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Perampanel chronic treatment does not induce tolerance and decreases tolerance to clobazam in genetically epilepsy prone rats

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ABSTRACT

Tolerance to some therapeutic effects of antiepileptic drugs may account for the development of pharmacoresistance in patients with epilepsy. In the present study, following oral acute pretreatment with the new antiepileptic drug perampanel (0.1, 0.3, 1 or 3 mg/kg), we observed that the drug significantly and dose-dependently attenuated the seizure phases (clonus and tonus) of audiogenic seizures in genetically epilepsy prone rats (GEPR-9 s), a genetic model of reflex generalized epilepsy. In addition, the GEPR-9 s were administered orally with perampanel (1 or 3 mg/kg) once daily for 10 weeks in order to study the possible development of tolerance, and when animals were subjected to auditory stimulation we observed that the ED_{50} values against clonus or tonus were not significantly different from those observed after single administration. Furthermore, the duration of anticonvulsant effects observed between 60 min and 9 h following oral administration of perampanel (1 mg/kg) were similar in acute and after chronic treatment. In another group of experiments, clobazam (0.75, 1.5, 3, 6, 9, 12 and 15 mg/kg) after acute administration was able to dose-dependently reduce the severity of the audiogenic seizures in GEPR-9 s. When clobazam (6 or 12 mg/kg) was administered alone for 10 consecutive weeks a clear development of tolerance to its anticonvulsant effects within approximately seven weeks was observed. In addition, we observed that when clobazam (6 mg/kg) was co-administered with perampanel (1 mg/kg), the latter drug was able to attenuate the development of tolerance to the antiseizure activity of clobazam. The present data indicate that both perampanel and clobazam are effective against audiogenic seizures, however, clobazam effects are hampered by the development of tolerance. Furthermore, concomitant treatment with perampanel slows development of tolerance to the anticonvulsant effects of clobazam in GEPR-9 s.

1. Introduction

Pharmacoresistance to anticonvulsant therapy remains one of the major problems in the treatment of epilepsy (Franco et al., 2014). Approximately one-third of all patients with epilepsy does not become seizure free, despite treatment with two or more antiepileptic drugs (AEDs) at a maximal tolerated dose (Canevini et al., 2010; Luoni et al., 2011, 2015). Tolerance to the antiseizure effects of various antiepileptic drugs (AEDs) has been studied in humans and animals and represents one of the mechanisms influencing long-term outcome and pharmacoresistance among others (Kwan and Brodie, 2006; Kwan et al., 2011; Loscher and Schmidt, 2006; Remy and Beck, 2006; Rogawski, 2013b).

Perampanel (PER; Fycompa, Eisai) has a novel mechanism of action and is the first clinically available AED selectively inhibiting excitatory postsynaptic function acting as a non-selective AMPA receptor antagonist (Citraro et al., 2014; Rogawski and Hanada, 2013). PER demonstrated antiseizure activity in several animal models of seizures and epilepsy (Citraro et al., 2014, 2017; Hanada et al., 2014; Rogawski and Hanada, 2013; Russmann et al., 2016) including models of epileptogenesis and pharmacoresistant seizures (Citraro et al., 2017; Hanada et al., 2011, 2014). AMPA receptors represent an historical target for the development of potential AEDs and this was based on the role played by these receptors in the brain, the finding that AMPA receptor antagonists (both competitive and non-competitive) are very effective drugs in animal models of seizures and epilepsy and finally, that they were found to be overexpressed and/or involved in the brain of rodents and patients with epilepsy (Citraro et al., 2014; Rogawski, 2013a).

Genetically epilepsy prone rats (GEPRs), a genetic model that exhibits repeated clonic-tonic seizures over time after a suitable

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audiogenic stimulus, represent a suitable model to test the potential development of tolerance after prolonged treatment and can be easily monitored without being influenced by disease progression. Furthermore, 1) some AMPA receptors antagonists are effective in this animal model in which, among others, excitatory neurotransmission is involved in seizures generation and maintenance and 2) tolerance is developed following benzodiazepine (acting on $GABA_A$ receptors) repeated administration (De Sarro et al., 1999a, 2017; De Sarro et al., 1992).

We hypothesized that PER, similarly to other non-competitive AMPA antagonists (De Sarro et al., 1999a), will not induce tolerance to its effects in GEPRs, furthermore, we decided to study the potential interaction between PER and clobazam which is known to induce tolerance in this rat strain and therefore determine whether a pharmacodynamic or pharmacokinetic interaction may exist (De Sarro et al., 1996, 1992). Therefore, we studied the effects of PER in this model and determined whether tolerance would develop during a 10 weeks chronic treatment and how PER would influence the development of tolerance to clobazam over the same length period of administration.

2. Materials and methods

2.1. Animals

Male GEPR-9 s were obtained from our breeding stock (Pharmacology Unit, Department of Health Sciences, University of Catanzaro, Italy). GEPR-9 s were tested 3 times at weekly intervals between 6 and 8 weeks of age, and only animals that showed a stage 8–9 (GEPR-9 s) audiogenic seizure in all 3 exposures to sound stimulation were used for these experiments (for details, see the audiogenic stimulation protocol paragraph (Citraro et al., 2015b; De Sarro et al., 2017). Rats were housed 3 or 4 per cage under stable conditions of humidity (60 ± 5%) and temperature (21 ± 2 °C), and were kept under a reversed light/dark (12/12 h) cycle (light on at 19:00). Procedures involving animals and their care were conducted in conformity with the international and national law and policies (European Union Directive 2010/63/EU for animal experiments; Animal Research: Reporting of In Vivo Experiments guidelines; and the Basel declaration, including the 3R concept). The experimental protocols and procedures described in this manuscript were approved by the local ethical committee of the University of Catanzaro, Italy. All efforts were made to minimize animal suffering and to reduce the number of animals used obtaining reliable scientific results.

