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Evaluation of the pentylenetetrazole seizure threshold test in epileptic mice as surrogate model for drug testing against pharmacoresistant seizures

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ABSTRACT

Resistance to antiepileptic drugs (AEDs) is a major problem in epilepsy therapy, so that development of more effective AEDs is an unmet clinical need. Several rat and mouse models of epilepsy with spontaneous difficult-to-treat seizures exist, but because testing of antiseizure drug efficacy is extremely laborious in such models, they are only rarely used in the development of novel AEDs. Recently, the use of acute seizure tests in epileptic rats or mice has been proposed as a novel strategy for evaluating novel AEDs for increased antiseizure efficacy. In the present study, we compared the effects of five AEDs (valproate, phenobarbital, diazepam, lamotrigine, levetiracetam) on the pentylenetetrazole (PTZ) seizure threshold in mice that were made epileptic by pilocarpine. Experiments were started 6 weeks after a pilocarpine-induced status epilepticus. At this time, control seizure threshold was significantly lower in epileptic than in nonepileptic animals. Unexpectedly, only one AED (valproate) was less effective to increase seizure threshold in epileptic vs. nonepileptic mice, and this difference was restricted to doses of 200 and 300 mg/kg, whereas the difference disappeared at 400 mg/kg. All other AEDs exerted similar seizure threshold increases in epileptic and nonepileptic mice. Thus, induction of acute seizures with PTZ in mice pretreated with pilocarpine does not provide an effective and valuable surrogate method to screen drugs for antiseizure efficacy in a model of difficult-to-treat chronic epilepsy as previously suggested from experiments with this approach in rats.

1. Introduction

Despite the need for more effective antiepileptic drugs (AEDs) for about 30% of all patients with treated epilepsy with seizures that are resistant to currently available drugs, only a few animal models of pharmacoresistant seizures are routinely used in preclinical AED development [1,2]. These include the 6-Hz mouse psychomotor seizure model and the lamotrigine-resistant kindled rat, which are part of the identification and initial differentiation phases of the National Institute of Neurological Disorders and Stroke (NINDS)-funded Anticonvulsant Screening Program (ASP) in the U.S. [2]. However, pharmacoresistant epilepsy in patients is characterized by spontaneous recurrent seizures (SRS), and several novel drugs that were effective against induced seizures in the 6-Hz or kindling models were not effective in patients with AED-resistant SRS [1]. A number of rodent models with difficult-to-treat SRS exist, including models in which epilepsy is induced by systemic administration of pilocarpine and kainate, but these models are not frequently used in the search for more effective AEDs, because studies to evaluate the antiseizure efficacy of drugs in such models are lengthy and expensive, not least because the frequency of SRS is variable [1–5].

Blanco et al. [6] presented the interesting idea that using acute seizure tests in epileptic rats might overcome the limitations associated with continuous monitoring of SRS in models of chronic epilepsy. By using the pilocarpine model of temporal lobe epilepsy (TLE) in rats, they reported that the clinically established AEDs valproate, phenytoin, and phenobarbital are less effective to suppress seizures induced by pentylenetetrazole (PTZ) in epileptic rats compared with that in nonepileptic controls, suggesting that the pharmacoresistance of SRS in this model translates to a lowered antiseizure efficacy of AEDs on PTZ-induced acute seizures, so that these induced seizures may serve as a surrogate model for difficult-to-treat spontaneous seizures [6]. If

Abbreviations: AED, antiepileptic drug; ASP, Anticonvulsant Screening Program; EEG, electroencephalogram; MES, maximal electroshock seizure; NINDS, National Institute of Neurological Disorders and Stroke; NMRI, Naval Medical Research Institute; PTZ, pentylenetetrazole; SE, status epilepticus; SRS, spontaneous recurrent seizures; TLE, temporal lobe epilepsy.

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this finding can be reproduced and extended to more recent AEDs, the induction of acute seizures with PTZ in animals pretreated with pilocarpine might constitute an effective and valuable surrogate method to screen novel compounds and to study mechanisms involved in pharmacoresistant TLE.

In the present study, we evaluated whether the findings of Blanco et al. [6] can be reproduced in the pilocarpine model of TLE in mice. Instead of using one fixed dose (50 mg/kg s.c.) of PTZ as in the studies of Blanco et al. [6], we determined the individual PTZ seizure threshold before and after drug treatment by timed i.v. infusion of the convulsant as previously reported [7–10], which is more sensitive to determine antiseizure effects of drugs than the traditional s.c. PTZ test [1,8]. The efficacy of 5 clinically established AEDs (valproate, phenobarbital, diazepam, levetiracetam, lamotrigine) to increase PTZ seizure threshold was studied in epileptic vs. nonepileptic mice. Phenytoin, which was one of the AEDs examined in the study of Blanco et al. [6], was not included because it does not increase the PTZ seizure threshold in mice [7].

