ETORICOXIB AS A PROBE OF COX-2 MECHANISMS IN LEARNING AND MEMORY

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Summary

In an attempt to study the COX-2 mechanisms in learning and memory, we used etoricoxib as the biological probe and elevated plus maze as the cognitive task. The study examined the role of COX-2 in leraning and memory and its interaction with nicotine, pilocarpine, gabapentin, and donepezil.Adult, female, Wistar rats (n=48) were trained in the elevated plus maze task for three days. Within 15 min post-training on day 3 rats received etoricoxib (n =40) or vehicle (n = 8). After 30 min, the etoricoxib treated animals received donepezil, gabapentin, pilocarpine, or nicotine. One day later, transfer latency was re-assesed.Etoricoxib impaired learning and memory. Nicotine was found to reverse the memory deficits caused by COX-2 inhibitor, etoricoxib.Memory impairment produced by etoricoxib was seen to be reversed by nicotine. Nicotine is thought to produce its action by modulating the NMDA signaling and in turn upregulating LTP; synaptic representation of learning and memory.

Key words: learning, memory, etoricoxib, COX-2, glutamate, NMDA

Introduction

Prostaglandins play a regulatory role in several forms of neural plasticity, including long-term potentiation, a cellular model for certain forms of learning and memory.

Prostaglandins and thromboxanes, collectively known as prostanoids, are metabolites of arachidonic acid. Phospholipases initiate prostanoid synthesis by liberating arachidonic acid from membrane fatty acids, cyclooxygenase (COX) enzymes catalyse the first two committed steps in the biosynthesis of prostanoids. Three COX isoforms have been identified. COX-2 is often referred to as the inducible isoform of COX, as levels of COX-2 increase in response to several forms of stimulation in various types of tissue. COX-3 is a COX-1 variant which is not expressed in humans or rats.[1]Although COX-2 is undetectable in most tissues under basal conditions, marked basal expression has been observed in the dendrites and cell bodies of neurons in the central nervous system.[2-5] The physiological role of COX-2 is a focus of attention in the field of cognitive neuropsychology. It was shown that spatial learning was impaired by celecoxib, a COX-2-selective inhibitor.[6] In another study it was shown that the retention of spatial learning was impaired by celecoxib, but not by indomethacin, which is a nonselective COX inhibitor.[7] It was also shown that COX-nonselective and COX-2-selective inhibitors both impaired the consolidation of hippocampal-dependent but not dorsal striate-dependent memory; this was so when the anti-inflammatory drugs were injected immediately after the training but not when they were administered 2h later.[8] In this study, COX-1 selective inhibition was not associated with impaired recall on either task. The importance of COX-2 selectivity in cognition was further emphasized in a study that observed that LTP is normal in C0X-1 knockout mice.[9] COX mechanisms have complex interactions with learning as well as memory processes as evident from the above mentioned experiments. COX -2 interacts with glutamate to modulate the hardwiring of learning and memory processes.

Normally, glutamate released during learning stimulates the NMDA receptor, launching a cascade of downstream events. These events include activation of COX-2 and thence a conversion of membrane arachidonic acid to endogenous prostanoids, as well as a conversion of endogenous cannabinoids (which dampen NMDA signaling) into novel prostaglandins (which increase NMDA activity). These events also include the activation of platelet activation factor (PAF). The COX-2 and PAF activation result in feedback augmentation of the NMDA signal.[10] Other reverberating circuits (including those that involve retrograde neurotransmission through arachidonic acid) that amplify the NMDA signal also develop.[11] The net effect is glutamate- and NMDA- dependent initiation of long term potentiation (LTP) in the hippocampus, and thence the hardwiring of learning and memory through neuroplasticity changes.

