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Effect of cyclosporin A on morphine-induced place conditioning in mice: involvement of nitric oxide

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Abstract

Cyclosporin A is shown to attenuate antinociceptive effects of morphine, development and expression of morphine-induced tolerance and dependency via nitric oxide (NO) pathway. In the present study, the effect of systemic cyclosporin A on morphine-induced conditioned place preference (CPP) and the probable involvement of nitric oxide were assessed in mice. Our data showed that administration of morphine (1, 2.5, 5, 7.5, 10 mg/kg) significantly increased the time spent in the drug-paired compartment in a dose-dependent manner. The maximum response was obtained with 5 mg/kg of morphine. Cyclosporin A (5, 10 mg/kg) and N^G-nitro-L-arginine methyl ester (L-NAME; 2.5, 5, 10 mg/kg), a nonselective nitric oxide synthase (NOS) inhibitor, did not induce either conditioned place preference or conditioned place aversion (CPA), while cyclosporin A (20 mg/kg) induced CPA. Both cyclosporin A (10, 20 mg/kg) and L-NAME (5, 10 mg/kg), in combination with morphine (5 mg/kg) during conditioning, significantly suppressed acquisition of morphine-induced place preference. Lower and per se noneffective doses of Cyclosporin A (1, 2.5, 5 mg/kg) and L-NAME (2.5 mg/kg), when coadministered, exerted a significant potentiating effect on the attenuation of morphine-induced place preference. Aminoguanidine (50, 100 mg/kg), the specific inducible nitric oxide synthase (iNOS) inhibitor, whether alone or in combination with cyclosporin A failed to show this inhibitory effect on morphine-induced place preference. In conclusion, decreasing nitric oxide production through inhibiting constitutive nitric oxide synthase may be a mechanism through which cyclosporin A attenuates morphine-induced place preference.

Keywords: Cyclosporin A; Morphine; L-NAME (N^G-nitro-L-arginine methyl ester); Aminoguanidine; Nitric oxide; Neuroimmunophilin ligands; Conditioned place preference

1. Introduction

Cyclosporin A is a lipophilic undecapeptide of fungal origin, used clinically as a potent immunosuppressant (Borel et al., 1996). It has been shown that the receptors for cyclosporin A, immunophilins are 50 times more abundant in the nervous system than in the immune system (Steiner et al., 1992). Hence, cyclosporin A and other agents of this family (like Tacrolimus (FK506)) are defined as neuroimmunophilin ligands (Snyder et al., 1998a; Gold, 2002). Immunophilin-binding ligands such as cyclosporin A have been suggested to modulate the release of certain neurotransmitters (Steiner et al., 1996), exert neurotrophic effects, and regulate intracellular Ca^{2+} release (Cameron et al., 1997; Snyder et al., 1998b) and nitric oxide (NO) neurotoxicity (Trajković et al., 1999).

It has been shown that cyclosporin A reduces catalytic activity of neuronal nitric oxide synthase (nNOS) via

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inhibition of calcineurin-mediated dephosphorylation of nNOS, thus inhibiting nitric oxide release (Rao et al., 1996a; Sabatini et al., 1997; Snyder et al., 1998b). This mechanism has been implicated in some of the important functions of cyclosporin A in the nervous system such as neuroprotection (Sabatini et al., 1997). Cyclosporin A is known to exert similar inhibitory effects on nitric oxide production in other tissues such as kidney (Rao et al., 1996b), lung (Mathieu et al., 1997), vascular (Lee et al., 1999), and some other tissues.

In addition, some interesting interactions between cyclosporin A and opioid system have been shown. Cyclosporin A attenuates the antinociceptive effects of morphine (Homayoun et al., 2002a) and diminishes development and expression of morphine-induced tolerance and expression of morphine-induced dependency (Homayoun et al., 2002c). Nitric oxide pathway has been proposed as the responsible mechanism in these interactions.

Some effects of cyclosporin A in reward system have been investigated. Suzuki et al. (1993) have shown that cyclosporin A suppresses the reinforcing effect induced by morphine. In addition, it has been shown that cyclosporin A antagonises spontaneous place preference in rats (Borlongan et al., 1999).

The role of nitric oxide in modulation of opioid antinociception (Brignola et al., 1994), tolerance (Kolesnikov et al., 1992; Dambisya and Lee, 1996), and dependence (Adams et al., 1993; Dambisya and Lee, 1996) has been defined. The inhibition of nitric oxide synthase has been shown to reduce the intensity of naloxone-precipitated withdrawal syndrome (Adams et al., 1993; Cappendijk et al., 1993) and the development and expression of tolerance to morphine-induced antinociception (Kolesnikov et al., 1992). Moreover, it has been shown that the nitric oxide synthase inhibitor, L-*N*-nitroarginine (L-NOARG), attenuates morphine-induced place preference (Kivastik et al., 1996).

Conditioned place preference (CPP) has been widely used to assess the rewarding effect of different systems including opioids (Tzschentke, 1998; Bardo and Bevins, 2000). 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. Morphine produces a significant dose-dependent effect on the magnitude of place preference. The effects of different drugs on acquisition and expression of morphine-induced place preference have also been assessed (Bardo et al., 1984; Bardo and Bevins, 2000).

