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Antiepileptic efficacy of lamotrigine in phenobarbital-resistant and -responsive epileptic rats: A pilot study

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Summary About 25% of patients with epilepsy are refractory to treatment, so that new, more effective antiepileptic drugs (AEDs) are urgently needed. Animal models that simulate the clinical situation with individuals responding and not responding to treatment are important to determine mechanisms of AED resistance and develop novel more effective treatments. We have previously developed and characterized such a model in which spontaneous recurrent seizures (SRS) develop after a status epilepticus induced by sustained electrical stimulation of the basolateral amygdala. In this model, prolonged treatment of epileptic rats with phenobarbital (PB) results in two subgroups, PB responders and PB nonresponders. When PB nonresponders were treated in previous experiments with phenytoin (PHT), 83% of the PB-resistant rats were also resistant to PHT. In the present study we examined if rats with PB resistant seizures are also resistant to lamotrigine (LTG), using continuous EEG/video recording of spontaneous seizures over 10 consecutive weeks. For this purpose, a new group of epileptic rats was produced and selected by treatment with PB into responders and nonresponders. As in previous studies, PB nonresponders had a significantly higher seizure frequency before onset of treatment. During subsequent treatment with LTG, all PB nonresponders and 60% of the PB responders exhibited

Abbreviations: AED, antiepileptic drug; ANOVA, analysis of variance; BLA, basolteral amygdala; EEG, electroencephalogram; HPLC, high-performance liquid chromatography; LTG, lamotrigine; NIH, National Institutes of Health; PB, phenobarbital; PHT, phenytoin; SE, status epilepticus; SRS, spontaneous recurrent seizures; TLE, temporal lobe epilepsy.

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>75% reduction of seizure frequency and were therefore considered as LTG responders. Plasma levels of LTG did not differ significantly between responders and nonresponders. The data of this pilot study indicate that LTG is more effective than PHT to suppress seizures in PB nonresponders in this model, but that not all PB responders also respond to LTG. Overall, our data provide further evidence that AED studies in post-SE TLE models are useful in determining and comparing AED efficacy and investigating predictors and mechanisms of pharmacoresistance.

Introduction

Resistance to antiepileptic drugs (AEDs) remains one of the major problems in the treatment of epilepsy, affecting about a quarter of patients with seizures (Kwan et al., 2011; Löscher and Schmidt, 2011; Simonato et al., 2012). Animal models of pharmacoresistant epilepsy can provide an important tool to help understanding the molecular basis underlying therapy resistance to AEDs and to develop new, more effective treatments (Löscher, 2006, 2011; Galanopoulou et al., 2012; Potschka, 2012). According to Kwan et al. (2010), drug resistant epilepsy is defined as failure of adequate trials of two tolerated, appropriately chosen and used AED schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom, so that animal models of pharmacoresistant epilepsy should meet this definition. In 2004, we reported that the AED phenobarbital (PB) differs markedly in its antiepileptic effects in rats with spontaneous recurrent seizures (SRS) that developed after a status epilepticus (SE) induced by sustained electrical stimulation of the basolateral amygdala (BLA; Brandt et al., 2004). PB was chosen for these experiments because its long half-life in rats allows maintenance of high plasma levels during chronic treatment. In a prospective trial in epileptic rats, in which SRS were continuously (24/7) recorded by video-EEG over a period of 2 weeks before onset of PB treatment (predrug control), followed by treatment with maximal tolerated doses of PB for 2 weeks, and then a 2-week postdrug control period, 64% of the rats responded to treatment with complete control or >90% reduction of seizures, while 36% did not respond and were therefore considered PB-resistant (Brandt et al., 2004). This finding was reproduced in a subsequent trial with PB in another group of epileptic rats (Bethmann et al., 2007), in which we found that 83% of the PB-resistant rats were also resistant to phenytoin (PHT), thus demonstrating that the AED-resistant rats of our model of temporal lobe epilepsy (TLE) meet the definition of pharmacoresistance in animal models, that is, persistent seizure activity not responding to at least two AEDs at maximum tolerated doses (Stables et al., 2003).

Alternative monotherapy, as performed in our study with PB and PHT in epileptic rats (Bethmann et al., 2007), is a relatively effective method in patients with seizures uncontrolled with a single AED. Probabilities of achieving seizure freedom by monotherapy with an alternative AED have been variably estimated at 44% (Tanganelli and Regesta, 1996), 34% (Hakkarainen, 1980), 32% (Mohanraj and Brodie, 2005), 24% (Kwan and Brodie, 2000a), 17% (Kwan and Brodie, 2000b), and 14% (Beghi et al., 2003), variability in reported rates being probably related to differences in patients' characteristics, choice of AED, dosing strategies, duration of follow-up and, not least, small sample size in most studies (Beghi et al., 2003). In our study in epileptic rats, 17% of the PB nonresponders responded to PHT, which is within the efficacy range reported for alternative monotherapy in clinical trials. In the present study, we performed a third prospective trial in our post-SE TLE model and examined the antiepileptic efficacy of lamotrigine (LTG) in rats with seizures uncontrolled by PB. For comparison, rats responding to PB were also treated with LTG. LTG was chosen because it was the most effective AED in patients with partial epilepsy in a large long-term observational study (Mohanraj and Brodie, 2005).

Materials and methods

Animals

As in our previous experiments in rats with SRS developing after SE induced by prolonged electrical stimulation of the BLA (Brandt et al., 2004; Bethmann et al., 2007), adult female Sprague-Dawley rats (Harlan-Winkelmann, Borchen, Germany) were used for this study. Following arrival in our laboratory, the rats were kept under controlled environmental conditions (22—24 °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, Lage, Germany) and tap water. Rats were maintained individually in transparent Makrolon polycarbonate type III cages (38 cm × 22 cm × 20 cm) without environmental enrichment. Transfer to new cages was once per week. 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). In previous studies of our group in female rats, no relationship between estrous cycle and susceptibility to seizure induction or frequency of spontaneous seizures was observed (Bankstahl et al., 2012; Rattka et al., 2013).

All rats were adapted to the laboratory and habituated to handling and injections for at least one week before starting the experiments. All animal experiments were carried out in accordance with the European Communities Council Directive of 24. November 1986 (86/609/EEC) and were formally approved by the animal subjects review board of our institution. All efforts were made to minimize the number of animals used and their suffering.

Electrode implantation and SE induction

Electrodes were stereotactically implanted into the right anterior BLA under anaesthesia as described in detail