Under physiological conditions, COX-2 inhibitors inhibit learning and memory by dampening NMDA signaling and thus inhibiting the processes of learning and memory. It was also seen that post-training intrahippocampal infusion of nicotine prevents spatial memory retention deficits induced by the cyclo-oxygenase-2-specific inhibitor.[12] Cholinergic hippocampal neurons are considered to be critical for memory formation. In particular, nicotinic acetylcholine (ACh) receptors localized on these neurons have been widely shown to be important for memory and cognition.[13] Nicotine increases COX-2 expression and subsequent prostaglandin (PG) release in the central and the peripheral nervous systems.[14] The present study was also designed to evaluate the possible roles of muscarinic ACh receptor, γ- amino butyric acid (GABA) receptor, and nicotinic ACh receptor in the COX-2 dependent processes of learning & memory.

Methodology

Animals

The study was conducted on adult, female, Wistar rats (150-200g; n = 60) obtained from Central Animal Research Facility,

National Institute of Mental Health and Neurosciences, Bangalore. The rats were housed four per cage with free access to water and standard laboratory diet, and were maintained and studied under ambient temperature and humidity conditions, in a disturbance-free environment. The project was approved by the Institutional animal ethics committee of Visveswarapura Institute of Pharmaceutical Sciences, Bangalore.

Drugs

Etoricoxib (Etoshine®, Sun Pharmaceuticals, Dadra, India), donepezil (Donecept®, Cipla, India), gabapentin (Neurontin®, Pfizer, India), pilocarpine (Pilomax®, Sun Pharma, India), nicotine hydrogen tartrate (Sigma, India), Carboxy methyl cellulose (SD chemicals, India) were used in the study.

To investigate the role of COX- 2 in memory.

The role of COX – 2 in the processes of learning and memory was studied using etoricoxib as a biological probe. Elevated maze was used as the cognitive task and the transfer latency was evaluated for the cognitive parameter.

Table 1: Study flow chart

Day 1	Acclimatization in the elevated plus maze
Days 2	Assessment of acquisition transfer latency score.
Day 3	Assessment of acquisition transfer latency score.
	Administration of etoricoxib or vehicle within 15 min of assessment on day 3
	Administration of the drugs; nicotine, pilocarpine, gabapentin, donepezil, after 30 min of etoricoxib administration to the respective treatment group animals as shown in Table-1.
Day 4	Assessment of retention transfer latency.

Bases for dose selection

The doses of drugs used in this experiment were based on previous animal studies. Donepezil (1mg/kg body weight),[15] pilocarpine (5mg/kg body weight),[15] gabapentin (10mg/kg body weight),[16] nicotine (1mg/kg body weight),[15] were used in the study.

Table – 2: Experimental groups

Group 1	n = 8, rats were treated with vehicle (1% CMC *p.o*)
Group 2	n = 8, rats were treated with etoricoxib (10mg/kg b.w., *p.o*)
Group 3	n = 8, rats were treated with etoricoxib (10mg/kg b.w., *p.o*) and donepezil (1mg/kg b.w., *p.o*)
Group 4	n = 8, rats were treated with etoricoxib (10mg/kg b.w., *p.o*) and pilocarpine (5mg/kg b.w., *p.o*)
Group 5	n = 8, rats were treated with etoricoxib (10mg/kg b.w., *p.o*) and gabapentin (10mg/kg b.w., *p.o*)
Group 6	n = 8, rats were treated with etoricoxib (10mg/kg b.w., *p.o*) and nicotine (1mg/kg b.w., *p.o*)

Acclimatization in the elevated plus maze

The animals were acclimatized in elevated plus maze for them to learn to enter the closed arm. The animal was placed on end of one of the open arms, facing away from the central platform. The time taken by the animal to reach the closed arm is taken as the transfer latency (TL). In case the animal did not enter the closed arm within 90s it was gently led to the closed arm and the TL was given a cut-off value of 90s. At the end of 90 sec the animal was

allowed to explore the maze for additional 30 s. The apparatus was made of plywood and consisted of two open arms (50cm x 10cm x 40 cm) and closed arms (50cm x 10cm x 40 cm). The maze was elevated to a height of 50 cm from the floor.

Assessment of acquisition transfer latency

Acquisition scores were recorded on days 2 and 3. The same cut-off criterion of 90 sec was used.

Medication

Five groups of animals received etoricoxib (10 mg/kg) and one group received vehicle (1% CMC) within 15 min post-training. After 30 min of etoricoxib administration, rats received the following drugs: nicotine, pilocarpine, gabapentin, donepezil as mentioned in Table 2. The above drugs were given to animals which previously received etoricoxib.

