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Enhancement of memory following repeated administration of NSAIDs in rats

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Abstract: Memory deficits have been observed following many inflammatory states including stress, infection, traumatic brain injury (TBI), normal aging, and Alzheimer's disease (AD). Prostaglandins (PGs), a class of lipid mediators which can have inflammatory actions, are up regulated by these inflammatory challenges and can impair memory. PGs are elevated under inflammatory conditions within the hippocampus. In present study effects of NSAIDs, Indomethacin and Diclofenac sodium on memory in rats was monitored. Memory was assessed in Morris water maze (MWM) as well as by transfer latency in Elevated plus Maze (EPM). Results of Morris water maze showed that rats treated with Indomethacin and Diclofenac sodium take less time to reach platform as compared to control however, this time is more in Diclofenac treated rats than indomethacin. Decrease in transfer latency is more in rats treated with indomethacin as compared to Diclofenac sodium. These results show that Indomethacin and Diclofenac sodium improve memory and this enhancement of memory by Indomethacin is more as compared to Diclofenac sodium.

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INTRODUCTION

Nonsteroidal anti-inflammatory drugs, usually abbreviated as NSAIDs or NAIDs, but also referred to as nonsteroidal anti-inflammatory agents/analgesics (NSAIDs) are drugs with analgesic and antipyretic effects and anti-inflammatory effects[1].NSAIDs produces their analgesic as well as anti-inflammatory effects through inhibition of cyclooxygenase (COX) enzyme[2].Cyclooxygenase (COX) enzyme is responsible for the biosynthesis of the prostaglandins and certain related autacoids and is considered to be a major component of the mechanism of NSAIDs. Anti-inflammatory drugs achieve their therapeutic actions at least in part by regulation of cytokine formation. A "cytokine hypothesis" of depression is supported by the observation that depressed individuals have elevated plasma levels of certain cytokines compared with healthy controls. Widely used anti-inflammatory drugs antagonize both biochemical and behavioral responses to selective serotonin reuptake inhibitors (SSRIs)[3]. Prior studies also suggested that non-steroidal anti-inflammatory drugs (NSAIDs) may lower the incidence of Alzheimer's disease (AD) and delay onset or slow progression of symptoms in mouse models of AD[4].PGs are elevated under inflammatory conditions within the hippocampus. In order for PGs to be responsible for disrupting memory in neuroinflammatory states, several conditions must be met including the increased PGs levels with inflammation in brain regions responsible for memory most eminently within the hippocampus and there must be increase in PGs and receptors present at specific plasticity sites, e.g. neuronally, either at pre-or post synaptic sites. Also a mechanism should exist by which PGs could impair learning and memory processes. Role of protein kinase in neuronal function is reported. Neuronal Ca^{++}calmodulin Kinase II(CaMKII) regulates important neuronal functions including neurotransmitter synthesis and release, modulation of ion channel activity, cellular transport and learning and memory.[5]Present work was undertaken to investigate the possible role of NSAIDs on memory in rats.

MATERIALS AND METHODS

Animals

Locally bred male Albino Wistar rats weighing 150-200g, purchased from Aga Khan University Hospital, Karachi were caged individually under 12:12h light: dark cycle (light on at 6:00h) and controlled room temperature (25±2°C) with cubes of standard rodent diet and water for 5 days before the experimentation. All experiments were performed according to a protocol approved by local animal care ethical committee.

Drugs

NSAIDS, Indomethacin and Diclofenac sodium were purchased from local market were used during the experiment. Drugs were given intra peritoneally at a dose of 0.5ml/kg for 5 days.

Experimental protocol

Eighteen animals were divided into three groups (six animals in each group) (i) control; (ii) Indomethacin; (iii) Diclofenac sodium. The animals of indomethacin and Diclofenac groups were injected with 0.5ml/kg of drug for 5 days and equal volume of saline was injected to the animals of control group for 5 days. The Morris water maze test (MWM) and Elevated plus maze (EPM) was performed following the drug administration to monitor the spatial learning and memory.

