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Short Communication

Rofecoxib, a selective cyclooxygenase-2 (COX-2) inhibitor potentiates the anticonvulsant activity of tiagabine against pentylenetetrazol-induced convulsions in mice

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Abstract. Numerous studies have implicated prostaglandins as potential modulators in seizure activity. The objective of the present study was to elucidate the effect of rofecoxib (selective COX-2 inhibitor) alone or in combination with newer antiepileptic drug tiagabine (γ-amino acid reuptake inhibitor) against pentylenetetrazol (PTZ) (80 mg/kg, i. p.)-induced chemoconvulsions in mice. Rofecoxib or tiagabine was administered 45 min prior to the PTZ challenge. In combination study, rofecoxib was administered 10 min before tiagabine and after 35 min the animals were challenged with convulsive dose of PTZ. Mean onset time of jerks, clonus and extensor phases following PTZ challenge was noted. Pretreatment with rofecoxib (1–5.0 mg/kg, i. p.) or tiagabine (1–10 mg/kg, i. p.) dose dependently protected the animals against PTZ-induced convulsions. The mean onset time of jerks, clonus and extensor phase were increased. A subeffective dose of rofecoxib (0.5 mg/kg, i. p.) potentiated the effect of subprotective dose of tiagabine (0.5 mg/kg, i. p.). The results of the present study suggested that the protective effect of rofecoxib, a COX-2 inhibitor against PTZ-induced convulsions may be possibly through the GABAergic modulation. Rofecoxib may have a place as adjuvant therapy with standard antiepileptic drugs such as tiagabine in the treatment of epilepsy.

Key words: Cyclooxygenase; COX-2 inhibitors; Rofecoxib; Tiagabine; Brain; Epilepsy

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Introduction

In recent years, increasing evidences has indicated neuroinflammation as cause in various central nervous systems (CNS) diseases (Vezzani, 2005). Cyclooxygenase is the key enzyme that converts arachidonic acid, derived from membrane phospholipids to prostaglandins, which have important signaling and housekeeping functions (Griffin, 1999). Cyclooxygenase inhibitors are used in the treatment of pain and inflammatory conditions. 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-κβ, 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 (Minghetti, 2004). 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 standard antiepileptic drug such as phenytoin against electroshock-induced convulsions (Shafiq et al., 2003). In one model of lithium chloride and tacrine induced status epilepticus seizures, there was an increased expression of COX-2 enzyme protein particularly, in dorsal hippocampus, further resulting in elevated brain prostaglandin E_2 (PGE_2) levels (Paoletti et al., 1998). In another study, COX-2 induction was found to be responsible for epileptic neuronal injury and that selective COX-2 inhibi-

tors are neuroprotective (Kawaquchi, 2005; Kunz and Oliw, 2001). The data from our laboratory have shown the protective effect of COX-inhibitors in different animal models of epilepsy (Dhir et al., 2005, 2006a, 2006b and 2006c) and its possible GABAergic modulation. COX- inhibitors potentiated the effect of various GABAergic agonists and reversed the effect of various $GABA_A$ antagonists (Dhir et al., 2006a). However, there are some contradictory reports on the role of cyclooxygenase in epilepsy (Hjalmar and George, 1981). 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). With this background, the present study investigates the effect of pretreatment with rofecoxib alone, or in combination with tiagabine, against PTZ-induced convulsions in mice.

Materials and methods

Animals

Male Albino mice (Laka strain) weighing between 22–30 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 09.00 and 15.00 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.

Drugs

The following drugs were used in the present study**.** PTZ (Sigma, USA), rofecoxib (Panacea Biotec. Ltd, New Delhi, India), tiagabine (Sun Pharma Advanced Research Centre). The drugs, doses were selected according to the previous studies conducted in our laboratory and the literature available (Jain et al., 2001; Dhir et al., 2006 a, b, c; Nielsen et al., 1991). PTZ was dissolved in sterile water.

