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ScienceDirect
Pharmacology, Biochemistry and Behavior 85 (2006) 705–712
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
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Intra-hippocampal inhibition of protein kinase AII attenuates morphine-induced conditioned place preference

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Received 6 February 2006; received in revised form 29 October 2006; accepted 31 October 2006
Available online 15 December 2006

Abstract

Morphine and other drugs of abuse modulate protein kinase A (PKA) signaling within the mesolimbic reward pathway. Using a balanced conditioned place preference (CPP) paradigm, we studied the possible involvement of protein kinase AII (PKA II) on the acquisition, expression and consolidation of morphine place conditioning in male Wistar rats. Subcutaneous administration of various doses of morphine sulfate (1–9 mg/kg) induced CPP in a dose-dependent manner. H-89, a selective PKA II inhibitor, was administered into CA1 region of the hippocampus at 1, 2.5 and 5 μM/rat. Using a 3-day schedule of conditioning, it was found that the H-89 did not produce a significant place preference or place aversion. H-89 (1, 2.5 and 5 μM/rat) significantly reduced the time spent by rats in the morphine compartment when given immediately after each conditioning session (consolidation), whereas it had no effect when administered before morphine during the conditioning phase (acquisition) or before testing for place preference in the absence of morphine (expression). It is concluded that the PKA II may play an active role in the consolidation of reward-related memory of morphine in CA1 region of the hippocampus.

Keywords: PKA; H-89; Morphine; Conditioned place preference; Hippocampus; CA1; Rat

1. Introduction

The reinforcing effects of opiates have long been known and indicate that the brain mesolimbic dopaminergic system is involved in these reinforcement effects (Wise, 1998). Previous studies have shown that chronic exposure to opiates produces specific biochemical adaptations in the ventral tegmental area (VTA) and nucleus accumbens (NAc). It has been suggested that morphine raises the extra-cellular concentrations of dopamine (DA) and glutamate in the NAc (Kebabian and Calne, 1979; Smith et al., 1995). Accordingly, heroin exposure is able to increase cAMP-PKA activity in the NAc (Self et al., 1995). Moreover, it has been shown that PKA inhibitor, Rp-cAMPS, is able to block the place preference induced by morphine when given into the VTA immediately after morphine conditioning. Also, Rp-cAMPS immediately prior to the preference test blocks the expression of morphine CPP when microinjected into the VTA (Harris et al., 2004).

The hippocampal formation is now believed to be an essential component of the learning and memory systems of the brain (Packard and White, 1991; Squire, 1992; LeDoux, 1993; Wan et al., 1994; Shen et al., 1996; Nguyen et al., 1994; Huang et al., 1996). Moreover, it has been suggested that the hippocampus is necessary for complex reward-related learning, particularly when animals must remember the place and cues associated with drug administration (McBride et al., 1999; Ferbinteanu and McDonald, 2001). Previous studies have indicated that chronic opiate treatment can significantly modulate synaptic plasticity in the hippocampus, leading to an opiate dependence of the plasticity and it has been suggested

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doi:10.1016/j.pbb.2006.10.027

that upregulation of cAMP pathway is likely one of the underlying mechanisms of the observed phenomena (Pu et al., 2002). The dorsal hippocampus contains the highest concentration of hippocampal dopamine (Ishikawa et al., 1982), which is shown to have an important role in mediating morphine reward (Rezayof et al., 2003). In addition, unilateral microinjection of morphine into the rat hippocampus has been found to produce a conditioned place preference (Corrigall and Linseman, 1988).

While various studies have associated changes in PKA with adaptive phenomena consequent to chronic use of opioids (Nestler et al., 1993), there is little information on the role of this pathway in the memory related to rewarding properties of morphine. Conditioned place preference (CPP) paradigm used in this study represents an animal model to assess the rewarding effect of different systems including opioids (Bardo, 1998). In the current study, we sought to determine if PKA activity in the rat hippocampal CA1 region is involved in learning to associate morphine exposure with a specific environment, using a balanced CPP paradigm.