Behavioral assessment

Morris water maze test (MWM)

The Morris water maze (MWM) is a known, conventional cognitive test that requires an animal to use spatial learning and memory to find a hidden platform just below the surface of a circular pool of water, and to remember locationof platform from the previous trial[6]. It is reported that the animal uses cues in order to locate the hidden platform. The maze used for rats is same as described by Srikumar *et al.*, with some modifications[7].MWM is a circular pool of water with a diameter of 45cm, height 37cm and depth of water is 12cm.The pool is a metal cylinder painted white on the inner surface and the escape platform is also made of metal cylinder with flat metallic top having a surface diameter of 8cm and is 2cm below the surface of water during water maze training. The pool is filled with water (23±2°C) and made opaque with milk in order to obscure the platform and to allow proficient tracking of the swim paths of the rats. In this study long term memory in terms of latency to locate the hidden platform was assessed. The test is based upon two phases; the training phase and the test phase. Memory functions of rats were tested by noting down the retention latency. The cut off time was 2 minutes for each session. Initially the training session was performed during which each rat was placed into the water in such a way that their face was towards the wall of the tank. After placing 120 seconds were given each animal to find and mount onto the hidden platform, if the rat positioned the platform it was allowed to stay on it for 10 seconds. If it failed to locate the platform during the allocated time then it was guided quietly onto the platform[8]. Then test was performed to assess long term memory which was measured after 24 hours.

Elevated plus maze (EPM)

The Elevated plus maze was used to assessed short term memory in rats. The apparatus constructed of Perspex plastic with 4 arms of 50×10cm area. The two enclosed arms had side walls of 40cm high. The open and closed arms were connected with a central square (5 × 5 cm) to give the apparatus a plus sign appearance. The whole maze was raised 60 cm above the floor. The maze was placed in the same position throughout the experiment in laboratory where extra maze cues were there to help learning. The procedure and technique was same as reported earlier by Haider *et al*[9]. To evaluate memory through the Elevated plus Maze, the experimental session comprised of two trials (Learning and retention of memory). In the training session rats were individually placed at one end of the open arm, facing away from the central platform and the transfer latency (TL; time taken in seconds for the rat to move into one end of the closed arms with all four paws) was recorded. The cut off time during the training session was 5 minutes for the rat to explore the maze. The test session to evaluate the retention of memory was performed after 1 hour and the same procedure was repeated with a cut off time of 60 seconds. During test sessions time spent in open arm was recorded. A significant decrease in time spent in open arm on subsequent EPM exposure was taken as an index of successful memory retention. This is based on the idea that during repeating testing on EPM rat acquires information about the spatial environment and avoids the elevated and open arms of the maze and prefers to stay in the closed arms where it could be safe on the maze. Total time spent in the open arm measured served as an index of learning and acquisition[10].

Statistical analysis

Values are presented as mean±SD. Statistical analysis was performed by one way ANOVA. Post hoc comparison was done by Newman-Keuls test. Values of $p<0.01$ were considered as significant.

RESULTS

Figure 1 shows the effect of repeated administration of NSAIDs on long term memory in rats. Data analyzed by one way ANOVA show the significant effect of NSAIDs ($F=24.15$; $df=1, 15$; $P<0.01$). Post hoc analysis by Neumann Keul test show significant decrease ($P< 0.01$) in time to reach the platform in rats treated with indomethacin and diclofenac sodium as compared to control.

Figure 2 shows the effect of repeated administration of NSAIDs on short term memory in rats. Data analyzed by one way ANOVA show the significant effect of NSAIDs ($F=15.71$;$df=1,15$; $P<0.01$). Post hoc analysis by Neuman Keul test show significant decrease ($P<0.01$) in transfer latency in rats treated with indomethacin and diclofenac sodium as compared to control.

