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$GABA_B$ receptors within the ventral tegmental area are involved in the expression and acquisition of morphine-induced place preference in morphine-sensitized rats

Hedayat Sahraei [a,*], Leila Etemadi [b], Parvin Rostami [c], Ali Pourmotabbed [d], Mohammad-Reza Zarrindast [e], Jamal Shams [f], Hassan Ghoshooni [g], Ali Noroozzadeh [h], Behnaz Esfandiari [b], Seyed Hossein Salimi [i]

[a] Department of Physiology and Biophysics and Applied Neuroscience Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
[b] Department of Biology, School of Science, North Branch of Tehran, Azad University, Iran
[c] Department of Biology, Tarbiat Moalem University, Tehran, Iran
[d] Department of Physiology, Kermanshah University of Medical Sciences, Kermanshah, Iran
[e] Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Iran
[f] Neuroscience Research Center, Shaheed Beheshti University of Medical Sciences, Iran
[g] Department of Physiology and Biophysics and Behavioral Sciences Research Center, Baqiyatallah University of Medical Sciences, Iran
[h] Department of Physiology and Biophysics and Military Health Research Center, Baqiyatallah University of Medical Sciences, Iran
[i] Department of Psychology and Research Center of Exercise Physiology, Baqiyatallah University of Medical Sciences, Iran

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ABSTRACT

The influence of intra-ventral tegmental area administration of gamma-amino-butyric-acid-B ($GABA_B$) receptor agonist and antagonist on the expression and acquisition of morphine-induced conditioned place preference (CPP) in morphine-sensitized female rats was examined. Our pilot experiment showed that subcutaneous administration of morphine-(2.5, 5 and 7.5 mg/kg) induced CPP. Administration of one dose daily morphine (5 mg/kg) for 3 days followed by 5 days rest, enhanced the conditioning induced by ineffective doses of morphine (0.25, 0.5 and 1 mg/kg). Injections of $GABA_B$ receptor agonist, baclofen, (1.5 and 12 μg/rat) reduced the expression of morphine CPP whereas the dose of 6 μg/rat of the drug increased it. Baclofen also significantly reduced the acquisition of morphine CPP in morphine-sensitized animals. Administration of $GABA_B$ receptor antagonist, CGP 35348, significantly reduced the expression (12 μg/rat) and acquisition (1.5, 6 and 12 μg/rat) of morphine CPP in morphine-sensitized animals.
In conclusion, results confirmed the importance of $GABA_B$ receptors within the ventral tegmental area of morphine CPP in morphine-sensitized female rats.

1. Introduction

The long-term effects of exposure to morphine are a major point on the pathophysiology of drug addiction. Several studies have shown that repeated morphine administration increases its rewarding and behavioral effects known as sensitization. The molecular mechanisms underlying morphine sensitization are not fully understood (For rev see: Robinson and Berridge, 2003). The ventral tegmental area (VTA) of the midbrain contains the cell bodies of the mesolimbic dopamine system as a major target for morphine (Spanagel and Weiss, 1999; Wise, 1998; Koob, 1992; Di Chiara and Imperato, 1988). The VTA dopaminergic neurons are under tonic inhibition of gamma-amino-butyric-acid- (GABA)-ergic interneurons within the VTA (Kalivas et al., 1990; Johnson and North, 1992; Koob, 1992). μ-Opioid receptors are localized on the GABAergic neurons where their activity (by morphine—for example) leads to a decrease of GABAergic neuronal activity and consequently disinhibits dopamine neurons and also increases extra-cellular dopamine concentrations in the targets of neurons including nucleus accumbens, amygdala, hippocampus and the prefrontal cortex (Spanagel and Weiss, 1999; Wise, 1998; Johnson and North, 1992; Koob, 1992). This mechanism has been considered as a major cause of the reward property of morphine.

Considering the role of GABA in morphine reward, the $GABA_B$ receptor subtypes within the VTA play a role in morphine reinforcement. In this respect, administration of the $GABA_B$ receptor agonist, baclofen, into the VTA reduces the acquisition (Tsuji et al., 1996) and expression (Sahraei et al., 2005) of morphine-induced place preference in the rat and morphine-induced place preference and also morphine-induced Fos expression in nucleus accumbens in mice (Kaplan et al., 2003). Moreover, intra-VTA baclofen administration reduces heroin-induced dopamine release in the nucleus accumbens and blocks opioid-induced self-administration in rats (Xi and Stein, 1999; 2000). In addition, baclofen also inhibits nicotine, cocaine and

* Corresponding author. Department of Physiology and Biophysics, School of Medicine, Baqiyatallah (a.s.) University of Medical Sciences, Tehran P.O. Box: 19395-6558, Iran. Tel./fax: +9821 22281561.
E-mail address: h.sahraei@bmsu.ac.ir (H. Sahraei).

