Neurobiology of Disease 91 (2016) 194–208

Contents lists available at ScienceDirect

Neurobiology of Disease

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

Continuous bilateral infusion of vigabatrin into the subthalamic nucleus: Effects on seizure threshold and GABA metabolism in two rat models☆

Laura Gey, Manuela Gernert *, Wolfgang Löscher *

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

ARTICLE INFO

Article history:
Received 15 December 2015
Revised 7 March 2016
Accepted 10 March 2016
Available online 11 March 2016

Keywords:
Pentylenetetrazole
GABA aminotransferase
Substantia nigra
Basal ganglia
Pharmacoresistance

ABSTRACT

The subthalamic nucleus (STN) plays a crucial role as a regulator of basal ganglia outflow but also influences the activity of cortical and limbic structures, so that it is widely used as a therapeutic target in different brain diseases, including epilepsy. In addition to electrical stimulation of the STN, targeted delivery of anti-seizure drugs to the STN may constitute an alternative treatment approach in patients with pharmacoresistant epilepsy. In the present experimental study, we investigated the anti-seizure and adverse effects of chronic infusion of vigabatrin into the STN of rats. Vigabatrin is a clinically approved anti-seizure drug, which acts by increasing brain GABA levels by irreversibly inhibiting GABA-aminotransferase (GABA-T). Based on functional and neurochemical effects of acute STN microinjection, doses for continuous infusion were calculated and administered, using an innovative drug infusion technology. Bilateral infusion of only 10 μg/day vigabatrin over 3 weeks into the STN resulted in an almost complete inhibition of GABA-T and 4-fold increase in GABA in the target region, which was associated with a significant increase in seizure threshold, determined once weekly by i.v. infusion of pentylenetetrazole (PTZ). Lower doses or unilateral infusion were less effective, both on PTZ seizures and on kindled seizures. Bilateral infusion into substantia nigra pars reticulata was less effective and more toxic than STN infusion. In part of the rats, tolerance to the anti-seizure effect developed. The data demonstrate that chronic administration of very low, nontoxic doses of vigabatrin into STN is an effective means of increasing local GABA concentrations and seizure threshold.

1. Introduction

Approximately one third of patients with epilepsy have persistent seizures despite trials of multiple anti-seizure medications (Löscher et al., 2013). For some of these patients, epilepsy surgery may provide freedom from seizures, but in many cases this is not a viable treatment option (Nilsen and Cock, 2004). Adjunctive therapies, such as ketogenic diet, deep brain stimulation (DBS), or vagus nerve stimulation, add an important and evolving dimension to the management of difficult to control epilepsy (Sharma et al., 2015). Furthermore, several alternative treatment approaches are being evaluated, including cell grafting, focal cooling, and targeted gene or drug delivery (Jacobs et al., 2001; Nilsen and Cock, 2004; Löscher et al., 2008; Rogawski, 2009; Ludvig et al., 2010; Van Dycke et al., 2011; Sharma et al., 2015).

Use of novel (targeted) drug delivery methods might enhance efficacy and reduce toxicity, in comparison with currently existing oral anti-seizure medication (Nilsen and Cock, 2004: Rogawski, 2009; Al Otaibi et al., 2011). Drugs can be delivered to a seizure focus by an implanted catheter and subcutaneous pump or other delivery devices, such as controlled-release polymers. However, for the many patients with multiple foci, or without a clear focal onset of seizures, local drug delivery to key propagation pathways might present the most suitable strategy. Various lines of evidence suggest that subcortical structures such as the substantia nigra pars reticulata (SNr) and the subthalamic nucleus (STN) are of particular interest in this respect (cf., Gale et al., 2008). Focal delivery of drugs that either increase GABAergic inhibitory transmission or block glutamatergic excitatory transmission in the SNr or STN block or attenuate diverse types of experimentally induced seizures, indicating that both regions are part of a seizure-suppressing circuit that becomes engaged by seizure discharge (Gale et al., 2008). However, most studies on focal drug delivery into SNr or STN in seizure models were designed as acute experiments with single dose

Abbreviations: ADT, after discharge threshold; BLA, basolateral amygdala; CSF, cerebrospinal fluid; GABA-T, GABA aminotransferase; GAD, glutamic acid decarboxylase; GST, generalized seizure threshold; HPLC, high performance liquid chromatography; PTZ, pentylenetetrazole; SNr, substantia nigra pars reticulata; SSA, succinic semialdehyde; STN, subthalamic nucleus.

