Experimental Neurology 234 (2012) 70–84

Contents lists available at SciVerse ScienceDirect

Experimental Neurology

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

Inter-individual variation in the anticonvulsant effect of phenobarbital in the pilocarpine rat model of temporal lobe epilepsy

Marion Bankstahl [1], Jens P. Bankstahl [1], Wolfgang Löscher *

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

ARTICLE INFO

Article history:
Received 8 July 2011
Revised 2 December 2011
Accepted 9 December 2011
Available online 19 December 2011

Keywords:
Antiepileptic drugs
Refractory seizures
Hippocampal damage
Seizure severity

ABSTRACT

Despite a large therapeutic arsenal of old and new antiepileptic drugs (AEDs), there remains a substantial unmet need for the patients with refractory (AED-resistant) epilepsy. Animal models of refractory epilepsy are needed for at least two goals; (1) better understanding of the mechanisms underlying resistance to AEDs, and (2) development of more efficacious AEDs for patients with refractory seizures. It is only incompletely understood why two patients with seemingly identical types of epilepsy and seizures may respond differently to the same AED. Prompted by this well-known clinical phenomenon, we tested whether epileptic rats from the same epilepsy model respond differently to AEDs and previously discovered phenobarbital (PB) responsive and resistant animals in groups of rats in which epilepsy had been induced by sustained electrical stimulation of the basolateral amygdala (BLA). In the present study, we used the same approach for the widely used pilocarpine model of temporal lobe epilepsy. Epileptic rats from this model were continuously video/EEG monitored over seven consecutive weeks, starting with a predrug control period of two weeks, then two weeks of daily treatment with PB at maximum tolerated doses, and finally a postdrug control period of three weeks. In those rats that were included in response selection, 50% did not adequately respond to PB, whereas PB significantly decreased seizure frequency and severity in another 50% of the animals. Responders and nonresponders did not differ in predrug seizure frequency, PB plasma levels or hippocampal neurodegeneration, but behavioral differences were observed in anxiety models. These findings demonstrate that in the pilocarpine model, similar to epilepsy patients, epileptic rats differ in their response to an AED, which is most likely due to as yet unknown genetic factors.

Introduction

Despite the development of various new antiepileptic drugs (AEDs) over the recent 20 years, the available evidence indicates that the efficacy of drug treatment of epilepsy has not substantially improved, but that still about 30–40% of patients suffer from AED-resistant seizures (Bialer and White, 2010; Kwan and Brodie, 2006; Löscher and Schmidt, 2011; Perucca et al., 2007). Thus, there is a need to identify and incorporate animal models of refractory epilepsy into preclinical development of new AEDs (Löscher, 2006, 2011; White et al., 2006). This idea is not new (Löscher, 1986) but, surprisingly, has not been fully appreciated for almost two decades. Based on the operational definition of AED resistance in patients with epilepsy (Kwan et al., 2010), the term "pharmacoresistant" applied in the context of animal models can be defined as persistent seizure activity not responding or with very poor response to monotherapy with at least two current AEDs at maximum tolerated doses (Stables et al., 2003). Several models which fulfill this definition have been developed in the last 20 years (Löscher, 2006, 2011). In this respect, two different approaches have been employed. One is to use models of seizures or epilepsy, such as the 6-Hz seizure model in mice, that per se are resistant to antiepileptic effects of AEDs such as phenytoin (Bialer and White, 2010; Löscher, 2011). The other approach, which has been initiated by our group in the early 1990s, is to use chronic epilepsy models such as kindling or post-status epilepticus (SE) models of temporal lobe epilepsy (TLE) and select animals which either respond or do not respond to AED treatment from such models (cf., Löscher, 2006, 2011). Repeated treatment of large groups of amygdala-kindled rats with phenytoin resulted in the discovery and characterization of the phenytoin-resistant kindled rat (Löscher and Rundfeldt, 1991), in which resistance extends to various other major AEDs (Löscher, 2006). Similarly, prolonged treatment with phenobarbital (PB) in rats that developed epilepsy after a SE induced by sustained electrical stimulation of the basolateral amygdala (BLA) resulted in the discovery and characterization of

Abbreviations: AED, antiepileptic drug; ANOVA, analysis of variance; BLA, basolateral nucleus of the amygdala; EEG, electroencephalogram; NeuN, neuronal nuclear antigen; PB, phenobarbital; SE, status epilepticus; SRS, spontaneous recurrent seizures; TLE, temporal lobe epilepsy; Pgp, P-glycoprotein.

