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Cyclooxygenase-2 inhibitors differentially attenuate pentylenetetrazol-induced seizures and increase of pro- and anti-inflammatory cytokine levels in the cerebral cortex and hippocampus of mice

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Celecoxib (PubChem CID: 2662)
Etoricoxib (PubChem CID: 123619)
Carboxymethylcellulose (PubChem CID: 23706213)
Tween 80 (PubChem CID: 443315)
Sodium Chloride (PubChem CID: 5234)
Phosphate-buffered saline (PBS) (PubChem CID: 24978514)
Ethylenediaminetetraacetic acid (PubChem CID: 6049)
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ABSTRACT

Seizures increase prostaglandin and cytokine levels in the brain. However, it remains to be determined whether cyclooxygenase-2 (COX-2) derived metabolites play a role in seizure-induced cytokine increase in the brain and whether anticonvulsant activity is shared by all COX-2 inhibitors. In this study we investigated whether three different COX-2 inhibitors alter pentylenetetrazol (PTZ)-induced seizures and increase of interleukin-1β (IL-1β), interleukin-6 (IL-6), interferon-γ (INF-γ), tumor necrosis factor-α (TNF-α) and interleukin-10 (IL-10) levels in the hippocampus and cerebral cortex of mice. Adult male albino Swiss mice received nimesulide, celecoxib or etoricoxib (0.2, 2 or 20 mg/kg in 0.1% carboxymethylcellulose (CMC) in 5% Tween 80, p.o.). Sixty minutes thereafter the animals were injected with PTZ (50 mg/kg, i.p.) and the latency to myoclonic jerks and to generalized tonic-clonic seizures were recorded. Twenty minutes after PTZ injection animals were killed and cytokine levels were measured. PTZ increased cytokine levels in the cerebral cortex and hippocampus. While celecoxib and nimesulide attenuated PTZ -induced increase of proinflammatory cytokines in the cerebral cortex, etoricoxib did not. Nimesulide was the only COX-2 inhibitors that attenuated PTZ-induced seizures. This effect coincided with an increase of IL-10 levels in the cerebral cortex and hippocampus, constituting circumstantial evidence that IL-10 increase may be involved in the anticonvulsant effect of nimesulide.

1. Introduction

Accumulating clinical and experimental evidence has been gathered over the past few years associating neuroinflammation with epilepsy and/or increased seizure susceptibility. In line with this view, while acute and chronic seizures increase key inflammatory mediators in the brain (Vezzani and Granata, 2005), inflammatory mediators synthesized and released in response to pathogen and tissue damage facilitate seizures (Gomez et al., 2014; Ho et al., 2015).

Inflammation, as part of the innate immune response, is commonly described as sequential events triggered by the activation of pattern-recognition receptors by pathogen- and damage-associated molecular patterns, such as the Toll-like receptors (TLRs) (Maroso et al., 2010). The activation of TLRs leads to the induction of numerous genes that function in inflammatory responses. These include cytokines

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(e.g., tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6)), inflammatory chemokines, such as CCL2 (Cazareth et al., 2014) and, sequentially, key enzymes involved in the synthesis of proinflammatory lipid mediators and nitric oxide (Hsiao et al., 2007). Accordingly, TNF-α induces prostaglandin G/H synthase-2 in microvascular vessels and infiltrating neutrophils (Tsao et al., 1999) and IL-1β stimulates prostaglandin E_2 (PGE_2) production in murine astrocytes (O'Banion et al., 1996). Cyclooxygenases (COXs) 1 and 2 convert arachidonic acid to PGH_2, and specific thromboxane or PG synthases convert PGH_2 to thromboxane A_2 (TXA_2), $PGF_{2\alpha}$, PGE_2, PGI_2 or PGD_2 (Medeiros et al., 2012).

While this sequential view of the inflammatory process (TLR activation, cytokine synthesis, lipid mediator production) has been relatively well established for the peripheral production of prostanoids (Kawai and Akira, 2008) and lipopolysaccharide (LPS)-induced neuroinflammation (Chu et al., 2015; Ho et al., 2015), there is evidence that the prostaglandins produced in response to seizures are initially synthesized by cytokine- independent mechanisms, which involve constitutive COX-2 (involved in $PGF_{2\alpha}$, PGE_2, 6-keto-$PGF_{1\alpha}$, TXA_2 and PGD_2 production) and COX-1 (involved in PGD_2 and TXA_2 production) (Choi et al., 2009). Therefore, the sequential production of cytokines and prostaglandins that has consolidated in the literature as a logical sequence for the peripheral inflammatory response, may not occur exactly in the same way in the central nervous system (CNS). As a consequence, it is possible that constitutive COX-2-derived prostaglandins modulate seizure-induced cytokine production and release in the CNS. This would be particularly relevant considering the well-known proconvulsant action of TNF-α (Zare-Shahabadi et al., 2015), IL-1β (Arisi et al., 2015; Balosso et al., 2008) and IL-6 (Campbell et al., 1993). If prostaglandins increased proconvulsant (or decreased anticonvulsant) cytokine production and release, this could constitute an additional mechanism by which proconvulsant prostaglandins, such as PGE_2 (Oliveira et al., 2008a, 2008b), facilitate seizures. This putative feedforward proinflammatory and proconvulsant mechanism would sum to the already described PGE_2-induced increase of astrocytic glutamate release (Takemiya et al., 2003), Na^+,K^+-ATPase activity inhibition (Oliveira et al., 2009) and inhibition of potassium currents (Chen and Bazan, 2005) in the CNS. Therefore, in this study we investigated whether different COX-2 inhibitors alter pentylenetetrazol (PTZ)-induced seizures and increase of IL-1β, IL-6, interferon-γ (INF-ɣ), TNF-α, and IL-10 levels in mice.