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morphine-induced dopamine release in the nucleus accumbens of rats (Fadda et al., 2003). The effects of $GABA_B$ receptors located in the VTA on morphine-induced behavioral sensitization were also investigated in several studies. In this regard, it has been shown that intra-VTA baclofen administration reduces the acquisition and expression of morphine-induced locomotor sensitization in mice (Leite-Morris et al., 2002, 2004; Woo et al., 2001). Similarly, morphine sensitization leads to down regulation of $GABA_B$ receptor function to activate G-protein in the mouse VTA (Narita et al., 2003), which further indicates the interaction of $GABA_B$ receptors on morphine sensitization.

However, the role of $GABA_B$ receptors within the VTA of morphine-induced place preference (CPP) in morphine-sensitized rats has not been demonstrated. In the present study, we attempt to further investigate the role of the $GABA_B$ receptors within the ventral tegmental area on the acquisition and expression of morphine-induced CPP in morphine-sensitized rats. The CPP paradigm was chosen because it has been widely used as a model for studying the reinforcing effects of drugs of abuse and localization of brain reinforcement mechanisms (McBride et al., 1999; Tzschentke, 1998).

2. Materials and methods

2.1. Animals

Female Wistar rats (250±20 g, Pasteure Institute, Tehran, IRAN) were used throughout the study (6–8 rats for each experiment). Animals were housed in groups of 4 per cage in a 12/12 h light-cycle (lights on at 07.00), with *ad libitum* food and water available. The animals were randomly allocated to different groups of the experiment. All experiments were conducted in accordance with standard ethical guidelines and approved by the local ethical committee (The Baqiyatallah (a.s.) University of Medical Committee on the Use and Care of Animals, 81/021, July 10, 2002).

2.2. Drugs

The following drugs were used in the experiments: morphine sulfate (TEMAD, IRAN), baclofen and CGP35348 (Novartis Basel, Switzerland), ketamine hydrochloride and xylazine (Alfasan Worden, Holland). The drugs were dissolved in sterile saline and their pH was adjusted to 7.0±0.2. The temperature of the solutions was 37±0.2 °C. Morphine, ketamine and xylazine were injected subcutaneously in a volume of 1 ml/kg. Baclofen and CGP35348 were given intra-VTA in a volume of '0.5 µl/rat' and were prepared before use. The control groups received saline.

2.3. Surgical procedures

Rats were anesthetized with ketamine hydrochloride (70 mg/kg, i.p.) + xylazine (10 mg/kg, i.p.) and two stainless steel cannulas (23-gauge) were placed stereotaxically (Stolting instruments, USA) into the VTA. Stereotaxic coordinates according to Paxinos and Watson (1987) were: incisor bar (−3.3 mm), 4.8 mm posterior to the bregma, ±1.2 mm lateral to the sagittal suture and 7.6 mm from top of the skull. Guide cannulas were secured to anchor the jewelers' screws with dental acrylic. All animals were allowed 1 week to recover from surgery and clear anesthetic.

2.4. Injection into the ventral tegmental area

During intra-VTA injections, each animal received an injection of 0.5 µl of the drugs dissolved in saline via a 30-gauge (0.5 mm below the tip of the guide cannula) blunt tapered needle (0.25 µl/side), at a rate of 0.5 µl/min. This was possible by using an especial double injection apparatus made by our lab connected to a 2 µl Hamilton Syringe which was operated by an infusion pump. After the completion of each injection, the needle remained in the guide cannula for 1 additional min and then was removed from guide cannula and after 2 min the animal was placed in the apparatus.

2.5. Histology

After the completion of testing, all animals were anesthetized and received a transcardiac perfusion of 0.9% normal saline followed by 10% buffered formalin. The brains were removed, blocked and cut coronally in 40 µm sections through the cannula placement. The tissues were stained with cresyl violet and were examined by light microscopy by an observer unfamiliar with the behavioral data. Only animals with correct cannula placements were included in the data analysis (Fig. 1).

2.6. Apparatus

A two compartment CPP apparatus (30×60×30 cm) was used in these experiments. The apparatus was made of wood. Both compartments were identical in size (the apparatus was divided into two equal-sized compartments by means of a removable white wall) and shading (both were white), but distinguishable by texture and olfactory cue. To provide the tactile difference between the compartments, one of the compartments had a smooth floor, while the other compartment had a nylon white mesh floor. A drop of menthol was placed at the center of the compartment with a textured (nylon mesh) floor, to provide the olfactory difference between the compartments. The compartments' walls were differently striped black (one compartment horizontal and the other one vertical) on their sides. In this apparatus, rats showed no consistent preference for either compartment.