2. Materials and methods

2.1. Animals

Male Wistar rats (Animal housing office, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran) weighing 200–240 g, at the time of surgery, were used. The animals were kept in standard polycarbonate cages in groups of three with food in a temperature-controlled room (22±2 °C) on a 12-h light/12-h dark cycle (lights on at 07:00) with free access to food and water. All animals were naïve and acclimatized to the laboratory conditions for at least 1 week before surgery and were handled for 5 min/day during this adaptation period. Experiments were conducted during the period between 9:00 a.m. and 3:00 p.m. All procedures were carried out in accordance with institutional guidelines for animal care and use and possible measures were undertaken to minimize the number of animals used and also to minimize animals' discomfort. The protocol has been approved by the committee of ethics of the faculty of Sciences of Tehran University (357; 8 November 2000). Each rat was used only once and each treatment group consisted of six animals.

2.2. Drugs

Morphine sulfate was purchased from Temad, Tehran, Iran. H-89 [*N*-(2-aminoethyl)-5-isoquinoline-sulfonamide], a selective PKA II inhibitor, ketamine and xylazine were purchased from Sigma, Germany. Morphine was dissolved in sterile 0.9% saline, just before the experiment, and injected subcutaneously in a volume of 0.1 ml. H-89 was prepared freshly in DMSO 0.2% and bilaterally injected into CA1 region of the hippocampus; the volume of the drug injections was 1.0 μl/rat (0.5 μl/side). Control animals received saline subcutaneously and DMSO 0.2% intra-hippocampal CA1 region.

2.3. Apparatus

The place conditioning apparatus is based on that used by Carr and White (1983) with minor modifications and consisted of three square-based wooden compartments. In order to distinguish the compartments, visual and sensory texture cues were used. Compartments A and B were identical in size (40×30×30 cm) but differed in shading. The compartment A was white with black horizontal stripes 2 cm wide on the walls and also had a textured floor. The compartment B was black with vertical white stripes 2 cm wide and also had a smooth floor. Compartment C (40×15×30 cm) was painted red and was attached to the rear of compartments A and B; it had removable wooden partitions that separated it from the other compartments and was used as the start box during pre- and post-conditioning sessions. During the conditioning phases, the two compartments were separated by the partitions. When the partitions were removed, the animals could freely move between the two compartments (A and B) via compartment C.

2.4. Surgery

Rats were anesthetized with intraperitoneal injection of ketamine hydrochloride (100 mg/kg) plus xylazine (25 mg/kg) and placed in a stereotaxic apparatus, while maintaining the incisor bar at approximately 3.3 mm below horizontal zero to achieve a flat skull position. Two stainless-steel, 22-gauge (0.4 mm inner diameter, 0.7 mm outer diameter) guide cannulae were placed (bilaterally) 1 mm above the intended site of injection according to the atlas of Paxinos and Watson (1997). Stereotaxic coordinates for the CA1 regions of the dorsal hippocampi were: 3.8 mm posterior to bregma, ±2.2 mm lateral to the midline, and −2.4 mm ventral of the dorsal surface of the skull. Cannulae were secured to anchor jewelers' screws with dental acrylic. Stainless steel stylets (27 gauges, 0.2 mm inner diameter, 0.4 mm outer diameter) were inserted into the guide cannulae to keep them free of debris. All animals were allowed 1 week to recover from surgery and clear anesthetic.

2.5. Intra-hippocampal CA1 infusion

The animals were gently restrained by hand and the stylets were removed from the guide cannulae. For intra-hippocampal CA1 injections of drugs, a 10-μl glass Hamilton syringe was used. The injection (inner) cannulae (27-gauge, 0.2 mm inner diameter, 0.4 mm outer diameter), which projected a further 1 mm ventral to the tip of the guides, were attached with polyethylene tubing to the Hamilton syringe. Each dose of drug used/rat was dissolved in 1.0 μl and the injection volume of drugs was 1.0 μl (0.5 μl/side) for all groups. The injections were made over a 60-s period, and the injection cannulae were left in the guide cannulae for an additional 60 s to facilitate diffusion of the drugs.

2.6. Measurement of conditioned place preference

Place conditioning was conducted using an unbiased procedure. The CPP paradigm took place on 5 consecutive

days consisted of three phases: preconditioning, conditioning, and post-conditioning.

2.6.1. Pre-conditioning

On the first day of the trials (i.e., pre-conditioning), each animal was placed separately into the apparatus for 15 min, while they could freely access all compartments. The time spent in each compartment was recorded to determine any individual innate preference for either of the compartments. Placement in each compartment was considered as placement of the front paws and the head. In the particular experimental setup used in the present study, the animals did not show an unconditioned preference for either of the compartments, which supported our unbiased method (compartment A: 174.66±21.15 s and compartment B: 199.33±23.15 s). Thus, in one of compartments A or B, randomly chosen, the animals received morphine and in the other they were administered with saline.