2. Materials and methods

2.1. Animals

Adult male Swiss mice (28 ± 3 g; n = 168), housed ten to a cage, and maintained under controlled light and environment (12-h light/dark cycle, lights on at 7:00, 22 ± 1 °C, 55% relative humidity) with free access to food (Guabi, Santa Maria, Brazil) and water were used. All animals were obtained from the Animal House of the Federal University of Santa Maria. Behavioral tests were conducted during the light phase of the cycle (between 10:00 a.m. and 14:00 p.m.). All experiments reported in this study were conducted in accordance with the policies of the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications no. 80-23), revised in 1996, and with the Institutional and National regulations for animal research (#024/2014). All efforts were made to reduce the number of animals used to a minimum, as well as to minimize their suffering.

2.2. Reagents

The following drugs were used in the present study. PTZ (Sigma, USA), nimesulide (Nisulid©, Aché), celecoxib (Celebra©, Pfizer) or etoricoxib (Arcoxia©, MSD). Commercial ELISA (enzyme-linked immunosorbent assay) kits for cytokines assays were purchased from eBIOSCIENCE (San Diego, USA). PTZ was dissolved in 0.9% NaCl and administered intraperitoneally (i.p.). Nimesulide, celecoxib and etoricoxib were suspended in 0.1% carboxymethylcellulose (CMC) plus 5% Tween 80 and administered by oral gavage (volume: 10 ml/kg).

2.3. Experimental protocol: effect of nimesulide, celecoxib or etoricoxib on PTZ-induced seizures

Since the three COX-2 inhibitors permeate the blood-brain barrier (Dembo et al., 2005; Ferrario and Bianchi, 2003; Renner et al., 2010) and their IC_{50}s are similar (around 1 μM) (Shi and Klotz, 2008), the effect of nimesulide, celecoxib or etoricoxib on PTZ-induced seizures was investigated by oral administering each drug (0.2, 2 or 20 mg/kg in 0.1% CMC in 5% Tween 80), or vehicle (0.1% CMC in 5% Tween 80, 10 ml/kg) 60 min before PTZ (50 mg/kg, i.p.). After PTZ injection animals were observed for the appearance of behavioral seizures, as described below. PTZ has been widely used as a model of acute seizure and drug doses were chosen based on previous studies (Dhir et al., 2006; Jayaraman et al., 2010; Oliveira et al., 2008b; Salvadori et al., 2012).

2.4. Seizure evaluation

After PTZ injection the animals were followed up (and video monitored) for 20 min and the latency to myoclonic jerks and generalized tonic-clonic seizures were recorded, according to Ferraro et al. (1999). The severity of PTZ-induced seizures was scored by a modified Racine scale (Luttjohann et al., 2009), as follows: (1) sudden behavioral arrest and/or motionless staring; (2) facial jerking with muzzle or muzzle and eye; (3) neck jerks; (4) clonic seizure in a sitting position; (5) convulsion including clonic and/or tonic-clonic seizure while lying on the belly and/or pure tonic seizure; (6) convulsion including clonic and/or tonic-clonic seizure while lying on the side and/or wild jumping. We have considered the current experimental model as a model of acute individual seizure (but not a model of *status epilepticus*) because the Commission on Classification and Terminology and the Commission on Epidemiology of the International League Against Epilepsy (ILAE) defined status epilepticus (SE) as "... a condition resulting either from the failure of the mechanisms responsible for seizure termination or from the initiation of mechanisms, which lead to abnormally, prolonged seizures (after time point t1). It is a condition, which can have long-term consequences (after time point t2), including neuronal death, neuronal injury, and alteration of neuronal networks, depending on the type and duration of seizures. This definition is conceptual, with two operational dimensions: the first is the length of the seizure and the time point (t1) beyond which the seizure should be regarded as "continuous seizure activity." The second time point (t2) is the time of ongoing seizure activity after which there is a risk of long-term consequences. In the case of convulsive (tonic–clonic) SE, both time points (t1 at 5 min and t2 at 30 min) are based on animal experiments and clinical research" (Trinka et al., 2015). Since our animals did not achieve 5 min of continuous seizure activity, it is not possible to classify their seizures as "*status epilepticus*".

2.5. Cytokine assay

Animals were treated with vehicle (0.1% CMC in 5% Tween 80, p.o., 10 ml/kg) or nimesulide (20 mg/kg) or celecoxib (20 mg/kg) or etoricoxib (20 mg/kg) by oral gavage and, 60 min thereafter were injected with PTZ (50 mg/kg, i.p.) or 0.9% NaCl (10 ml/kg, i.p.). Animals were killed by decapitation 20 min after PTZ (or 0.9% NaCl) injection and the hippocampi and cerebral cortex were dissected and homogenized in 10 mM PBS containing 1 mM ethylenediaminetetraacetic acid, 0.1 mM phenylmethylsulfonyl fluoride and 0.5% bovine serum albumin, pH 7.4. Samples were centrifuged at 25,000*g* for 10 min and the supernatant was used to measure IL-1β, IL-6, TNF-α,