Experimental Neurology 253 (2014) 82–90

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Prostaglandin D_2 is crucial for seizure suppression and postictal sleep

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ARTICLE INFO

Article history:
Received 23 August 2013
Revised 22 November 2013
Accepted 1 December 2013
Available online 10 December 2013

Keywords:
CRTH2 (DP_2) receptor
Electrographic seizures
Postictal EEG depression
Prostanoid
Convulsion
REM sleep
Prostaglandin E_2
Prostaglandin $F_2\alpha$

ABSTRACT

Epilepsy is a neurological disorder with the occurrence of seizures, which are often accompanied by sleep. Prostaglandin (PG) D_2 is produced by hematopoietic or lipocalin-type PGD synthase (H- or L-PGDS) and involved in the regulation of physiological sleep. Here, we show that H-PGDS, L/H-PGDS or DP_1 receptor (DP_1R) KO mice exhibited more intense pentylenetetrazole (PTZ)-induced seizures in terms of latency of seizure onset, duration of generalized tonic–clonic seizures, and number of seizure spikes. Seizures significantly increased the PGD_2 content of the brain in wild-type mice. This PTZ-induced increase in PGD_2 was attenuated in the brains of L- or H-PGDS KO and abolished in L/H-PGDS KO mice. Postictal non-rapid eye movement sleep was observed in the wild-type and H-PGDS or DP_2R KO, but not in the L-, L/H-PGDS or DP_1R KO, mice. These findings demonstrate that PGD_2 produced by H-PGDS and acting on DP_1R is essential for seizure suppression and that the L-PGDS/PGD_2/DP_1R system regulates sleep that follows seizures.

Introduction

Epilepsy is a common and devastating neurological disorder affecting more than 50 million people around the world and is observed across all age groups and with varying levels of severity. Epilepsy is a chronic disease characterized by spontaneous recurrent seizures in the absence of any metabolic intoxication or fever and by a period of sleepiness or sleep after the seizures (Derry and Duncan, 2013). When homeostasis of a biological system is jeopardized by trauma or disease, defense hormones like prostaglandins (PGs) are released locally to protect the integrity of the organism. PGD_2 is a major prostanoid produced in the central nervous system (CNS) of rats, mice, and humans and functions as a neuromodulator of sleep–wake regulation and neuroinflammation (Eguchi et al., 1999, Mohri et al., 2006, Urade and Hayaishi, 2011). PGD_2 is synthesized by 2 different types of PGD synthase (PGDS) (Smith et al., 2011), i.e., lipocalin-type PGDS (L-PGDS) (Urade et al., 1985a, 1985b) and hematopoietic PGDS (H-PGDS) (Urade et al., 1985a, 1985b, 1987); and it elicits its action through binding to DP_1 receptor (DP_1R) (Kabashima and Narumiya, 2003) or chemoattractant receptor CRTH2 (DP_2R) (Nagata and Hirai, 2003). The somnogenic activity of PGD_2 was discovered when PGD_2 was microinjected in nanomolar amounts into rats (Ueno et al., 1982) and non-human primates (Onoe et al., 1988), and it is now well accepted that PGD_2 produced by L-PGDS and acting through DP_1Rs is involved in the regulation of physiological sleep (Qu et al., 2006). On the other hand, PGD_2 produced by H-PGDS has been implicated in the neuroinflammation associated with the activation of microglia and astrocytes after neuronal injury/infection (Taniguchi et al., 2007) and in a mouse model of demyelination *twitcher* mice (Mohri et al., 2006). The molecular mechanism of the action of PGD_2 during seizures is, however, poorly understood.

