physiol. 2005;562:27–36.
26. Kaila K, Price TJ, Payne JA, Puskarjov M, Voipio J. Cation-chloride cotransporters in neuronal development, plasticity and disease. Nat Rev Neurosci. 2014;15:637–54.
27. Kaila K, Ruusuvuori E, Seja P, Voipio J, Puskarjov M. GABA actions and ionic plasticity in epilepsy. Curr Opin Neurobiol. 2014;26:34–41.
28. Dzhala VI, Talos DM, Sdrulla DA, Brumback AC, Mathews GC, Benke TA, et al. NKCC1 transporter facilitates seizures in the developing brain. Nat Med. 2005;11:1205–13.
29. Ben Ari Y, Gaiarsa JL, Tyzio R, Khazipov R. GABA: a pioneer transmitter that excites immature neurons and generates primitive oscillations. Physiol Rev. 2007;87:1215–84.
30. Clancy B, Darlington RB, Finlay BL. Translating developmental time across mammalian species. Neuroscience. 2001;105:7–17.
31. Erecinska M, Cherian S, Silver IA. Energy metabolism in mammalian brain during development. Prog Neurobiol. 2004;73:397–445.
32. Spoljaric A, Seja P, Spoljaric I, Virtanen MA, Lindfors J, Uvarov P, et al. Vasopressin excites interneurons to suppress hippocampal network activity across a broad span of brain maturity at birth. Proc Natl Acad Sci U S A. 2017;114:E10819–28.
33. Hyde TM, Lipska BK, Ali T, Mathew SV, Law AJ, Metitiri OE, et al. Expression of GABA signaling molecules KCC2, NKCC1, and GAD1 in cortical development and schizophrenia. J Neurosci. 2011;31:11088–95.
34. Vanhatalo S, Palva JM, Andersson S, Rivera C, Voipio J, Kaila K. Slow endogenous activity transients and developmental expression of K+-Cl-cotransporter 2 in the immature human cortex. Eur J Neurosci. 2005;22:2799–804.
35. Robinson S, Mikolaenko I, Thompson I, Cohen ML, Goyal M. Loss of cation-chloride cotransporter expression in preterm infants with white matter lesions: implications for the pathogenesis of epilepsy. J Neuropathol Exp Neurol. 2010;69:565–72.
36. Sedmak G, Jovanov-Milosevic N, Puskarjov M, Kaila K, Judas M. Expression patterns of KCl cotransporter KCC2 in the human fetal and adult brain. FENS Abstr. 2014;2398.
37. Virtanen MA, Uvarov P, Mavrovic M, Poncer JC, Kaila K. The multifaceted roles of KCC2 in cortical development. Trends Neurosci. 2021. in press. https://doi.org/10.1016/j.tins.2021.01.004
38. Rivera C, Li H, Thomas-Crusells J, Lahtinen H, Viitanen T, Nanobashvili A, et al. BDNF-induced TrkB activation down-regulates the K+-Cl- cotransporter KCC2 and impairs neuronal Cl- extrusion. J Cell Biol. 2002;159:747–52.
39. Soul JS, Bergin AM, Stopp C, Hayes B, Singh A, Fortuno CR, et al. A pilot randomized, controlled, double-blind trial of bumetanide to treat neonatal seizures. Ann Neurol. 2021;89:327–40.
40. Painter MJ, Scher MS, Stein AD, Armatti S, Wang Z, Gardiner JC, et al. Phenobarbital compared with phenytoin for the treatment of neonatal seizures. N Engl J Med. 1999;341:485–9.
41. Perucca E. Antiepileptic drugs: evolution of our knowledge and changes in drug trials. Epileptic Disord. 2019;21:319–29.
42. Vanhatalo S, Hellstrom-Westas L, De Vries LS. Bumetanide for neonatal seizures: based on evidence or enthusiasm? Epilepsia. 2009;50:1292–3.
43. Hampel H, Johne M, Gailus B, Vogel A, Schidlitzki A, Gericke B, et al. Deletion of the Na-K-2Cl cotransporter NKCC1 results in a more severe epileptic phenotype in the intrahippocampal kainate mouse model of temporal lobe epilepsy. Neurobiol Dis. 2021;152:e105297.
44. Tóth K, Lénárt N, Szabadits E, Pósfai B, Fekete R, Cserép C, et al. Microglial NKCC1 shapes microglial phenotype, cerebral inflammatory responses and brain injury. bioRxiv. 2021. 2021.01.21.427597.

45. Krystal AD, Sutherland J, Hochman DW. Loop diuretics have anxiolytic effects in rat models of conditioned anxiety. PLoS One. 2012;7:e35417.
46. Marguet SL, Le Schulte VT, Merseburg A, Neu A, Eichler R, Jakovcevski I, et al. Treatment during a vulnerable developmental period rescues a genetic epilepsy. Nat Med. 2015;21:1436–44.
47. Lykke K, Töllner K, Römermann K, Feit PW, Erker T, Macaulay N, et al. Structure-activity relationships of bumetanide derivatives: correlation between diuretic activity in dogs and inhibition of human NKCC2 variant A. Br J Pharmacol. 2015;172:4469–80.
48. Lykke K, Töllner K, Feit PW, Erker T, Macaulay N, Löscher W. The search for NKCC1-selective drugs for the treatment of epilepsy: structure-function relationship of bumetanide and various bumetanide derivatives in inhibiting the human cation-chloride cotransporter NKCC1A. Epilepsy Behav. 2016;59:42–9.
49. Johne M, Römermann K, Hampel P, Theilmann W, Ala-Kurikka T, Kaila K, et al. Anticonvulsant drug actions in a rat model of birth asphyxia. Program No. 041. 07 / C42. 2019 Neuroscience Meeting Planner. Chicago, IL: Society for Neuroscience; 2019.
50. Virtanen MA, Uvarov P, Hübner CA, Kaila K. NKCC1, an elusive molecular target in brain development: making sense of the existing data. Cells. 2020;9(12):2607.
51. Henneberger C, Bard L, Panatier A, Reynolds JP, Kopach O, Medvedev NI, et al. LTP induction boosts glutamate spillover by driving withdrawal of perisynaptic astroglia. Neuron. 2020;108:919–36.
52. Xu H, Fame RM, Sadegh C, Sutin J, Naranjo C, Della S, et al. Choroid plexus NKCC1 mediates cerebrospinal fluid clearance during mouse early postnatal development. Nat Commun. 2021;12:447.

How to cite this article: Löscher W, Kaila K. Reply to the commentary by Ben-Ari and Delpire: Bumetanide and neonatal seizures: Fiction versus reality. *Epilepsia*. 2021;62:941–946. https://doi.org/10.1111/epi.16866