* Corresponding author at: Department of Pharmacology, Toxicology and Pharmacy, University of Veterinary Medicine, Bünteweg 17, D-30559 Hannover, Germany. Fax: +49 511 953 8581.
E-mail address: wolfgang.loescher@tiho-hannover.de (W. Löscher).

[1] Contributed equally to this study.

0014-4886/$ – see front matter © 2011 Published by Elsevier Inc.
doi:10.1016/j.expneurol.2011.12.014

the PB-resistant epileptic rat, in which resistance extends to phenytoin (Bethmann et al., 2007; Brandt et al., 2004). The advantage of these models is that the mechanisms of AED-resistance can be explored by directly comparing AED-nonresponders and -responders in the same model. By using this approach, we demonstrated that AED-resistance is a multifactorial phenomenon in epileptic rats (Löscher, 2011).

One of the most widely used chronic models of TLE is the pilocarpine model in rats (Curia et al., 2008). In this model, prolonged administration of levetiracetam via osmotic minipumps resulted in a large inter-individual variation in drug response (Glien et al., 2002). About 40% of the epileptic rats were responders with complete or almost complete control of spontaneous seizures, another 40% were nonresponders, and the remaining rats could not clearly be included in either group because of variation between pre- and postdrug control seizure frequency. However, based on the restricted dose range that can be administered via osmotic minipumps, it was not clear whether the levetiracetam nonresponders would have responded at higher doses of this AED. In the present study in the pilocarpine model, we evaluated whether epileptic rats in this model respond differently to prolonged treatment with PB at maximally tolerated doses, using the same dosing protocol by which responders and nonresponders had previously been selected from an electrically induced post-SE model of TLE (Brandt et al., 2004). The aims of our study were to investigate (1) whether PB responders and nonresponders can be selected from the pilocarpine model; (2) whether these subgroups differ in the severity of epilepsy; (3) whether these subgroups differ in anxiety models; and (4) whether these subgroups exhibit differences in hippocampal damage.

Materials and methods

Animals

Thirty-two female Sprague–Dawley rats were purchased from Harlan Netherlands (Horst, Netherlands) at an age of 9 weeks (body weight of 200 to 220 g) and kept under controlled environmental conditions (23 ± 1 °C; 50–60% humidity; 12-h light/dark cycle; light on at 6:00 a.m.) with free access to standard laboratory chow (Altromin 1324 standard diet, Altromin Spezialfutter GmbH, Lage, Germany) and tap water. Female rats were used to allow comparing the present data with those of our previous studies with pilocarpine and the BLA SE models in rats (e.g., Bethmann et al., 2007; Brandt et al., 2004; Glien et al., 2001; 2002). Female rats were housed without males in order to keep them acyclic or asynchronous with respect to their estrous cycle (cf., Kücker et al., 2010; Rattka et al., 2011). Furthermore, in recent, yet unpublished studies in the pilocarpine and intrahippocampal kainate models of post-SE TLE in 44 female Sprague–Dawley rats, we did not determine any significant relationship between SE induction and estrous cycle (Marta Rattka, Katrin Becker and Kathrin Töllner, unpublished data). Previous data from the kindling model of TLE indicated that the estrous cycle does not affect seizure threshold or response of seizures to AEDs (Rundfeldt et al., 1990; Wahnschaffe and Löscher, 1992). However, we cannot rule out that the estrous cycle has an effect on the occurrence of spontaneous seizures in the model used in the present study, so that we examined whether SRS exhibit any cyclicity that resembles the estrous cycle (see Results).

Before being used in the experiments, the rats were allowed to adapt to the new conditions for ≥1 week. All experiments were done in accordance with the European Communities Council Directive of November 24, 1986 (86/609/EEC) and were formally approved by the animal subjects review board of our institution. All efforts were made to minimize pain or discomfort as well as the number of animals.