2. Materials and methods

2.1. Animals

Naval Medical Research Institute (NMRI) mice were obtained from Charles River (Sulzfeld, Germany) at a weight of 20–22 g and adapted at least one week to the laboratory before being used in experiments. Experiments were performed according to the EU council directive 210/63/EU and the German Law on Animal Protection ("Tierschutzgesetz"). Ethical approval for the study was granted by an ethical committee (according to §15 of the Tierschutzgesetz) and the government agency (Lower Saxony State Office for Consumer Protection and Food Safety, LAVES) responsible for approval of animal experiments in Lower Saxony (reference number for this project: 09/1769). All efforts were made to minimize both the suffering and the number of animals. Animals were housed in groups of 10 under controlled conditions (temperature: 23 ± 1 °C, humidity: 50%–60%), under a 12-h light–dark cycle (lights on at 6:00 h). Standard laboratory chow (Altromin 1324 standard diet, Altromin Spezialfutter GmbH, Lage, Germany) and tap water were provided ad libitum. As in previous studies on the pilocarpine model in mice [9,11–13], female mice were used because they are easier to maintain and handle during prolonged experiments on chronic epilepsy. We did not study the estrous cycle of the female mice during the experiments because previous studies have shown that it does not affect the PTZ seizure threshold [14,15]. Furthermore, females were housed without males in order to keep them acyclic or asynchronous with respect to their estrous cycle. The experimental protocol of the present study is illustrated in Fig. 1.

2.2. Pilocarpine model of temporal lobe epilepsy in mice

Three groups with 160 mice in total were used for this study of which 110 were used for induction of status epilepticus (SE) and 50 as sham controls. As previously described for induction of SE by pilocarpine in NMRI mice [11], we used a ramping-up dosing protocol that allows a more individual dosing of pilocarpine compared with injection of the same high dose in each animal per group, thereby reducing interindividual variability in SE induction and mortality. In order to avoid peripheral cholinergic effects, methylscopolamine (1 mg/kg i.p.) was administered 30 min before the application of pilocarpine. For induction of seizures, 100 mg/kg pilocarpine were injected i.p. every 20 min until onset of SE. Status epilepticus was defined as continuous limbic seizure activity, which was occasionally interrupted by clonic forelimb seizures, generalized clonic–tonic seizures, or running and jumping seizures. The experiments were performed subsequently in three groups of mice with 30–40 animals per group (plus additional groups of 10–20 mice as sham controls) over a period of 10 months. In a total of 110 mice in which pilocarpine was injected, 108/110 (98%) animals developed SE. All mice that developed SE received diazepam (10 mg/kg i.p.) after 90 min of SE to decrease mortality. Despite receiving diazepam, 55/110 (50%) mice died during or after SE, so only 55 survivors could be used for further experiments. On the days following SE, mice received subcutaneous saline injections (1 ml/day) and moist chow to facilitate recovery.

About 6 weeks following SE, i.e., at a time where all mice have developed epilepsy with spontaneous recurrent seizures in this model [11], we started to test drugs for their effects on the PTZ seizure threshold (see below). Age-matched, sham-treated control mice, which received all treatments except pilocarpine (for which NaCl was repeatedly injected instead), were used in parallel for comparison. The epileptic mice (and age-matched controls) were repeatedly used in the PTZ seizure threshold test at intervals of at least one week. As previously shown, repeated determination of this seizure threshold at such intervals does not affect seizure threshold in this model [16].

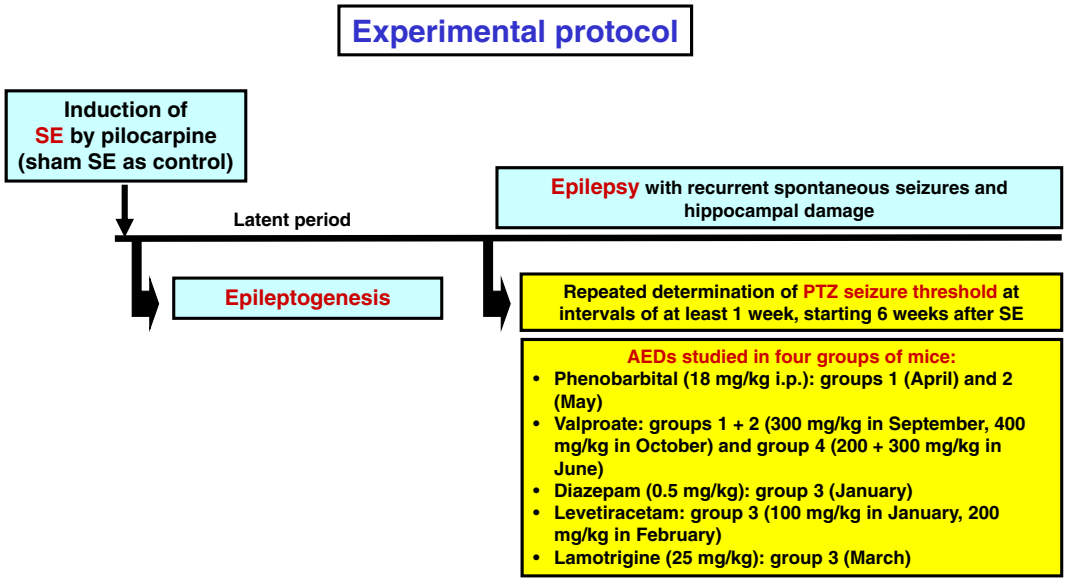

Fig. 1. Schematic illustration of the experimental protocol of the present study. In view of seasonal variation in seizure thresholds and drug effects on seizure thresholds (see text), the month in which an experiment was performed is indicated for the drug experiments.