Involvement of GABAB receptors of the dorsal hippocampus on the acquisition and expression of morphine-induced place preference in rats

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Abstract

In the present study, effects of intra-hippocampal CA1 (intra-CA1) injections of GABAB receptor agonist and antagonist on the acquisition and expression of morphine-induced place preference in male Wistar rats have been investigated. Subcutaneous administration of different doses of morphine sulphate (0.5–6 mg/kg) produced a dose-dependent conditioned place preference (CPP). Using a 3-day schedule of conditioning, it was found that the GABAB receptor agonist, baclofen (0.5–2 μg/rat; intra-CA1), or the GABAB receptor antagonist, phaclofen (1–3 μg/rat; intra-CA1), did not produce a significant place preference or place aversion. Intra-CA1 administration of baclofen (1 and 2 μg/rat; intra-CA1) decreased the acquisition of CPP induced by morphine (3 mg/kg; s.c.). On the other hand, intra-CA1 injection of phaclofen (1 and 2 μg/rat; intra-CA1) in combination with a lower dose of morphine (1 mg/kg; s.c.) elicited a significant CPP. The response of baclofen (2 μg/rat; intra-CA1) was reversed by phaclofen (4 and 6 μg/rat; intra-CA1). Furthermore, intra-CA1 administration of baclofen but not phaclofen before testing significantly decreased the expression of morphine (3 mg/kg; s.c.)-induced place preference. Baclofen or phaclofen injections had no effects on locomotor activity on the testing sessions. It is concluded that the GABAB receptors in dorsal hippocampus may play an active role in morphine reward.

Keywords: GABAB receptors; Morphine; Conditioned place preference; Hippocampus; CA1; Rat

1. Introduction

The behavioral evidence shows that opiate addiction is a complex phenomenon involving many biological and social factors [1], and it is also suggested that learning and memory play an important role in the development of opiate tolerance and dependence [2,3]. It has been shown that the administration of GABAergic agonists impair memory and their antagonists facilitate recall [4–6]. The inhibitory functions of GABA in the brain are mediated by two distinct classes of receptors, namely the GABA_A/C and GABA_B receptors. The GABA_A/C receptors are ligand-gated Cl⁻ channel that mediate the fast synaptic inhibition. The GABA_B receptors are linked to K⁺ channel via G protein and mediate slow on-set and prolonged effects of GABA in the CNS [7–9]. In a number of mammalian species, including rats, mice and monkeys have been shown that GABA_B receptors modulate memory processes in learning paradigms [5,10,11].

Conditioned place preference (CPP) has been widely used to study the rewarding effects of morphine [12–15]. The mesocorticolimbic dopaminergic system, which originates in the ventral tegmental area (VTA) to the nucleus accumbens (Nac) and other forebrain regions play an important role in mediating the rewarding activities of morphine [16]. Various lines of evidence suggest that dopamine neuronal activity in the VTA is modulated by GABAergic inhibitory input [17,18]. Examination of dopaminergic cell firing concomitant with stimulation of GABA_B receptors indicated that dopaminergic cell firing in the VTA can be inhibited by activation of GABA receptors [19,20]. On the other hand, CPP is a learning paradigm requiring formation of associations between reward and particular locations [21,22]. It has been suggested that there might exist some correlation between opiate reward and...
certain kinds of learning and memory processes [1]. Furthermore, the CPP paradigm has been used extensively to assess reward-related learning in rats [23]. Some studies also showed that hippocampal lesions in rats disrupt long-term retention of stimulus–stimulus associations [24]. Our previous experiments indicate that dopamine [25] and nitric oxide [14,26] may be implicated in dorsal hippocampus-dependent mediation of stimulus-reward associations. Moreover, multiple models of hippocampal function have suggested that the hippocampus is involved in the formation of arbitrary associations that may be critical for paired-associate learning [27,28]. Based on previous reports, the aim of the present study was to investigate the role of the dorsal hippocampal GABA<sub>B</sub> receptors on the rewarding effect of morphine using the conditioned place preference paradigm and the microinjection technique.

2. Materials and methods

2.1. Animals

Male Wistar rats weighting 220–250 g were used. The animals were housed in a colony maintained at 22±2°C with 12:12-h light-dark cycle (lights on at 07:00 h) and allowed free access to food and water inside standard polypropylene cages. The animals were allowed to adapt laboratory conditions for at least 1 week before surgery. Each animal was used once only. Eight animals were used in each group of experiments. The experiments were carried out during the light phase of the cycle. All procedures were carried out in accordance with institutional guidelines for animal care and use.