Assessment of retention transfer latency

On day 4 the rats were re-exposed to the elevated plus maze and retention transfer latency was assessed.

Statistical analysis

Transfer latency values are expressed as mean ± SEM. Final transfer latencies were compared between two groups (acquisition and retention) and across four groups (drug treatments) using one way analysis of variance (ANOVA) with the Tukey HSD post hoc test. $P < 0.05$ was considered statistically significant.

Results

Table 3: Acquistion and retention transfer latencies of rats in the elevated plus maze

Group	Treatment	Acquisition TL (seconds)	Retention TL (seconds)	n
1	Vehicle	23.75 ± 3.46	18.21 ± 3.04	8
2	Etoricoxib	14.67 ± 1.82	31.98 ±4.68[a]	8
3	Etoricoxib+ Donepezil	20.96 ± 5.45	33.62 ± 6.71	8
4	Etoricoxib+ Pilocarpine	18.21 ± 3.04	25.20 ± 4.32	8
5	Etoricoxib+ Gabapentin	19.50 ± 3.98	27.17 ± 5.11	8
6	Etoricoxib+ Nicotine	23.28 ± 5.22	5.63±1.03***	8

Values are expressed as mean ± SEM. Data was analysed by one- way ANOVA followed Tukey HSD post hoc analysis. [a] $P < 0.05$ vs vehicle retention TL, *** $P< 0.001$ vs etoricoxib retention TL.

The retention transfer latency data are presented in Table 3. There was significant difference in the retention transfer latency between the vehicle and the etoricoxib groups ([a] $p< 0.05$). This indicates validity of the model. The retention transfer latency of the nicotine-treated group was significantly different from that of the etoricoxib treated group (*** $p< 0.001$).

Discussion

The present study evaluated the effect of post-training administration of nicotine, donepezil, gabapentin, pilocarpine on memory retention deficits induced by etoricoxib. Similar study has been carried out in rats using celecoxib and water maze and nicotine was found to

reverse the memory deficits induced by celecoxib.[12] Nicotine also appears to increase the release of GABA, ACh in brain areas associated with cognitive function.[17-20] Therefore gabapentin, donepezil, and pilocarpine were included in the study to find whether drugs that act directly on the GABA and the Ach receptors would modulate the cognitive functions by interacting with COX-2 in some way. However, statistically significant result was seen only in the nicotine treated group (Table 3).

A considerable body of evidence has shown the critical role of hippocampal NMDA glutamate receptors in memory function.[20-25] The direct interactions of nicotinic and glutamateergic systems have also been previously reported.[25,26] Nicotine increases COX- 2 expression, PGE2 release, as well as activation of extracellular signal-related protein kinase,[27,28] all of which are prevented by COX – 2 inhibitor.[27] PGE2 is involved in hippocampal synaptic signaling which leads to LTP.[29]

PGE2 dynamically regulates membrane excitability, synaptic transmission, and plasticity; the PGE2-induced synaptic modulation is mediated via cAMP-protein kinase A and protein kinase C pathways in rat hippocampal CA1 pyramidal neurons.[29,30] COX-2 is also involved in the conversion of endogenous cannabinoids into novel prostaglandins which are signalling factors in synaptic transmission and plasticity in the hippocampus.[9]

The present experiment re-affirms glutamatergic modulation by nicotine in reversing or blocking the memory deficits induced by a COX-2 selective inhibitor. Understanding the involvement COX-2 in learning and memory might open up the possibility of newer drug molecules to treat disorders of learning and memory. This study does not indicate any role of muscarinic receptor, and GABA receptor and acetylcholine esterase in COX-2 mediated pathways of learning and memory.

However this does not rule out the possibility of the above mentioned receptors and enzyme in COX-2 mediated processes of cognition. The dose chosen, the model, and other experimental conditions would not have detected the interaction. Further experiments are required to establish the molecular interaction between neurotransmitters such as acetyl choline and GABA in modulating the physiological role of COX-2 in cognition.

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