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Effect of cyclooxygenase-2 (COX-2) inhibitors in various animal models (bicuculline, picrotoxin, maximal electroshock-induced convulsions) of epilepsy with possible mechanism of action

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Enzyme cyclooxygenase (COX) is reported to play a significant role in neurodegeneration and may play a significant role in the pathogenesis of epilepsy. Bicuculline (4 mg/kg; ip), picrotoxin (8 mg/kg; ip) and electroshock (60 mA for 0.2 sec) significantly induced convulsions in male Laka mice. COX-inhibitors viz. nimesulide (2.5 mg/kg; ip) and rofecoxib (2 mg/kg, ip) administered 45 minutes prior to an epileptic challenge prolonged mean onset time of convulsions, decreased duration of clonus and decreased % mortality rate against bicuculline- and picrotoxin-induced convulsions in mice. COX-2 inhibitors were ineffective towards maximal electroshock-induced convulsions. Nimesulide (1 mg/kg) and rofecoxib (1 mg/kg) also enhanced the effect of subprotective dose of muscimol against picrotoxin-induced convulsions. The result of the present study strongly suggests for a possible role of cyclooxygenase isoenzymes particularly, COX-2 in the pathophysiology of epilepsy and its GABAergic modulation.

Keywords: Bicuculline, Convulsions, Cyclooxygenases, Epilepsy, Nimesulide, Picrotoxin, Rofecoxib

Epilepsy is a common neurological disorder affecting about 0.5-1% of the world's population[1]. Clinical signs of epilepsy arise from the intermittent, excessively synchronized activity of group of neurons. Different neurotransmitters and neuromodulators are known to play a significant role in the system of excitation[2]. Therefore, there is always a continuous research being done to unravel the pathophysiology of epilepsy and the development of newer therapeutic strategies. Cyclooxygenase is the key enzyme that converts arachidonic acid, derived from membrane phospholipids to prostaglandins (PGs) which have important signaling and house keeping functions particularly in platelets, gastrointestinal tract, lungs and kidneys[3]. The neuromodulation by prostaglandins and their possible role in epileptogenesis has been reported[4]. The involvement of COX-2 in acute and chronic neurodegenerative syndromes has promoted the development of neuroprotective treatment strategies involving COX-inhibitors, such as non-steroidal anti-inflammatory drugs (NSAIDs). Although studies suggest that NSAIDs may be protective in chronic neurodegenerative conditions, little is known of their clinical efficacy in treating acute neurodegeneration.

It has been reported that COX-2 mRNA and protein are specifically and rapidly increased in brain by electroconvulsive seizure activity[5]. There are recent reports regarding the up-regulation of cyclooxygenase enzyme particularly COX-2 isoform following seizures activity[6]. But there are some contradictory results on the role of cyclooxygenase in epilepsy[7] 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 non-selective COX-inhibitor such as indomethacin, aggravated kainic acid-induced seizure activity and the following hippocampal neuronal death[8] and there is no mentioning regarding the mechanism of action of COX-inhibitors in epilepsy. With this background, the present study has been undertaken to elucidate the effect of cyclooxygenase inhibitors in various animal models of epilepsy and the possible mechanism of action.

Materials and Methods

Animals—Male Albino mice (Laka strain) weighing 22-30 g and bred in Central Animal House

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(CAH) facility of Panjab University, Chandigarh were used. The animals were housed under standard laboratory conditions, maintained on a natural light and dark cycle and had free access to food and water. Animals were acclimatized to laboratory conditions before the experiment. All the experiments were carried out between 0900 and 1500 hrs. The experimental protocols were approved by 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 and treatment schedule

The following drugs were used: picrotoxin (Sigma, USA), bicuculline (Sigma, USA), nimesulide and rofecoxib (Panacea Biotec, India). The drugs doses were selected according to the previous studies[9]. Picrotoxin was dissolved in normal saline. Bicuculline was dissolved in saline with the aid of 0.1 *N* HCl and the COX-inhibitors were suspended in 0.25% carboxymethylcellulose (CMC). Different COX-inhibitors were administered 1 hr before bicuculline or picrotoxin challenge. To see the mechanism of action, COX-inhibitors were given 30 min before subprotective dose of muscimol and after 30 min challenged with picrotoxin.