We hypothesized that endogenous PGD_2 plays an important role in the pathology of seizures and postictal sleep. By using knockout (KO) mice for L-PGDS, H-PGDS, L/H-PGDS, DP_1R, and DP_2R, we investigated the molecular mechanisms of PGD_2 in pentylenetetrazole (PTZ)-induced seizures and postictal sleep. We used KO mice rather than pharmacological approaches, including antagonists and inhibitors for the PGD_2 system, because 1) DP_1R antagonist (ONO-4127Na) has limited solubility in saline or artificial CSF, 2) PGD_2/DPR inhibitors and antagonists have low blood–brain barrier permeability, and 3) PGDS inhibitors (AT-56 and HQL-79) have anti-histamine effects as well as DP_2R antagonist (Ramatroban) also acts on thromboxane receptors (Urade and Lazarus,

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0014-4886/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.expneurol.2013.12.002

2013). We found that PGD_2 produced by H-PGDS suppressed seizures by acting through DP_1R, whereas L-PGDS-derived PGD_2, also acting through this receptor, was involved in postictal sleep.

Materials and methods

Animals

L-PGDS KO and H-PGDS KO mice were generated at Osaka Bioscience Institute (Osaka, Japan), DP_1R KO mice at Kyoto University (Kyoto, Japan), and DP_2R KO mice at Tokyo Medical and Dental University, as previously reported (Eguchi et al., 1999, Matsuoka et al., 2000, Satoh et al., 2006, Trivedi et al., 2006). All of these KO mice were backcrossed to the C57BL/6 strain for more than 20 generations. Heterozygous mice for either the L- or H-PGDS gene mutation were cross-bred to generate double-heterozygous mice. These double-heterozygous mice were then cross-bred to produce the WT, H-PGDS KO, L-PGDS KO, and L/H-PGDS double KO mice used in this work. Male WT, L-, H-, and L/H-PGDS, and DP_1R and DP_2R KO mice of the inbred C57BL/6 strain were maintained at Oriental Bioservice (Kyoto, Japan). For behavioral studies, mice, weighing 25–28 g (10–13 weeks), were housed in an insulated soundproofed recording room maintained at an ambient temperature of 24 ± 0.5 °C with a relative humidity of 60 ± 2% on an automatically controlled 12-h light/dark cycle (light on at 0800), and they had free access to food and water. The experimental protocols were approved by the Animal Care Committee of Osaka Bioscience Institute, and every effort was made to minimize the number of animals used as well as any pain and discomfort.

Surgery for placement of electroencephalogram (EEG)/electromyogram (EMG) electrodes and polygraphic recording

Surgeries were performed under anesthesia using pentobarbital (50 mg/kg, intraperitoneally). Under aseptic conditions, mice were chronically implanted with EEG and EMG electrodes for polysomnography, as previously described (Qu et al., 2006). Briefly, the implant consisted of 2 stainless steel screws (1 mm diameter) serving as EEG electrodes, one of which was placed epidurally over the right frontal cortex (1.0 mm anterior and 1.5 mm lateral to bregma) and the other over the right parietal cortex (1.0 mm anterior and 1.5 mm lateral to lambda). Two insulated stainless steel, Teflon-coated wires (0.2 mm in diameter), which were placed bilaterally into the trapezius muscles, served as EMG electrodes. Both EEG and EMG electrodes were connected to a microconnector, and the whole assembly was then fixed to the skull with self-curing dental acrylic resin.

After 8–10 days of postoperative recovery, the mice were placed in experimental cages for a 4-day habituation/acclimatization period and connected with counterbalanced recording leads. All mice that were subjected to EEG/EMG recordings received vehicle and drug treatment on 2 consecutive days. On day 1, the mice were treated with vehicle (saline, i.p.) at 9:00 PM; and EEG/EMG signals were recorded for 24 h. On day 2, they were then treated with PTZ (Sigma Aldrich; 50 mg/kg, i.p., in a volume of 10 ml/kg body weight); EEG/EMG signals were again recorded for 24 h and behavioral seizures were observed by using an infrared video camera.