2.2. Apparatus

The place conditioning apparatus is based on that used by Carr and White [29] with a minor modification and consisted of three wooden compartments. Compartments A and B were identical in size (40 x 30 x 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 other compartment (B) was black with vertical white stripes 2 cm wide and also had a smooth floor. Compartment C (40 x 15 x 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. When the partitions were removed, the animal could freely move between the two compartments (A and B) via compartment C.

2.3. Surgery

Rats were anesthetized with intraperitoneal injection of ketamine hydrochloride (100 mg/kg) plus xylazine (4 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 guide cannulae were placed (bilaterally) 1 mm above the intended site of injection according to the atlas of Paxinos and Watson [30]. Stereotaxic coordinates for the CA1 regions of the dorsal hippocampi were: −3 to −3.5 mm (depending on body weight) posterior to bregma, ±1.8 to 2 mm lateral to the midline, and −2.8 to −3 mm ventral of the dorsal surface of the skull. Cannulae were secured to anchor jewelers’ screws with dental acrylic. Stainless steel stylets (27 gauge) were inserted into the guide cannulae to keep them free of debris. All animals were allowed one week to recover from surgery and clear anesthetic.

2.4. Intrahippocampal CA1 injection

The animals were gently restrained by hand; the stylets were removed from the guide cannulae. For intrahippocampal CA1 injections of drugs, a 1.0-μl glass Hamilton syringe was used. The injection (inner) cannula (27-gauge), which projected a further 1 mm ventral to the tip of the guides, were attached with polyethylene tubing to the Hamilton syringe. The injection volume of drugs was 1.0 μl (0.5 μl per side) for all groups. Each dose of drug used/rat was dissolved in 1.0 μl. 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.

2.5. Experimental procedure

The CPP paradigm took place on 5 consecutive days by using an unbiased procedure. The experiment consisted of the three following phases.

2.5.1. Pre-conditioning

On day 1, the animals were accustomed to the conditioned place preference apparatus for 15 min. The removable wall was raised, thereby allowing each rat to freely explore the three compartments. The amount of time spent in each compartment was measured to assess unconditioned preference (the position of the rat was defined by the position of its front paws). In the particular experimental setup used in the study, the animals did not show an unconditioned preference for either of the compartments. Animals were then randomly assigned to one of two groups for place conditioning and a total of eight animals were used for all subsequent experiments.

2.5.2. Conditioning

Place conditioning phase started 1 day after pre-conditioning phase. This phase consisted of six, 45-min sessions (three saline and three drug pairing). 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. Conditioning was con-
ducted as previously described in detail, using an unbiased procedure [31].

2.5.3. Testing

The testing phase was carried out on day 5, 1 day after the last conditioning session, in a morphine-free state. Each animal was tested only once. For testing, the removable wall was raised, and the animals had a free choice in the apparatus for 15 min. The time spent in drug-paired compartment was recorded for each animal and the change of preference was calculated as the difference (in seconds) between the time spent in the drug-paired compartment on the testing day, and the time spent in this compartment in the pre-conditioning day [31]. In this phase, an observer who was unaware of the treatment group for each rat recorded the time spent in each compartment.

2.6. Drugs

The drugs used in the present study were morphine sulfate (Temad Co., Tehran, Iran), baclofen and phaclofen (Tocris Cookson Ltd, UK). All drugs were dissolved in 0.9% saline, just before the experiment. Baclofen and phaclofen were administered intrahippocampal CA1 and morphine was injected subcutaneously (s.c.). Control animals received saline.

2.7. Drug treatments

2.7.1. Morphine dose–response analysis

In a pilot study, we studied the effects of s.c. administration of different doses of morphine (0.5, 1, 3 and 6 mg/kg) on the induction of a CPP (Fig. 2). Morphine or saline was injected in a 3-day schedule of conditioning as described in details in Experimental procedure. The time spent in the drug-paired compartment on the testing day minus to that spent in this compartment in the pre-conditioning day was calculated to assess the CPP induction. Animals were tested in a morphine-free state. This may eliminate the possibility that morphine-induced motor effects influence the response [13,32].

