Original paper

Folia Neuropathologica

Interactive involvement of hippocampal cAMP/PKA and cyclooxygenase-2 signaling pathways in spatial learning in the Morris water maze

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Folia Neuropathol 2018; 56 (1): 58-66 *DOI: https://doi.org/10.5114/fn.2018.74660*

Abstract

Introduction: *Accumulated evidence shows that the cAMP-PKA signaling pathway plays a key role in memory functions. Cyclooxygenase-2, a critical player in neuroinflammation, has been confirmed in the pathogenesis of neurodegenerative diseases. This study is aimed to assess the effect of the interaction of cAMP-PKA and cyclooxygenase pathways on spatial memory acquisition in animal models.*

Material and methods: *In the present study, the effects of the four-day bilateral intra-hippocampal infusions of H-89 as a protein kinase AII inhibitor (10 µM/side), celecoxib (0.1 M/side) as a selective cyclooxygenase-2 inhibitor, celecoxib/H-89 and bucladesine (10 µM/side)/celecoxib/H-89 on spatial memory acquisition in the Morris water maze were investigated. Control animals received bilateral intra-hippocampal infusions of dimethyl sulfoxide. Rats were trained for 4 days; each day included one block of four trials. Post-training probe trial tests were performed on day five.*

Results: *A bilateral intra-hippocampal infusion of H-89 and celecoxib led to a significant impairment in spatial learning compared to the controls through a notable decrease in escape latency and traveled distance. But, combination treatment of animals with celecoxib/H-89 and bucladesine/celecoxib/H-89 could considerably reverse celecoxib and H-89-induced spatial memory acquisition impairments in the Morris water maze.*

Conclusions: *These results indicate the probable regulatory effects of cAMP/PKA and cyclooxygenase-2 signaling pathways on spatial memory acquisition in the Morris water maze.*

Key words: *COX-2, cAMP/PKA, Morris water maze, spatial memory acquisition, hippocampus.*

Introduction

The hippocampus and the adjacent cortex are broadly acknowledged structures for memory processes [31]. A considerable body of evidence about hippocampal lesion-induced learning and memory deficits has confirmed the vital role of the hippocampus as represented by synaptic plasticity in formation of memory [1,31].

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Recent studies have supported the essential function of cAMP/PKA signaling pathway in the final phases of memory consolidation [12]. PKA-induced CREB phosphorylation in the hippocampus accompanied by sensitivity to inhibitors of gene transcription and protein synthesis during PKA active periods, suggest the important role of this signaling pathway in long-term memory formation [12]. Moreover, previous published data on intra-hippocampal infusion of H-89 as a selective PKA II inhibitor and dibutyryl cyclic AMP (bucladesine) as a membrane permeable selective activator of PKA into the CA1 region of the hippocampus, plus behavioral and immuno-histochemical findings have suggested the importance of PKA in spatial memory retention and its regulation of cholinergic gene expression [2,27,30]. Several studies indicated that PKA signaling pathway inhibition led to learning and memory deficiency [8,21] and enhancement of apoptosis and autophagy [14]. Cyclooxygenase (COX) is the key enzyme that converts arachidonic acid to prostaglandins [33]. Two unique cyclooxygenase genes (COX-1 and COX-2) and a putative third isoform (COX-3) as a splice variant of the COX-1 transcript have been identified [13]. Of the three isozymes of COX that have been known, COX-2 has attracted a growing attention because of its involvement in cognitive functions and inflammatory processes [33]. Investigations of the inflammatory signaling pathways to determine potential interaction of COX with learning and memory have demonstrated that celecoxib as a COX-2 selective inhibitor could impair the spatial memory acquisition and retention in rodents [1,26]. COX-2 protein has been recognized particularly in the cortex, hippocampus, amygdala and dorsal horn of the spinal cord of both rodent's and human central nervous system (CNS) [13].

On the other hand, glutamate is a major excitatory neurotransmitter in the brain involved in numerous brain functions, such as synaptic plasticity, learning and memory [11]. It is important to note that the co-localization of COX-2 protein with glutaminergic neurons in dentate gyrus and CA1–CA3 pyramidal layers of the hippocampus confirms the link of neuronal COX-2 expression with excitatory activity in CNS [13].

In the present study, the intra-hippocampal effects of H-89, celecoxib, and also interactive effects of celecoxib/H-89 and bucladesine/celecoxib/H-89 on spatial learning acquisition in the Morris water maze (MWM) were investigated.

