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Effect of cyclooxygenase inhibitors on pentylenetetrazol (PTZ)-induced convulsions: Possible mechanism of action

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Abstract

Cyclooxygenase (COX) is reported to play a significant role in neurodegenerative and neuropsychiatric disorders, and may play a significant role in the pathogenesis of epilepsy. Various neurotransmitter abnormalities, especially of GABA and glutamate, have been reported to play a key role in the pathophysiology of epilepsy. The objective of the present study was to elucidate the effect of cyclooxygenase inhibitors on pentylenetetrazol (PTZ)-induced (80 mg/kg) convulsions in mice with possible mechanism of action. Various COX-inhibitors were administered 45 min prior to the PTZ administration. Onset, duration of clonic convulsions and percentage mortality/recovery were recorded. Pretreatment with COX-inhibitors aspirin (10 and 20 mg/kg, p.o.), naproxen (7 and 14 mg/kg, p.o.), nimesulide (1–5 mg/kg, p.o.) or rofecoxib (1–4 mg/kg, p.o.) dose-dependently showed protection against PTZ-induced convulsions. COX-2 inhibitors were more effective as compared to non-selective COX-inhibitors. Rofecoxib (1 mg/kg) or nimesulide (1 mg/kg) also enhanced the sub-protective effect of diazepam or muscimol showing GABAergic modulation of COX-2 inhibitors. COX-2 inhibitors also antagonized the effect of flumazenil (4 mg/kg)- against PTZ-induced convulsions further confirming the GABAergic mechanism.

In conclusion, the results of the present study strongly suggest the possible role of cyclooxygenase isoenzymes in the pathophysiology of epilepsy and the use of COX-inhibitors as an adjuvant therapy in the treatment of epilepsy.

Keywords: Convulsions; Cyclooxygenases; Epilepsy; NSIADs; PTZ

1. Introduction

Epilepsy is a common neurological disorder affecting about 0.5–1% of the world's population (Hackinski, 1998). Different neurotransmitters and neuro-modulators are known to play a significant role in the system of excitation. Cyclooxygenase is the key enzyme that converts arachidonic acid, derived from membrane phospholipids to prostaglandins, which have important signaling and housekeeping functions (Griffin, 1999). Recent reports indicated the up-regulation of cyclooxygenase enzyme particularly COX-2 isoform, following seizure activity (Takemiya et al., 2003). In one study, seizures related IL-1β, NF-*K*β and COX-2 expression might contribute to the pathophysiology of epilepsy by inducing neuronal death and astrocytic activation (Voutsinos-Porche et al., 2004). COX-2 is expressed in the layers of neocortex and the pyramidal cells of hippocampus, the area that plays a significant role in onset of seizures activity. Takemiya et al. (2003) also reported the expression of cyclooxygenase-2 isoenzyme in the mouse brain after rapid kindling. The expression of COX-2 is dramatically increased in the granule cells and modestly increased in the pyramidal cells of the hippocampus right after rapid kindling as compared to unstimulated control (Takemiya et al., 2003). There were some recent evidences, in which Shafiq et al. (2003) showed that there was an increase in percentage protection when celecoxib was combined with phenytoin against electroshock-induced convulsions (Shafiq et al., 2003). In one model of lithium chloride and tacrine induced status epilepticus seizures, there was also an expression of COX-2 enzyme protein particularly, in dorsal hippocampus and elevated brain prostaglandin

Abbreviations: CMC, carboxymethylcellulose; COX, cyclooxygenases; COX-1, cyclooxygenases-1; COX-2, cyclooxygenases-2; DMSO, dimethylsulfoxide; EPSC, excitatory postsynaptic current; NSAIDs, non-steroidal anti-inflammatory drugs; PGD2, prostaglandin D_2; PGE1, prostaglandin E_1; PGE2, prostaglandin E_2; PTZ, pentylenetetrazol.

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E2 (PGE2) levels (Paoletti et al., 1998). But there are some contradictory results on the role of cyclooxygenase in epilepsy (Hjalmar and George, 1981) as there was lowering of the convulsive threshold by non-steroidal anti-inflammatory drugs like aspirin and paracetamol. In one report, COX-2 selective inhibitor as well as nonselective COX-inhibitor such as indomethacin, aggravated kainic acid-induced seizure activity and the following hippocampal neuronal death (Baik et al., 1999), and there is no mentioning regarding the mechanism of action of COX-inhibitors in epilepsy. With this background, the present study was carried out to elucidate the effect of cyclooxygenase inhibitors in epilepsy and the possible GABAergic mechanism regarding its role in epilepsy.