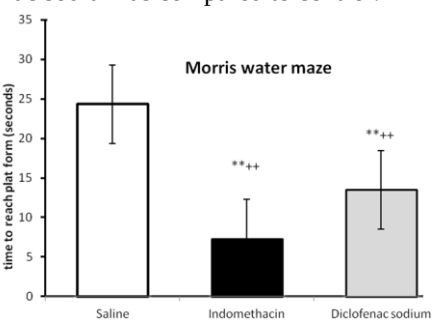

Figure 1: Effects of Indomethacin and Diclofenac sodium (0.5ml/kg) on long term memory in Morris water Maze. Values are the mean±SD (n=6). Significant differences by Neuman Keul test, ** $p<0.01$ from control animals; following One way ANOVA.

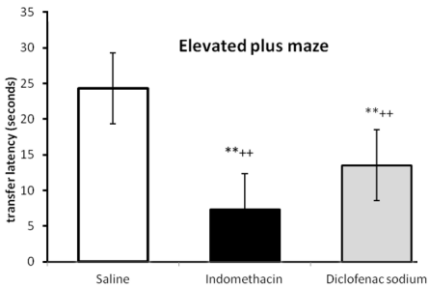

Figure 2: Effects of Indomethacin and Diclofenac sodium (0.5ml/kg) on short term memory in Elevated plus maze. Values are the mean±SD (n=6).Significant differences by Neuman Keul test, ** p<0.01 from control animals; following One way ANOVA.

DISCUSSION

Traumatic brain injury (TBI) is a neurological impairment and the most common consequences of TBI include memory impairment, apathy, aggressiveness and mood disorder. NSAIDs are widely used for the management of rheumatological disorder and as analgesic and antipyretic agent. Anti-inflammatory activity of NSAIDs is mediated through inhibition of biosynthesis of prostaglandin by inhibiting the Cyclooxygenase 1 and Cyclooxygenase 2 enzymes.

NSAIDs are associated with a marked reduction in the risk of Alzheimer's disease. Blockade of COX2 mediated PGE_2 response in synapse involves in protection against Alzheimer by NSAIDs**[11]**.In the present study effects of two NSAIDs, Indomethacin and Diclofenac sodium on memory is monitored. Two components of memory were determined, the short-term memory, which endures for a few hours, and long-term memory, which persists for several days and often much longer. The finding of primary interest in the present experiment is that the administration of NSAIDS produces enhancing effect on memory function. This improved memory retention was evident from the significant decrease in transfer latency in EPM. Results of MWM test also emphasized the same assumption as there was significant decrease in time to reach the platform. It is reported previously that NSAIDs acts in the CNS and has positive effects in reducing and delaying the onset of various CNS disorders including Parkinson's, Alzheimer's disease, depression, anxiety and nociception**[12]**.These disorders leads to impairment in memory, NSAIDs act on the CNS to delay the progression of disease which ultimately leads to the enhancement of memory.

Central serotonin is one of the neurotransmitter involved in the regulation of cognitive functions. It is also reported from the previous studies that NSAIDs like Ibuprofen, piroxicam, celecoxib and nimesulide treated rats completed the task in Morris water maze in a statistically significant shorter time when compared with control group**[13]**. The results support that COX-2 is probably involved in the physiological mechanisms underlying memory formation[14,15,16].Enhancement of memory by NSAIDs observed in the present study shows that these enhancement is more by Indomethacin as compare to Diclofenac sodium. Indomethacin serve as a potential inhibitor of protein Phosphatase and this inhibiting effects of Indomethacin could cause activation of Calcium calmodulin dependent protein kinaseII (CaMKII)**[17].** CaMKII involve in many signaling cascades and play important role in learning and memory**[5,18].** It is therefore suggested that increased memory enhancement by indomethacin as compared to Diclofenac sodium may be due to role of Indomethacin in activation of CaMKII.

CONCLUSION

In conclusion present study demonstrated that indomethacin and diclofenac sodium enhanced the spatial learning and memory which indicates that prostanoids, such as 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.

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