Material and methods

Animals

Male Wistar rats (180-220 g, purchased from the Faculty of Pharmacy, Zabol University of Medical Sciences, were housed at 25 ± 2°C with a 12-h light/dark cycle and free access to food and water. All animal procedures were done in accordance with the Ethical Committee guidelines for the care and use of laboratory animals of the Zabol University of Medical Sciences.

Drugs

Celecoxib, H-89 [n-(2-aminoethyl)-5-isoquinoline-sulfonamide] and bucladesine (dibutyryl cAMP, DB-cAMP) were purchased from Sigma (St. Louis, MO) and dissolved in DMSO (dimethyl sulfoxide) to obtain desirable concentrations (0.1 M, 10 µM and 10 µM, respectively). Ketamine (100 mg/kg, i.p.) and xylazine (25 mg/kg, i.p.) were used for induction of anesthesia for stereotaxic surgery.

Surgery and drug infusions

After induction of anesthesia, bilateral cannulation in the CA1 region of the hippocampus was performed using a stereotaxic instrument according to Paxinos and Watsons atlas of the rat brain [9,24]. Bilateral intra-hippocampal infusions of 1 µl/side of celecoxib (0.1 M), H-89 (10 µM), bucladesine (10 µM) and DMSO were carried out through the guide cannula. All infusions were done 30 minutes before training each day. In combination treatments, celecoxib + H-89 or bucladesine + celecoxib + H-89, the infusion procedure involved a 5 minutes' interval between each microinjection.

Behavioral trainings and learning assessments

Four-day training (including one block of four trials) in MWM was performed according to our previous studies [16,27]. Spatial acquisition was assessed through measuring escape latency, traveled distance, and swimming speed by the EthoVision video tracking system (Noldus Information Technology, Wageningen, the Netherlands) as described previously [3,26-28,30]. Post-training probe trial tests were performed on day 5 to examine the time spent in the target quadrant (the quadrant included a hidden platform during training trials). In the probe trial test, the hidden platform was removed and animals were allowed to swim freely for 90 seconds.

Experiments

Experiment 1

In the first experiment, the effects of bilateral intra-hippocampal infusions of H-89 (10 μM/side) and celecoxib (0.1 M/side) in a volume of 1 μl/side on spatial learning acquisition were explored. Control animals received 1 μl/side DMSO.

Experiment 2

The purpose of this experiment was to examine (i): the interactive effects of celecoxib (0.1 M/side) and H-89 (10 μM/side), and (ii): effects of interaction of bucladesine, celecoxib and H-89 on spatial learning acquisition in MWM. The animals received bilaterally combination doses of bucladesine (10 μM/side), celecoxib (0.1 M/side) and H-89 (10 μM/side) respectively in the CA1 region of the hippocampus. The time interval between each microinjection was 5 minutes. Post-training probe trial tests were performed in both experiments on day 5 to determine the time spent in the target quadrant.

Statistical analysis

Unpaired *t*-test, one-way analysis of variance (ANOVA) and two-way ANOVA followed by Bonferroni's post hoc test, using Graph Pad Prism 5 were used to compare the results of our behavioral studies. The statistical significance was considered at *p* values less than 0.05.

Results

Experiment 1: Effects of celecoxib and H-89 on spatial learning in MWM

The results of the four-day training program in H-89 (10 μM/side)- and celecoxib (0.1 M/side)-treated animals and their related controls are presented in Figures 1 and 2. The rats receiving bilateral intra-hippocampal infusion of either H-89 or celecoxib showed a significant increase in both traveled distance (**$p < 0.01$, Figs. 1A and 2A) and escape latency (**$p < 0.01$, Fig. 1B and ***$p < 0.001$, Fig. 2B) parameters compared to control groups, while no significant differences were found in the swimming speed