In the present study, we assessed the effect of cyclosporin A on acquisition of morphine-induced conditioned place preference in mice and then examined the probable involvement of nitric oxide in this effect.

2. Materials and methods

2.1. Subjects

Male NMRI mice (Institute Pasteur of Iran), weighing 20–30 g were used. The animals were housed six per cage in a temperature-controlled (22±3 °C) colony room and all had free access to water and food. They were maintained in a 12 h/12 h light/dark cycle and all trials were carried out in the light phase. All animals were experimentally naïve. Animals were allowed 7 days to acclimatise to the laboratory environment before testing began. The protocol has been approved by the committee of ethics of the faculty of Sciences of Tehran University (357; 8 November 2000).

2.2. Drugs

The Drugs used in the present study were Morphine sulphate (Temad Pharmaceutical, Tehran, Iran), Cyclosporin A (Zakaria Pharmaceutical, Tabriz, Iran), N^G-nitro-L-arginine methyl ester (L-NAME; Sigma, UK), N^G-nitro-D-arginine methyl ester (D-NAME; Sigma, UK), and Aminoguanidine (Sigma, USA). Morphine sulphate, L-NAME, D-NAME, and aminoguanidine were prepared freshly in sterile normal saline (0.9% NaCl solution) and cyclosporin A was prepared daily in Dimethylsulfoxid, DMSO (Merck, Germany). Morphine was injected subcutaneously (s.c.) and cyclosporin A, L-NAME, D-NAME, and aminoguanidine were injected intraperitoneally (i.p.) in all experiments. Drugs were all injected in a 5 ml/kg volume.

2.3. Apparatus

Two compartment place preference apparatus were used. Place conditioning was conducted using a biased procedure. The apparatus were made of wood and consisted of two square-based compartments (15×15×30 H cm each). In order to distinguish the two compartments, visual and sensory texture cues were used; one compartment was painted in vertical black and white shadings (except the floors) and the other compartment was painted white. A black texture covered the floor of the black and white compartment. During the conditioning phases, the two compartments were separated by a guillotine door and covered with a transparent Plexiglas ceiling. In such apparatus, the mice preferred the black–white compartment significantly.

2.4. Experimental procedure

2.4.1. Measurement of conditioned place preference

Conditioned place preference consisted of three phases: Familiarization and Preconditioning, Conditioning, and Postconditioning.

2.4.1.1. Familiarization and preconditioning. On the first day of the trials (i.e., familiarization) and the second day

(i.e., Preconditioning), each mouse was placed separately into the apparatus for 10 min, while they could freely access both compartments. The time spent in each compartment was recorded on the preconditioning day. Placement in each compartment was considered as placement of the front paws and the head. The preconditioning score was measured as the subtraction of the preferred compartment staying time from the nonpreferred compartment staying time. After the test, the animals were grouped randomly (six per cage).

2.4.1.2. Conditioning. This phase consisted of six conditioning sessions held in six consecutive days. The duration of each session was 40 min and the mice were confined to the considered compartment, by isolating the compartment using a removable partition. The mice received the considered drugs (Morphine, Cyclosporin A, L-NAME, D-NAME, and Aminoguanidine) in the nonpreferred compartment (defined in the preconditioning phase, which was the white compartment in our experiments) on days 1, 3, and 5 of the conditioning phase, and the vehicles (saline and DMSO) in the preferred compartment on days 2, 4, and 6 of the conditioning phase.

2.4.1.3. Postconditioning. This phase was carried out in the ninth day of the trials (24 h after the last conditioning session, with no preceding injections) in a drug-free state. As in the preconditioning phase, the partition was raised and the animals were placed in the apparatus for 10 min, with free access to both compartments and the time spent in each compartment was recorded. The postconditioning score was measured in the same way as the preconditioning score. The difference between post- and preconditioning scores was considered as Change in Preference score (CIP).

2.4.2. Measurement of locomotor activity

The locomotor activity was measured in the postconditioning day. To measure the locomotor activity, the ground areas of the two compartments were divided in two equal segments by a transverse line and locomotion was measured as the number of crossings from one half to the other. The results are demonstrated as counts per animal over 10-min testing in the postconditioning day.

2.5. Experimental design

2.5.1. Experiment 1: dose–response effects of place conditioning produced by morphine

In this experiment, we established a dose–response function for morphine place conditioning. Six different doses of morphine sulphate (0.5, 1, 2.5, 5, 7.5, 10 mg/kg, s.c.) were tested for producing place preference. A control group that received saline (5 ml/kg, s.c.) in all sessions was included in order to confirm that the injection and conditioning schedule did not affect the time spent in the compartments.

2.5.2. Experiment 2: effects of cyclosporin A on the acquisition of place preference conditioning in the absence or presence of morphine

2.5.2.1. Effects of cyclosporin A on the acquisition of place preference. Three doses of cyclosporin A (5, 10, 20 mg/kg, i.p.) were injected 25–30 min prior to placement in the nonpreferred compartment, under the schedule described above. One additional group received DMSO (5 ml/kg, i.p.) 25–30 min prior to placement in apparatus and served as a control.