Cortical EEG and EMG signals were amplified and filtered (EEG, 0.5–30 Hz; EMG, 20–200 Hz), then digitized at a sampling rate of 128 Hz, and recorded by using SleepSign software (Kissei Comtec, Nagano, Japan) as previously described (Kohtoh et al., 2008). Polysomnographic recordings were scored with automated analysis, off-line, in 4-s epochs as wakefulness, rapid eye movement (REM) and non-REM (NREM) sleep by SleepSign software, using standard criteria (Huang et al., 2001, Kohtoh et al., 2008). Seizures were induced by injecting PTZ (50 mg/kg, i.p.) and latency to seizure onset, which was defined as the time from injection to the first seizure spike, and the duration of generalized tonic–clonic seizures (GTCS) were calculated based on video and EGG recordings. Postictal EEG depression is characterized by low-amplitude, slow-wave EEG signals that occur following behavioral seizures (Buckmaster and Wong, 2002); and, thus, the duration of this depression was calculated as time between the termination of behavioral seizures and reappearance of EEG signals with an amplitude exceeding 100 μV. Seizure spikes were detected and counted by using the peak analysis function of OriginLab v8.5 Pro software (Data Analysis and Graphing Software, USA). Postictal sleep was contaminated with electrographic seizure spikes characterized by sharp, low frequency (4 Hz) and high amplitude (more than twice the baseline) EEG waves that appeared similar to slow-wave activity. The EEG signals exceeding twice the baseline amplitude were scored as seizure spikes and counted during behavioral seizures and during 1 h following postictal EEG depression (Ziemann et al., 2008). The defined sleep–wake stages were visually examined and corrected, wherever necessary, so that the epochs with 50% or more contamination with seizure spikes were categorized as seizure. Spectral analysis of EEG by fast Fourier transformation (FFT) was performed, and the EEG power densities of each 0.5-Hz bin were averaged by calculating the percentage of each bin with respect to the total power (0.5–35 Hz).

Measurement of prostanoids following PTZ injection

Prostanoid levels were measured in the mouse brain at different time points after PTZ injection, i.e., 5, 10, 15, 30, 60, and 120 min after i.p. injection of PTZ (50 mg/kg, i.p.), to determine the time course of changes in PG content in WT and KO mice. The brains were harvested and immediately frozen in liquid nitrogen at the respective time points. They were then homogenized in acidified ethanol (containing 0.02% HCl at pH 2.0). Deuterium-labeled $PGF_{2\alpha}$ (100 ng) was added to each sample as tracers for estimation of the recovery, and the samples were then centrifuged at 500 ×*g* for 30 min. The ethanol extracts were applied onto Sep-Pak C18 cartridges (Waters Associates, Milford, MA), washed with hexane, eluted with ethyl acetate, and evaporated to dryness in vacuo. The amounts of PGD_2, PGE_2, $PGF_{2\alpha}$, TXB_2 and 6-keto $PGF_{2\alpha}$ were measured by using a liquid chromatography–tandem mass spectrometer, as previously described (Qu et al., 2006).

Statistical analysis

All data were expressed as the mean ± SEM. Time course data of the PGD_2 content in WT and L/H-PGDS KO mice were compared using independent sample t-test, on the other hand time-course data of the NREM sleep were analyzed by the paired t-test, with each animal serving as its own control. All inter-group comparisons were done by one-way ANOVA followed by the Fisher probable least-squares difference test where sample size was similar in all groups, and Scheffe's post-hoc test was applied with uneven sample size across groups. In all cases, $p \leq 0.05$ was considered as significant. Statistical analysis was performed with SPSS 16.0 (SPSS Inc., Chicago, IL, U.S.).

Results

Mice lacking H-PGDS and DP_1R are highly sensitive to chemically-induced seizures

We first examined the effect of a PTZ injection on the seizure severity by performing video analysis of events during seizures and EEG/EMG recordings for epileptic brain activity. The injection of the epileptogenic dose of PTZ (50 mg/kg, i.p.) induced generalized tonic–clonic seizures (GTCS) in wild-type (WT) as well as in KO mice for L-PGDS, H-PGDS, L/H-PGDS, DP_1R or DP_2R. Video analysis showed that animals first became motionless and then progressed to a rigid posture with forelimb and tail extension after the injection of PTZ. Initial seizure behavior was followed by head nodding, myoclonic jerks with brief twitching,