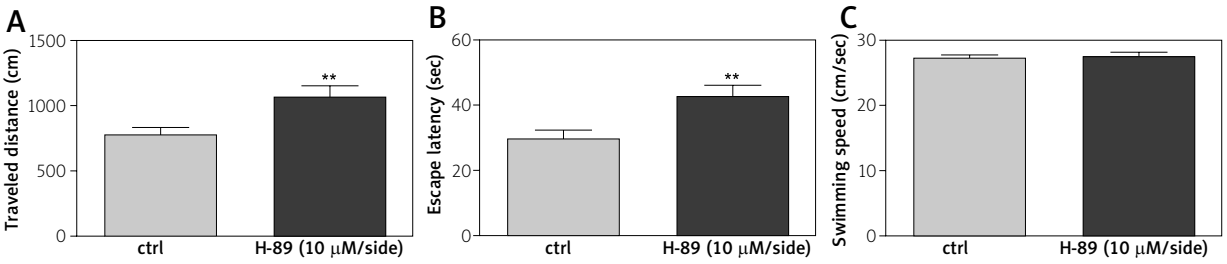

Fig. 1. An average of traveled distance (**A**), escape latency (**B**), and swimming speed (**C**) during four training days in the Morris water maze task in H-89-treated animals. **$p < 0.01$ shows a considerable difference as compared to the control (DMSO) group. Values represent means ± SEM of 6 animals in each group.

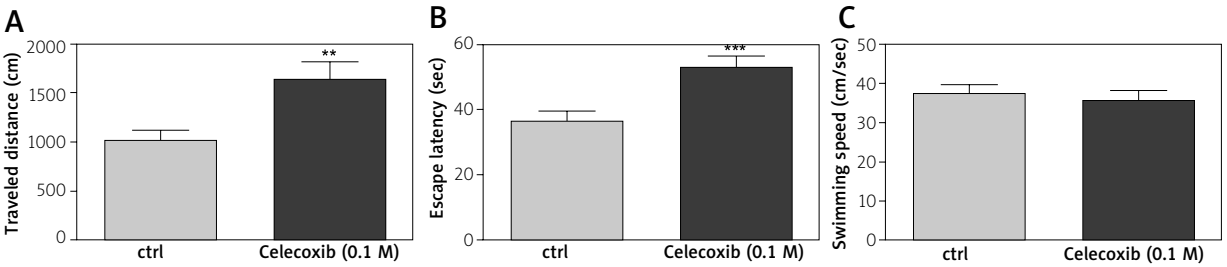

Fig. 2. An average of traveled distance (**A**), escape latency (**B**) and swimming speed (**C**) during four training days in the Morris water maze task in celecoxib-treated animals. **$p < 0.01$ and *** $p < 0.001$ show a significant difference as compared to the control (DMSO) group. Values represent means ± SEM of 6 animals in each group.

between these animals ($p > 0.05$) in comparison to controls (Figs. 1C and 2C). Considering the same training days in these treated animals, infusion of H-89 caused significant memory impairments via increasing traveled distance and escape latency at the fourth ($^{*}p < 0.05$) day of training (Fig. 3A and 3B) in comparison to the controls (DMSO-treated). In addition, a significant increase in the traveled distance and escape latency at the third and fourth ($^{*}p < 0.05$) day of training were observed in celecoxib-treated animals in comparison to the controls (Fig. 4A and 4B).

Experiment 2: Effect of co-administration of celecoxib/H-89 and bucladesine/celecoxib/H-89 on spatial learning in MWM

Figure 5 demonstrates the results obtained from (i): intra-hippocampal infusion of H-89 (10 µM/side), 5 minutes after celecoxib (0.1 M/side) administration and (ii): infusion of bucladesine (10 µM/side), celecoxib (0.1 M/side, i.h.) and H-89 (10 µM/side, i.h.) with a time interval of 5 minutes, respectively. There were significant differences in traveled distance ($^{\#}p < 0.05$, Fig. 5A) between the celecoxib/H-89 or bucladesine/celecoxib/H-89 groups compared to H-89-treated animals during four days of training. Similarly, there was a significant increase in the traveled distance (Fig. 5A) between the celecoxib/H-89 ($^{+}p < 0.05$) and bucladesine/celecoxib/H-89 ($^{++}p < 0.01$) group compared to celecoxib-treated animals during four days of training.

Regarding the escape latency parameter, there was a significant difference between the celecoxib/H-89 or bucladesine/celecoxib/H-89 groups compared to H-89- ($^{\#}p < 0.05$, Fig. 5B) and also celecoxib ($^{+++}p < 0.001$, Fig. 5B)-treated animals during four days of training.

Considering the swimming speed, no significant differences were found ($p > 0.05$, Fig. 5C) between control, celecoxib/H-89- and bucladesine/celecoxib/H-89-treated animals.