2.6.2. Conditioning

This phase consisted of six, 45 min conditioning sessions held in three consecutive days (three saline and three drug pairings). These sessions were conducted twice each day (from day 2 to day 4) with a 6-h interval. On each of these days, separate groups of animals received one conditioning session with morphine and one with saline. During these sessions, the animals were confined to one compartment by closing the removable wall. Animals of each group were injected with morphine and were immediately confined to one compartment of the apparatus for 45 min. Following administration of saline, the animals were confined to the other compartment for 45 min. Treatment compartment and order of presentation of morphine and saline were counterbalanced for either group.

2.6.3. Post-conditioning

This phase was carried out in the fifth day of the trials (24 h after the last conditioning session, with no preceding injections) in a drug-free state. As in the pre-conditioning phase, the guillotine door was raised and the animals were allowed free access to all compartments for 15 min. An observer who was unaware of rats and treatments recorded the time spent in drug-paired compartment for each animal. Change in preference (CIP) was calculated as the difference (in seconds) between the time spent in the drug-paired compartment on the post-conditioning day and the time spent in this compartment in the pre-conditioning session.

2.7. Measurement of locomotor activity

Locomotion was measured, based on a method used previously by Tzschentke and Schmidt (1997), during the test sessions (Belzung and Barreau, 2000) in the drug-paired compartment. To measure the locomotor activity, the ground areas of compartments A and B were divided into four equal-sized squares by two transverse lines and the number of squares rats entered during the 15 min test of CPP was measured by another observer and used as an index of locomotor activity.

2.8. CPP experimental design

2.8.1. Experiment 1: dose-response effects of place conditioning produced by morphine

In this experiment, we established a dose–response function for morphine place conditioning. Different doses of morphine (1, 3, 6 and 9 mg/kg) were tested for their ability to produce a place conditioning. Four groups of animals were injected with morphine and saline (s.c.) on alternate sessions in a 3-day schedule of conditioning as described above. A separate group of animals received saline (1 ml/kg, s.c.) in both compartments (A and B) in order to confirm that the injection and conditioning schedule did not affect the time spent in the compartments, and served as control. Locomotor activity was also measured in the post-conditioning session.

2.8.2. Experiment 2: effect of selective PKA II inhibitor, H-89, on the acquisition of place preference conditioning

Three different doses of the selective PKA II inhibitor, H-89 (1, 2.5 and 5 μM/rat, intra-CA1), was given to three distinct groups of animals just before the administration of saline (1 ml/kg), during the conditioning phase. One additional group received DMSO 0.2% (1 μl/rat, intra-CA1) just before saline (1 ml/kg) administration during the conditioning phase and served as a control. All groups were tested 24 h after the last conditioning session, with no preceding injection. Locomotor activity was measured in the post-conditioning session.

2.8.3. Experiments 3–5: the effects of H-89 on the expression, acquisition and consolidation of morphine-induced place preference

In experiment 3, in order to test the effects of H-89 on the expression of morphine-induced conditioned place preference, H-89 (1, 2.5 and 5 μM/rat; intra-CA1) was injected once on the day of testing (day 5), 5 min prior to the conditioned place preference testing. The respective control groups received DMSO 0.2% in a volume of 0.5 μl/rat/side, intra-CA1. During

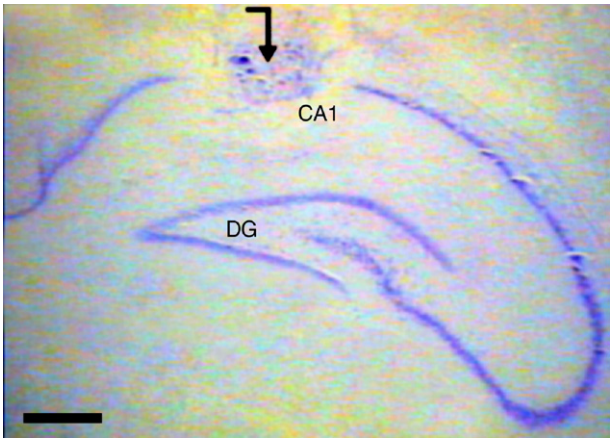

Fig. 1. A representative photomicrograph of an infusion site into the rat hippocampal CA1 region. The figure shows the site of infusion in the dorsal hippocampus with the arrowhead pointing to the infusion cannula tract. CA1, Ammon's horn region 1; DG, dentate gyrus. Scale bar=0.5 mm.

