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The COX-2 inhibitor parecoxib is neuroprotective but not antiepileptogenic in the pilocarpine model of temporal lobe epilepsy

Nadine Polascheck, Marion Bankstahl, Wolfgang Löscher *

Department of Pharmacology, Toxicology, and Pharmacy, University of Veterinary Medicine Hannover, Germany
Center for Systems Neuroscience, Hannover, Germany

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ABSTRACT

The enzyme cyclooxygenase-2 (COX-2), which catalyzes the production of pro-inflammatory prostaglandins, is induced in the brain after various insults, thus contributing to brain inflammatory processes involved in the long-term consequences of such insults. Mounting evidence supports that inflammation may contribute to epileptogenesis and neuronal injury developing after brain insults. Anti-inflammatory treatments, such as selective COX-2 inhibitors, may thus constitute a novel approach for anti-epileptogenesis or disease-modification after brain injuries such as head trauma, cerebral ischemia or status epilepticus (SE). However, recent rat experiments with prophylactic administration of two different COX-2 inhibitors after SE resulted in conflicting results. In the present study, we evaluated whether treatment with parecoxib, a pro-drug of the highly potent and selective COX-2 inhibitor valdecoxib, alters the long-term consequences of a pilocarpine-induced SE in rats. Parecoxib was administered twice daily at 10 mg/kg for 18 days following SE. Five weeks after termination of treatment, spontaneous recurrent seizures were recorded by continuous video/EEG monitoring. Prophylactic treatment with parecoxib prevented the SE-induced increase in prostaglandin E_2 and reduced neuronal damage in the hippocampus and piriform cortex. However, the incidence, frequency or duration of spontaneous seizures developing after SE or the behavioral and cognitive alterations associated with epilepsy were not affected by parecoxib. Only the severity of spontaneous seizures was reduced, indicating a disease-modifying effect. These results substantiate that COX-2 contributes to neuronal injury developing after SE, but inhibition of COX-2 is no effective means to modify epileptogenesis.

Introduction

There is accumulating experimental and clinical evidence that activation of inflammatory pathways is a common factor contributing to the pathogenesis of seizures in various forms of epilepsy of different etiologies (Vezzani and Granata, 2005; Vezzani and Baram, 2007; Choi and Koh, 2008; Vezzani et al., 2008). Several lines of evidence suggest a key role of inflammation in the development of epilepsy (a process termed epileptogenesis) and the initiation of seizures (Vezzani and Granata, 2005; Takemiya et al., 2007; Vezzani and Baram, 2007; Choi and Koh, 2008; Vezzani et al., 2008; Rijkers et al., 2009): (a) Common risk factors for symptomatic epilepsy (such as CNS tumors, head injury, stroke, complex febrile seizures, status epilepticus [SE]) are accompanied by variable levels of inflammation. (b) Infections (systemic or CNS) are well-recognized etiologic triggers for seizures. (c) Inflammation in epilepsy is not restricted to different types of encephalitis associated with seizures but occurs also in common types of human epilepsy such as temporal lobe epilepsy (TLE) and malformations of cortical development. (d) Both seizures and SE induce pro-inflammatory cytokines and down-stream inflammatory mediators. (e) Systemic or CNS inflammation affect the integrity of the blood–brain barrier (BBB), enhance neuronal excitability, decrease seizure threshold and may exacerbate seizure-induced brain injury. (f) BBB disruption by inflammatory processes may lead to epileptogenesis by disturbance of brain homeostasis. (g) And, finally, several anti-inflammatory drugs have been reported to exert antiepileptic actions. However, opposing results have been published as well, so that more research is needed to fully establish the role of inflammation in seizure generation and epilepsy (Rijkers et al., 2009).