Interestingly during the same training days, there were considerable differences among celecoxib/H-89 or bucladesine/celecoxib/H-89 groups in the traveled distance and escape latency parameters at the fourth day of training compared to celecoxib- ($^{+}p < 0.05$, Fig. 6A and 6B) and H-89 ($^{\#}p < 0.05$, Fig. 7A and 7B)-treated groups.

Evaluation of the probe trial test in H-89, celecoxib, celecoxib/H-89 and bucladesine/celecoxib/H-89-treated animals in MWM

Intra-hippocampal infusion of H-89 (10 µM/side) and celecoxib (0.1 M/side) caused a significant decrease ($^{***}p < 0.001$) in the time spent in the target quadrant compared to control groups. On the other hand, the time spent in the target quadrant was increased significantly in animals treated with a combination of celecoxib/H-89 or bucladesine/celecoxib/H-89 compared to H-89 ($^{\#\#\#}p < 0.001$) or celecoxib ($^{+++}p < 0.001$)-treated animals (Fig. 8).

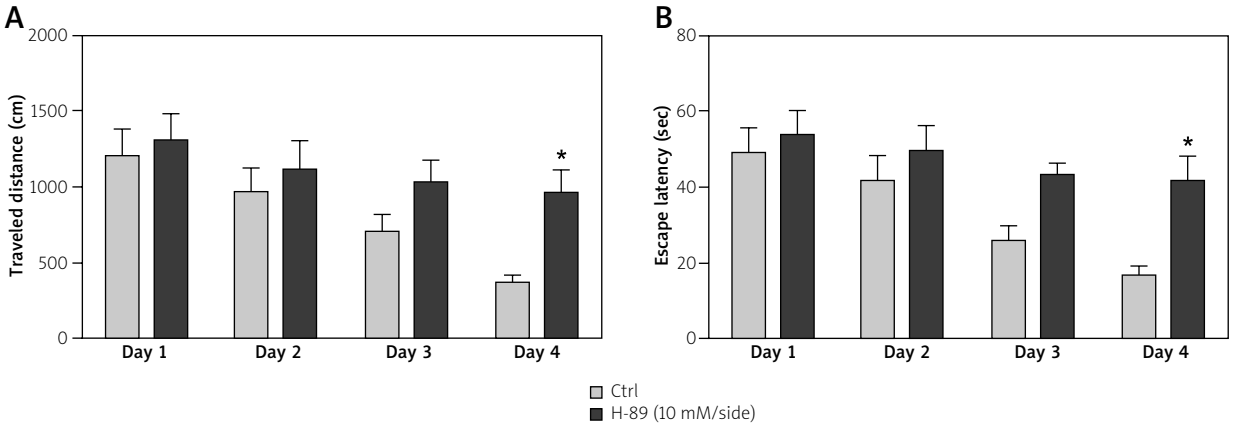

Fig. 3. Assessment of traveled distance (**A**) and escape latency (**B**) on the same training day in H-89-treated animals. A substantial increase in the traveled distance and escape latency was observed in the H-89 group ($^{*}p < 0.05$) on day 4. Data are presented as means ± SEM of 6 animals in each group.

