European Journal of Pharmacology 746 (2015) 78–88

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Neuropharmacology and analgesia

Bumetanide is not capable of terminating status epilepticus but enhances phenobarbital efficacy in different rat models

Kathrin Töllner [a,b,1], Claudia Brandt [a,b,1], Thomas Erker [c], Wolfgang Löscher [a,b,*]

[a] Department of Pharmacology, Toxicology, and Pharmacy, University of Veterinary Medicine Hannover, Germany
[b] Center for Systems Neuroscience, Hannover, Germany
[c] Department of Medicinal Chemistry, University of Vienna, Austria

ARTICLE INFO

Article history:
Received 8 July 2014
Received in revised form
21 October 2014
Accepted 27 October 2014
Available online 11 November 2014

Keywords:
Kainate
Pilocarpine
Amygdala
Cation cotransporters
GABA receptors

Chemical compounds studied in this article:
Pilocarpine (PubChem CID: 5910)

ABSTRACT

In about 20–40% of patients, status epilepticus (SE) is refractory to standard treatment with benzodiazepines, necessitating second- and third-line treatments that are not always successful, resulting in increased mortality. Rat models of refractory SE are instrumental in studying the changes underlying refractoriness and to develop more effective treatments for this severe medical emergency. Failure of GABAergic inhibition is a likely cause of the development of benzodiazepine resistance during SE. In addition to changes in $GABA_A$ receptor expression, trafficking, and function, alterations in Cl^- homeostasis with increased intraneuronal Cl^- levels may be involved. Bumetanide, which reduces intraneuronal Cl^- by inhibiting the Cl^- intruding Na^+, K^+, Cl^- cotransporter NKCC1, has been reported to interrupt SE induced by kainate in urethane-anesthetized rats, indicating that this diuretic drug may be an interesting candidate for treatment of refractory SE. In this study, we evaluated the effects of bumetanide in the kainate and lithium–pilocarpine models of SE as well as a model in which SE is induced by sustained electrical stimulation of the basolateral amygdala. Unexpectedly, bumetanide alone was ineffective to terminate SE in both conscious and anesthetized adult rats. However, it potentiated the anticonvulsant effect of low doses of phenobarbital, although this was only seen in part of the animals; higher doses of phenobarbital, particularly in combination with diazepam, were more effective to terminate SE than bumetanide/phenobarbital combinations. These data do not suggest that bumetanide, alone or in combination with phenobarbital, is a valuable option in the treatment of refractory SE in adult patients.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Status epilepticus (SE), a life-threatening neurologic emergency requiring prompt treatment, has been shown to induce alterations in $GABA_A$ receptor function and neuronal chloride homeostasis that may underlie resistance to the anticonvulsant effect of benzodiazepines (Macdonald and Kapur, 1999; Chen and Wasterlain, 2006; Löscher, 2007; Deeb et al., 2012; Löscher et al., 2013). Benzodiazepines such as diazepam and lorazepam, which act by enhancing the inhibitory effect of GABA, are standard first-line treatments for SE; however, patients often become refractory to benzodiazepines when seizures are prolonged (Rossetti and Lowenstein, 2011).

The regulation of intracellular chloride (Cl^-) determines the polarity of $GABA_A$-induced neuronal Cl^- currents. In neurons, Cl^- concentrations depend on the activity of the Cl^- extruding K^+, Cl^- cotransporter KCC2 and the activity of the Cl^- intruding Na^+, K^+, Cl^- cotransporter NKCC1 (Blaesse et al., 2009). Alterations in the balance of NKCC1 and KCC2 activities may induce a switch from hyperpolarizing to depolarizing effects of GABA that may contribute to prolongation of SE and AED resistance (Deeb et al., 2012). In line with this hypothesis, Kapur and Coulter (1995), using the lithium/pilocarpine SE model in rats, reported a loss of GABA-mediated inhibition of hippocampal CA1 neurons after 45 min of SE, which resulted from a positive shift in E_{GABA} in response to marked elevation in intracellular Cl^- concentration. At about the same time following onset of SE, KCC2 expression was reported to markedly decrease in the hippocampus (Lee et al., 2010). We found that a pilocarpine-induced SE increases NKCC1 expression in the rat hippocampus, but the earliest time point studied was 24 h after onset of SE (Brandt et al., 2010).