Among the various inflammatory mediators that may be involved in the processes leading to epilepsy, cytokines such as interleukin 1β (IL-1β) and prostaglandins (PGs) such as PGE_2 are thought to play a particular role (Cole-Edwards and Bazan, 2005; Vezzani and Granata, 2005; Takemiya et al., 2007; Vezzani and Baram, 2007; Choi and Koh, 2008; Vezzani et al., 2008). Cyclooxygenase (COX) is the rate-limiting enzyme in PG synthesis and is a major target of nonsteroidal anti-inflammatory drugs (NSAIDs) (Takemiya et al., 2007). Two isoforms of COX enzymes have been identified: the constitutively expressed COX-1 and the inducible, highly regulated COX-2, which is the predominant

* Corresponding author. Department of Pharmacology, Toxicology and Pharmacy, University of Veterinary Medicine, Bünteweg 17, D-30559 Hannover, Germany. Fax: +49 511 953 8581.
E-mail address: wolfgang.loescher@tiho-hannover.de (W. Löscher).

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COX isoform expressed in the brain. Induction of COX-2 in the brain has been shown to facilitate epileptogenesis and contribute to neuronal damage in rat models of TLE (Cole-Edwards and Bazan, 2005; Takemiya et al., 2007; Kulkarni and Dhir, 2009). It is thus tempting to speculate that COX-2 inhibitors exert antiepileptogenic and neuroprotective effects in such models. If so, COX-2 inhibitors may offer a new strategy for preventing neuronal damage and epileptogenesis after brain insults such as traumatic brain injury, focal cerebral ischemia or SE. Such brain insults are typically followed by a latent (or silent) period, during which pharmacological modulation of epileptogenic processes may allow to prevent or modify the development of epilepsy, which is a major clinical goal in people at risk (Kelley et al., 2009).

The first evidence that COX-2 inhibitors may be interesting in this regard was reported by Jung et al. in 2006. They showed that prolonged administration of the COX-2 inhibitor celecoxib after a pilocarpine-induced SE in rats prevents neuronal damage in the hippocampus and, more importantly, reduces the incidence, frequency and duration of spontaneous recurrent seizures, indicating an antiepileptogenic or disease-modifying effect. The study of Jung et al. (2006) prompted us to perform similar experiments with the highly selective COX-2 inhibitor parecoxib because celecoxib has been reported to exert also COX-2 independent actions (Kang et al., 2005; Grosch et al., 2006; Miyamoto et al., 2006), which may have been involved in the antiepileptogenic effects reported by Jung et al. (2006). During the course of our experiments with parecoxib in the pilocarpine model of TLE (first data were presented at a conference in March 2009 and published as an abstract: Polascheck et al., 2009), a second study using a COX-2 inhibitor in a rat TLE model was published (Holtman et al., 2009). In apparent contrast to the study of Jung et al. (2006), Holtman et al. (2009) did not find any evidence of antiepileptogenic, disease-modifying or neuroprotective effects of prolonged treatment with the COX-2 inhibitor SC58236 after an electrically-induced SE. In the present study, we partially confirm the findings of Jung et al. (2006), thus allowing to evaluate which experimental factors may affect the outcome of such studies. Furthermore, in view of the well-known neuroprotective effects of COX-2 inhibitors such as celecoxib and parecoxib (Kunz and Oliw, 2001; Candelario-Jalil et al., 2003; Scali et al., 2003; Kunz et al., 2005, 2006; Hewett et al., 2006; Jung et al., 2006; Kelsen et al., 2006; Reksidler et al., 2007), we included a behavioral and cognitive test battery in the present study because TLE is known to be associated with psychopathology and cognitive impairment which, at least in part, are thought to be a consequence of neuronal damage in the hippocampal formation and other parts of the limbic system (LaFrance et al., 2008).

Materials and methods

Animals

Female Sprague–Dawley rats were purchased at a body weight of 200–220 g (Harlan, Horst, The Netherlands). Following arrival, the rats were kept under controlled environmental conditions (24–25 °C; 50–60% humidity; 12:12-h light/dark cycle; light on at 6:00 h) with free access to standard laboratory chow (Altromin 1324 standard diet) and tap water. The female rats were housed without males in order to keep them acyclic or asynchronous with respect to their estrous cycle (Kücker et al., 2010). We have shown previously that this avoids any significant effects of estrous cycle on seizure susceptibility or severity (Wahnschaffe and Löscher, 1992). All experiments were done in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). All efforts were made to minimize pain or discomfort of the animals used.