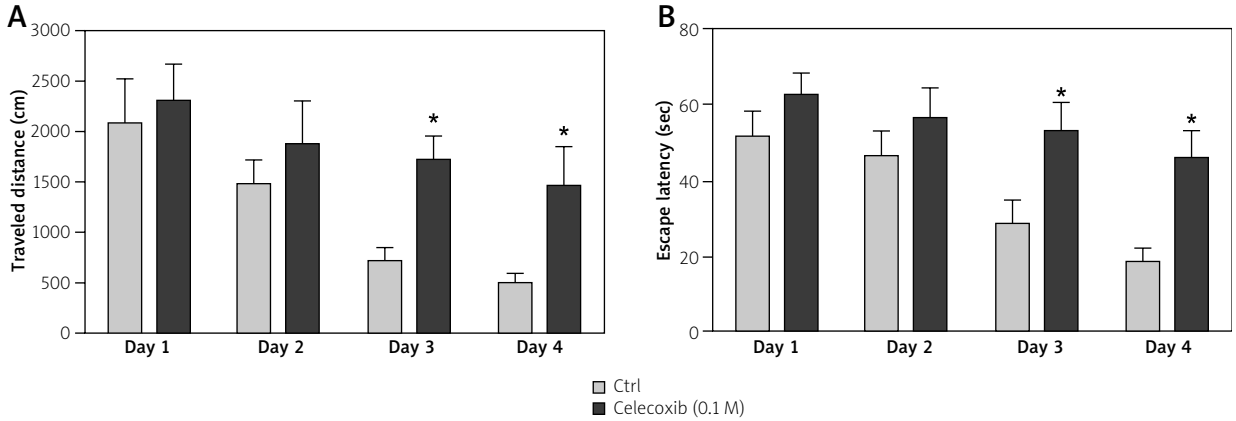

Fig. 4. Evaluation of traveled distance (**A**) and escape latency (**B**) on the same training day in celecoxib-treated animals. A considerable increase in the traveled distance and escape latency was observed in the celecoxib group (*$p < 0.05$) on days 3 and 4. Data are presented as means ± SEM of 6 animals in each group.