2.7. Induction of morphine sensitization

Animals received a daily injection of morphine (5 mg/kg, s.c.) for three consecutive days in a room distinct from that where the conditioning occurred. Five days later, the CPP paradigm was induced by an ineffective dose of morphine (0.5 mg/kg, s.c.) (which was effective after sensitization to morphine) as described in the methods section.

2.8. Experimental procedure

2.8.1. Measurement of conditioned place preference

Place conditioning was conducted using an unbiased procedure, with minor changes to the design previously described (Karami et al., 2002). Conditioned place preference consisted of three phases: pre-conditioning, conditioning and post conditioning.

2.8.1.1. Pre-conditioning. On day 1 (pre-exposure), each rat was placed separately into the apparatus for 10 min, with free access to all compartments.

2.8.1.2. Conditioning. This phase consisted of a 3-day schedule of conditioning sessions. In this phase, the animals received the first three trials in which they experienced the effects of the morphine while confined in one compartment for 45 min. In the second three trials, they experienced the effects of saline while confined in the other compartment. In the first day of conditioning sessions animals received morphine at 9.00 and saline at 16.00. On day 2, the time order of receiving morphine and saline was reversed. On day 3, the animals received morphine and saline as day 1. Access to the other compartment was blocked on these days. In addition, morphine and saline compartments were randomly assigned for each animal in a counterbalanced way.

2.8.1.3. Post conditioning phase. On the 5th day (the preference test day) the partition was removed, and the rats could access the entire

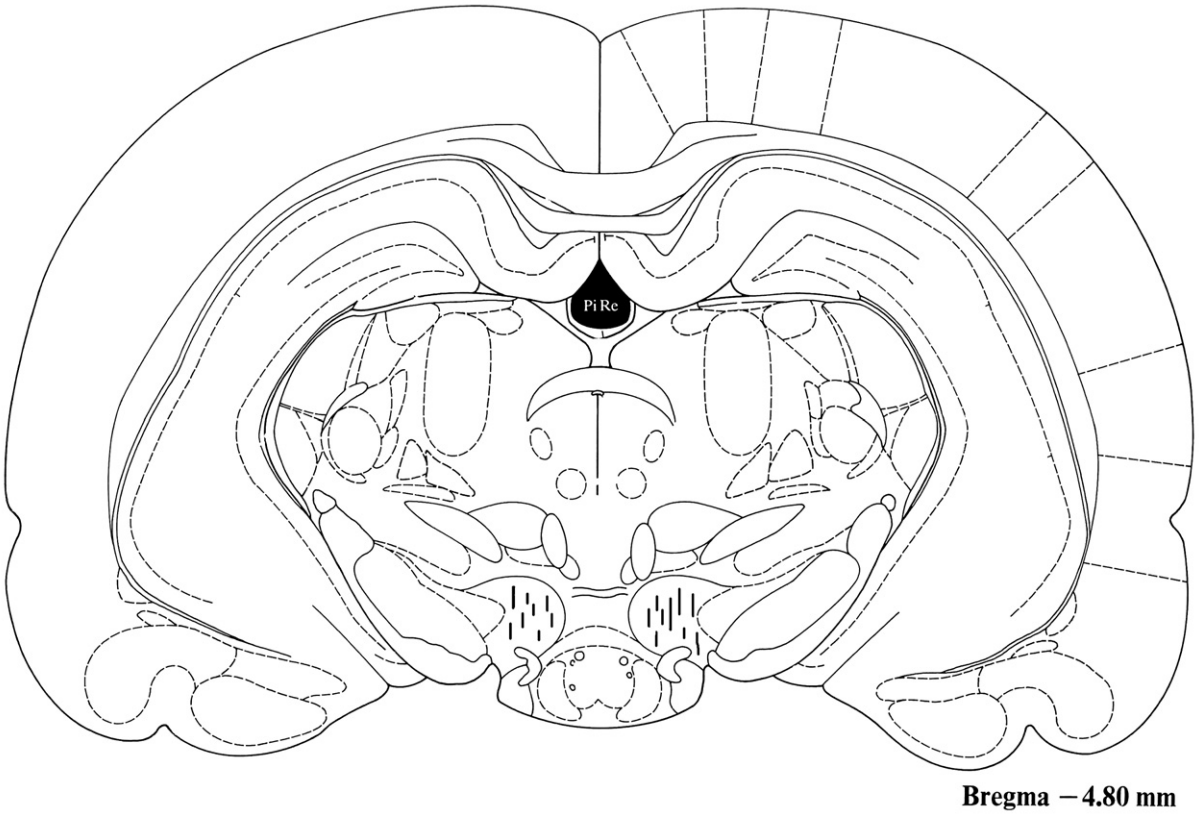

Fig. 1. Location of cannula tips in the ventral tegmental area of animals used in the dose-response studies and experiments involving GABAergic agents. Symbols (|) indicate where the cannula tips are placed.

