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Status epilepticus induction has prolonged effects on the efficacy of antiepileptic drugs in the 6-Hz seizure model

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ABSTRACT

Several factors may influence the efficacy of antiepileptic drugs (AEDs) in patients with epilepsy, and treatment resistance could be related to genetics, neuronal network alterations, and modification of drug transporters or targets. Consequently, preclinical models used for the identification of potential new, more efficacious AEDs should reflect at least a few of these factors. Previous studies indicate that induction of status epilepticus (SE) may alter drug efficacy and that this effect could be long-lasting. In this context, we wanted to assess the protective effects of mechanistically diverse AEDs in mice subjected to pilocarpine-induced SE in another seizure model. We first determined seizure thresholds in mice subjected to pilocarpine-induced SE in the 6-Hz model, 2 weeks and 8 weeks following SE. We then evaluated the protective effects of mechanistically diverse AEDs in post-SE and control animals.

No major differences in 6-Hz seizure susceptibility were observed between control groups, while the seizure threshold of pilocarpine mice at 8 weeks after SE was higher than at 2 weeks and higher than in control groups. Treatment with AEDs revealed major differences in drug response depending on their mechanism of action. Diazepam produced a dose-dependent protection against 6-Hz seizures in control and pilocarpine mice, both at 2 weeks and 8 weeks after SE, but with a more pronounced increase in potency in post-SE animals at 2 weeks. Levetiracetam induced a potent and dose-dependent protection in pilocarpine mice, 2 weeks after SE, while its protective effects were observed only at much higher doses in control mice. Its potency decreased in post-SE mice at 8 weeks and was very limited (30% protection at the highest tested dose) in the control group. Carbamazepine induced a dose-dependent protection at 2 weeks in control mice but only limited effect (50% at the highest tested dose) in pilocarpine mice. Its efficacy deeply decreased in post-SE mice at 8 weeks after SE. Perampanel and phenytoin showed almost comparable protective effects in all groups of mice.

These experiments confirm that prior SE may have an impact on both potency and efficacy of AEDs and indicate that this effect may be dependent on the underlying epileptogenic processes.

This article is part of a Special Issue entitled "Status Epilepticus".

1. Introduction

Treatment-resistant epilepsy remains a major therapeutic challenge, and different hypotheses have been proposed to explain the mechanisms underlying this phenomenon [1,2]. Alterations in drug targets or transporters and impact of intrinsic seizure severity have been linked with the decreased efficacy of antiepileptic drugs (AEDs) [3]. In this respect, preclinical assessment of AEDs in naïve experimental animals may be questionable since it does not show the pathophysiological changes associated with epilepsy [2]. Previous reports suggested a potential influence of the inciting event leading to epilepsy development, e.g., traumatic brain injury (TBI) or early-life seizures, on the efficacy of anticonvulsant treatments evaluated at later time points [4,5]. This led to the development of the so called "two-hit model" approach, which was described in a handful of experimental studies [6–9].

Post-status epilepticus (SE) models are characterized by a high level of resistance to AEDs [10]. The induction of SE by means of an acute injection of pilocarpine in both mice [11] and rats [12] is increasingly recognized as a valuable tool to study the pathophysiological changes associated with temporal lobe epilepsy (TLE), which is one of the major treatment-resistant types of epilepsy [13]. The differences in seizure susceptibility of both mice and rats following pilocarpine- or kainate-induced SE have been assessed in different acute seizure models [9,14,15]. Interestingly, both the potency and the efficacy of AEDs were altered under these experimental conditions compared with naïve animals [9]. Therefore, a "two-hit model" approach using

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post-SE animals and then testing AEDs with another seizure model could help evaluate the efficacy and potency of AEDs in an experimental setting that more closely resembles clinical conditions. This could lead to the development of future treatments that may be active against drug-resistant epilepsies.

In this work, we evaluated the seizure susceptibility of pilocarpine-treated mice using the 6-Hz seizure model during early and late time points after SE induction, namely, 2 weeks post-SE and 8 weeks post-SE, respectively. In the next step, using the same model, we compared the anticonvulsant efficacy of several clinically used AEDs in post-SE mice and matched controls (sham mice) at these selected time points. Similar experiments were previously performed [9], but drug testing was done only at one time point (5 months post-SE), so a potential progressive fluctuation in AED efficacy was not studied.

2. Materials and methods

2.1. Animals

Male NMRI mice (28–35 g) (Charles River, France) were used for all experiments. They were maintained on a 12/12-h light/dark cycle with lights on at 06:00 h and had free access to standard laboratory chow and tap water. The temperature in the husbandry was maintained at 20–21 °C, and the humidity was maintained at about 40%. All procedures were carried out according to the Declaration of Helsinki and were conducted according to the guidelines of the European Community Council Directive 2010/63/EU. A local Ethics Committee approved all experimental protocols.