2. Materials and methods

2.1. Animals

Male Albino mice (Laka strain) weighing between 22 and 30 g bred in Central Animal House (CAH) facility of Panjab University, Chandigarh were used. The animals were housed under standard laboratory conditions maintained under a natural light and dark cycle, and had free access to food and water. Animals were acclimatized to laboratory conditions before the experiment. Each animal was used only once. All the experiments were carried out between 0900 and 1500 h. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) and conducted according to the Indian National Science Academy guidelines (icmr.nic.in/bioethics/INSA_Guidelines.pdf) for the use and care of experimental animals.

2.2. Drugs

The following drugs were used in the present study. PTZ (Sigma, USA), aspirin, naproxen, nimesulide and rofecoxib (Panacea Biotec Ltd., New Delhi, India). The drug doses were selected according to the previous studies conducted in our laboratory (Jain et al., 2001) and from the literature. PTZ was dissolved in normal saline.

2.3. Drug administration

COX-inhibitors were suspended in 0.25% carboxymethylcellulose (CMC) and given orally. Diazepam (Sigma, USA) was dissolved in one drop of Tween 80 and solution was made with sterile water, and muscimol (Sigma, USA) was dissolved in sterile water and administered intraperitoneally. Flumazenil (Sigma, USA) was dissolved in 10% dimethylsulfoxide (DMSO) and administered intraperitoneally. The experiment protocol was comprised of the following groups, each consisting of 6–10 animals: group 1—control group treated with vehicle (0.25% CMC); group 2 given graded doses of aspirin (10 and 20 mg/kg), naproxen (7 and 14 mg/kg), nimesulide (1, 2.5 and 5 mg/kg) or rofecoxib (1, 2 and 4 mg/kg); group 3 given sub-protective dose of diazepam (0.1 mg/kg, i.p.); group 4 given a combination of sub-protective dose of diazepam and sub-protective dose of rofecoxib (1 mg/kg) or nimesulide (1 mg/kg, i.p.); group 5 given sub-protective dose of muscimol (0.05 mg/kg); group 6 given a combination of sub-protective dose of muscimol and sub-protective dose of rofecoxib (1 mg/kg) or nimesulide (1 mg/kg); group 7 given flumazenil (4 mg/kg, s.c.); group 8 given combination of flumazenil and rofecoxib (1 and 2 mg/kg); or nimesulide (1 and 2.5 mg/kg) followed by PTZ.

2.4. Pentylenetetrazol-induced seizures (Ghosh, 1984)

Pentylenetetrazol (PTZ) (Sigma Co.) (80 mg/kg) intraperitoneally was administered to induce clonic convulsions (Ghosh, 1984). Animals were observed for a period of 30 min post-PTZ administration. The parameters noted were mean onset time of convulsions, duration of clonus and recovery/death (% recovery or % survival) due to PTZ. Various COX-inhibitors were administered 45 min before the PTZ challenge. In mechanistic study, various agonists/antagonists were administered 15 min before COX-2 inhibitors and after 45 min were challenged with convulsive dose of PTZ (i.e., agonists/antagonists-T0, COX-inhibitors-T15 and PTZ-T60).

2.5. Statistical analysis

One specific group of mice was assigned to one specific drug treatment condition and each group comprised 6–10 mice. All the values were expressed as mean±S.E.M. The data was analyzed by using analysis of variance followed by Dunnett's test. In all tests, the criterion for statistical significance was $P<0.05$.

3. Results

3.1. Effect of non-selective COX-inhibitors against PTZ-induced convulsions

Pretreatment with aspirin (20 mg/kg) or naproxen (7 and 14 mg/kg) significantly decreased the susceptibility to PTZ-induced seizures in mice as these drugs increased the mean onset time of clonus phase (Fig. 1A). Aspirin (20 mg/kg) or naproxen (14 mg/kg) also decreased the duration of clonus against PTZ-induced convulsions (Fig. 2A). Aspirin per se at lower dose, i.e., 10 mg/kg did not increase the mean onset time of convulsion but significantly increased the percent recovery in mice (Fig. 3A).

3.2. Effect of selective COX-2 inhibitors on PTZ-induced convulsions

Pretreatment with nimesulide and rofecoxib significantly and dose-dependently decreased the incidence of PTZ-induced seizures as there was an increase in the mean onset time of clonus (Fig. 1B) and decrease in the duration of clonus (Fig. 2B). Rofecoxib or nimesulide at lower doses, i.e., 1 mg/kg showed only a very subtle decrease in the duration of clonus phase. So these doses were chosen as the sub-protectant doses. Rofecoxib (2 and 4 mg/kg) showed 100% recovery of animals after PTZ challenge (Fig. 3B).