apparatus. The mean time that each rat spent in each compartment during a 10 min period was determined as the preference criteria. The animals (6–8/group) received an intra-VTA injection of baclofen, CGP35348 or saline on the test day when the effects of the GABAergic agents on the expression of morphine-induced CPP were investigated. However, when the effects of the GABAergic drugs on the acquisition of morphine CPP were examined, no injection was given in the post conditioning phase as described in the results section.

2.9. Animals activity

During testing phase, total crossings between the compartments were calculated as index of animal's activity.

2.10. Statistical analysis

The conditioning scores represent the time spent in the drug-paired place minus the time spent in the saline-paired place, given as the mean±S.E.M. for 6–8 animals. Animal's activity was calculated as described in the method section and expressed as mean±S.E.M. of the same animals. In order to test the hypothesis, one- or two-way analysis of variance (ANOVA) followed by the Tukey test was performed to assess specific group comparisons. Differences with $P<0.05$ were considered statistically significant.

3. Results

3.1. Morphine dose-response on CPP paradigm

The effects of morphine have been shown in Fig. 2A. Injection of different doses of morphine sulfate (2.5, 5 and 7.5 mg/kg, s.c.) to rats caused a significant increase in time spent in the drug-paired compartment compared to that spent in the saline-paired compartment [$F(6, 41)=10.125$, $P<0.0001$]. Subcutaneous injection of saline to the animals (the saline control group) in the conditioning compartments did not produce any preference or aversion for either place. Based on these data, the doses of 0.25, 0.5 and 1 mg/kg of morphine were selected as ineffective doses for the rest of the experiments. However, this part of the experiments indicated that the apparatus and the paradigm are sufficient.

3.2. Morphine dose-response on CPP paradigm in morphine-sensitized rats

The effects of morphine on CPP in morphine-sensitized rats have been shown in Fig. 2B. The animals were sensitized to morphine (5 mg/kg, s.c., once daily for three consecutive days, followed by five days rest) and then were conditioned with ineffective doses of morphine (0.25, 0.5 and 1 mg/kg, s.c.). Results showed that injection of ineffective doses of morphine induced a predominant CPP in the morphine-sensitized animals (one-way ANOVA, [$F(3, 24)=8.32$, $P<0.0001$]. However, the response was not dose-dependent and the dose of 0.5 mg/kg was chosen in the subsequent experiments.

3.3. Effects of intra-VTA injections of $GABA_B$ receptor agents on the expression of morphine-induced CPP in morphine-sensitized rats

The animals were sensitized to morphine (5 mg/kg, s.c., once daily for three consecutive days, followed by five days rest) and then were conditioned with an ineffective dose of morphine (0.5 mg/kg, s.c.). Baclofen (1.5, 6 and 12 μg/rat) was injected into the VTA on the test day, 5 min before the test. Analysis by two-way ANOVA showed that baclofen in doses of 1.5 and 12 μg/rat reduced the expression of morphine CPP whereas the drug in a dose of 6 μg/rat increased it [within-group comparison: baclofen effect: $F(9, 68)=3.45$, $P<0.001$, morphine effect: $F(1, 69)=5.21$, $P<0.001$, baclofen×morphine: $F(9, 68)=5.74$, $P<0.0001$] (Fig. 3A). However, in order to measure the animals' activity a similar analysis was conducted and the results revealed that baclofen

