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Evaluation of pro-convulsant risk in the rat: Spontaneous and provoked convulsions

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ABSTRACT

Introduction: The aim of the present study was to evaluate the utility of different tests performed in the absence or presence of factors promoting seizures in order to evaluate the pro-convulsant effects of drugs. We studied the effects of theophylline in the rat since this is a well-known pro-convulsant substance in humans.
Methods: The occurrence of spontaneous convulsions following administration of theophylline was evaluated by observation in the Irwin Test and by measuring brain activity using video-EEG recording in conscious telemetered animals. Theophylline was also tested in the electroconvulsive shock (ECS) threshold and pentylenetetrazole (PTZ)-induced convulsions tests, two commonly used models of provoked convulsions.
Results: In the Irwin test, theophylline induced convulsions in 1 out of 6 rats at 128 mg/kg. Paroxysmal/seizure activity was also observed by video-EEG recording in 4 out of the 12 animals tested at 128 mg/kg, in presence of clonic convulsions in 3 out of the 4 rats. Paroxysmal activity was observed in two rats in the absence of clear behavioral symptoms, indicating that some precursor signs can be detected using video-EEG. Clear pro-convulsant activity was shown over the dose-range 32–128 mg/kg in the ECS threshold and PTZ-induced convulsions tests.
Discussion: Evaluation of spontaneous convulsions provides information on the therapeutic window of a drug and the translational value of the approach is increased by the use of video-EEG. Tests based on provoked convulsions further complement the evaluation since they try to mimic high risk situations. Measurement of both spontaneous and provoked convulsions improves the evaluation of the pro-convulsant risk of novel pharmacological substances.

1. Introduction

Despite the fact that ICHS7A (Anonymous, 2000) does not include studies of pro-convulsant activity in its recommendations for a core battery, we consider that such procedures can usefully be included at an early stage of the drug development process (Castagné et al., 2013), as convulsions represent a serious safety risk and can even be fatal (Hamdam et al., 2013).

Pro-convulsant drugs include both CNS stimulants and CNS depressants. Among stimulant substances, amphetamines, methylxanthines (theophylline) and PTZ may lower the seizure threshold at subconvulsive doses and produce convulsions at higher doses (see for review Loscher, 2009). It has also been reported that certain neuroleptics (chlorpromazine, clozapine), opioids (tramadol) or even some anti-convulsants (ketamine) may possess pro-convulsant activity (see for review Loscher, 2009). A biphasic (anti/pro-convulsant) effect can be observed for some substances which are anticonvulsant at low doses but display pro-convulsant effects at high doses, as for example carbamazepine (Perucca, Gram, Avanzini, & Dulac, 1998).

Clinically, the terms "pro-convulsant" and "convulsant" are often used without any clear distinction because it is ethically difficult to evaluate the effects of a pro-convulsant drug in humans (Loscher, 2009). In preclinical studies, pro-convulsant or convulsant risk may be evaluated by measurement of the occurrence of spontaneous or provoked convulsions. Spontaneous convulsions may be assessed by direct observation of the animals or by measuring brain activity via electroencephalography (EEG). The primary observation test described by Irwin is often used to evaluate the global behavioral effects and potential toxicity of a molecule (Irwin, 1968). It also permits to identify the highest dose that can be administered without inducing adverse effects, the active dose-range and the first convulsive dose. EEG, in contrast, possesses high translational validity and permits pro-convulsant and convulsant phenomena to be recorded continuously over longer periods of time.

A pro-convulsant drug may also facilitate the apparition of convulsions in combination with factors promoting seizures (see for review Easter et al., 2009). In pre-clinical studies, drug seizure liability is generally assessed in small animals such as the mouse or the rat following electrical stimulation (for example, the ECS threshold test) or administration

Abbreviations: CNS, Central Nervous System; DSI, Data Sciences International; ECS, Electroconvulsive shock; EEG, Electroencephalogram; GABA, Gamma-aminobutyric acid; PTZ, Pentylenetetrazole.

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of chemical agents with known convulsive effects (for example, the PTZ-induced convulsion test) (Porsolt, Lemaire, Durmuller, & Roux, 2002). Use of both electrically- and chemically-induced convulsions is recommended to assess the potential pro-convulsant activity of novel substances as the neurobiological mechanism involved in seizures induced by ECS and chemical agents are different (Bankstahl, Bankstahl, Bloms-Funke, & Loscher, 2012).

The aim of the present study was to evaluate the utility of different tests, either based on the observation of spontaneous convulsions or based on the facilitation of provoked convulsions, in the evaluation of the pro-convulsant profiles of drugs. Tests based on spontaneous convulsions seem relevant to the use of pharmacological substances in the general population whereas tests on provoked convulsions are closer to the use of substances in specific populations presenting increased risks. We firstly assessed the effects of a pro-convulsant substance in normal conditions, i.e. in absence of any factors promoting seizures, using the Irwin and video-EEG tests. We then assessed the same substance in tests of convulsions provoked electrically (ECS threshold test) or chemically (PTZ-induced convulsion). As prototypic positive control, we used theophylline, a well-known pro-convulsant in humans (Chrościńska-Krawczyk, Radzik, Miziak, & Czuczwar, 2014).