Drug administration

Rofecoxib was suspended in 0.25 % carboxymethylcellulose (CMC) and given intraperitoneally. Tiagabine was dissolved in sterile water and also administered intraperitoneally. The drugs were given at a constant dose volume of 10 ml/kg. In one study, tiagabine when administered intraperitoneally dose-dependently increases extracellular GABA overflow in the globus pallidus and substantia nigra. The maximal increase in extracellular GABA levels occurs at 40 min (Sujdak and Jansen, 1995). Therefore, we have administered the PTZ after 45 min of tiagabine or rofecoxib administration. In combination studies, the subeffective doses of rofecoxib was administered 10 min before tiagabine and after 35 min of treatment, animals were challenged with convulsive dose of PTZ. 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 tiagabine (0.5, 1, 5 and 10 mg/kg., i. p.). Group-3 given graded doses of rofecoxib (0.5, 1 and 5 mg/kg., i. p.), Group-4 given a combination of sub-protective dose of tiagabine (0.5 mg/kg., i. p.) and sub-protective dose of rofecoxib (0.5 mg/kg., i. p.).

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 jerks, full-blown convulsions, extensor due to PTZ (Kulkarni, 2005).

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 ±SEM. The data was analyzed by using analysis of variance (ANOVA) followed by Dunnett's test. In all tests, the criterion for statistical significance was $P < 0.05$.

Results

Pretreatment with rofecoxib (5 mg/kg, i. p.) significantly increased the mean onset time of all the three phases viz. jerks (Fig. 1.), clonus (Fig. 2) and extensor phases (Fig. 3) due to PTZ. A lower dose (1 mg/kg, i. p.) significantly enhanced the onset time of two phases i. e. clonus and extensor (Figs. 2 and 3) but was ineffective in reversing the onset of jerks (Fig. 1), whereas a still lower doses i. e., 0.5 mg/kg, i. p. did not significantly affect the onset of all the three phases due to PTZ (Figs. 1, 2 and 3). Similarly**,** acute treatment with tiagabine (1, 5 and 10 mg/kg, i. p.) significantly and dose-dependently decreased the PTZ-induced seizures as there was an increase in the mean onset time of jerks (Fig. 1.), clonus (Fig. 2) and extensor phase (Fig. 3). However, tiagabine at lower doses i. e., 0.5 mg/kg, i. p. was not effective in reversing the different parameters of PTZ-induced convulsions. When rofecoxib at subprotectant dose was combined with the subeffective dose of tiagabine, resulted in pronounced anticonvulsant effect.

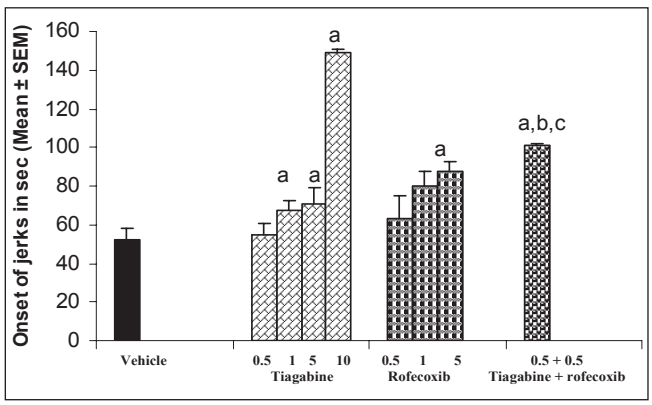

Fig. 1. Effect of rofecoxib (0.5–5 mg/kg i. p.), tiagabine (0.5–10 mg/kg i. p.) or its combination (0.5 mg/kg + 0.5 mg/kg) on mean onset time of jerks against PTZ- induced convulsions. Rofecoxib or tiagabine was administered 45 min before PTZ challenge. In combination studies rofecoxib was administered 10 min before tiagabine and after 35 min challenged with convulsive dose of PTZ. [a]$P < 0.05$ as compared to vehicle treated group. [b]$P < 0.001$ as compared to tiagabine (0.5 mg/kg) treated group. [c]$P < 0.05$ as compared to rofecoxib (0.5 mg/kg) treated group (ANOVA followed by Dunnett's test).

The combination markedly increased the mean onset time of clonus and extensor phase (Figs. 2 and 3). Also, the combination increased the onset time of jerks (Fig. 1).