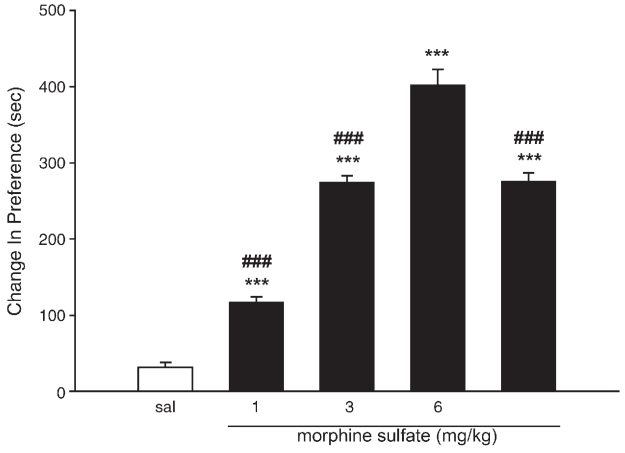

Fig. 2. Effects of morphine on conditioned place preference (CPP) induction in opioid-naïve mice. Different doses of morphine (1, 3, 6 and 9 mg/kg) and saline (1 ml/kg) were administered subcutaneously (s.c.) in a 3-day schedule of conditioning. On the test day, the animals were observed for a 15-min period. The change of preference was assessed as the difference (in seconds) between the time spent in the drug-paired compartment on the post-conditioning day and the time spent in this compartment in the pre-conditioning session. Data are expressed as mean±S.E.M. of 6 animals per group. *** $P<0.001$ different from the saline control group; ### $P<0.001$ different from morphine 6 mg/kg group (Tukey–Kramer's multiple comparison tests).

the post-conditioning session locomotor activity of the animals was measured.

In the fourth experiment, the effects of H-89 on the acquisition of morphine-induced conditioned place preference were investigated. Four groups of animals were injected with different doses of H-89 (1, 2.5 and 5 μM/rat, intra-CA1) or DMSO 0.2% (1 μl/rat, intra-CA1), just before morphine administration (6 mg/kg, s.c.) during the conditioning sessions and were tested on the fifth day of the schedule with no preceding injection. Locomotor activity of the animals was measured in the post-conditioning phase.

In experiment 5, in order to study the effects of H-89 on the consolidation of morphine-induced conditioned place preference, four groups of animals were injected with different doses of H-89 (1, 2.5 and 5 μM/rat, intra-CA1) or DMSO 0.2% (1 μl/rat, intra-CA1), immediately after each conditioning session and were tested on the fifth day of the schedule with no preceding injection. During the post-conditioning session locomotion of animals was also measured.

2.9. Histological verification

After completion of behavioral trials, each animal was killed by decapitation and the brains were removed. For histological examination of cannulas and needle placement in the CA1 region, the brains were cut into sections 30–40 μm thick on a cryostat, mounted on glass slides, and stained with cresyl violet. The sections were then examined under a light microscope to find the bilateral sites of cannulas and infusion tracts and sites to ascertain whether the sites were in the dorsal hippocampus. The cannulae placements were verified using the atlas of Paxinos and Watson (1997). Fig. 1 shows the location of the cannula in the CA1 of the hippocampus in a representative animal. Only data from rats that received histological verified injections were included for analyses.

2.10. Statistical analysis

All results are presented as mean±S.E.M. Data were assessed by one-way analysis of variance (ANOVA). If a significant F value was obtained, post hoc analyses (Tukey–Kramer's multiple comparison tests) were performed to determine the effects of various treatments on induction of place preference, and changes in locomotion. Calculations were performed using the SPSS statistical package (version 11.5).