The experiment protocol comprises the following groups, each constitutes 6-18 animals:

Bicuculline-induced convulsions—Following groups were used: -

Group-1 control i.e. treated with vehicle (0.25% CMC, ip); Group-2 treated with rofecoxib (2 mg/kg; ip) or nimesulide (2.5 mg/kg; ip).

Picrotoxin-induced convulsions—Group-1 control treated with vehicle (0.25% CMC ip); Group-2 treated rofecoxib (1 and 2 mg/kg; ip) or nimesulide (1 and 2.5 mg/kg; ip); Group-3 given sub-protective dose of muscimol (0.05 mg/kg; i.p.); Group-4 given a combination of sub-protective dose of muscimol and sub-protective dose of rofecoxib (1 mg/kg; ip) or nimesulide (1 mg/kg; ip) against picrotoxin-induced convulsions.

Maximal electro-shock induced seizures—The following groups were used: Group-1 control treated with vehicle (0.25% CMC, ip); Group-2 treated rofecoxib (2 mg/kg; ip) or nimesulide (2.5 mg/kg; ip).

Experimental models

Bicuculline- and picrotoxin-induced convulsions—Bicuculline (4 mg/kg; ip) and picrotoxin (8 mg/kg; ip) were used to induce chemoconvulsions in mice. Different parameters of chemoconvulsions were noted like onset time of convulsions, duration of convulsions and recovery/death. Each animal was observed individually for the presence of absence of above phases of convulsions up to 2 hr and thereafter at 24 hr for mortality. Bicuculline and picrotoxin produced severe tonic-clonic convulsions followed by 100% mortality at respective doses[10].

Maximal electro-shock induced seizures (MES)—The mice were subjected to maximal electroshock (MES) using an electroconvulsometer. A current of 60 mA for 0.2 sec was used to induce convulsions. The parameters observed were tonic hind limb extension of mice and percentage mortality of animals[11].

Statistical analysis—One specific group of mice was assigned to one specific drug treatment condition and each group comprised 6-18 mice (n=6-18). All the values are expressed as mean ± SE. The data were analyzed by using analysis of variance followed by Dunnett's test. In all tests, the criterion for statistical significance was $P< 0.05$.

Results

Effect of selective COX-2 inhibitors on bicuculline-induced convulsions—Bicuculline (4 mg/kg; ip) produced severe tonic-clonic convulsions followed by 100% mortality. Pretreatment with rofecoxib (2 mg/kg; ip) or nimesulide (2.5 mg/kg; ip) significantly decreased the severity of bicuculline-induced seizures as these drugs prolonged the mean onset time of convulsions and decreased the duration of clonus (Fig. 1A). Pretreatment with rofecoxib (2 mg/kg; ip) or nimesulide (2.5 mg/kg; ip) decreased the % mortality (Fig.1B) after bicuculline challenge.

Effect of selective COX-2 inhibitors on picrotoxin-induced convulsions—Pretreatment with rofecoxib (2 mg/kg; ip) or nimesulide (2.5 mg/kg; ip) significantly decreased the severity of picrotoxin-induced convulsions (Fig. 2A). Rofecoxib (1 mg/kg; ip) or nimesulide (1 mg/kg; ip) was not effective against picrotoxin-induced convulsions, as rofecoxib and nimesulide at 1 mg/kg did not prolonged the mean onset time of convulsions. Pretreatment with either rofecoxib or nimesulide decreased the % mortality in mice. (Fig. 2B).