2. Methods

2.1. Animals

Experiments were carried out on male Wistar rats (Janvier-Labs, Le Genest-Saint-Isle, France) housed on wood litter and nesting material with free access to food and water up to administration of drugs. Animals were housed singly for EEG experiments and in groups of 3 or 5 by cage for the other tests. Animals weighed between 174 and 284 g on the testing day for the Irwin, ECS threshold and PTZ-induced convulsion tests and between 379 and 453 g on the testing days for video-EEG recordings. The animal house was maintained under artificial lighting (12 h with lights on at 7:00) with constant temperature and humidity. Experiments were performed during the light period of the day.

All procedures described in the present paper have been approved by the ethical committee of Porsolt S.A.S. The experiments were performed in accordance with French legislation and European Directive No 2010/63/UE concerning the protection of laboratory animals and in accordance with a currently valid license for experiments on vertebrate animals, issued by the French Ministry for Agriculture and Fisheries.

2.2. Treatment

Theophylline anhydrous (Sigma-Aldrich, France) was dispersed in 0.2% hydroxypropylmethyl-cellulose in physiological saline which served as vehicle. It was administered intraperitoneally (i.p.) as a single injection in a volume of 5 ml/kg body weight. The suspensions were prepared freshly on each testing day.

The doses of theophylline were selected on the basis of internal data obtained in epilepsy studies to evaluate pro-convulsant activity. Testing was performed blinded for the ECS threshold and PTZ-induced convulsion tests.

2.3. Primary observation (Irwin) test

The primary observation test in the rat was that described by Castagné et al. (2013). The rat method represents an adaptation of the method originally described by Irwin (1968) in the mouse. Theophylline was administered at 5 different doses (8, 16, 32, 64 and 128 mg/kg i.p.) immediately before the test. Two or 3 treated groups were observed in simultaneous comparison with a vehicle control group at each time (non-blind conditions). Observations were performed continuously from 0 to 15 min then at 15, 30, 60, 120 and 180 min after administration and also 24 and 48 h later. Behavioral modifications, physiological and neurotoxicity symptoms, rectal temperature and pupil diameter were recorded according to a standardized observation grid derived from that of Irwin. The different items were listed in the Fig. 1. Results obtained were reported in large categories related to effects on activity (sedation/excitation), on motor behavior, stereotypies, pain and autonomic signs. Six rats were studied per group.

2.4. Measurement of video-EEG

Rats were placed under isoflurane anesthesia (5% for induction and 2% for maintenance, under 100% O_2). Following a midline incision in the abdomen, a DSI CTA-F40 (Data Sciences International) implantable telemetric device was introduced into the peritoneal cavity and the bio-potential positive and negative leads were passed subcutaneously to emerge close to the skull. The abdominal and skin incisions were then sutured. Two depth electrodes, consisting of twisted platinum-iridium wires, were placed stereotaxically into the hippocampus CA1 area (Paxinos and Watson coordinates inter-aural: AP +5.0 mm, L ±2.5 mm, V +7.0 mm). The leads of the telemetry device were soldered to the electrodes and the whole assembly was secured on the skull with dental acrylate. The leads were coiled into a loop and anchored to surrounding tissue. To reduce pain and infection risk, rats were treated with carprofen (Rimadyl®) at a subcutaneous dose of 7.5 mg/kg prior to the surgery and with amoxicillin (100 mg/kg s.c.) after surgery. Animals returned individually to their home cages. Approximately 2 weeks later, the animals were placed individually in a testing cage placed in proximity of a telemetry receiver (DSI). Animals were also video-recorded in parallel. Theophylline was tested at 2 different doses (64 and 128 mg/kg i.p.). Each animal received increasing doses of theophylline with a washout period of 7 days between the treatments. Each week, the rats received an administration of vehicle (baseline session) on the first day followed by administration of theophylline on the second day (test session).

The animals were continuously recorded during 120 min after administration. All generated data were acquired and analyzed using the EMKA Technologies software (IOX version 2.8.2.10 and ECG-Auto version 2.6.0.20).

Paroxysmal activity was defined as an event including repeated spikes or spikes-and-waves of high amplitude (clearly above normal EEG signal) over at least one second in absence of clear convulsive symptoms. Seizure activity was defined as an event including repeated spikes or spikes-and-waves accompanied with convulsive symptoms (clonic or tonic convulsions).

Six animals were tested in parallel during each testing session and 12 rats were used in the experiment.

2.5. ECS threshold test

The test was performed according to the method described by Swinyard, Brown, & Goodman, 1952. Theophylline was administered at 4 different doses (16, 32, 64 and 128 mg/kg i.p.), 30 min before the test. Rats were administered ECS (rectangular current: 0.6 ms pulse width, 1.5 s duration, 200 Hz) via ear-clip electrodes connected to a constant current shock generator (Ugo Basile: Type 57800). The first animal of each group was exposed to 30 mA of ECS. Then, the intensity of the stimulation was decreased or increased for the next animal to be tested based on the response of the previous animal (up-and-down method). For example, the current was increased by 5 mA until the first tonic convulsion was observed. Then, the intensity was decreased by 2 mA for the next animal and was then decreased or increased by 2 mA from animal to animal depending on whether the preceding animal convulsed or not. Inversely, when the first animal convulsed, the current was decreased by 5 mA in the following animals until the absence of tonic convulsions was confirmed. Then, the intensity was increased by 2 mA for the next animal. This up-and-down method permits to rapidly approach the threshold current within a given group of rats. Fifteen rats were

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