Assuming that both decreased KCC2 and increased NKCC1 contribute to the shift from hyperpolarizing to depolarizing GABA during SE, a selective NKCC blocker such as the loop diuretic bumetanide may be useful for treatment of intractable SE. The first

* Corresponding author at: Department of Pharmacology, Toxicology and Pharmacy, University of Veterinary Medicine, Bünteweg 17, D-30559 Hannover, Germany.
Tel.: +49 511 856 8721; fax: +49 511 953 8581.
E-mail address: wolfgang.loescher@tiho-hannover.de (W. Löscher).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.ejphar.2014.10.056
0014-2999/© 2014 Elsevier B.V. All rights reserved.

indication that blockade of Cl^- cotransport interrupts SE was reported by Hochman et al. (1995), showing that the loop diuretic furosemide terminates a kainate-induced SE in urethane-anesthesized rats. Subsequently, they reported a similar effect for bumetanide (Schwartzkroin et al., 1998), which is more selective for NKCC1 than furosemide. Since both studies were performed in anesthetized rats, it was not clear whether the SE-blocking effect of the diuretics was due to an interaction with urethane, which is known to enhance GABA (Hara and Harris, 2002), or whether the diuretics alone exerted this effect. Holtkamp et al. (2003) reported that high doses of furosemide terminate an electrically induced SE in awake rats, but, to our knowledge, bumetanide has not yet been evaluated for such an effect in conscious animals. This prompted us to evaluate the effects of bumetanide in SE models in anesthetized and non-anesthetized rats. Our hypothesis was that the anticonvulsant effect reported by Schwartzkroin et al. (1998) was primarily due to an interaction between bumetanide and urethane, similar to the interaction between bumetanide and the GABA-potentiating drug phenobarbital reported for other seizure models (Löscher et al., 2013). Thus, in addition to testing bumetanide's effects on SE in anesthetized and non-anesthetized rats, we also investigated whether bumetanide increases the anticonvulsant effect of phenobarbital in SE models.

2. Materials and methods

2.1. Animals

As in the experiments of Schwartkroin's group (Hochman et al., 1995; Schwartkroin et al., 1998), male Sprague–Dawley rats were used in all experiments unless otherwise indicated. They were obtained from Harlan (Horst, Netherlands) at a body weight of 200–224 g and were adapted to the laboratory for at least one week before starting the experiments. Animals were housed under controlled conditions (ambient temperature 22–24 °C, humidity 30–50%, lights on from 6:00 a.m. to 6:00 p.m.). Food (Altromin 1324 standard diet) and water were freely available. All experiments were done in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and had institutional approval (LAVES, Oldenburg, Germany). All efforts were made to minimize both the suffering and the number of animals.

2.2. Experiments with kainate

In order to replicate the experiments of Schwartkroin's group in the kainate model as closely as possible, we asked Daryl Hochman to provide details of the experiments that were published by Hochman et al. (1995) and Schwartzkroin et al. (1998) with furosemide and bumetanide in urethane-anesthesized rats. 3–4 weeks before the experiment, rats were implanted with electrodes under anesthesia with chloral hydrate as described previously (Rattka et al., 2012) to record electroencephalographic (EEG) activity in the fronto–parietal cortex (mm from bregma: AP, −2.2; L, ±1.5; Paxinos and Watson, 2007) as in the experiments of Hochman et al. (1995). Some rats were also implanted with electrodes into the dentate gyrus (AP, −3.9; L, −1.7; and V, −3.5).

For the experiments with urethane anesthesia, rats received 1.25–1.4 g/kg urethane (Sigma-Aldrich; Taufkirchen, Germany) i.p. and, about 60 min later, they received an i.v. injection of 10–12 mg/kg kainate [(−)-(α)-kainic acid; Cayman Chemical; Ann Arbor, MI, USA]. If no SE was induced, additional kainate doses (4–8 mg/kg) were administered. Bumetanide (Sigma-Aldrich; dissolved in distilled water by means of dilute NaOH) was then injected at two boluses of 5 mg/kg i.v. each, at 80 and 115 min after the last kainate injection as described by Schwartzkroin et al. (1998). All i.v. injections were done in a tail vein.