2.7.2. Effects of baclofen and/or phaclofen with or without morphine on the acquisition of CPP

Effects of intrahippocampal CA1 (intra-CA1) injection of different doses of baclofen or phaclofen on the acquisition of the conditioned place preference induced by morphine were determined as follows. Rats received morphine or saline (s.c.) once daily in a 3-day schedule of conditioning. Baclofen (0.5, 1 and 2 µg/rat; intra-CA1) and phaclofen (1, 2 and 4 µg/rat; intra-CA1) was administered into the hippocampal CA1 regions once per day for 3 days, 5 min before the administration of morphine (three sessions); the conditioning scores then were measured in a drug-free state (testing day). Intra-CA1 injections of the same (above mentioned) doses of all drugs without morphine, during conditioning, were also used to assess their effects on CPP. The conditioning scores were then measured in a drug-free state on the test day.

To determine the probable reversal effect of phaclofen on the response induced by baclofen, phaclofen (2, 4 and 6 µg/rat; intra-CA1) was administered intra-CA1, 5 min prior to the administration of baclofen at 2 µg/rat in same experiment (Fig. 5).

2.7.3. Effects of baclofen and/or phaclofen on the expression of conditioned place preference induced by morphine

In order to test the effects of baclofen or phaclofen on the expression of morphine-induced conditioned place preference, both baclofen (0.5, 1 and 2 µg/rat; intra-CA1) and phaclofen (1, 2 and 4 µg/rat; intra-CA1) were injected once on the day of testing (day 5), 5 min prior to the conditioned place preference testing. The respective control groups received saline in a volume of 0.5 µl per side (1 µl/rat), intra-CA1 (Fig. 6).

2.8. Measurement of effects of drug treatments on locomotor activity

Locomotor activity was measured, based on a method used previously by Tzschentke and Schmidt [33], during the testing phase [34,35], in a morphine-free state. An observer, unaware of the treatments, measured locomotor activity in the two main compartments. For this purpose the ground area of A and B compartments were divided into 4 equal sized squares. Locomotion was measured as the number of crossings from one square to another during 15 min.

2.9. Histological verification

After completion of behavioral testing, each animal was killed with an overdose of chloroform. Animals received a 0.5-µl side injection of ink (1% aquatic methylene blue solution). The brains were then removed and fixed in a 10% formalin solution for 10 days before sectioning. Sections were examined to determine location of the cannulae aimed for the CA1. The cannulae placements were verified using the atlas of Paxinos and Watson [30]. Only data from rats that received histologically verified injections were included for analyses.

2.10. Statistical analysis

The experimental data were expressed as mean ± S.E.M. Group differences were tested by one- or two-way analyses of variance (ANOVA) followed by Tukey post-hoc test. P < 0.05 was taken as the significant level of difference. Calculations were performed using the SPSS statistical package.

3. Results

3.1. Histology

Fig. 1A shows location of the injection cannulae tips in the CA1 regions of the dorsal hippocampus for all rats included in the data analyses. The approximate spread of drug assessed by dye (1% aquatic methylene blue solution) injection indicated in this histological verification. Fig. 1B also shows a representative section taken from the rat brain atlas of Paxinos and Watson [30]. Each dot represents the approximate point in
which the cannulae were positioned for each animal. Data from the animals with injection sites located outside the CA1 were not used in the analysis.

3.2. 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. Animals, which received saline (1 ml/kg) twice per day, during six sessions, exhibited no preference for either compartment. Administration of different doses of morphine (0.5, 1, 3 and 6 mg/kg), during conditioning, induced CPP [one-way ANOVA; $F(4,35) = 13.2, P < 0.0001$]. Maximum response was observed by 3 mg/kg of the opioid.

3.3. Effects of GABA$_B$ receptor agonist with or without morphine on the acquisition of CPP

Fig. 3 shows the effects of GABA$_B$ receptor agonist, baclofen, with or without morphine (3 mg/kg), on the acquisition of CPP. Two-way ANOVA indicates a significant difference between the response to baclofen (0.5, 1 and 2 µg/rat, intra-CA1) and that to baclofen plus morphine (3 mg/kg) [Factor morphine, $F(1,56) = 69.56, P < 0.001$; Factor baclofen, $F(3,56) = 5.2, P < 0.01$; Factor morphinebaclofen, $F(3,56) = 5.9$, $P < 0.0001$]. In addition, one-way ANOVA revealed that baclofen alone induced neither a significant place preference nor place aversion [$F(3,28) = 0.3, P > 0.05$]. Furthermore, baclofen dose-dependently inhibited the morphine-induced place preference [one-way ANOVA: $F(3,28) = 13.2, P < 0.0001$].