2.5.2.2. Effects of cyclosporin A on the acquisition of morphine-induced place preference. Six groups of animals received different doses of cyclosporin A (2.5, 5, 10, 20 mg/kg, i.p.), DMSO (5 ml/kg, i.p.), and saline (5 ml/kg, i.p.) 25–30 min before the administration of the most potent dose of morphine, obtained from experiment 1, under the schedule.

2.5.3. Experiment 3: effects of L-NAME on the acquisition of place preference conditioning in the absence or presence of morphine

2.5.3.1. Effects of L-NAME on the acquisition of place preference. Three doses of L-NAME (2.5, 5, 10 mg/kg, i.p.), D-NAME (the inactive enantiomer of L-NAME; 5 mg/kg, i.p.), and saline (5 ml/kg, i.p.) were injected 25–30 min prior to placement in apparatus under the schedule and the ability of L-NAME to induce place conditioning on the postconditioning day was evaluated.

2.5.3.2. Effects of L-NAME on the acquisition of morphine-induced place preference. In this experiment, five groups of animals received different doses of L-NAME (2.5, 5, 10 mg/kg, i.p.), D-NAME (5 mg/kg, i.p.), and saline (5 ml/kg, i.p.) 25–30 min before the administration of the most potent dose of morphine under the schedule.

2.5.4. Experiment 4: effects of coadministration of L-NAME and cyclosporin A on morphine-induced place preference

Six groups of animals were used in this experiment to observe the possible involvement of nitric oxide in cyclosporin A influence on morphine-induced place preference. Three groups received three noneffective doses of cyclosporin A (1, 2.5, 5 mg/kg, i.p.) plus a noneffective dose of L-NAME (2.5 mg/kg, i.p.) 25–30 min prior to administration of the most potent dose of morphine (5 mg/kg, s.c.) under the schedule. Three control groups received a noneffective dose of cyclosporin A (2.5 mg/kg, i.p.) plus saline (5 ml/kg, i.p.), DMSO (5 ml/kg, i.p.) plus saline (5 ml/kg, i.p.), and DMSO (5 ml/kg, i.p.) plus a noneffective dose of L-NAME (2.5 mg/kg, i.p.), using the same conditioning schedule. Noneffective doses of cyclosporin A and L-NAME were obtained from experiments 2 and 3, respectively.

2.5.5. Experiment 5: effects of aminoguanidine on the acquisition of place preference conditioning in the presence of morphine

Three groups of animals received different doses of aminoguanidine (50, 100 mg/kg, i.p.) and saline (5 ml/kg, i.p.) 25–30 min before the administration of the most potent dose of morphine, obtained from experiment 1, under the schedule.

2.5.6. Experiment 6: effects of coadministration of cyclosporin A and aminoguanidine on morphine-induced place preference

Six groups of animals were used in this experiment to observe the possible involvement of inducible nitric oxide synthase (iNOS) inhibition in cyclosporin A influence on morphine-induced place preference. Two noneffective doses of cyclosporin A (2.5, 5 mg/kg, i.p.) in combination with two noneffective doses of aminoguanidine (50, 100 mg/kg, i.p.) were injected 25–30 min prior to administration of the most potent dose of morphine (5 mg/kg, s.c.) under the schedule (which led to four groups). Two control groups received a noneffective dose of cyclosporin A (2.5 mg/kg, i.p.) plus saline (5 ml/kg, i.p.) and DMSO (5 ml/kg, i.p.) plus saline (5 ml/kg, i.p.) 25–30 min before morphine, using the same conditioning schedule. Noneffective doses of cyclosporin A and aminoguanidine were obtained from experiments 2 and 5, respectively.

2.6. Statistical analysis

All results are presented as mean±S.E.M. One-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparison tests were used to determine the effect of various treatments on induction of place preference (by comparing mean CIP values of different groups) and also changes in locomotion. P values less than 0.05 were considered statistically significant.

3. Results

3.1. Effect of morphine sulphate on conditioned place preference

Fig. 1 shows a dose–response effect curve for morphine on conditioned place preference. In preconditioning session, mean staying time in the white compartment was 233±20.6 s (mean±S.D.) out of 600 s, thus the white compartment was chosen as the drug-paired compartment. Statistical analysis indicated that morphine induced place preference [one-way ANOVA; $F(6,34)=16.785$, $P<0.001$] but did not change locomotion significantly [one-way ANOVA; $F(6,34)=12.361$, $P>0.05$]. Tukey–Kramer multiple comparison tests revealed that the doses of 1–10 mg/kg of morphine induced place preference, but saline (5 ml/kg, s.c.) or morphine (0.5 mg/kg, s.c.) failed to produce significant conditioning in animals and no preference for either of the compartments was seen. The maximum response was obtained with 5.0 mg/kg of morphine (Fig. 1); therefore, this dose was employed in all subsequent experiments.