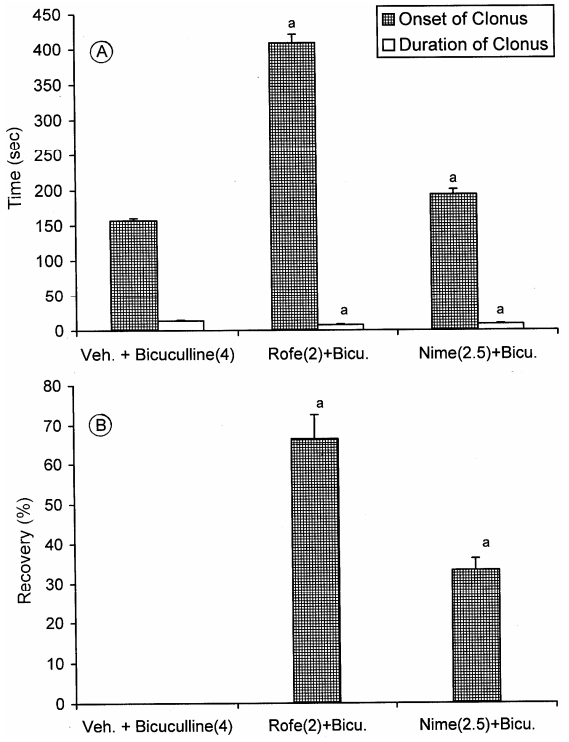

Fig. 1—Effect of COX-2 inhibitors on (A) onset of convulsions or duration of clonus and (B) recovery (%) against bicuculline-induced convulsions (n=6-18). [a]*P*<0.05 as compared to bicuculline treated group (ANOVA followed by Dunnett's test).

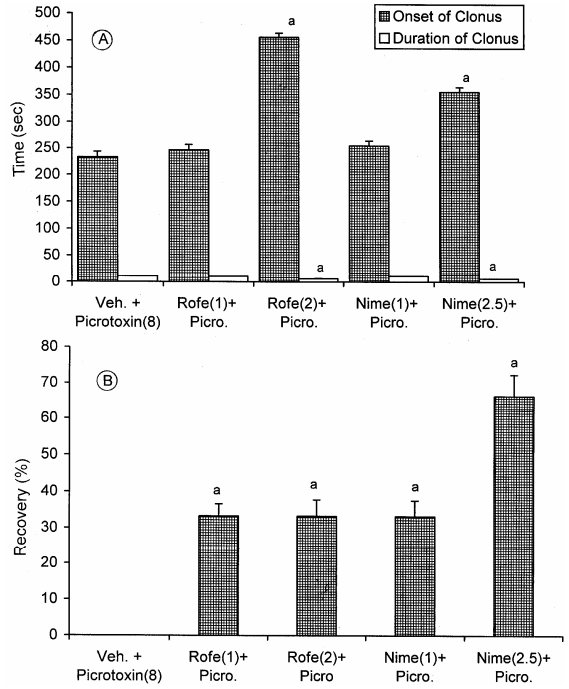

Fig. 2—Effect of COX-2 inhibitors on (A) onset time of convulsions and duration of clonus and (B) recovery (%) against picrotoxin-induced convulsions (n=6-18). [a]*P*<0.05 as compared to picrotoxin treated group (ANOVA followed by Dunnett's test).

Effect of combination of sub-effective dose of selective COX-2 inhibitors and sub-effective dose of muscimol against picrotoxin-induced convulsions—Muscimol (0.05 mg/kg) *per se* was not effective in preventing seizures against picrotoxin-induced convulsions. Pretreatment with rofecoxib (1 mg/kg) or nimesulide (1 mg/kg; ip) potentiated the effect of sub-protective dose of muscimol (0.05 mg/kg) against picrotoxin-induced convulsions (Fig. 3) and the combination showed 100% recovery against picrotoxin-induced convulsions (Fig. 4).

Effect of selective COX-2 inhibitors on maximal electroshock-induced convulsions—Rofecoxib (2 mg/kg) or nimesulide (2.5 mg/kg) were selected as these doses were effective against bicuculline or picrotoxin