3. Results

3.1. Dose–response curve for place preference conditioning produced by morphine in rats

Fig. 2 shows the dose–response curve for place conditioning induced by morphine in rats. Statistical analysis indicated that morphine-induced place preference (one-way ANOVA;

Table 1
Locomotor activity of rats on post-conditioning day

Groups	Locomotor activity (counts/15 min)
Experiment 1	
Saline	51.66±1.82
Morphine 1 mg/kg	49.33±2.60
Morphine 3 mg/kg	50.50±3.09
Morphine 6 mg/kg	51.33±1.74
Morphine 9 mg/kg	49.83±2.71
Experiment 2	
DMSO	49.66±1.80
H89 1 μg/rat	51.16±2.78
H89 2.5 μg/rat	50.00±2.84
H89 5 μg/rat	51.83±4.20
Experiment 3 (expression)	
DMSO+morphine 6 mg/kg	51.50±2.40
H89 1 μg/rat+morphine 6 mg/kg	51.00±2.28
H89 2.5 μg/rat+morphine 6 mg/kg	51.16±1.92
H89 5 μg/rat+morphine 6 mg/kg	52.66±2.44
Experiment 4 (acquisition)	
DMSO+morphine 6 mg/kg	46.33±1.22
H89 1 μg/rat+morphine 6 mg/kg	49.50±1.64
H89 2.5 μg/rat+morphine 6 mg/kg	54.33±2.09
H89 5 μg/rat+morphine 6 mg/kg	51.33±2.59
Experiment 5 (consolidation)	
DMSO+morphine 6 mg/kg	51.33±1.74
H89 1 μg/kg+morphine 6 mg/kg	47.50±2.74
H89 2.5 μg/kg+morphine 6 mg/kg	49.66±2.37
H89 5 μg/kg+morphine 6 mg/kg	52.83±1.57

Data are expressed as mean±S.E.M.

Locomotion was measured in the drug-paired compartment in the post-conditioning session. To measure the locomotor activity, the ground areas of compartments A and B were divided into four equal-sized squares by two transverse lines and the number of squares rats entered during the 15 min test of CPP was measured ($n=6$ for each group). Analysis revealed that none of the treatments altered locomotor activity significantly.

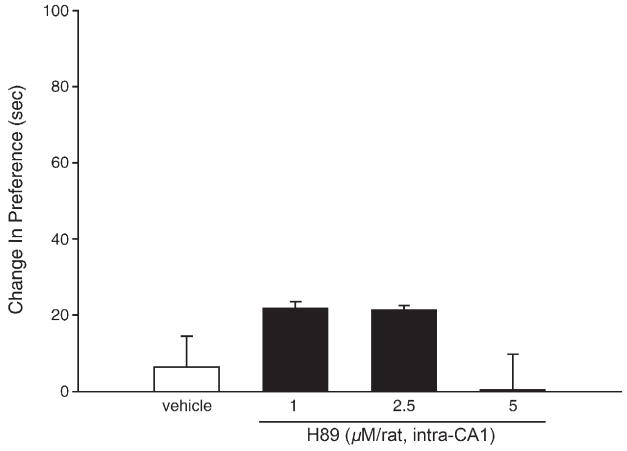

Fig. 3. The effects of intra-hippocampal H-89 infusion on the acquisition of CPP in the absence of morphine. Four groups of animals received intra-hippocampal infusion of either DMSO 0.2% (1 μl/rat) or three doses of H-89 (1, 2.5 and 5 μM/rat), just before the administration of saline (1 ml/kg, s.c.), during the conditioning phase in a 3-day schedule of conditioning. Change of preference for all of the groups was tested 24 h after the last conditioning session for all of six rats per group. The data are shown as means of change in preference±S.E.M. Analysis revealed that no group showed a statistical significant difference.

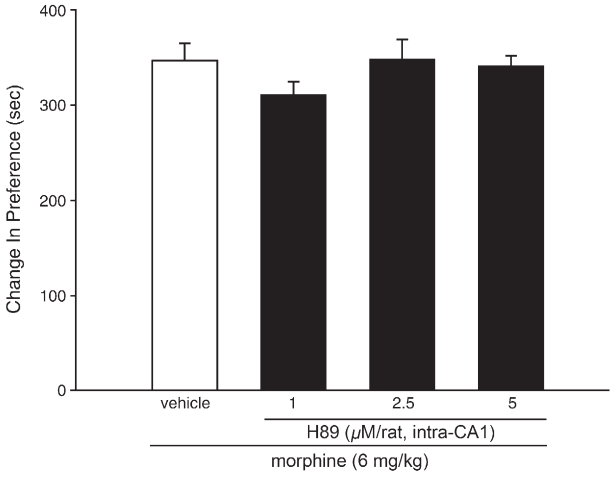

Fig. 4. The effects of intra-hippocampal injection of H-89 on the expression of morphine-induced place preference. All animals were conditioned with morphine (6 mg/kg, s.c.) in a 3-day schedule of conditioning. On the test day, different doses of H-89 (1, 2.5 and 5 μM/rat) or its vehicle (1 μl/rat) were injected into the CA1. Changes of preference were tested immediately after the intra-CA1 injection of H-89 or its respective vehicle. Data are expressed as mean±S.E.M. of 6 animals per group. Analysis revealed that no group showed a statistical significant difference.