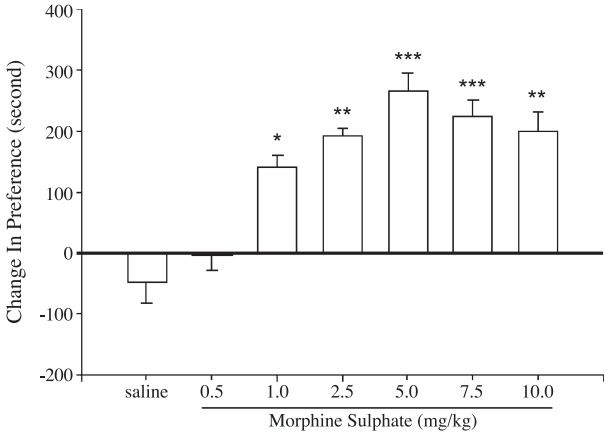

Fig. 1. Effects of morphine on conditioned place preference (CPP) in mice. In a 6-day schedule, animals received saline (5 ml/kg, s.c.) or morphine (0.5, 1, 2.5, 5, 7.5, 10 mg/kg, s.c.) in the drug-paired compartment in the 1st, 3rd, and 5th days. The data are shown as means of change in preference±S.E.M. *$P<0.01$, **$P<0.005$, ***$P<0.001$ different from the group treated with saline (Tukey–Kramer multiple comparison test).

3.2. Effects of cyclosporin A on the acquisition of place preference conditioning in the absence or presence of morphine

Fig. 2 shows the effect of different doses of cyclosporin A (5, 10, 20 mg/kg, i.p.) and its vehicle, DMSO (5 ml/kg, i.p.), on place preference. Analysis showed a significant effect for cyclosporin A on place preference [one-way ANOVA; $F(3,17)=5.325$, $P<0.01$; mean preconditioning drug-paired staying time=239±40.8 s]. Although DMSO (5 ml/kg, i.p.) and cyclosporin A (5, 10 mg/kg, i.p.) reduced the staying time by −5.8, −63.0, −71.2 s, respectively, they were not significant statistically [$P>0.5$]. Cyclosporin A (20 mg/kg, i.p.) induced conditioned place aversion (CPA) [mean=−409.2 s, $P<0.05$]. As Fig. 3 shows, a significant interaction was seen between cyclosporin A and morphine [one-way ANOVA; $F(5,28)=7.656$, $P<0.001$; mean preconditioning drug-paired staying time=205.5±51.5 s]. Groups pretreated with DMSO (5 ml/kg, i.p.), saline (5 ml/kg, i.p.), and cyclosporin A (2.5, 5 mg/kg, i.p.) showed no significant effect on morphine-induced place preference, while cyclosporin A (10, 20 mg/kg, i.p.) significantly attenuated acquisition of morphine-induced place preference [$P<0.01$]. The maximum response was observed with 10 mg/kg of cyclosporin A. As cyclosporin A (10 mg/kg, i.p.) did not show any significant effect on place preference while injected alone, it can be implicated that the attenuation noticed in Fig. 3, is produced due to interaction between cyclosporin A and morphine. Cyclosporin A changed

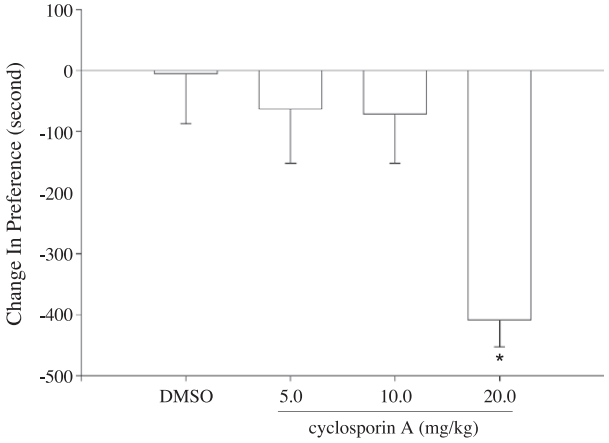

Fig. 2. Effects of cyclosporin A on conditioned place preference (CPP) in mice. In a 6-day schedule, animals received DMSO (5 ml/kg, i.p.) or cyclosporin A (5, 10, 20 mg/kg, i.p.) in the drug-paired compartment in the 1st, 3rd, and 5th days. The data are shown as means of change in preference±S.E.M. *P<0.05 different from the group treated with DMSO (Tukey–Kramer multiple comparison test).

