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Targeting the prostaglandin E2 EP1 receptor and cyclooxygenase-2 in the amygdala kindling model in mice

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Summary The prostaglandin E2 EP1 receptor as well as the inflammatory enzyme cyclooxygenase-2 have been suggested as targets for disease modulation, improvement of therapeutic response, and restoration of pharmacosensitivity in epilepsies. Translational development of respective add-on approaches requires careful analysis of putative effects on ictogenesis.

Therefore we evaluated the impact of the EP1 receptor antagonist SC-51089, the EP1 receptor agonist misoprostol and the COX-2 inhibitors celecoxib and NS-398 in the mouse amygdala kindling model of temporal lobe epilepsy.

Neither celecoxib nor NS-398 affected the generation, spread and termination of seizure activity. Whereas SC-51089 did not affect the seizure threshold, the highest dose (30 mg/kg) significantly decreased the seizure severity when administered 60 min before stimulation. Moreover, SC-51089 significantly prolonged seizure duration at the highest dose. The EP1 receptor agonist misoprostol exerted contrasting effects on seizure duration with a significant decrease in the duration of motor seizure activity.

The data suggest that doses of COX-2 inhibitors and EP1 receptor antagonists which exert disease modulating or antiepileptic drug potentiating effects do not negatively affect seizure control in temporal lobe epilepsy. The contrasting impact of the EP1 receptor antagonist and agonist suggests that EP1 receptors can influence endogenous mechanisms involved in termination of seizure activity.

Introduction

Based on a series of investigations we suggested key signalling factors of the P-glycoprotein regulatory cascade as novel targets to restore pharmacosensitivity in drug-refractory epilepsies (Potschka, 2010). These targets include

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factors of the arachidonic acid signalling cascade including the prostaglandin E2 EP1 receptor and the inflammatory enzyme cyclooxygenase-2 (COX-2) (Zibell et al., 2009; Pekcec et al., 2009; van Vliet et al., 2010). Moreover, evidence exists that targeting EP1 receptors and COX-2 can protect from excitatory neuronal cell damage (Abe et al., 2009; McCullough et al., 2004; Kawaguchi et al., 2005; Kunz and Oliw, 2001; Takemiya et al., 2006; Kunz et al., 2005; Kawano et al., 2006). Further development of respective therapeutic approaches requires careful tolerability and safety considerations. In addition to peripheral side effects associated with the specific targets putative effects on seizure thresholds and seizure development need to be taken into account.

Recently, we demonstrated that sub-chronic treatment with the EP1 receptor antagonist SC-51089 during a massive kindling paradigm did not affect seizure development with the supra-threshold stimulation condition used in the paradigm (Pekcec et al., 2009). However, these data do not rule out that SC-51089 might affect seizure thresholds or seizure development at threshold stimulation. Therefore, we aimed to further evaluate the impact of EP1 receptor antagonism in the amygdala kindling model in mice. The model was chosen based on its excellent predictive validity for temporal lobe epilepsy allowing detection of anticonvulsant and proconvulsant effects (Loscher, 2002; McIntyre and Gilby, 2009; Potschka et al., 2000).

Whereas there is an obvious lack of studies evaluating the effects of EP1 receptor antagonists on seizure generation and spread, the situation is completely different for COX-2 inhibitors. Multiple studies determined the impact of COX-2 inhibitors with different selectivity in a variety of rodent models (Kulkarni and Dhir, 2009). Most of these studies focused on the effects of COX-2 inhibition in acute seizure and status epilepticus models, i.e. models in which seizures are elicited in naïve mice or rats. Whereas the majority of studies revealed beneficial effects of COX-2 inhibitors including inhibition of seizure development or indicated no impact of COX-2 inhibition, a small subset of studies described seizure aggravation and lowering of seizure thresholds in response to COX-2 inhibition (Baik et al., 1999; Kim et al., 2001, 2008; Akarsu et al., 2006). These latter studies leave some concern regarding further translational development of COX-2 inhibitor based approaches in epilepsy therapy. Therefore, we aimed to thoroughly evaluate the effects of COX-2 inhibition in a chronic model with a high predictive validity for temporal lobe epilepsy, i.e. the amygdala kindling model.