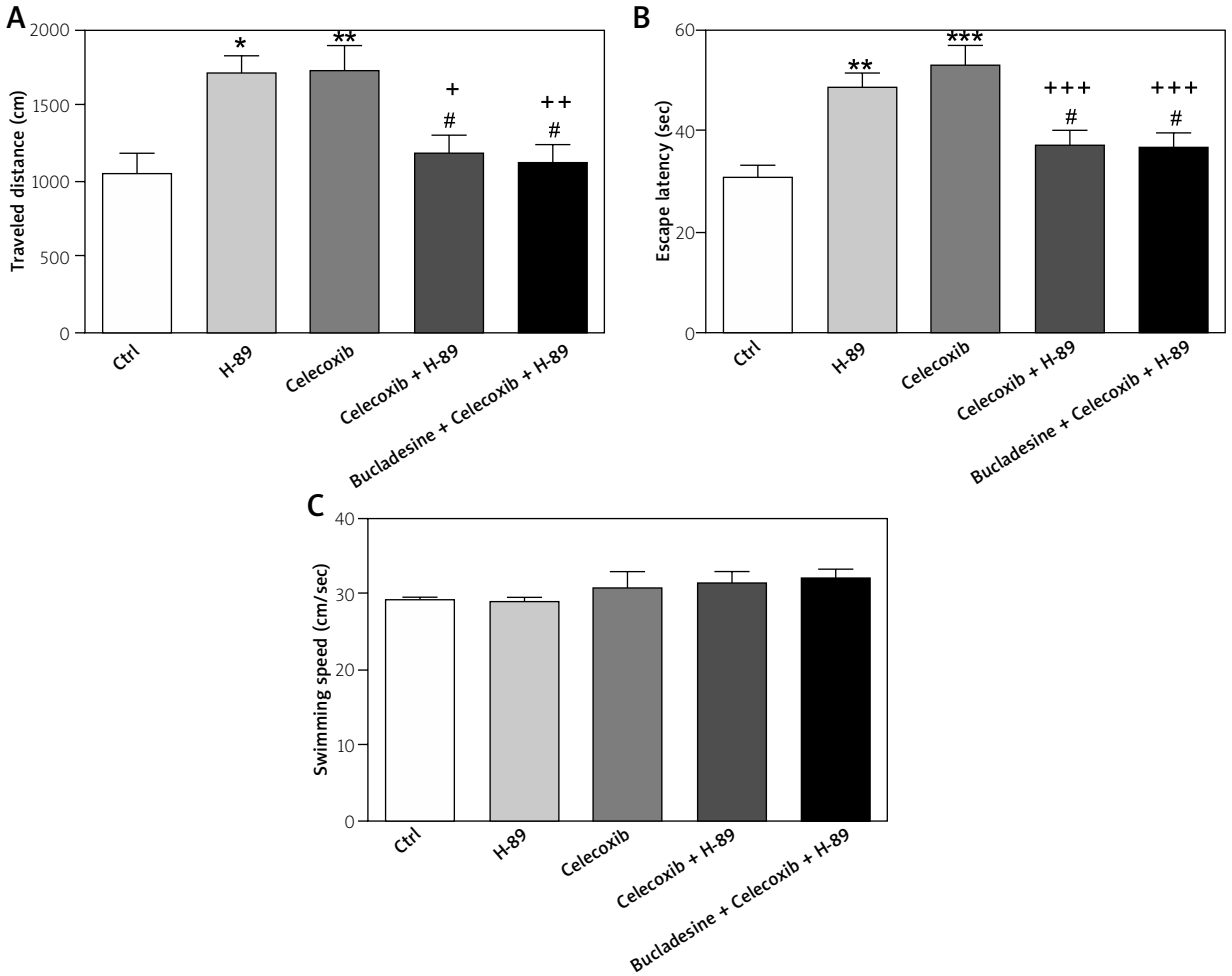

Fig. 5. An average of traveled distance (**A**), escape latency (**B**) and swimming speed (**C**) during four training days in the Morris water maze task for H-89-, celecoxib-, celecoxib/H-89- and bucladesine/celecoxib/H-89-treated animals. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ show a notable difference as compared to the control (DMSO) group. $^{\#}p < 0.05$ shows a considerable difference as compared to the H-89-treated group. $^{+}p < 0.05$, $^{++}p < 0.01$ and $^{+++}p < 0.001$ demonstrates a remarkable difference as compared to the celecoxib-treated group. Values represent means ± SEM of 6 animals in each group.

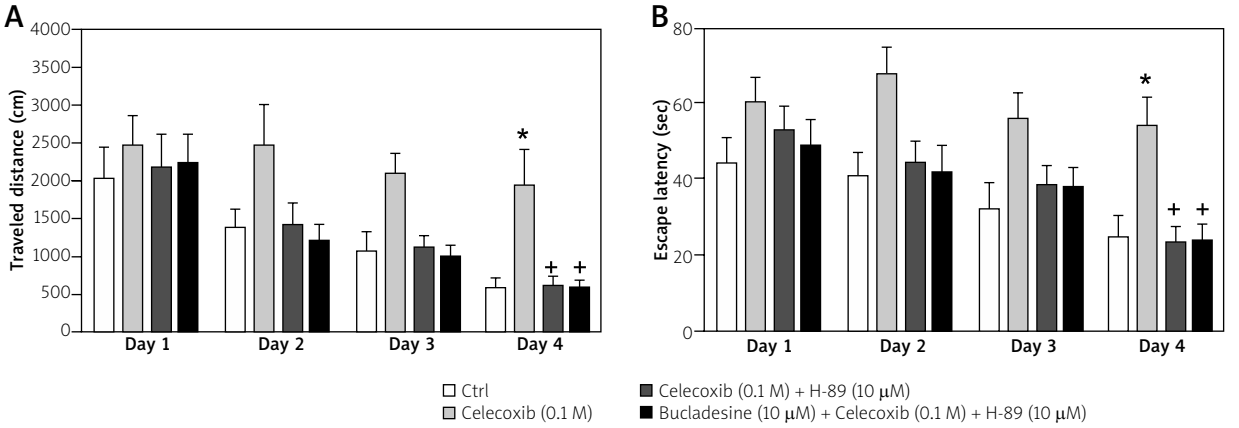

Fig. 6. Investigation of traveled distance (**A**) and escape latency (**B**) on the same training day in celecoxib-, celecoxib/H-89- and bucladesine/celecoxib/H-89-treated animals. *$p < 0.05$, describes a considerable difference as compared to the control (DMSO) group. $^{+}p < 0.05$ shows a meaningful difference as compared to the celecoxib-treated group. Values represent means ± SEM of 6 animals in each group.