Discussion

The core finding of the present study is that administration of a relevant dose of rofecoxib, a selective cyclooxygenase-2 (COX-2) inhibitor delays the onset time of all the three phases (jerks, clonus and extensor) due to PTZ. Also rofecoxib potentiated the effect of subprotective dose of tiagabine against PTZ-induced convulsions, probably acting through GABAergic modulation. The ED_{50} of tiagabine to inhibit clonic convulsions in mice has been reported to be 1.3 mg/kg i.p. (Nielsen et al., 1991). Therefore, we have chosen the different doses (0.5, 1 and 5 mg/kg) of tiagabine according to the ED_{50} studies reported in the literature. Moreover, the different doses of rofecoxib were chosen according to the previous studies done in our laboratory (Dhir et al., 2006a, 2006b). In the present study, rofecoxib attenuated the PTZ-induced convulsions, potentiated the effect of subprotective dose of tiagabine, thus providing the first evidence regarding its beneficial role in epilepsy.

Inflammatory processes have been implicated in both acute and chronic neurodegenerative conditions. Accumulating evidence has shown that any insult to the brain is accompanied by a marked inflammatory reaction, which is characterized by increased expression of various proinflammatory molecules, activation of microglia, and infiltration of monocytes/macrophages (Allan and Rothwell, 2001). Inflammatory processes, such as the activation of cyclooxygenase isoenzyme and the prostaglandin formation have been described in brain after seizures induced in experimental models and in clinical cases of epilepsy.

The presence of prostaglandins in the mammalian brain is well-documented (Kim et al., 2001) and prostaglandins are either directly or indirectly involved with neuronal activity (Haris and Ramwell, 1979). A role of prostaglandins in seizures has been reported. Increased levels of PGD_2 and PGE_2 following PTZ-induced seizures were observed (Takemiya et al., 2003). PG_1 and PGE_2 have excitatory effects on the cerebral cortex, the area that plays an important role in the onset of seizure activity. $PGF_{2\alpha}$ is the predominant prostaglandin identified in the experimentally induced as well as spontaneous seizure activity (Tandon et al., 2003). We hypothetized that COX-inhibitors reduce PTZ-induced seizures by inhibiting the synthesis of prostaglandins. Also PGE_2, which is preferentially formed during the activity of COX-2 rather than COX-1, could participate through several mechanisms, including modulation of glutamatergic neurotransmission (Bazan, 2003). Also there are reports that cyclooxygenase levels and PGs tend to rise in both chemically and electrically induced seizures.

Tiagabine is a potent, and specific, GABA reuptake inhibitor in a variety of brain tissue preparations (Braestrup et al., 1990). It is reported to be a potent anticonvulsant agent against methyl-6,7-dimethyoxy-4-ethyl-β-carboline-3-carboxylate (DMCM)-induced clonic convulsions and subcutaneous PTZ-induced tonic convulsions, and electrically induced convulsions in kindled rats (Sujdak and Jansen, 1995). Tiagabine is also effective against subcutaneous PTZ-induced clonic convulsions. Tiagabine is weakly efficacious in the intravenous PTZ seizure threshold test (Picardo et al., 1990) and the maximal electroshock-induced convulsions (MES) test and produced only partial protection against bicuculline-induced convulsions in rats (Nielsen et al., 1991).

Arachidonic acid which is metabolized to prostaglandins by cyclooxygenases and has been proposed to be a diffusible second messenger in the CNS with a pathophysiological role in epilepsy. This is possibly due to the ability of arachidonic acid to enhance extra-neuronal glutamate concentration (Dicke et al., 1994). Cyclooxygenase induction lead to the increase in prostaglandins levels particularly PGE_2, which