☆ This study is dedicated to the late Karen Gale, who ignited our original interest in the critical roles of subcortical structures, including the substantia nigra and subthalamic nucleus, in seizure control and propagation

* Corresponding authors at: Department of Pharmacology, Toxicology and Pharmacy, University of Veterinary Medicine, Bünteweg 17, D-30559 Hannover, Germany.
E-mail addresses: manuela.gernert@tiho-hannover.de (M. Gernert), wolfgang.loescher@tiho-hannover.de (W. Löscher).

Available online on ScienceDirect (www.sciencedirect.com).

http://dx.doi.org/10.1016/j.nbd.2016.03.012
0969-9961/

administration, while continuous administration would be required for the clinical setting. Furthermore, many of the experimental studies used drugs that are not clinically available.

We have recently reported that single dose injection of the clinically approved anti-seizure drug vigabatrin into the STN was more effective to increase seizure threshold than injection into SNr or systemic administration (Bröer et al., 2012). The anti-seizure effect of vigabatrin is a result of enhanced brain GABA levels caused by irreversible inhibition of the GABA-degrading enzyme GABA aminotransferase (GABA-T; Sabers and Gram, 1992). In the present study, we used a programmable microinfusion pump for continuous intracerebral delivery of vigabatrin. Our hypotheses were that (1) chronic bilateral microinfusion of very low doses of vigabatrin into STN induces long-lasting increases in seizure threshold; (2) bilateral infusion is more effective than unilateral infusion; (3) chronic drug delivery into STN is more effective than delivery into SNr; (4) the anti-seizure effects of vigabatrin correlates with regional alterations in GABA metabolism; and (5) no serious adverse effects occur during the period of drug delivery. For determining the anti-seizure effect of vigabatrin in rats, we used timed i.v. infusion of the $GABA_A$ receptor antagonist pentylenetetrazole (PTZ), which allows to assess drug effects on seizure threshold in individual rats (Löscher, 2009; Bröer et al., 2012). Furthermore, vigabatrin was also locally delivered in the amygdala kindling model of epilepsy. To our knowledge, this is the first study that clearly demonstrates anti-seizure effects of vigabatrin when infused chronically into the STN and correlates the efficacy of vigabatrin under these conditions with its ability to inhibit GABA-T and elevate GABA in the STN.

2. Materials and methods

2.1. Animals

As in our previous study with vigabatrin (Bröer et al., 2012), adult female Wistar Unilever rats were used, which were purchased at a body weight of 200–220 g from Harlan Laboratories (Horst, Netherlands). The rats were housed in groups of five without males in order to keep them acyclic or asynchronous with respect to their estrous cycle (Kücker et al., 2010). Furthermore, we have previously shown that the PTZ seizure threshold is not affected by the estrous cycle in rats (Rattka et al., 2012).

Animals were kept under controlled environmental conditions with a 12/12 h light/dark cycle, light on at 7:00 a.m., for at least 2 weeks before the experiments. Standard laboratory chow (Altromin 1324 standard diet) and tap water were allowed ad libitum. All experiments were done in compliance with the EU council directive 210/63/EU and were formally approved by the animal subjects review board of our institution.