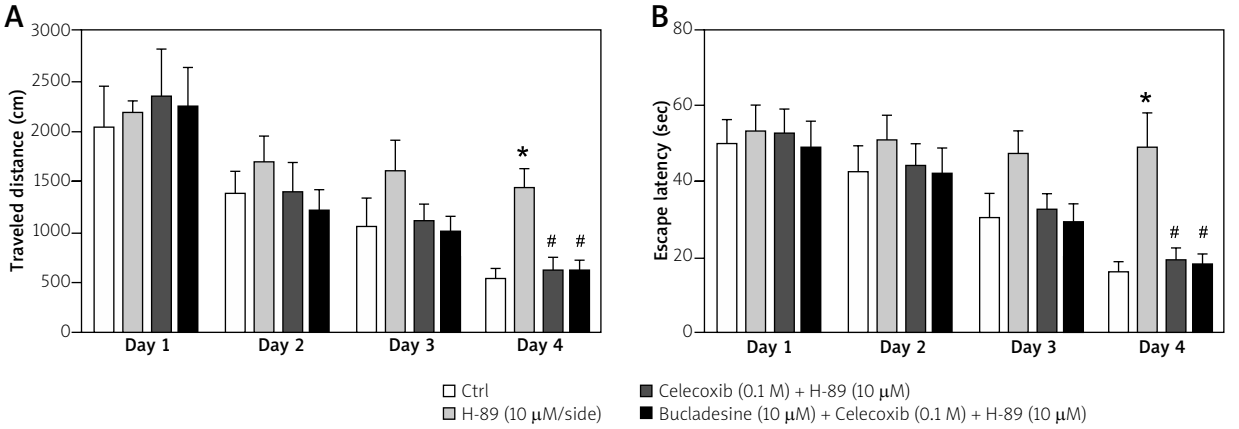

Fig. 7. Evaluation of traveled distance (**A**) and escape latency (**B**) on the same training day in H-89-, celecoxib/H-89- and bucladesine/celecoxib/H-89-treated animals. *$p < 0.05$ show a meaningful difference as compared to the control (DMSO) group. $^{\#}p < 0.05$ indicates a considerable difference as compared to the H-89-treated group. Values represent means ± SEM of 6 animals in each group.

Discussion

To evaluate the potential interaction of cAMP/PKA and COX-2 signaling pathways, the drugs – bucladesine, a selective activator of PKA, H-89 as a PKA II inhibitor, and celecoxib as a selective COX-2 inhibitor were used in this study. H-89- and celecoxib-treated animals were not trained well and showed a remarkable increase in time and distance to find the hidden platform. Moreover a significant decrease in the time spent in the target quadrant was observed in comparison to control animals.

The importance of cAMP-dependent PKA in the expression of long-term potentiation, long-term depression, hippocampal long-term memory, and synaptic plasticity had been reported previously [30]. Also it has been proposed that the hippocampal cAMP/PKA pathway could be activated following NMDA receptor-mediated Ca^{2+} influx via stimulation of adenylate cyclase activity, cAMP accumulation, and PKA and CREB phosphorylation [12].

It has been claimed that intra-hippocampal bucladesine infusion could improve spatial memory retention via synergistic interaction with nicotine as represented by significant increases in ChAT and VAChT protein expressions in the CA1 region and medial septal area of the hippocampus [2,30]. On the other hand, post training intra-hippocampal infusion

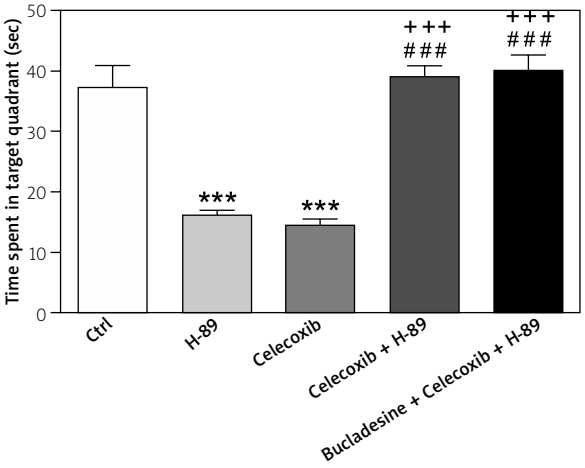

Fig. 8. Measurement of time spent in the target quadrant in the probe trial test. $^{***}p < 0.001$ shows a significant difference as compared to the control group. $^{###}p < 0.001$ illustrates a considerable difference as compared to the H-89-treated group. $^{+++}p < 0.001$ displays a considerable difference as compared to the celecoxib-treated group. Values represent means ± SEM of 6 animals in each group.

of H-89 in our previous studies [27] could damage spatial memory retention as evidenced by the qualitative and quantitative decrease in the density of ChAT-containing cholinergic nerve terminals in the dorsal hippocampus and medial septum. Similarly, bilateral intra-hippocampal infusion of H-89 in the present study impaired spatial learning in animals. These findings suggest the pivotal role of PKA II in regulation of cholinergic gene expression and spatial memory acquisition and retention in rats.

