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Effective termination of status epilepticus by rational polypharmacy in the lithium–pilocarpine model in rats: Window of opportunity to prevent epilepsy and prediction of epilepsy by biomarkers

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ABSTRACT

The pilocarpine rat model, in which status epilepticus (SE) leads to epilepsy with spontaneous recurrent seizures (SRS), is widely used to study the mechanisms of epileptogenesis and develop strategies for epilepsy prevention. SE is commonly interrupted after 30–90 min by high-dose diazepam or other anticonvulsants to reduce mortality. It is widely believed that SE duration of 30–60 min is sufficient to induce hippocampal damage and epilepsy. However, resistance to diazepam develops during SE, so that a SE that is longer than 30 min is difficult to terminate, and SE typically recurs several hours after diazepam, thus forming a bias for studies on epileptogenesis or antiepileptogenesis. We developed a drug cocktail, consisting of diazepam, phenobarbital, and scopolamine that allows complete and persistent SE termination in the lithium–pilocarpine model. A number of novel findings were obtained with this cocktail. (a) In contrast to previous reports with incomplete SE suppression, a SE of 60 min duration did not induce epilepsy, whereas epilepsy with SRS developed after 90 or 120 min SE; (b) by comparing groups of rats with 60 and 90 min of SE, development of epilepsy could be predicted by behavioral hyperexcitability and decrease in seizure threshold, indicating that these read-outs are suited as biomarkers of epileptogenesis; (c) CA1 damage was prevented by the cocktail, but rats exhibited cell loss in the dentate hilus, which was related to development of epilepsy. These data demonstrate that the duration of SE needed for induction of epileptogenesis in this model is longer than previously thought.

Introduction

Induction of status epilepticus (SE) by the cholinergic (muscarinic) agonist pilocarpine or lithium–pilocarpine in rats is widely used to study mechanisms of SE resistance and long-term consequences of SE, including neurodegenerative brain alterations, psychopathology, cognitive impairment and epilepsy, and how to prevent such sequelae with potentially antiepileptogenic compounds (Curia et al., 2008; Löscher and Brandt, 2010; Pitkänen et al., 2013). In order to reduce mortality and avoid inter-individual variation in SE length in studies on epileptogenesis and antiepileptogenesis, SE is typically interrupted by an anticonvulsant drug such as diazepam after a period considered sufficient to induce epilepsy and other long-term consequences (Löscher and Brandt, 2010). Several previous studies using the pilocarpine or lithium–pilocarpine models in rats have reported that an SE duration of 30–60 min is sufficient to induce hippocampal damage and epilepsy (e.g., Lemos and Cavalheiro, 1995; Glien et al., 2001; Klitgaard et al., 2002; Rigoulot et al., 2004; Francois et al., 2006; Jung et al., 2006; Chu et al., 2008).

However, similar as in humans, SE persisting for >0.5 h becomes increasingly resistant to drug treatment by benzodiazepines, barbiturates and other anticonvulsants, so that the treatment may suppress SE severity but does not terminate SE completely (Morrisett et al., 1987; Jones et al., 2002; Chen and Wasterlain, 2006; Wasterlain and Chen, 2008; Löscher, 2009a; Wasterlain et al., 2009). Even if the generalized convulsive seizures are temporarily suppressed by anticonvulsant treatment, they may recur later (Löscher and Brandt, 2010; Reddy and Kuruba, 2013), because most anticonvulsants are much more rapidly eliminated by rats than humans (Löscher, 2007). However, most studies using SE interruption by anticonvulsant drugs do not continuously record the animals' EEG during SE and in the 24 h following its interruption, so that SE recurrence is easily overseen (Löscher and Brandt, 2010; Reddy and Kuruba, 2013). SE recurrence is a well-known phenomenon and forms an important bias when studying development or prevention of epilepsy after SE (Löscher and Brandt, 2010; Reddy and Kuruba, 2013). The aim of the present study was therefore to develop a combination of drugs that completely and persistently terminates SE in the

Abbreviations: PTZ, pentylenetetrazole; SE, status epilepticus; SRS, spontaneous recurrent seizures; TLE, temporal lobe epilepsy.

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lithium–pilocarpine model in rats. We have previously shown that a combination of diazepam and phenobarbital is more efficacious to interrupt SE than either drug alone (Bankstahl and Löscher, 2008), but, as shown in the present study, SE recurrence is not prevented, so that we added a third compound, the muscarinic antagonist scopolamine. Scopolamine alone is not capable of blocking SE once it is established in this model (Buterbaugh et al., 1986; George and Kulkarni, 1996), because SE becomes rapidly self-sustaining and independent of muscarinic receptors (Morisett et al., 1987), but we thought that cholinergic activity may still contribute to SE maintenance, which formed the rationale for combining scopolamine with the GABA mimetic drugs diazepam and phenobarbital. Unexpectedly, we found not only that this drug combination effectively interrupts SE and prevents SE recurrence, but also prevents the development of epilepsy after an SE of 60 min, which previously was reported to induce epilepsy in the majority of rats (for review see Löscher and Brandt, 2010). Rats in which SE was terminated after 90 or 120 min developed epilepsy, so that SE groups that developed or did not develop epilepsy were used to study whether determination of seizure threshold and behavioral alterations after SE are predictive biomarkers of epilepsy. Furthermore, we studied whether early seizures or damage in the dentate hilus was related to the occurrence of epilepsy.