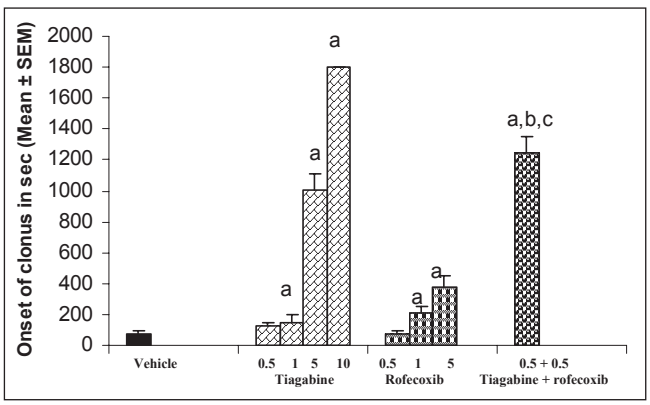

Fig. 2. Effect of rofecoxib (0.5–5 mg/kg i.p.), tiagabine (0.5–10 mg/kg i.p.) or its combination (0.5 mg/kg + 0.5 mg/kg) on mean onset time of clonus against PTZ-induced convulsions. Rofecoxib or tiagabine was administered 45 min before PTZ challenge. In combination studies rofecoxib was administered 10 min before tiagabine and after 35 min challenged with convulsive dose of PTZ. [a]$P < 0.001$ as compared to vehicle treated group. [b]$P < 0.001$ as compared to tiagabine (0.5 mg/kg) treated group. [c]$P < 0.001$ as compared to rofecoxib (0.5 mg/kg) treated group (ANOVA followed by Dunnett's test).

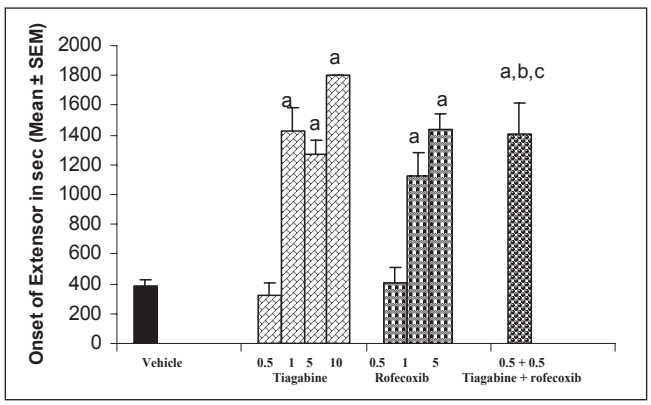

Fig. 3. Effect of rofecoxib (0.5–5 mg/kg i.p.), tiagabine (0.5–10 mg/kg i.p.) or its combination (0.5 mg/kg + 0.5 mg/kg) on mean onset time of extensor against PTZ-induced convulsions. Rofecoxib or tiagabine was administered 45 min before PTZ challenge. In combination studies rofecoxib was administered 10 min before tiagabine and after 35 min challenged with convulsive dose of PTZ. [a]$P < 0.001$ as compared to vehicle treated group. [b]$P < 0.001$ as compared to tiagabine (0.5 mg/kg) treated group. [c]$P < 0.001$ as compared to rofecoxib (0.5 mg/kg) treated group (ANOVA followed by Dunnett's test).

may facilitates glutamate release from the nerve terminal and astrocytes (Takemiya et al., 2003). Glutamate is an excitatory neurotransmitter that can leads to decrease in GABA input, results in convulsions. Here in the present study, rofecoxib, a COX-2 enzyme inhibitor has shown the antiepileptic activity against PTZ-induced convulsions. Rofecoxib also enhanced the effect of tiagabine, a GABA reuptake inhibitor. Therefore, it may be conceived that rofecoxib is acting through GABAergic modulation.

There may be some probability of pharmacokinetic interactions of rofecoxib with tiagabine. But, no clinically significant drug interaction has been reported for rofecoxib except with diuretics, where it reverses their salt-wasting effect and thus can be clinically exploited in electrolyte-wasting disorders. (Ahuja et al., 2003). Moreover, in one study done by Kaminski et al. (1998), non-steroidal anti-inflammatory agents (NSAIDs) increased the protective effect of valproate in epilepsy and when checked the plasma levels of drugs were studied, the total plasma levels of valproate and free plasma levels of diphenylhydantoin remained unchanged in the presence of all the NSAIDs examined, showing no pharmacokinetic interactions (Kaminski et al., 1998).

Conclusion

The result of the present study suggested significant protection by rofecoxib against the onset time of different phases of PTZ-induced convulsions. COX-2 inhibitors could be a useful adjuvant therapy with tiagabine for the treatment of epilepsy. Further studies are required to confirm the hypothesis.

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