2.2. Acute and chronic treatment protocols

2.2.1. Acute treatments

Immediately prior to use, each compound was diluted in an appropriate vehicle solution until the desired concentration and administered orally by gavage (o.s.). Clobazam (Martindale Pharmaceuticals Ltd, Essex, UK; 5 ml of clobazam suspension contains 1250 mg of Sorbitol, 10.3 mg of sodium methyl hydroxybenzoate and 1.12 mg of sodium propyl hydroxybenzoate, which were used as vehicle for its control group). PER (EISAI, Milan, Italy) was initially solubilized in a minimal amount of ethanol, which was then diluted in the drinking water to obtain a solution containing < 0.5% ethanol (Citraro et al., 2017). Tap water containing 0.5% ethanol was used as vehicle for control rats. To test drug effects over time after administration and determine ED_{50}s, the following doses (o.s.) were used: clobazam 0.75, 1.5, 3, 6, 9 and 12 mg/kg and PER 0.1, 0.3, 1 and 3 mg/kg. Control animals received equal volumes of vehicle at the respective times before the test. To evaluate the effect of PER on clobazam ED_{50}s, one group of rats was treated with PER at 1 mg/kg and one of the following doses of clobazam: 0.375, 0.75, 1.5, 3 mg/kg. For ED_{50}s determination, rats were tested 60 min after drugs' administration (n = 8 for each dose); whereas, the effects over time (time-course) were only measured for PER 1 or 3 mg/kg and clobazam 6 or 12 mg/kg and the combination of PER 1 mg/kg and clobazam 6 mg/kg every hour for 12 h after drug administration. Rats used for the acute protocol were then randomly assigned to continue in the chronic treatment protocol avoiding that rats treated with one drug would be included in a group treated with the other.

2.2.2. Chronic treatment

Both drugs were orally administered at the following doses, PER 1 or 3 mg/kg/day and clobazam 6 or 12 mg/kg/day (Citraro et al., 2017; De Sarro et al., 1992) and the combined treatment by PER 1 mg/kg/day and clobazam 6 mg/kg/day, by dissolving adequate samples of each drug in 120 ml of drinking water (e.g. PER 1 mg in 120 ml of water for the 1 mg/kg/day dose). Dosage was calculated on the basis of the knowledge that rats drink, on average, 10–12 ml/100 g/day; the volume drunk was also weekly checked (Citraro et al., 2015b, c). Drug solutions were freshly prepared and replaced 3 times a week, and bottles were wrapped in silver foil to exclude light (Citraro et al., 2015c, 2017; Russo et al., 2011). As a vehicle control group for the chronic treatment, a combination of the two vehicles has been used (see above).

Rats (32 animals for each dose and 10 for each vehicle) started treatment at ~P70 (10 weeks of age) and were kept on the drug for 10 additional weeks; treatment was then stopped and animals were normally housed for 4 more weeks in order to evaluate possible withdrawal effects (Scheme 1). Drugs' effects were weekly evaluated and ED_{50}s were again determined in the PER 1 mg/kg/day, clobazam 6 mg/kg/day and their combination 24 h after drug withdrawal at 10 weeks; the aim of this experiment was to study whether the chronic treatment would have had led to tolerance comparing the ED_{50}s and not only drug effects over time. ED_{50}s were calculated as reported above for the acute measurements. Finally, a 10 h' time-course as described above was also performed again in these 3 groups to ascertain any metabolic alteration.

In the groups of PER 1 mg/kg/day, clobazam 6 mg/kg/day, their combination and vehicle, 5 additional rats were included and sacrificed at 10 weeks of age for the binding study (see Section 2.4). In this groups, 1 ml blood sample was obtained every 5 weeks through the tail vein of 3 randomly selected rats for later analysis of drugs' serum concentrations (see below). Animals were only gently restrained during this process. In all groups, animals were weighed weekly every Monday between 9:00 and 11:00 a.m. (Citraro et al., 2015b, 2017).

2.3. Audiogenic seizure protocol

Rats were exposed to a mixed frequency sound of 12–16 kHz, 109 dB intensity under a hemispheric Plexiglas dome (diameter of 58 cm). Individual animals were placed into the dome box for habituation at least 2 min before sound stimulation. Auditory stimulation was applied for 1 min A full seizure response consisted of 1 or 2 running phases, followed by a convulsion (clonus of forelimbs, hindlimbs, head, pinnae, vibrissae, and tail) and tonic extension to give a score of 9 (Citraro et al., 2015b). In particular, the audiogenic seizure response was assessed on the following scale: 0 = no response; 1 = running only; 2 = 2 running phases, followed by a clonic convulsion (clonus of forelimbs, hindlimbs, head, pinnae, vibrissae, and tail); 3 = 1 running phase, followed by a clonic convulsion (clonus of forelimbs, hindlimbs, head, pinnae, vibrissae, and tail); 4 = 2 running phases followed by tonus of neck, trunk, and forelimb, and hindlimb clonus; 5 = 1 running phase followed by tonus of neck, trunk, and forelimb, and hindlimb clonus; 6 = 2 running phases followed by nearly complete tonic extension except hindfeet; 7 = 1 running phase followed by nearly complete tonic extension except hindfeet; 8 = 2 running phases followed by complete tonic extension; and 9 = 1 running phase followed by complete tonic extension (De Sarro et al., 2017). The maximum response was recorded for each animal by two independent blinded researchers expert in animal behavior evaluation for this model, as previously