2.2. Acute administration of vigabatrin

Vigabatrin was kindly provided by Sanofi (Berlin, Germany). Dose selection for the acute experiments was based on our previous experiments with microinjection of vigabatrin into SNr and STN (Bröer et al., 2012). The acute experiments were performed to develop a dosing protocol for continuous administration. For this purpose, animals were anesthetized with isoflurane (CP-Pharma, Burgdorf, Germany) via an inhalation mask adjusted to a stereotactic frame, through which the animals inhaled isoflurane (3%) until the surgical stage was reached; then isoflurane was reduced for maintenance of anesthesia to 1.5%. Animals were mounted to the stereotactic device and prepared for bilateral microinjection of vigabatrin or vehicle (sterile distilled water; Braun Melsungen; Germany). Stereotactic coordinates (in mm relative to bregma according to the atlas of Paxinos and Watson (2007)) for bilateral focal injection were as follows. STN: posterior, 3.5; lateral, ±2.6; and ventral, 8.0 mm. Anterior SNr: posterior, 5.0; lateral, ±2.0; and ventral, 8.3 mm. These coordinates were based on preliminary experiments in a large number of age-matched female Wistar rats with subsequent histological verification of injection site. Microinjection was performed during anesthesia using stainless-steel cannulae (outer diameter 350 μm, inner diameter 150 μm) connected to a 0.5-μl Hamilton syringe via about 30 cm of flexible tubing, which allowed to control the injection volume by the movement of a small air bubble in the tubing (for details see Gernert and Löscher, 2001). One minute after inserting the injection cannulae, 10 μg vigabatrin in 0.25 μl distilled water (or the same volume of distilled water in controls) were infused per hemisphere over a period of 4 min; the small injection volume (0.25 μl) was chosen to minimize volume effects, particularly in a relatively small region such as the STN. Following microinjection, the injection cannulae were left in place for 1 min and controlled for permeability after removal from the brain. Four and 24 h following injection of vigabatrin or distilled water, rats were killed for determination of vigabatrin, GABA, GABA-T and the GABA-synthesizing enzyme glutamic acid decarboxylase (GAD) in the target region and adjacent tissue (see below). In preliminary experiments we had excluded that isoflurane anesthesia exerts any effect on brain GABA levels (not illustrated).

We had recently shown that bilateral injection of 10 μg vigabatrin into either STN or anterior SNr significantly increases the PTZ seizure threshold for 48 h with a maximum increase after 24 h (Bröer et al., 2012), so that these experiments were not repeated. However, in an additional experiment we determined whether unilateral injection of 10 μg vigabatrin into STN also increases seizure threshold. Apart from the STN, the anterior rather than the posterior SNr was used in both acute and chronic experiments, because our recent study had shown that vigabatrin injection into the anterior SNr is more effective to enhance seizure threshold (Bröer et al., 2012).

2.3. Continuous administration of vigabatrin

Based on the data from the acute experiments (see Results), we decided to start the chronic experiments with a daily dose of 10 μg vigabatrin per hemisphere, using a flow rate of 0.2 μl/h. Subsequent experiments were performed with bilateral doses of 5, 1, and 0.1 μg/day. In vehicle controls, mock cerebrospinal fluid (CSF) was infused. A novel type of battery-powered implantable and programmable infusion pump (iPRECIO®, Model SMP-200; Data Sciences International [DSI], MC s-Hertogenbosch, The Netherlands) for small laboratory animals with a reservoir volume of 900 μl was used (Abe et al., 2009). The precision of these pumps has been demonstrated previously (Abe et al., 2009) and their advantages for CNS drug administration have been recently shown (Yamato et al., 2014; Matsubara et al., 2015). Because the pumps are refillable, they could be used for several experiments after cleaning and disinfection; for the present experiments each pump was used for a maximum of three rats and only for either drug or vehicle experiments. Battery life time of the pumps is 6 months.

Before starting with the pump experiments, we determined whether vigabatrin solutions as used in the pumps are stable over 5 weeks. Vigabatrin was dissolved in mock-CSF at concentrations of 0.4 and 4 mg/ml, respectively. pH (7.2–7.3) and osmolarity (310 mOsm) of the vigabatrin solution were adjusted to the physiological values of the brain's extracellular fluid. The solutions were stored at 37 °C and the concentration of vigabatrin and pH of the solution were measured once per week (see below). These experiments showed that vigabatrin solutions are stable under these conditions (Table 1). In order to evaluate whether vigabatrin solutions are also stable in the implanted pumps, we determined the drug in the pump at the end of each experiment, substantiating the stability of the drug solution (not illustrated).

For the chronic experiments, pumps were filled with the respective vigabatrin solution (which was freshly prepared before each experiment) and maintained at 37 °C for 24 h. Under anesthesia with isoflurane, pump connection cannulae (outer diameter 360 μm, inner diameter 180 μm; L-shaped stainless steel tube topped with a pedestal, length under pedestal 9 mm; Model 328OP, Plastics One Inc., Roanoke, USA) were bilaterally implanted into either STN or anterior SNr, using