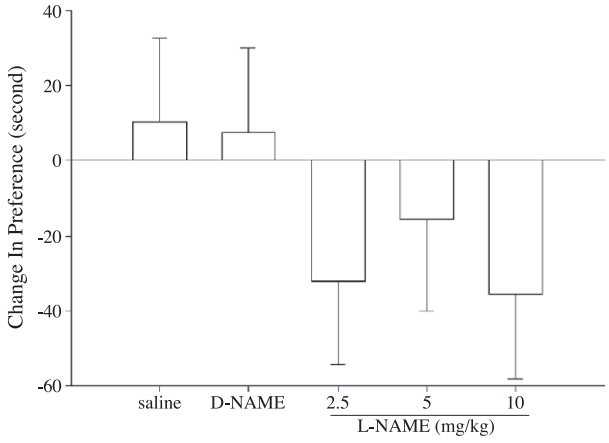

Fig. 4. Effects of L-NAME on conditioned place preference (CPP) in mice. In a 6-day schedule, animals received saline (5 ml/kg, i.p.), L-NAME (2.5, 5, 10 mg/kg, i.p.) or D-NAME (5 mg/kg, i.p.) in the drug-paired compartment in the 1st, 3rd, and 5th days. The data are shown as means of change in preference±S.E.M. Analysis revealed that no group showed a statistical significant difference.

locomotion neither alone [one-way ANOVA; F(3,17)=1.333, P>0.05] nor in the presence of morphine [one-way ANOVA; F(5,28)=0.466, P>0.5], so the observed effects could not be attributed to any changes in locomotion.

3.3. Effects of L-NAME on the acquisition of place preference conditioning in the absence or presence of morphine

Fig. 4 shows the effects of the nonselective NOS inhibitor, L-NAME, on place conditioning. No significant change in place preference was observed by administration of L-NAME (2.5, 5, 10 mg/kg, i.p.), D-NAME (5 mg/kg, i.p.), or saline (5 ml/kg, i.p.) alone [one-way ANOVA; F(4,24)=0.928, P>0.1]. The effects of L-NAME on acquisition of morphine-induced place preference is shown in Fig. 5, where a significant interaction was observed [one-way ANOVA; F(4,23)=13.903, P<0.001]. L-NAME (5, 10 mg/kg, i.p.) significantly suppressed morphine-induced place preference [P<0.05 and P<0.001, respectively] while L-NAME (2.5 mg/kg, i.p.) and D-NAME (5 mg/kg, i.p.) showed no significant effect [P>0.5]. No significant change in locomotion was seen in administration of L-NAME neither alone [one-way ANOVA; F(4,24)=0.512, P>0.5] nor in combination with morphine [one-way ANOVA; F(4,23)=2.112, P>0.05]. The results indicate that 5 and 10

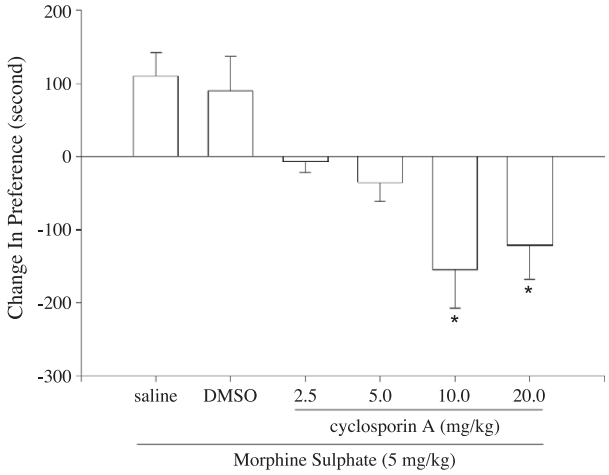

Fig. 3. Effects of cyclosporin A on acquisition of morphine-induced conditioned place preference (CPP) in mice. In a 6-day schedule, six groups of animals received saline (5 ml/kg, i.p.), DMSO (5 ml/kg, i.p.), and cyclosporin A (2.5, 5, 10, 20 mg/kg, i.p.) 25–30 min before injection of morphine (5 mg/kg, s.c.) and were placed in the drug-paired compartment in the 1st, 3rd, and 5th days. The data are shown as means of change in preference±S.E.M. *P<0.01 different from the groups pre-treated with saline and DMSO (Tukey–Kramer multiple comparison test).

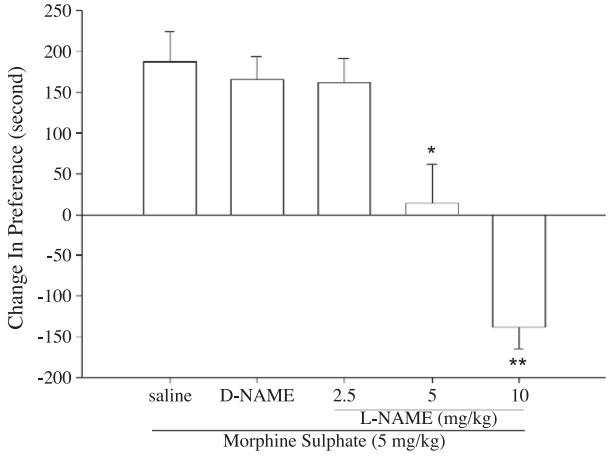

Fig. 5. Effects of L-NAME on acquisition of morphine-induced conditioned place preference (CPP) in mice. In a 6-day schedule, five groups of animals received saline (5 ml/kg, i.p.), D-NAME (5 mg/kg, i.p.) or L-NAME (2.5, 5, 10 mg/kg, i.p.) 25–30 min before injection of morphine (5 mg/kg, s.c.) and were placed in the drug-paired compartment in the 1st, 3rd, and 5th days. The data are shown as means of change in preference±S.E.M. *P<0.05, **P<0.001 different from the group pretreated with saline (Tukey–Kramer multiple comparison test).

mg/kg of L-NAME could suppress effects of morphine on place conditioning and this attenuation is not a motivational property of L-NAME itself.