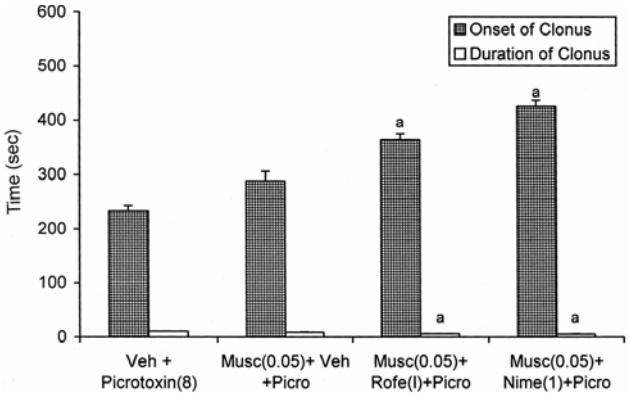

Fig. 3—Effect of subprotective dose of muscimol (0.05 mg/kg) and its combination with rofecoxib (1 mg/kg) or nimesulide (1 mg/kg) inhibitors on onset time of convulsions and duration of clonus against picrotoxin-induced convulsions (n=6-18). [a]*P*<0.05 as compared to picrotoxin treated group (ANOVA followed by Dunnett's test).

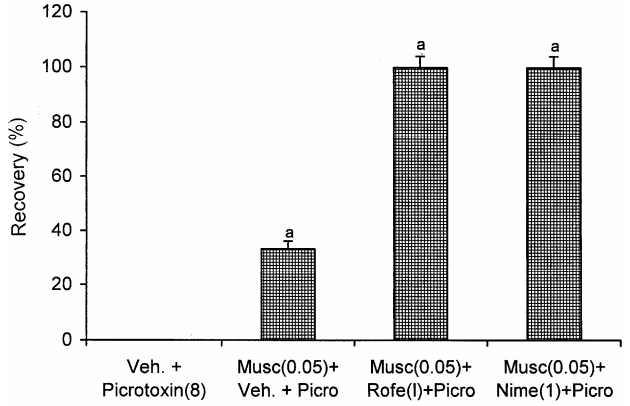

Fig. 4—Effect of sub protective dose of muscimol and its combination with COX-2 inhibitors on recovery (%) against picrotoxin-induced convulsions (n=6-18). [a]*P*<0.05 as compared to picrotoxin treated group (ANOVA followed by Dunnett's test).

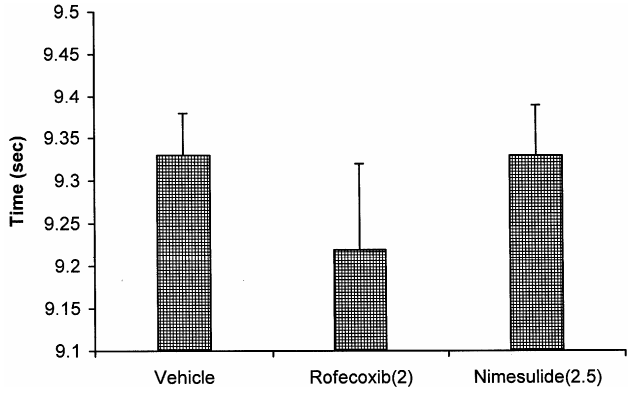

Fig. 5—Effect of COX-2 inhibitors on duration of extensor phase against maximal electroshock-induced convulsions (n=6-18).

induced convulsions. Pretreatment with rofecoxib (2.0 mg/kg) or nimesulide (2.5 mg/kg) did not affect the mean onset time of convulsions in maximal electroshock-induced convulsions. These drugs did not decrease the duration of extensor phase (Fig. 5) and also did not decrease the % mortality in mice.

Discussion

The presence of prostaglandins in the mammalian brain is well-documented[12] and prostaglandins are either directly or indirectly involved with neuronal activity[13]. The role of prostaglandins in seizures is known, as there are increased levels of PGD_2 and PGE_2 following PTZ-induced seizures. PGE_1 and PGE_2 have excitatory action on cerebral cortex[14]. Prostaglandins and cyclooxygenases, levels tend to rise in both chemically and electrically induced seizures[15]. PGE_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[16]. It was hypothetized that COX-inhibitors reduces 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 in through several mechanisms, including modulation of glutamatergic neurotransmission[17]. There was increased expression of COX-2 in the genetically epilepsy susceptible E1 mice[18]. In one model of lithium chloride and tacrine (5 mg/kg; ip) induced status epilepticus seizures, there was also an expression of COX-2 enzyme protein particularly in dorsal hippocampus and elevated brain PGE_2 levels[19]. Cyclooxygenase-2 expression is markedly and transiently up regulated in neurons in response to excitatory stimuli such as seizures and kainic acid[20]. COX-2 induction appears to be the characteristic of forebrain seizures since COX-2 was not induced in mid-brain seizures, but was dramatically up regulated in ipsilateral cortex following seizure generalization[5]. Takemiya *et al*[6]., studied the expression of cyclooxygenase-2 isoenzyme in the mouse brain after rapid kindling.