The key function of prostaglandin E2 (the predominant reaction product of COX-2) in neural plasticity as well as modulatory effects of prostaglandins on adrenergic, noradrenergic, and glutaminergic transmission have been investigated in previous studies. In addition, the regulatory effects of acetylcholine on COX-2 activity have suggested the interaction of the prostaglandin pathway and the cholinergic system in neuronal plasticity. However, there are some controversial findings about other physiological and pathological functions of COX-2 in the CNS [26]. It has been shown that endogenous basal levels of PGE2 production following COX-2 activity are essential for acquisition of memory and synaptic plasticity [7]. Inhibition of COX-2 suppresses glutamatergic neurotransmission and long-term potentiation in the hippocampus. These events are mostly mediated by prostaglandin E2 [6,20] which consequently increases glutamate release [22]. Celecoxib is a highly selective cell permeable COX-2 inhibitor [25] and might prevent glutamate release by reducing the levels of Ca^{2+} entry through the Cav2.2 (N-type) and Cav2.1 (P/Q-type) Ca^{2+} channels that are linked to glutamate exocytosis in the nerve endings [19].

It has also been proven that bilateral infusion of 0.1 M/side celecoxib could significantly damage spatial memory retention which persisted for 3 days, while nicotine could prevent the celecoxib-induced impairment by restoring the immunostaining pattern of COX-2 neurons in the rat dorsal hippocampus [26,28,29]. This study, in agreement with previous publications on spatial memory retention, demonstrates a probable role for COX-2 in spatial memory acquisition.

In contrast with our study, Yang *et al*. demonstrated that celecoxib treatment in diabetic rats could improve memory impairments through reduction of hippocampal COX-2 expression and increase in the BDNF-TrkB signaling pathway [34]. It has also been indicated that chronic unpredictable mild stress could increase COX2 expression and consequently learning and memory deficiency because of hippocampal cAMP/PKA-CREB-BDNF signaling activation [18]. Beneficial effects of COX2-specific inhibitors have been reported in treatment of the traumatic brain injury [10]. These findings may be obtained due to the use of COX2 inhibitors under the inflammation condition. But we investigated the effects of celecoxib in lack of inflammatory responses. Due to contradictory findings about the role of the cyclooxygenase pathway in learning and memory processes, it has been realized that different factors including the type of the experimental task, employed protocol, stage of memory formation and some pathological factors such as inflammatory conditions are involved in COX activity on memory functions.

In this work, co-administration of celecoxib/H-89 and bucladesine/celecoxib/H-89 could protect or reverse H-89- and celecoxib-induced spatial memory acquisition deficits in MWM.

It has been reported that salicylate can decrease apoptosis and autophagy induced by H-89 [4].

Interestingly, the protective effects of H-89 on Aβ-induced memory impairment via a reduction of NFκB, (factor of neuronal death), and decrease in oxidative stress factors have been proposed [8]. Also,

certain inflammatory cytokines have been identified which can induce COX-2 expression via an NF-kB pathway [15].

Based on previous records, NF-κB is involved in a number of brain functions, especially in neurodegenerative diseases and after injury. Glutamate (via both AMPA/KA and NMDA receptors) and neurotrophins are brain-specific activators of NF-κB with regards to participation in synaptic plasticity [23]. Likewise, NF-κB serves a role in synaptic transmission through regulation of COX-2 neuronal expression [32]. The COX-2 gene, induced by NF-κB, plays a key function in inflammation and carcinogenesis [5].

It has been asserted that celecoxib can prohibit release of glutamate from nerve endings through reduction of voltage-dependent Ca^{2+} entry via a signaling mechanism including EP2 (prostaglandin E2 (PGE2) receptors) and PKA. Therefore PKA inhibitors might prevent the inhibitory effect of celecoxib on the release of glutamate [17].

To investigate the role of dibutyryl cAMP in spatial memory, we used the drug bucladesine, a selective activator of PKA. Previous studies suggested that intrahippocampal infusions of bucladesine improve spatial memory retention in male rats and interact synergistically with nicotine to improve spatial memory [30]. Also, it has been reported that significant increases in ChAT and VAChT protein expressions, as cholinergic markers, in the CA1 region and medial septal area of the hippocampus are the possible mechanisms of spatial memory improvement induced by nicotine–bucladesine combination [2].

In conclusion, the improvement of spatial memory acquisition by celecoxib/H-89 and bucladesine/celecoxib/H-89 in the present study, suggest the probable regulatory effects of cAMP/PKA and COX-2 signaling pathways in spatial memory acquisition in MWM.

Disclosure

The authors report no conflict of interest.

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