3.4. Effects of coadministration of L-NAME and cyclosporin A on acquisition of morphine-induced place preference

The results, shown in Fig. 6, indicate significant suppression of morphine-induced conditioned place preference by coadministration of cyclosporin A (1, 2.5, 5 mg/kg, i.p.) and L-NAME (2.5 mg/kg, i.p.) [one-way ANOVA; $F(5,30)=24.054$, $P<0.001$; mean preconditioning drug-paired staying time=216.9±40.5 s], while having no effect on locomotion [one-way ANOVA; $F(5,30)=1.552$, $P>0.05$]. Cyclosporin A (2.5, 5 mg/kg, i.p.) showed more inhibitory effect than 1 mg/kg of cyclosporin A during coadministration with L-NAME (2.5 mg/kg, i.p.) on morphine-induced place preference [$P<0.001$ for cyclosporin A (2.5, 5 mg/kg, i.p.); $P<0.05$ for cyclosporin A (1 mg/kg, i.p.)].

3.5. Effects of aminoguanidine on the acquisition of place preference conditioning in the presence of morphine

The results, shown in Fig. 7, indicate that aminoguanidine (50, 100 mg/kg, i.p.) had no significant effect on morphine-induced place conditioning [one-way ANOVA; $F(2,12)=0.434$, $P>0.5$]. It also had no effect on locomotion [one-way ANOVA; $F(2,12)=0.571$, $P>0.5$].

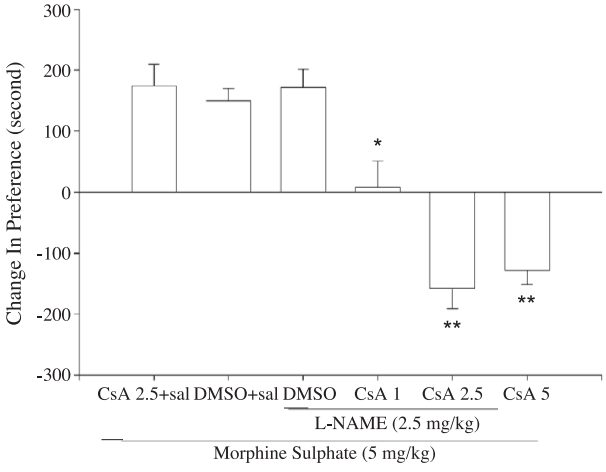

Fig. 6. Effects of coadministration of cyclosporin A and L-NAME on acquisition of morphine-induced conditioned place preference (CPP) in mice. In a 6-day schedule, six groups of animals received saline (5 ml/kg, i.p.) plus DMSO (5 ml/kg, i.p.), DMSO (5 ml/kg, i.p.) plus L-NAME (2.5 mg/kg, i.p.), cyclosporin A (2.5 mg/kg, i.p.) plus saline (5 ml/kg, i.p.), cyclosporin A (1 mg/kg, i.p.) plus L-NAME (2.5 mg/kg, i.p.), cyclosporin A (2.5 mg/kg, i.p.) plus L-NAME (2.5 mg/kg, i.p.), and cyclosporin A (5 mg/kg, i.p.) plus L-NAME (2.5 mg/kg, i.p.) 25–30 min before injection of morphine (5 mg/kg, s.c.) and were placed in the drug-paired compartment in the 1st, 3rd and 5th days. The first three groups served as the control groups. The data are shown as means of change in preference±S.E.M. CsA=Cyclosporin A, sal=saline. *$P<0.05$, **$P<0.001$ different from the three control groups (Tukey–Kramer multiple comparison test).

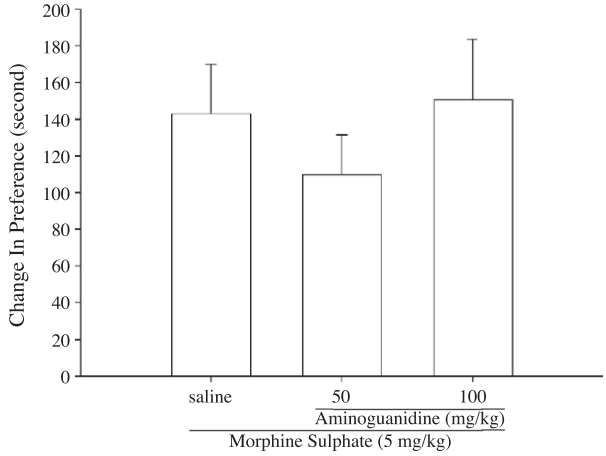

Fig. 7. Effects of aminoguanidine on acquisition of morphine-induced conditioned place preference (CPP) in mice. In a 6-day schedule, animals received saline (5 ml/kg, i.p.) or aminoguanidine (50, 100 mg/kg, i.p.) in the drug-paired compartment in the 1st, 3rd, and 5th days. The data are shown as means of change in preference±S.E.M. Analysis revealed that no group showed a statistical significant difference.

