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Striking differences in proconvulsant-induced alterations of seizure threshold in two rat models

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ABSTRACT

During drug development, seizure threshold tests are widely used to identify potential proconvulsant activity of investigational drugs. The most commonly used tests in this respect are the timed intravenous pentylenetetrazole (PTZ) infusion seizure test and the maximal electroshock seizure threshold (MEST) test in mice or rats. To our knowledge, no study is available in which proconvulsant drug activities in these models are directly compared, which prompted us to perform such experiments in male Wistar rats. Five drugs with reported proconvulsant activity were tested in the two models: D-amphetamine, chlorpromazine, caffeine, theophylline, and tramadol. Furthermore, the anticonvulsant drug phenobarbital was included in the experiments. While phenobarbital exerted anticonvulsant activity in both models, the five proconvulsant drugs markedly differed in their effects. In the dose range tested, D-amphetamine significantly lowered the PTZ seizure threshold but increased the MEST, caffeine and theophylline did not alter the PTZ seizure threshold but decreased the MEST, and tramadol reduced the PTZ threshold but increased the MEST. These marked differences between seizure threshold tests are most likely a consequence of the mechanisms underlying seizure induction in these tests. Our data indicate that using only one seizure threshold model during preclinical drug development may pose the risk that potential proconvulsant activity of an investigational drug is overseen. However, the label "proconvulsant" may be misleading if such activity only occurs at doses high above the therapeutic range, but the drug is not proconvulsant or even exerts anticonvulsant effects at lower, therapeutically relevant doses.

1. Introduction

Serious injury and/or death of volunteers and patients participating in early clinical trials on investigational drugs are rare and thus very disturbing when it occurs (Bass et al., 2004). Seizures arising from the central nervous system (CNS) are among the most frequent severe adverse events, so that it is important to identify any proconvulsant or convulsant activity of investigational drugs prior to any human clinical trials (Porsolt et al., 2002; Bass et al., 2004; Löscher, 2009). As a consequence, detection of (pro)convulsant activity in animal models is an important part of safety pharmacology studies designed to test the potential adverse effects of a compound in the therapeutic range and above (Porsolt et al., 2002; Kumar et al., 2007). The term "proconvulsant" means that a drug may promote convulsions, e.g., in patients with epilepsy or in combination with other potentially proconvulsant drugs (Porsolt et al., 2002; Löscher, 2009). Proconvulsants, such as amphetamines, cocaine, several neuroleptics and opioids, and methylxanthines, typically decrease seizure threshold at subconvulsive doses but cause or produce convulsions at higher, convulsant doses (Löscher, 2009). Clinically, the terms "proconvulsant" and "convulsant" are often mixed or even used synonymously, because it is difficult to identify a drug-induced decrease in seizure threshold in humans, whereas induction of seizures, particularly in association with drug intoxication, is an easily recognizable event. Thus, in order to protect humans from the risk associated with proconvulsant drug effects, the potential of an investigational drug to decrease seizure threshold needs to be determined preclinically. The most commonly used tests in this respect are the timed intravenous pentylenetetrazole (PTZ) infusion seizure test and the maximal electroshock seizure threshold (MEST) test in mice or rats (Porsolt et al., 2002; Kumar

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et al., 2007; Löscher, 2009). Both seizure threshold models allow determining anti- as well as proconvulsant drug activities and are therefore particularly useful for safety pharmacology purposes (Porsolt et al., 2002). However, a recent review of the literature on proconvulsant drug effects in these models indicated that the same drugs may act very differently in these tests (Löscher, 2009). At least in part, this may be due to different drug doses, routes of drug administration, rodent strains, and various other technical factors when performing experiments on proconvulsant drugs in either the PTZ seizure threshold or MEST tests in different laboratories. Surprisingly, to our knowledge, a direct model comparison of the effects of diverse drugs with presumed proconvulsant activity is not available in the literature. Such a comparison would be important to judge which of these seizure threshold models is best suited to predict proconvulsant drug effects in humans. This prompted us to perform experiments in which we tested various proconvulsant drugs in the PTZ and MEST models at the same doses and pretreatment intervals in age-matched rats of the same strain (Wistar) and gender (male).

2. Materials and methods

2.1. Animals

Adult male Wistar Unilever rats (HsdCpb:WU) were obtained from Harlan-Winkelmann (Harlan Laboratories GmbH, Horst, Netherlands) at an age of 6 weeks. After arrival, animals were housed in groups under controlled conditions (temperature: 23 ± 0.5 °C; humidity: 50–60%), under a 12-h light–dark cycle (lights on at 6.00 a.m.). Standard laboratory chow (Altromin 1324 standard diet) and tap water were provided ad libitum. The animals were allowed to adapt to laboratory conditions for at least one week before starting the experiments. All experiments in each seizure threshold model were performed within the same 4-h time period per day, thereby minimizing circadian bias in the seizure threshold determinations. 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 both the suffering and the number of animals.