3.4. Effects of GABA$_B$ receptor antagonist with or without morphine on the acquisition of CPP

Fig. 4 shows the effects of GABA$_B$ receptor antagonist, phaclofen, with or without morphine (1 mg/kg), on the acquisition of CPP. Two-way ANOVA indicates a significant difference between the response to phaclofen (1, 2 and 3 µg/rat,
intra-CA1) and that to phaclofen plus the lower dose of morphine (1 mg/kg) [Factor morphine, $F(1,56)=162.3$, $P<0.0001$; Factor phaclofen, $F(3,56)=4.3$, $P<0.01$; Factor morphine × phaclofen, $F(3,56)=1.8$, $P>0.05$]. In addition, one-way ANOVA revealed that phaclofen alone induced neither a significant place preference nor place aversion [$F(3,28)=0.4$, $P>0.05$]. Furthermore, phaclofen potentiated the morphine-induced place preference [$F(3,28)=6.3$, $P<0.01$].

3.5. Effects of phaclofen on baclofen response during morphine conditioning

Fig. 5 shows the effects of the drugs on morphine-induced place preference. One-way ANOVA showed that different doses of phaclofen (2, 4 and 6 µg/rat) altered the response induced by baclofen (2 µg/rat) plus morphine (3 mg/kg) [one-way ANOVA: $F(4,35)=11.1$, $P<0.0001$]. Post-hoc analysis showed that higher doses of phaclofen (2 and 4 µg/rat, intra-CA1) reversed the effect of baclofen on morphine response.

3.6. Effects of baclofen or phaclofen on the expression of morphine-induced place preference

Fig. 6 shows the effects of bilateral intra-CA1 injection of baclofen or phaclofen on the expression of morphine-induced CPP. One-way ANOVA indicates that baclofen (1 and 2 µg/rat) attenuated the expression of morphine-induced place preference [one-way ANOVA: $F(3,28)=28.7$, $P<0.0001$]. Furthermore, phaclofen (1, 2 and 4 µg/rat) had no effect on the expression of morphine-induced place preference [one-way ANOVA: $F(3,28)=1.1$, $P>0.05$].

3.7. The effect of the drugs on locomotor activity

One-way ANOVA indicated that intra-CA1 injection of the different doses of morphine (0.5, 1, 3 and 6 mg/kg) [$F(4,35)=1.9$, $P>0.05$], baclofen (0.5, 1 and 2 µg/rat) [$F(3,28)=0.57$, $P>0.05$] or phaclofen (1, 2 and 3 µg/rat) [$F(3,28)=1.2$, $P>0.05$], during conditioning phase, alone had no effect on the locomotor activity during the testing phase. Besides, the bilateral intra-CA1 injection of baclofen [$F(3,28)=0.59$, $P>0.05$] or phaclofen [$F(3,28)=1.7$, $P>0.05$]...
plus the subcutaneous injection of morphine, during conditioning phase, did not induce any effect on locomotor activity during the testing phase. Furthermore, intra-CA1 injection of baclofen or phaclofen, 5 min prior to the testing phase, had no effect on the locomotor activity during this phase \[ F(6,42)=1.1, P>0.05 \] (data not shown).

4. Discussion

The present study showed that subcutaneous injection of morphine produced a dose-related conditioned place preference (CPP) in rats. The data agree with those of previous reports [33,31,36,37], which suggested that the conditioning procedure in the present study can be used to investigate the rewarding effect of morphine. It has been indicated that opiate-induced place preference depends on activation of the mesolimbic dopaminergic system in a number of studies [38–41].

Opiates are known to interact with dopaminergic (DA) system by suppressing GABA inhibitory input to DA neurons in the ventral tegmental area (VTA), thereby augmenting DA system by suppressing GABA inhibitory input to DA neurons [38–41]. In a set of experiments, we also examined the effects of the administration of baclofen or phaclofen on the expression of morphine-induced place preference. The results showed that administration of baclofen, but not phaclofen, before testing prevented the expression of morphine-induced place preference. Since baclofen attenuated acquisition and expression of morphine-induced CPP, therefore, GABA\(B\) receptors in the CA1 may interact with dopaminergic system and thus reduce the expression of morphine reward.

Numerous studies have shown that the dorsal hippocampus mediates contextual conditioning (both acquisition and retrieval), in both fear conditioning [59,60] and place conditioning settings [61]. Therefore, GABA\(B\) receptor stimulation could interfere with both acquisition (contextual learning) and expression (retrieval of contextual information) of CPP. In addition, injection of morphine directly into the hippocampus has been shown to induce CPP [62], although low doses of morphine appeared ineffective [63]. Together, this indicates that intra-hippocampal baclofen and phaclofen could interfere with both the rewarding properties of morphine, as well as with the mnemonic processes underlying CPP.

References


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