Materials and methods

Animals

Sprague–Dawley rats were purchased from Harlan Netherlands (Horst, Netherlands) at an age of 9 weeks (body weight of 200 to 220 g) and kept under controlled environmental conditions (23 ± 1 °C; 50–60% humidity; 12-h light/dark cycle; light on at 6:00 a.m.) with free access to standard laboratory chow (Altromin 1324 standard diet, Altromin Spezialfutter GmbH, Lage, Germany) and tap water. After most of the experiments described in this study were completed, some additional experiments (see below) had to be performed with Sprague–Dawley rats from Janvier (Le Genest-St-Isle, France), because Harlan closed their Sprague–Dawley colony in Horst. Female rats were used in all experiments to allow comparing the present data with those of our previous studies with the lithium–pilocarpine model in rats (see Results). Female rats were housed without males in order to keep them acyclic or asynchronous with respect to their estrous cycle (cf., Kücker et al., 2010; Rattka et al., 2011). Before being used in the experiments, the rats were allowed to adapt to the new conditions for ≥1 week. All experiments were done in accordance with the European Communities Council Directive of November 24, 1986 (86/609/EEC) and were formally approved by the animal subjects review board of our institution. All efforts were made to minimize pain or discomfort as well as the number of animals.

Electrode implantation and SE induction

Teflon-isolated bipolar stainless steel electrodes were stereotactically implanted into the right dentate gyrus of the hippocampus (AP −3.9; L −1.7; V −3.5, according to the atlas of Paxinos and Watson, 2007) under anesthesia with chloral hydrate (400 mg/kg, i.p.) and served for the recording of the electroencephalogram (EEG). One screw, placed above the left parietal cortex, served as the indifferent reference electrode. Additional skull screws and dental acrylic cement anchored the entire headset. To prevent postoperative infection, rats were treated with marbofloxacin (4–5 mg/kg s.c., twice daily) for 7 days starting two days before electrode implantation. After 2 weeks of post-surgical recovery, lithium chloride (127 mg/kg p.o.) was administered 14–16 h before pilocarpine treatment. In order to ensure the occurrence of SE and decrease mortality, individual dosing of pilocarpine was performed by a modified version of a dose escalation protocol described previously (Glien et al., 2001). For this purpose, pilocarpine was administered i.p. at a bolus dose of 30 mg/kg, followed, if needed, by repeated i.p. injection of 10 mg/kg every 30 min until the onset of a SE. SE was characterized either by continuous limbic seizure activity, consisting of head nodding, stereotyped chewing, and bilateral forelimb clonus, interrupted by generalized convulsive seizures with rearing and falling, or by intermittent generalized convulsive seizure activity (with inter-seizure intervals of less than 3 min) without that the animals normalized in-between the seizures. In the EEG, limbic SE onset was characterized by continuous spike activity of ≥1 Hz without intervals of baseline activity; usually episodes with low frequency spiking (about 1–2 Hz) were followed by episodes with high frequency spiking (≥4 Hz). Intermittent generalized convulsive seizures were associated with high-voltage spiking. Typical paroxysmal EEG activity during SE is shown in Fig. 1. The peripherally acting anticholinergic, methyl-scopolamine (1 mg/kg i.p.) was administered 30 min prior to the first pilocarpine injection to reduce peripheral muscarinic adverse effects of pilocarpine (Curia et al., 2008). The average dose of pilocarpine for inducing SE was 46.2 ± 8.2 mg/kg (mean ± SD; range 30–60 mg/kg). Only rats that developed a self-sustained SE with continuous limbic and/or generalized convulsive seizures were used for further experiments, which was the case in 80% of the rats used for this study. Heating devices were used to prevent hypothermia by the drugs used to terminate SE (see below). Overall mortality in SE rats was 10%. Following SE, all rats were fed with baby food and injected with saline over a couple of days until they resumed normal feeding behavior.

Termination of SE

Three protocols of SE termination were compared. (1) Administration of diazepam (10 mg/kg i.p.) alone; depending on seizure suppression, injection was repeated once or twice at 15-min intervals because of the short half-life of diazepam in rats (Löscher, 2007); (2) administration of a combination of diazepam (10 mg/kg i.p.; repeated as described above for diazepam alone) and phenobarbital (25 mg/kg i.p.) as recently described (Bankstahl and Löscher, 2008; Brandt et al., 2010; Bankstahl et al., 2012); and (3) a combination of diazepam, phenobarbital, and scopolamine. Following a series of preliminary experiments with different doses and routes of administration, the following protocol was used for the latter drug combination. After either 60, 90, or 120 min of SE, diazepam (10 mg/kg), phenobarbital (25 mg/kg), and scopolamine (1 mg/kg) were administered i.v. via a tail vein. Four and 8 h later, the same doses of drugs were administered i.p. In these experiments, rats were randomly assigned to the 60, 90, or 120 min groups after onset of SE, to avoid differences in SE severity between groups. In some animals (n = 4), we omitted the third administration of phenobarbital, to reduce the long-lasting marked sedation associated with repeated administration of this drug. In all rats, the EEG was continuously recorded during and up to 8 days following onset of SE. Furthermore, the behavior of the rats was recorded by video monitoring. SE termination was defined as complete suppression of behavioral seizures and paroxysmal EEG activity (which was the case in most rats) or suppression of spike frequency to less than 1 Hz (without any intervals of higher frequency spiking) and spike amplitude to ≤3 times baseline.

Video–EEG monitoring

The video–EEG monitoring protocol chosen for the present study was based on previous findings in the lithium–pilocarpine model. Systemic administration of pilocarpine in rats promotes sequential behavioral and electrographic changes that can be divided into 3 distinct periods: (a) an acute period that builds up progressively into a limbic SE that lasts for about 24 h if not interrupted earlier, (b) a latent period with a progressive normalization of EEG and behavior which varies from 1 to 3 weeks, and (c) a chronic period with SRS characterizing the development of epilepsy (Cavalheiro et al., 1991; Curia et al., 2008; Scorza et al., 2009). Early, insult-associated seizures may occur

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