Evidences support the involvement of GABA in seizures paradigm. $GABA_A$ receptor agonist as well as drugs, which allosterically modulate the receptor channel complex, is therapeutically active against convulsive seizures[21]. Both picrotoxin and bicuculline affect GABA neurotransmission. Picrotoxin appears to interfere indirectly with the tonic presynaptic inhibition action of GABA, thus induced convulsions through rapid summation of synaptic activity[22]. Bicuculline is a pure GABA receptor antagonist, as assessed by ligand binding studies[23]. Zath and Roth[24] observed that electroconvulsive stimuli increase PGF in rat cerebral cortex. In the present study, rofecoxib (2 mg/kg) or nimesulide (2.5 mg/kg) significantly decreased the incidence of bicuculline-induced convulsions. γ-amino butyric acid (GABA), the principal inhibitory neurotransmitter in the cerebral cortex maintained the inhibitory tone that counterbalances neurons excitation. When this balance is disturbed, seizures ensue. Reduction in GABA-mediated inhibitory activity of glutamate decarboxylase has been reported in studies of human epileptic brain tissues[25]. Glutamate concentration increased before seizure onset and found to be highest in the epileptic hippocampus than non-epileptic hippocampus while GABA levels increased during seizures was greater in non-epileptic hippocampus than in epileptic hippocampus showing decreased levels of GABA in epilepsy[26]. The $GABA_A$ receptor is responsible for most fast inhibitory neurotransmission in the central nervous system. Consequently, this receptor has been targeted for the pharmacological control of anxiety, sleep, and epilepsy. Numerous natural and synthetic compounds interact with the $GABA_A$ receptor at distinct, yet incompletely defined, sites. These compounds include barbiturates, benzodiazepines, neurosteroids, and picrotoxin[27,28]. The drugs, which enhance the anti-epileptic activity of $GABA_A$ agonist such as muscimol, are thought to act through $GABA_A$ receptors. In the present study, pretreatment with rofecoxib (2 mg/kg) or nimesulide (2.5 mg/kg) significantly decreased the incidence of

picrotoxin-induced convulsions while at rofecoxib (1 mg/kg) and nimesulide (1 mg/kg) were not effective against picrotoxin-induced convulsions. Rofecoxib (1 mg/kg) or nimesulide (1 mg/kg) potentiated the effect of sub-effective dose of muscimol (0.05 mg/kg) against picrotoxin-induced convulsions. These combinations also decreased the duration of clonus and combining rofecoxib (1 mg/kg) and nimesulide (1 mg/kg) with sub-effective dose of muscimol (0.05 mg/kg) showed 100% recovery in animals against picrotoxin-induced convulsions. This further proves the GABAergic mechanism of action of COX-inhibitors. Arachidonic acid which by cyclooxygenases action formed prostaglandins has been proposed a diffusible second messenger in CNS with a pathophysiological role in epilepsy. This is possibly due to ability of arachidonic acid to enhance extraneuronal glutamate concentration[29]. Also arachidonic acid cannot be only considered as a facilitatory neuromodulator in the hippocampus as the arachidonic acid facilitation of the release of the main inhibitory neurotransmitter GABA. Also majority of cyclooxygenases are excitatory and express in glutamatergic neurons[30]. Increased in glutamate levels may leads to decrease in GABAergic output, which may be responsible for increased seizures susceptibility. In the present study, COX-inhibitors proved to be ineffective against maximal-electroshock induced convulsions. Further studies are needed at higher doses to see the effect of COX-inhibitors against maximal electroshock induced seizures paradigm. Finally, the result of the present study suggested that COX-2 have significant protection against the bicuculline- and picrotoxin-induced convulsions via GABA/Benzodiazepine receptor mechanism.

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