3.6. Experiment 6: effects of coadministration of cyclosporin A and aminoguanidine on morphine-induced place preference

The results are shown in Fig. 8. In comparison to the control groups, coadministration of cyclosporin A (2.5, 5 mg/kg, i.p.) and aminoguanidine (50, 100 mg/kg, i.p.) had

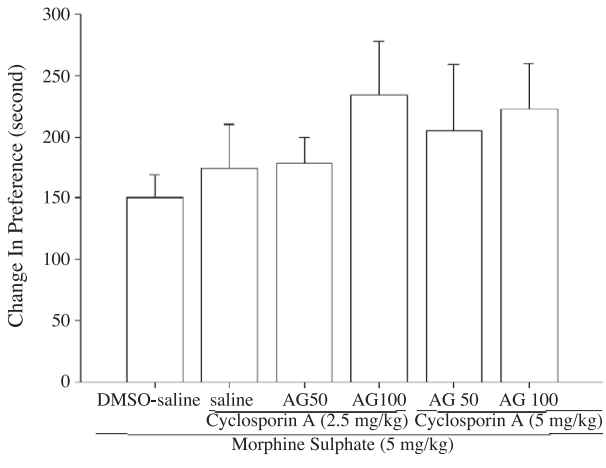

Fig. 8. Effects of coadministration of cyclosporin A and aminoguanidine on acquisition of morphine-induced conditioned place preference (CPP) in mice. In a 6-day schedule, six groups of animals received saline (5 ml/kg, i.p.) plus DMSO (5 ml/kg, i.p.), cyclosporin A (2.5 mg/kg, i.p.) plus saline (5 ml/kg, i.p.), cyclosporin A (2.5 mg/kg, i.p.) plus aminoguanidine (50 mg/kg, i.p.), cyclosporin A (2.5 mg/kg, i.p.) plus aminoguanidine (100 mg/kg, i.p.), cyclosporin A (5 mg/kg, i.p.) plus aminoguanidine (50 mg/kg, i.p.), and cyclosporin A (5 mg/kg, i.p.) plus aminoguanidine (100 mg/kg, i.p.) 25–30 min before injection of morphine (5 mg/kg, s.c.) and were placed in the drug-paired compartment in the 1st, 3rd, and 5th days. The first two groups served as the control groups. The data are shown as means of change in preference±S.E.M. AG=aminoguanidine. Analysis revealed that no group showed a statistical significant difference.

no significant effect on morphine-induced place conditioning [one-way ANOVA; $F(5,30)=0.414$, $P>0.5$] and also showed no significant effect on locomotion [one-way ANOVA; $F(5,30)=1.108$, $P>0.05$].

4. Discussion

The present study concerned the effects of administration of cyclosporin A, a potent immunosuppressive agent, on the acquisition of morphine-induced place preference. Moreover, the possible involvement of nitric oxide in this regard has been studied by using L-NAME, a nonselective nitric oxide synthase (NOS) inhibitor and aminoguanidine, a specific inducible nitric oxide synthase (iNOS) inhibitor.

Neuroimmunophilins are a family of proteins which are highly conserved in the nature and mediate the actions of neuroimmunophilin ligands (such as tacrolimus and cyclosporin A) in the central nervous system (Gold, 2000). It has been found that neuroimmunophilin ligands are able to modulate some effects of opioid drugs. Dafny et al. (1985) have shown that cyclosporin A considerably modifies the behavioural signs of naloxone-induced opioid withdrawal in morphine dependent rats and also can attenuate the opioid withdrawal syndrome precipitated by naloxone following intracerebroventricular administration (Dougherty and Dafny, 1988). Acute administration of cyclosporin A induces an antinociceptive effect that involves the L-arginine/nitric oxide pathway and is not mediated by opioid receptors (Homayoun et al., 2002a). Cyclosporin A diminishes the development and expression of morphine-induced tolerance and expression of morphine-induced dependence by decreasing nitric oxide production through inhibition of neuronal nitric oxide synthase (Homayoun et al., 2002c). Moreover, immunophilin ligands at very low concentrations can reduce the induction of acute tolerance to and dependence on morphine in the myenteric plexus of guinea pig ileum (Ejtemaei mehr et al., 2003). Several early studies had tried to relate such effects of cyclosporin A to its modulatory properties on the immune system (Dafny et al., 1985; Dougherty et al., 1987; Dougherty and Dafny, 1988). However, the surprising finding that the levels of immunophilins in the nervous system are 50 times higher than those in tissues of the immune system implied a neural role for cyclosporin A (Steiner et al., 1992). Further investigations revealed that cyclosporin A and its protein receptor, cyclophilin A, bind to and inhibit the activity of calcineurin phosphates in the nervous system, which in turn leads to an increase in phosphorylated levels of several important proteins including neuronal nNOS (Snyder et al., 1998b; Rao et al., 1996a). Therefore, neuroimmunophilin ligands are able to diminish the catalytic activity of nNOS.