2.2. Induction of SE in mice

A dose of 300 mg/kg of pilocarpine (Sigma), selected as optimal for induction of SE with minimal lethality [11], was injected intraperitoneally (ip), following ip administration of N-methylscopolamine bromide (1 mg/kg), with the latter being known to limit the peripheral muscarinic effects due to pilocarpine injection [16,17]. Behavioral observations were performed during SE, and the occurrence of the different seizure stages was scored according to Racine's scale [18]. The so called SE state typically appeared within the first hour following pilocarpine injection and was characterized by recurrent generalized convulsive seizures associated with loss of the righting reflex. Diazepam (10 mg/kg) was administered ip in order to limit the duration of the SE state to a maximum of 3 h. Control mice (sham groups) were subjected to the same experimental conditions but received vehicle instead of pilocarpine. After the completion of the behavioral observations during SE, the mice were single-housed with rodent nesting material (Envirodri®, Shepherd Specialty Papers) as environmental enrichment. Sham mice were also single-housed using the same environmental enrichment material in order to ensure fully comparable experimental conditions. Naïve mice, group-housed in standard experimental conditions, were also used for comparison.

2.3. Induction of 6-Hz focal seizures

The mice were stimulated through corneal electrodes connected to a stimulator (ECT Unit 57800, Ugo-Basile, Comerio, Italy). A drop of Unicaïne (0.4% oxybuprocainum in saline) was placed on the eyes before the stimulation to induce local anesthesia and ensure good conductivity. During the stimulation, each mouse was manually restrained and then gently released into a plastic bowl (diameter = 19 cm) immediately after the current application. The seizures were often preceded by a brief period (~2–3 s) of locomotor agitation (running and jumping). The animals then exhibited immobility associated with rearing, automatisms, forelimb clonus, twitching of the vibrissae, and, sometimes, Straub tail. The animals were observed for 30 s following the electrical stimulation. The main seizure endpoint was the duration of immobility. Mice resuming a normal behavior within 7 s after the end of the stimulation were considered as not displaying the seizure behavior.

For determination of the seizure threshold, an injection of saline was performed 30 min before application of the stimulus. Different groups of mice (N = 8–14) were stimulated for 3 s using different current intensities ranging from 9 to 26 mA, increasing current steps by 25% each time. The proportion of animals showing a seizure behavior in each group was calculated, and the CS_{50} value (stimulation current inducing seizures in 50% of mice) was determined in post-SE and sham mice.

Each tested AED was injected ip at its optimal preadministration time [19,20]. Different groups of mice (N = 7–10) were used in both post-SE mice and matching sham groups, 2 weeks or 8 weeks after SE induction. The same AEDs were also evaluated in naïve mice. A 6-Hz electrical stimulation at 44 mA was applied for 3 s, and the number of mice protected against seizures was noted to calculate the proportion of protected mice at each dose, and ED_{50} values (doses predicted to protect 50% of animals) were determined.

2.4. Statistical analysis

Both CS_{50} and ED_{50} values and their 95% confidence intervals were determined by a nonlinear curve fitting performed with GraphPad Prism 4.0 software (GraphPad Software, Inc., La Jolla, CA, USA). Statistical comparison of ED_{50s} and CS_{50s} was performed with the same software using the sum of squares F-test.

3. Results

3.1. Seizure thresholds in the 6-Hz model at 2 weeks and 8 weeks post-SE

The seizure susceptibility in the post-SE and sham mice 2 weeks after SE was comparable with CS_{50} values of 13.1 mA and 13.9 mA, respectively (Fig. 1A). It is worth noting that this value in sham mice was significantly lower than the seizure threshold determined in naïve NMRI mice under the same experimental conditions [21]. At 8 weeks post-SE, the seizure threshold in sham mice was comparable (CS_{50} = 15.2 mA) with the one determined at 2 weeks. The threshold in post-SE mice at the 8-week time point tended to increase, with a CS_{50} value of 17.7 mA, but this was not statistically significant in comparison with the respective sham group (Fig. 1B).

3.2. The efficacy of AEDs in post-SE mice challenged with 6-Hz seizures

Phenytoin treatment induced very limited protective effects in both post-SE and sham mice at 2 weeks, with ED_{50} values >60 mg/kg (Fig. 2A). It was more potent in sham mice at 8 weeks post-SE (ED_{50} value of 32 mg/kg) and showed almost no protective effects up to 40 mg/kg in post-SE mice (Fig. 2B). This drug also showed limited efficacy in naïve NMRI mice, with only 60% protection observed at the high tested dose of 80 mg/kg (Table 1). Surprisingly, carbamazepine which also acts on sodium channels afforded a clear-cut dose-dependent protection against seizures in naïve mice with an ED_{50} value of 10 mg/kg (Table 1) and, to a lesser extent, in the two sham groups (Figs. 3A and B), with respective ED_{50} values of 27 and 25 mg/kg. Nearly complete loss of efficacy was observed in post-SE mice at 2 weeks and 8 weeks (Figs. 3A and B), and it was not possible to calculate ED_{50} values for this drug.

Levetiracetam was dose-dependently effective against 6-Hz seizures in naïve mice (Table 1) but drastically lost its protective effects in sham mice (Figs. 4A and B). Its potency in post-SE mice was very good at 2 weeks (Fig. 4A), while levetiracetam also showed limited protective effects (60% protection at the dose of 540 mg/kg) at 8 weeks post-SE (Fig. 4B).

Diazepam was also less potent in sham mice (Figs. 5A and B) in comparison with its potency in naïve mice (Table 1). This compound was