$F_{4,25}=166.874$, $P<0.001$) but did not change locomotion significantly (one-way ANOVA; $F_{4,25}=0.160$, $P=0.956$) (Table 1). Tukey–Kramer multiple comparison tests revealed that the doses of 1–9 mg/kg of morphine-induced place preference, but saline (1 ml/kg) failed to produce significant conditioning in animals and no preference for either of the compartments was seen. The maximum response was obtained with 6 mg/kg of morphine; therefore, this dose was employed in all subsequent experiments.

3.2. Effects of PKA II inhibitor H-89 on the acquisition of conditioned place preference

Fig. 3 shows the effect of intra-CA1 injection of H-89 (1, 2.5 and 5 μM/rat), a selective PKA II inhibitor, and its vehicle (1 μl/rat, intra-CA1) on the induction of place preference conditioning. Statistical analyses did not show any significant effect for H-89 on place preference (One-way ANOVA; $F_{3,20}=2.972$, $P>0.05$). It also had no effect on locomotion of animals (One-way ANOVA; $F_{3,20}=0.111$, $P=0.953$) (Table 1).

3.3. Effects of H-89 on the expression, acquisition and consolidation of morphine-induced place preference

Fig. 4 shows the effects of bilateral intra-CA1 injection of H-89 on the expression of morphine-induced CPP. One-way ANOVA indicated that H-89 (1, 2.5 and 5 μM/rat) or its vehicle had no effect on the expression of morphine-induced place preference (One-way ANOVA; $F_{3,20}=1.180$, $P=0.342$) and also did not show a significant effect on locomotor activity of rats (One-way ANOVA; $F_{3,20}=0.109$, $P=>0.954$) (Table 1).

The effect of bilateral intra-CA1 injection of H-89 on the acquisition of morphine CPP is shown in Fig. 5. One-way ANOVA indicated that H-89 (1, 2.5 and 5 μM/rat) or DMSO 0.2% (1 μl/rat) had no effect on the acquisition of morphine-induced place preference ($F_{3,20}=0.317$, $P=0.813$). Also, H-89 did not induce a significant effect on locomotor activity during the testing phase (One-way ANOVA; $F_{3,20}=2.933$, $P=0.058$) (Table 1).

As Fig. 6 shows, a significant main effect was seen for the action of H-89 (1, 2.5 and 5 μM/l rat) on the consolidation of conditioned place preference (One-way ANOVA; $F_{3,20}=110.022$, $P<0.001$). Further analyses revealed that groups that received DMSO 0.2% (1 μl/rat, intra-CA1), immediately after each conditioning session showed no significant effect on morphine-induced place

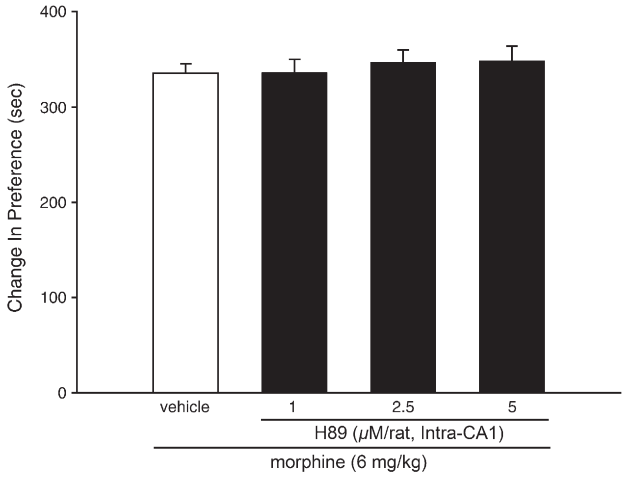

Fig. 5. The effects of bilateral intra-CA1 injection of H-89 on the acquisition of conditioned place preference induced by morphine. Four groups of animals received intra-CA1 injection of either DMSO 0.2% (1 μl/rat) or three doses of H-89 (1, 2.5 and 5 μM/μl/rat), just prior to morphine (6 mg/kg, s.c.) administration, during the conditioning phase, in a 3-day schedule. Change of preference for all of the groups was tested 24 h after the last conditioning session for all of six rats per group. Data are expressed as mean±S.E.M. of 6 animals per group. Analysis revealed that no group showed a statistical significant difference.