Since in most experiments, it was not possible to induce SE activity in the EEG with kainate in urethane-anesthetized rats (see Section 3), additional experiments with kainate were performed without anesthesia. In these experiments, SE was induced with 10–12 mg/kg kainate i.v.; in some rats an additional dose of 4 mg/kg had to be administered to induce SE. Bumetanide (5 mg/kg i.v.) was administered repeatedly after SE onset (see Section 3). In some experiments, additional injections of bumetanide were combined with 10 mg/kg i.v. phenobarbital (Serva; Heidelberg, Germany). The dose of phenobarbital, which was dissolved as sodium salt in distilled water, was chosen from previous experiments with bumetanide and phenobarbital in the amygdala-kindling model of epilepsy (Töllner et al., 2014), in which phenobarbital injected alone at a dose of 10 mg/kg did not exert significant anticonvulsant activity, but combined treatment with bumetanide resulted in anticonvulsant effects. Some experiments were also performed with female Sprague–Dawley rats, but the results did not differ from male rats. At the end of the experiment, we examined whether SE could be terminated by a higher dose (20–25 mg/kg i.p.) of phenobarbital injected alone or in combination with diazepam (10 mg/kg i.p.). For injection of diazepam, a commercial ethanol-containing aqueous solution (Faustan®; Temmler Pharma, Marburg, Germany), which contains 5 mg diazepam per ml (the ethanol concentration is 18.6%), was used.

2.3. Experiments with lithium/pilocarpine

As with kainate, lithium/pilocarpine was used to induce SE in conscious and anesthetized rats. Since in our hand urethane did not induce surgical anesthesia (see Section 3), chloral hydrate (400 mg/kg i.p.) was used in these experiments. EEG was recorded from cortical electrodes as in the kainate experiments (for electrode locations see above). Lithium chloride (127 mg/kg p.o.) was administered 12–18 h and methyl-scopolamine (1 mg/kg i.p.) 30 min before pilocarpine. In both conscious and anesthetized rats, pilocarpine was administered as an i.p. bolus of 30 mg/kg, followed by repeated injections of 10 mg/kg at intervals of 30 min until onset of SE. In conscious rats, one additional injection of pilocarpine was sufficient to induce SE, where following chloral hydrate, 5 additional injections were needed. Bumetanide (5 mg/kg i.v.) was injected 30, 60, 90, and 150 min following SE onset, either alone or in combination with phenobarbital (10 mg/kg i.v.).

2.4. Experiments with electrical SE induction

In view of the resistance of chemically-induced SE to bumetanide (see Section 3), further experiments were performed with electrical SE induction, using a model previously described and characterized in detail by us (Brandt et al., 2003). In this model, a self-sustained SE is induced by electrical stimulation of the basolateral amygdala (BLA). For this purpose, electrodes were stereotactically implanted into the right anterior BLA under anesthesia as described in detail previously (Brandt et al., 2003) and served for electrical stimulation and recording of the EEG. About 2 weeks after electrode implantation, 28 rats were electrically stimulated via the BLA electrode for induction of a self-sustained SE as described previously (Brandt et al., 2003; Bethmann et al., 2007). The following stimulus parameters were chosen: stimulus duration 25 min; stimulus consisting of 100 ms trains of 1 ms alternating positive and negative square wave pulses. The trains were given at a frequency of 2/s and the intra-train pulse frequency was 50/s. Peak pulse intensity was 700 μA. For this pulsed-train stimulation, an Accupulser A310C stimulator connected with a Stimulus Isolator A365 (World Precision Instruments, Berlin, Germany) was used. In all rats, the EEG was recorded via the BLA electrode during self-sustained SE, which typically lasts up to 8 h in

Download English Version:

https://daneshyari.com/en/article/2531559

Download Persian Version:

https://daneshyari.com/article/2531559

Daneshyari.com