2.2. Drugs

The following compounds and doses were tested to assess their ability to modify seizure thresholds in the PTZ and MEST tests: phenobarbital, 20 mg/kg; D-amphetamine, 5 mg/kg; chlorpromazine, 3 mg/kg; caffeine, 60 and 80 mg/kg; theophylline, 30 and 50 mg/kg; tramadol, 2.5, 5, 10, and 20 mg/kg. Doses were based on the literature (cf., Löscher, 2009) and preliminary dose–response experiments with these drugs in rats. The aim of these preliminary experiments was to determine doses of proconvulsants that decrease seizure threshold in at least one seizure threshold test and then to use these doses for comparison of drug effects in the PTZ and MEST tests in larger groups of animals. For determination of control thresholds, rats received saline injections. All injections were performed i.p. Unless otherwise indicated, pretreatment time for saline or drug administration was 30 min before PTZ infusion or current application in the two seizure threshold models. Phenobarbital (sodium salt) was purchased from Serva (Heidelberg, Germany), D-amphetamine (sulfate), chlorpromazine (hydrochloride), caffeine and theophylline were purchased from Sigma–Aldrich (Taufkirchen, Germany). Tramadol was received from Grünenthal (Aachen, Germany). All compounds were freshly dissolved in isotonic saline prior to each experiment. Administration volume varied from 2 to 10 ml/kg, depending on the solubility of a given compound. All doses of drugs given in this study refer to the free acid or base of the respective drug.

2.3. Timed intravenous PTZ infusion seizure test

The timed i.v. PTZ infusion seizure model in unrestricted rats was performed essentially as described by Pollack and Shen (1985) except that we infused PTZ in the tail rather than the jugular vein. The threshold for PTZ-induced myoclonic and clonic seizures was determined by infusion of a 0.8% solution of PTZ via a 26 G needle into the lateral tail vein of conscious, freely moving (i.e., unrestricted) rats. The needle was secured to the tail vein by a piece of adhesive tape and the animal was permitted to move freely inside a Makrolon® cage type III. The needle was connected to a syringe by polyethylene tubing (Kleinfeld Labortechnik, Gehrden, Germany) and the PTZ solution was infused at a constant rate of 1.0 ml/min using an infusion pump (PHD 2000 Infusion, Harvard Apparatus Holliston, Massachusetts). The following two seizure types were recorded during PTZ infusion: (1) the first myoclonic twitch, and (2) the onset of the first clonic seizure of fore- and/or hindlimbs without loss of righting reflexes. Using cortical and hippocampal depth electrode recordings of the electroencephalogram (EEG), we have recently shown that the first myoclonic twitch during PTZ infusion was not associated with obvious paroxysmal EEG alterations, but convulsive discharges were observed shortly before and during the clonic seizure that immediately followed the myoclonic twitch (Rattka et al., 2011). Infusion was terminated at the time of this first clonic seizure to prevent the onset of tonic seizures and respiratory arrest. Nevertheless, several animals exhibited tonic seizures as well and some of these rats died thereafter. The threshold for each endpoint was calculated in mg/kg PTZ based on the time needed to induce the respective seizure endpoint, the body weight of the animal, and the rate of infusion and concentration of PTZ in the infusate.

For assessing pro- or anticonvulsant drug activity, each animal served as its own control, i.e., each rat was used at first for the determination of the individual control threshold after saline treatment and about 48 h later for seizure threshold determination after drug treatment. Pollack and Shen (1985) demonstrated that the PTZ pretest did not influence the results of the posttest when using an inter-test interval of 48 h. In a first series of the present experiments, a few rats were used for up to 8 seizure threshold determinations. To exclude changes in seizure threshold by means of previous drug administration or multiple PTZ infusions in such animals, the control threshold was determined again 48 h before each new drug experiment. In a separate experiment, six consecutive threshold determinations were performed in the same animals (n = 6–7) with intervals of at least 48 h between two consecutive threshold determinations to evaluate whether repeated seizure threshold determination leads to a change of seizure thresholds, e.g., by development of a kindling effect. In a second series of experiments, which was performed to study the reproducibility of drug effects in this model, each rat was used only twice, once for control threshold determination and 48 h later for drug threshold determination.

2.4. MEST test

The MEST was determined via bilateral transcorneal stimulation, using a stimulator (Witt GmbH, Berlin, Germany), which delivered a constant current (adjustable from 1 to 200 mA regardless of impedance of the tested rat; self-adjusting stimulus voltage max. 7000 V) with sinusoidal pulses (50 s^{-1}) for 0.2 s. To check the pattern of the applied stimulus, the stimulator was connected to an oscilloscope (DMM-Scope S2405, Reichelt