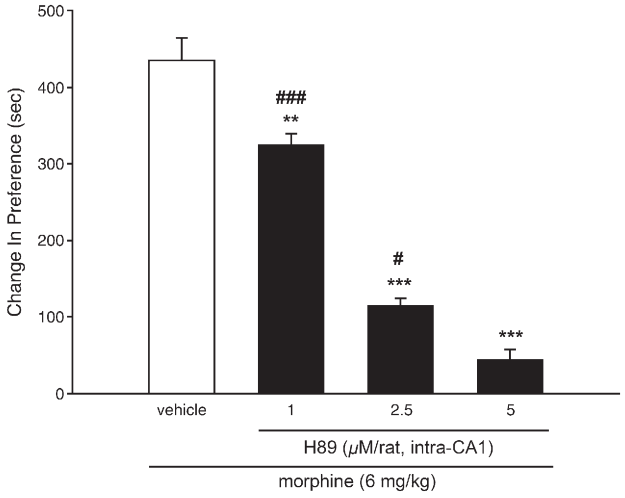

Fig. 6. The effects of bilateral intra-CA1 injection of H-89 on the consolidation of morphine CPP. Four groups of animals received intra-CA1 injection of either DMSO 0.2% (1 μl/rat) or H-89 (1, 2.5 and 5 μM/μl/rat) immediately after each conditioning session with morphine (6 mg/kg, s.c.) in a 3-day schedule. All of the groups were tested 24 h after the last conditioning session. Values are expressed as mean±S.E.M. ** and *** $P<0.005$ and $P<0.001$ different from the vehicle control group, respectively; # and ### $P<0.05$ and $P<0.001$ different from H-89 5 μM/rat/morphine 6 mg/kg group (Tukey–Kramer's multiple comparison tests).

preference, while H-89 (1, 2.5 and 5 μM/rat) significantly attenuated consolidation of morphine-induced place preference. The maximum response was observed with 5 μM/rat of H-89. However, none of the treatments altered locomotor activity in the post-conditioning session (One-way ANOVA; $F_{3,20}=1.121$, $P=0.364$) (Table 1).

4. Discussion

The current study concerns the effects of intra-hippocampal CA1 region administration of H-89, a selective inhibitor of PKA II activity, on the expression, acquisition and consolidation of morphine-induced conditioned place preference. Conditioned place preference (CPP) has been widely used to assess the rewarding effect of different systems including opioids (Tzschentke, 1998). The test is based upon the principle that, when a primary reinforcer is paired with a contextual stimulus, the contextual stimulus can acquire secondary reinforcing properties. These secondary reinforcing properties, which are presumably established due to a Pavlovian contingency, are thought to be capable of eliciting an operant approach response or place preference which results in a significant increase in the time spent in the drug-paired place. CPP has also been used to measure memory or learning of simple stimulus–reward associations (McIntyre et al., 1998). Our results indicate that subcutaneous injection of morphine-induced a CPP, which is accordant with previous studies (De Fonseca et al., 1995; Tzschentke and Schmidt, 1997; Belzung and Barreau, 2000). The maximum effect was obtained by 6 mg/kg of opioid.

It has been widely accepted that dopaminergic neurons projecting from ventral mesencephalic nuclei to forebrain targets play a critical role in reward-related incentive learning. The molecular mechanism underlying dopamine-mediated reward-related learning may involve the formation of cyclic adenosine monophosphate (cAMP) and the activation of cAMP-dependent protein kinase (PKA) and additional intracellular events leading to modification of cortico-striatal glutamatergic synapses activated by stimuli encountered in close temporal contiguity with reward (Beninger and Miller, 1998; Beninger et al., 2003; Sutton et al., 2000). Several lines of evidence suggest that the hippocampus mediates learning and memory processes (Wilkerson and Levin, 1999; Umegaki et al., 2001) and Packard and White (1991) indicated that the dopaminergic system is involved in modulating the memory processes of the hippocampus. The hippocampal CA1 region receives a dopaminergic input originating predominantly in the VTA (Scatton et al., 1980; Hersi et al., 1995) and has all five types of dopamine receptors (Meador-Woodruff, 1994; Hersi et al., 1995), which may be involved in reward-related learning (Rezayof et al., 2003).