Electrode implantation and SE induction

Electrodes were stereotactically implanted in all 32 rats (including 8 controls) into the right dentate gyrus of the hippocampus (AP −3.9; L −1.7; V −3.5, according to the atlas of Paxinos and Watson (2007)) under anesthesia with chloral hydrate (360 mg/kg, i.p.) and served for the recording of the electroencephalogram (EEG). To prevent postoperative infection, rats were treated with marbofloxacin (3 mg/kg s.c., twice daily) for 7 days starting two days before electrode implantation. After 2 weeks of post-surgical recovery, lithium chloride (127 mg/kg p.o.) was administered 16 h before pilocarpine treatment in 24 rats. In order to ensure the occurrence of SE and decrease mortality, individual dosing of pilocarpine was performed by ramping up the dose until onset of SE as described previously (Glien et al., 2001). For this purpose, pilocarpine was administered i.p. at a dose of 10 mg/kg every 30 min until the onset of a SE, consisting of ongoing limbic or generalized convulsive seizure activity. Methyl-scopolamine (1 mg/kg i.p.) was administered 30 min prior the first pilocarpine injection to prevent peripheral adverse effects of pilocarpine. The total number of pilocarpine injections was limited to five injections per animal.

Convulsive SE with generalized seizures could be induced in 18 (75%) of the 24 rats without mortality. The average dose of pilocarpine for inducing a convulsive SE was 32.8 ± 11.8 mg/kg (mean ± SD; range 10–50 mg/kg). Only rats that developed a self-sustained SE with generalized convulsive seizures were used for further experiments. Based on a previous study (Bankstahl and Löscher, 2008), SE was interrupted after 90 min by a combination of diazepam (10 mg/kg i.p., twice within a 10 min interval) and PB (25 m/kg i.p.). All rats were closely observed (including the EEG) during SE and in the hours after SE. Following SE, all rats were fed with baby food and injected with saline (4 ml i.p.) over a couple of days until they resumed normal feeding behavior.

Five of the 18 SE rats lost their electrode head assembly during subsequent weeks and were therefore excluded from the study. Starting 9–11 weeks after SE, the remaining 13 rats were continuously video- and EEG-monitored for selection of responders and nonresponders by prolonged treatment with PB (see below). One rat (EU 15) exhibited no seizure during the recording periods and was therefore excluded from any further evaluation. Eight electrode-implanted rats received all drugs except pilocarpine and served as a non-epileptic control group.

Treatment with phenobarbital

A schematic illustration of the experimental protocol is shown in Fig. 1. PB was chosen because it is an efficacious AED in rat models of TLE with an adequate elimination half-life in female Sprague–Dawley rats (16.9 ± 1.43 h; Brandt et al., 2004). As recently described, a dosing protocol with maximum tolerated doses of PB, leading to maintenance of plasma drug concentrations within or above the therapeutic range (10–40 μg/ml; Baulac, 2002) was established for female Sprague–Dawley rats in our lab (Brandt et al., 2004). Based on these experiments and previous selection trials in groups of epileptic rats (Bethmann et al., 2007; Brandt et al., 2004), treatment with PB was started by an i.p. bolus of 25 mg/kg PB in the evening, followed 14 h later by 15 mg/kg i.p., and then twice daily 15 mg/kg i.p. with an interval of 10 to 14 h in between. Before onset of drug treatment, baseline seizure frequency was determined over a 13.5 day period (predrug control), then PB was administered over a 14 day period, followed by a postdrug control period of 21 days, which started 24 h after the last PB administration to allow for some elimination of PB. In this way, each animal served as its own control, accounting for differences between animals, e.g., variability in baseline seizure frequency. The non-epileptic control rats received the same drug treatment but were not video-/EEG-monitored (see below).

Because the epileptic rats were extremely difficult to handle, i.p. administration of PB was performed under short CO_2 anesthesia (Kohlensäurewerk Hannover EG, Laatzen, Germany). We did not observe any EEG alterations or seizures provoked by this twice daily procedure. During pre- and postdrug control periods, we abstained