In addition, several lines of evidence suggest participation of NO–cGMP pathway in the mechanisms of tolerance to and dependence on opioids. The role of nitric oxide in the modulation of opioid antinociception (Brignola et al., 1994), tolerance (Kolesnikov et al., 1992; Dambisya and Lee, 1996), and dependence (Adams et al., 1993; Dambisya and Lee, 1996) has been defined. The inhibition of nitric oxide synthase has been shown to reduce the intensity of naloxone-precipitated withdrawal syndrome (Adams et al., 1993; Cappendijk et al., 1993) and the development and expression of tolerance to morphine-induced antinociception (Kolesnikov et al., 1992). In addition, it has been shown that the nitric oxide synthase inhibitor, L-*N*-nitroarginine, attenuates the morphine induced place preference (Kivastik et al., 1996). Studies on the rat hippocampal CA1 area, central amygdala, and nucleus accumbens have suggested a role for nitric oxide in the acquisition and expression of morphine-induced place preference (Gholami et al., 2002; Karami et al., 2002; Zarrindast et al., 2002).

Our data indicate that morphine induced a significant conditioned place preference in a dose dependent manner, which is in accordance with results of others in this respect. In agreement with Borlongan et al. (1999), the administration of cyclosporin A (20 mg/kg, i.p.) by itself induced CPA. L-NAME (i.p.) alone did not show any effect on place conditioning or place aversion. However, pretreatment of animals with L-NAME (5, 10 mg/kg, i.p.) during conditioning sessions significantly decreased place preference induced by morphine, while aminoguanidine (50, 100 mg/kg, i.p.) failed to exert the similar inhibitory effect on morphine-induced place preference. As cyclosporin A (5, 10 mg/kg, i.p.) and L-NAME (2.5, 5, 10 mg/kg, i.p.) did not elicit any response regarding conditioning, the observed suppressing effects on morphine-induced place conditioning could be attributed to their interaction with morphine and not their own motivational property. It has been previously shown that cyclosporin A blocks morphine-induced place preference in ddY mice, but not in μ1 receptor depleted CXBK mice (Suzuki et al., 1993) and was concluded that this suppression by cyclosporin A may be mediated by μ1 opioid receptors. In our study, lower and per se noneffective doses of cyclosporin A attenuated place preference induced by morphine when coadministered with L-NAME but not with aminoguanidine. Low doses of cyclosporin A (1, 2.5, 5 mg/kg, i.p.) and L-NAME (2.5 mg/kg, i.p.) exerted inhibitory effect on morphine-induced place preference, while having no effect alone. In addition, the observed inhibitory effect was comparable to the inhibitory effect of higher doses of each drug alone (cyclosporin A (10, 20 mg/kg, i.p.) and L-NAME (10 mg/kg, i.p.)). For instance, the mean CIP was −156.75 s for the group pretreated with cyclosporin A (2.5 mg/kg, i.p.) plus L-NAME (2.5 mg/kg, i.p.) which is comparable to the results observed in pretreatment with cyclosporin A (10 mg/kg, i.p.) or L-NAME (10 mg/kg, i.p.) alone, which were −154.20 s and −138.40 s, respectively, regarding the fact that the results for cyclosporin A (2.5 mg/kg, i.p.) or L-NAME (2.5 mg/kg, i.p.) alone were −17.20 s and 161.67 s, respectively. Therefore, this observation cannot be interpreted as a simple additive effect, but as a potentiating or synergistic effect.

Potentiation of the inhibitory effect of cyclosporin A on morphine-induced place preference by blockade of nitric oxide production suggests that the underlying mechanisms of action of these two agents may somehow cross. However, three different forms of NOS have been identified that include inducible NOS (iNOS), endothelial NOS (eNOS), and neuronal NOS (nNOS; Moncada and Higgs, 1993). Cyclosporin A is shown to exert inhibitory effects on nNOS and iNOS (Sanchez-Lozada et al., 2000) while enhancing the activity of eNOS (Stroes et al., 1997). However, in the present study, the irreversible inhibitor of iNOS, aminoguanidine (50, 100 mg/kg, i.p.), whether alone or in combination with cyclosporin A did not exert any significant effect on morphine-induced place preference, implying that previously reported interactions between cyclosporin A and iNOS are not involved in the modulation of morphine effects by cyclosporin A. In other words, the inability of aminoguanidine to show synergistic inhibitory effects with cyclosporin A, makes the probability of involvement of iNOS in these effects less possible. This result is in accordance with the previously shown synergism and potentiation of anitinociceptive and anticonvulsive effects of cyclosporin A by L-NAME but not by aminoguanidine (Homayoun et al., 2002a,b,c).

In conclusion, the present results show that cyclosporin A attenuates the effects of morphine on place preference conditioning. These effects may be due to a decrease in NO production induced by cyclosporin A through one or several mechanism(s) involving constitutive but not inducible isoforms of NOS. The underlying mechanism(s) for the interaction of immunophilins and the reward system warrant further investigation.

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