Drugs of abuse modulate cAMP-PKA signalling within the mesolimbic reward pathway (Wand et al., 2001). It has been shown that PKA activity levels are increased in the accumbens of cocaine trained rats (Lu et al., 2003). Also, it has been reported that the amphetamine CPP is blocked by co-administration of PKA inhibitor Rp-cAMPS, while Rp-cAMPS or the PKA activator Sp-cAMPS alone had no effect on side preference (Beninger et al., 2003). Accordingly, bilateral intra-NAc infusions of the PKA inhibitor Rp-cAMPS is shown to reduce baseline cocaine self-administration and shift the dose–response curve for cocaine self-administration to the left (Self et al., 1998). In contrast, pre-treatment with intra-NAc infusions of a PKA activator, Sp-cAMPS or dibutyryl cAMP, increased baseline cocaine self-administration during the second hour of testing and shifted the dose–response curve to the right (Self et al., 1998). Additionally, it has been suggested that mice with reduced neuronal PKA activity have decreased alcohol consumption compared with their wild-type littermates (Wand et al., 2001). Similar effects have been observed with PKA inhibition in the basolateral amygdala or medial prefrontal cortex (Beninger and Gerdjikov, 2004).

Protein kinases appear to play an important role in various plastic phenomena such as long-term potentiation, kindling and learning (Nguyen and Woo, 2003). In addition, large evidence suggests that PKA is a part of the molecular cascade of events leading to memory consolidation (Nguyen et al., 1994; Serrano et al., 1995; Marthis et al., 1992; Olds et al., 1990; Steven, 1994; Takashima et al., 1991; Carew, 1996), but its role in cue-dependent memories of drug use that may predispose to relapse is not clear. Recent studies have demonstrated a role for cAMP dependent PKA in the NAc in reward-related learning, suggesting that the activation of PKA plays a critical role in the process by which properties of drugs become associated with environmental stimuli (Sutton et al., 2000; Beninger et al., 2003). Changes in the activity of PKA is proposed to be part of the cascade of events that contribute to enhancing synaptic responses in the consolidation phase of cocaine CPP and determine rats' behavior associated with the memory of the rewarding effect of cocaine during cocaine CPP expression (Cervo et al., 1997). Also, stimulation of

PKA activity in the amygdala can facilitate reward-related learning (Jentsch et al., 2002).

Since cues associated with drugs of abuse may elicit place preference, with important implication for the study of drug craving and relapse (Markou et al., 1993; Robinson and Berridge, 1993), we evaluated the effects of bilateral intra-hippocampal microinjection of H-89 as a selective PKA II inhibitor on the morphine-induced place preference. Our present findings show that intra-CA1 administration of 1–5 μM/rat of H-89 significantly reduced the time spent in the morphine compartment when given immediately after each conditioning session, suggesting that protein kinases are activated transiently during a specific time window to allow consolidation of morphine CPP. As conditioned place preference is a learning paradigm, in that animals must remember the place and cues associated with drug administration. This finding indicates that the PKA system might play a role in mediating this type of learning. Nonetheless, H-89 had no such effect when administered before morphine during the training phase (acquisition) or before testing (expression) for place preference in the absence of morphine. The finding that H-89 injection before morphine during the training phase had no effect on morphine CPP may be due to the fact that it did not reach high enough concentration to interfere with the start of the consolidatory process shortly after training. In analogy with studies on memory formation in chicks (Serrano et al., 1995) and cocaine CPP (Cervo et al., 1997), it is possible that protein kinase inhibitors are effective only at a critical period which protein kinases are activated. The relatively short interval in which protein kinase inhibition affects morphine CPP suggests that their role is to activate a cascade of molecular processes underlying the cue-dependent memory of morphine use.

In summary, this study examined the possible role of the PKA system in the CA1 area of the rat hippocampus on the acquisition, expression and consolidation of conditioned place preference induced by morphine, and showed that the consolidation of the rewarding properties of morphine was reduced in animals infused with the PKA II inhibitor, H-89. The PKA system in the rat hippocampal CA1 area, therefore, may be involved in consolidation of morphine-induced conditioned place preference.

Acknowledgement

We thank Dr. Ali Roghani for help with the improvement of this manuscript. This work was supported in part by funds from Tehran University of Medical Sciences, National Center of Excellence in Toxicology and Pharmaceutical Sciences Research center to MS.

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