In fully kindled mice we tested whether the EP1 receptor antagonist SC-51089 and the COX-2 inhibitors celecoxib and NS-398 affect seizure thresholds and seizure parameters. To further explore the role of EP1 receptors in ictogenesis, we additionally analyzed the effects of the prostaglandin E2 derivative misoprostol which acts as an EP1 receptor agonist.

Materials and methods

Animals

Male NMRI mice were purchased at a body weight of 20—25 g (Harlan Netherlands, Horst, The Netherlands). Prior to surgery animals were housed in groups of five animals for 7 days under controlled environmental conditions (24—25 °C, 50—60% humidity, 12-h light/dark cycle) with free access to water and standard feed. To minimize the impact of circadian variations all experiments were performed at the same day time (12 a.m. to 16 p.m.). During the period of experiments animals had a body weight between 30 and 55 g, and were housed separately. Experimental procedures were performed according to the German Animal Welfare Act and were approved by the responsible governmental administration of Upper Bavaria.

Electrode implantation

For implantation of kindling electrodes, 30 mice were anesthetized with chloral hydrate (400 mg/kg, i.p.). Bupivacaine was applied s.c. for additional local anesthesia before exposure of the skull surface. The skull surface was exposed, and a bipolar electrode was implanted into the right hemisphere aimed at the amygdala using the following stereotaxic coordinates according to the atlas of Paxinos and Franklin (2004): +1.0 mm caudal, +3.2 mm lateral, +5.3 mm ventral (all respective to bregma). The electrodes consisted of two twisted Teflon-coated stainless steel wires (0.47 mm) separated by 0.55 mm at the tip. A screw, which served as grounding electrode, was positioned over the left parietal cortex. Bipolar and ground electrodes were connected to plugs, and the electrode assembly and anchor screws were held in place with dental acrylic cement applied to the skull surface.

Following a post-operative recovery period of 2 weeks, the pre-kindling afterdischarge threshold was determined by administering a series of stimulations (1 ms, monophasic square wave pulses, 50 Hz for 1 s) at intervals of 1 min increasing in steps of 20% of the previously applied current. The afterdischarge threshold was defined as the lowest current intensity discharges lasting at least 5 s. Constant current stimulations were then delivered to the amygdala once daily (five times per week) until at least 10 generalized stage 4 or 5 seizures were elicited. Animals were then considered fully kindled and subsequently used for drug testing. Seizure severity was scored according to Racine (1972): stage 1, immobility, eye closure, ear twitching, twitching of vibrissae, sniffing, facial clonus; stage 2, head nodding associated with more severe facial clonus; stage 3, clonus of one forelimb; stage 4, bilateral clonus of forelimbs accompanied by rearing; stage 5, rearing and falling accompanied by generalized clonic seizures. In fully kindled mice the post-kindling afterdischarge threshold was determined as described. Determination of afterdischarge thresholds was repeated until thresholds proved to be stable allowing subsequent drug testing.

Drugs

Celecoxib (Pfizer Pharma GmbH, Berlin, Germany) was dissolved in NaCl containing 10% DMSO (Sigma, Taufkirchen, Germany) and was administered at 7, 20 and 30 mg/kg (Kim et al., 2008; Zibell et al., 2009). NS-398 (Cayman Chemicals, Ann Arbor, MI, USA) was dissolved in aqua ad injectabilia (aq. inj.) containing 6% Cremophor EL (Sigma) at 35 °C and administered at 10, 30 and 90 mg/kg (Zibell et al., 2009). SC-51089 (Sigma) was dissolved in aq. inj. and administered at 3, 10 and 30 mg/kg (Abe et al., 2009; Pekcec et al., 2009). Misoprostol (Pfizer) was dissolved in 2% hydroxypropyl methylcellulose (Sigma) and administered at 0.3, 1 and 3 mg/kg (Li et al., 2008). Phenobarbital (Sigma) was dissolved in NaCl and administered at 20 mg/kg. Dose ranges have been chosen based on previous studies demonstrating different CNS effects including neuroprotection or control of blood—brain barrier P-glycoprotein expression.

Celecoxib, NS-398, SC-51089 and phenobarbital were injected i.p. in a volume of 10 ml/kg bodyweight prior to kindling procedure. Misoprostol was administered s.c. in a volume of 10 ml/kg bodyweight.