Induction of status epilepticus by pilocarpine

For the parecoxib trial, 40 rats received lithium chloride (127 mg/kg; Sigma-Aldrich, Germany) 12–17 h and methyl-scopolamine (1 mg/kg i.p.; Sigma-Aldrich) 30 min before pilocarpine treatment. In order to ensure the occurrence of SE and decrease mortality, individual dosing of pilocarpine was performed by ramping up the dose until onset of SE as described previously (Glien et al., 2001). For this purpose, pilocarpine (Sigma-Aldrich) was administered i.p. at a dose of 10 mg/kg every 30 min until the onset of an SE, consisting of ongoing limbic or generalized convulsive seizure activity. The total number of pilocarpine injections was limited to 5 injections per animal. In this way, a generalized convulsive SE could be induced in 36 of 40 rats administered with pilocarpine. The average dose of pilocarpine for inducing convulsive SE was 24.2 ± 1.2 mg/kg (range 20–50 mg/kg). Time to onset of SE was 67.9 ± 3.9 min (range 40–147 min). Diazepam (10 mg/kg i.p.; Temmler Pharma, Germany) was administered after 90 min of SE, leading to a suppression of generalized convulsive seizures. The application of diazepam was repeated in all rats after 15–20 min, which also suppressed partial seizures that were not blocked by the first injection of diazepam. Age-matched control rats ($n = 18$) received all treatments (lithium, methyl-scopolamine, diazepam), but saline instead of pilocarpine. All rats were closely observed during and after the SE to exclude differences among rats injected with pilocarpine alone vs. treatment with parecoxib (see below). Following SE, all rats were fed with baby food and injected with saline (4 ml i.p.) over a couple of days until they resumed normal feeding behavior.

Treatment with parecoxib

Immediately after the onset of SE, the 36 rats with SE were randomly allocated to two groups: (1) SE + parecoxib ($n = 20$), these rats received parecoxib immediately after interruption of SE, followed by twice daily parecoxib, 10 mg/kg i.p., for the subsequent 17 days; (2) SE + NaCl ($n = 16$), these rats received i.p. injections of NaCl instead of parecoxib twice daily for 18 days (see Fig. 1). The 18 age-matched control groups without SE were also randomly allocated to two groups and either treated with NaCl ($n = 8$) or parecoxib ($n = 10$), using the same treatment protocol as in the two SE groups. All groups were run in parallel throughout the experiments to allow direct comparisons. Blood was sampled by retro-orbital puncture (after local anesthesia with 2% tetracaine) 2 h after the injections of parecoxib on day 1, 7 and 14. The selection of dose and dosing interval of parecoxib was based on previous studies demonstrating a neuroprotective effect of this dose in ischemia and Parkinson rat models (Kelsen et al., 2006; Reksidler et al., 2007) and on pharmacokinetic experiments in rats (Talley et al., 2000). Parecoxib was used as commercial solution (Dynastat®; Pfizer), containing the water-soluble sodium salt of parecoxib, which was diluted with saline and injected at a volume of 1 ml/kg.

Several rats of the SE–parecoxib group (6/20) died after onset of treatment, most likely as a consequence of SE, because only one rat died in the parecoxib control group (1 day after the end of the 18-day treatment period). Furthermore, 3 rats (two of the SE-NaCl, one of the SE–parecoxib groups) were killed because they were moribund. Necropsy of the parecoxib-treated rats did not indicate any obvious adverse effects, such as gastrointestinal bleeding.

Determination of parecoxib and valdecoxib in plasma

Parecoxib and valdecoxib concentrations in plasma were determined by HPLC with UV detection. For this purpose, 50 µl of rat plasma was mixed with 150 µl of methanol, centrifuged and the supernatant injected into an HPLC apparatus equipped with a precolumn (Nucleosil 120-5 C18, 60 × 4 mm; Knauer, Berlin, Germany), a main column (Nucleosil 120-5 C18, 250 × 4.6 mm; Macherey & Nagel, Düren, Germany) and a UV detector (SPD-6A; Shimadzu, Duisburg, Germany). The mobile phase was composed of 0.05 M phosphate buffer (pH adjusted to 5.6) and acetonitrile in a volume ratio of 60 to 40. Using a