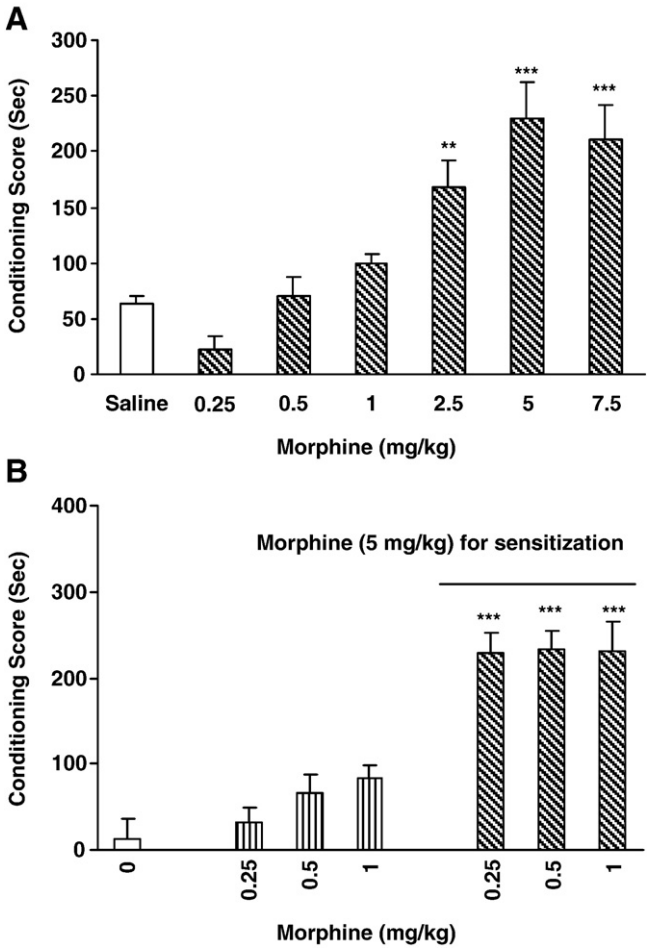

Fig. 2. (A) Conditioned place preference induced by morphine. Morphine-naive animals received different doses of morphine (0.25–7.5 mg/kg, s.c.). Each point shows the mean ± S.E.M. for 7–8 rats, $^{**}P<0.01$ and $^{***}P<0.001$ different from the respective saline control group. (B) Effects of repeated concomitant morphine administration on the animal responsivity to low doses of morphine (i.e. sensitization). Animals received three morphine (5 mg/kg, s.c.) injections in three consecutive days followed by 5 days of resting. After this period, these animals were conditioned to ineffective doses of morphine (0.25, 0.5 and 1 mg/kg, s.c.). As indicated in the figure, animals that have previous history of morphine, showed prominent response to low doses of morphine than those that do not have a previous history of morphine. Each point shows the mean ± S.E.M. for 7–8 rats, $^{***}P<0.001$ different from the saline control group.

administration increased total compartments crossing during the test in all doses [within-group comparison: baclofen effect: $F(9, 68)=2.1$, $P<0.05$, morphine effect: $F(1, 69)=0.941$, $P>0.05$, baclofen × morphine: $F(9, 68)=2.18$, $P<0.05$] (Fig. 4A).

Injection of CGP35348 also reduced the expression of morphine place preference in doses of 1.5 and 12 μg/rat but increased the morphine action in a dose of 6 μg/rat [Two-way ANOVA; within-group comparison: CGP35348 effect: $F(9, 68)=3.45$, $P<0.001$, morphine effect: $F(1, 69)=5.21$, $P<0.001$, CGP5348 × morphine: $F(9, 68)=5.74$, $P<0.0001$] (Fig. 3B).

Again, the animals' activities were increased in all doses [Two-way ANOVA; within-group comparison: CGP35348 effect: $F(9, 68)=1.8$, $P<0.05$, morphine effect: $F(1, 69)=0.941$, $P>0.05$, CGP5348 × morphine: $F(9, 68)=4.30$, $P<0.01$] (Fig. 4B).

3.4. Effects of baclofen and CGP35348 on the acquisition of morphine CPP in morphine-sensitized rats

To determine the effects of $GABA_B$ receptor drugs on the acquisition of morphine-induced CPP in morphine-sensitized rats, baclofen (a $GABA_B$ receptor agonist) and CGP35348 (a $GABA_B$ receptor antagonist) were administered 5 min before the morphine (0.5 mg/kg) injection on the conditioning phase of the experiments. Administration of baclofen (1.5, 6 and 12 μg/rat) into the VTA, significantly decreased the acquisition of morphine CPP [Two-way ANOVA; within-group comparison: baclofen effect: $F(9, 70)=4.12$, $P<0.001$, morphine effect: $F(1, 70)=7.9$, $P<0.001$, baclofen × morphine: $F(9, 70)=4.31$, $P<0.001$] (Table 1A). However, measurement of the animals' activities revealed that baclofen administration decreases total compartments crossing during the test in all doses [Two-way ANOVA; within-group comparison: baclofen effect: $F(9, 70)=0.94$, $P>0.05$, morphine effect: $F(1, 70)=1.02$, $P>0.05$, baclofen × morphine: $F(9, 70)=4.12$, $P<0.01$] (Table 2A). Similarly, intra-VTA

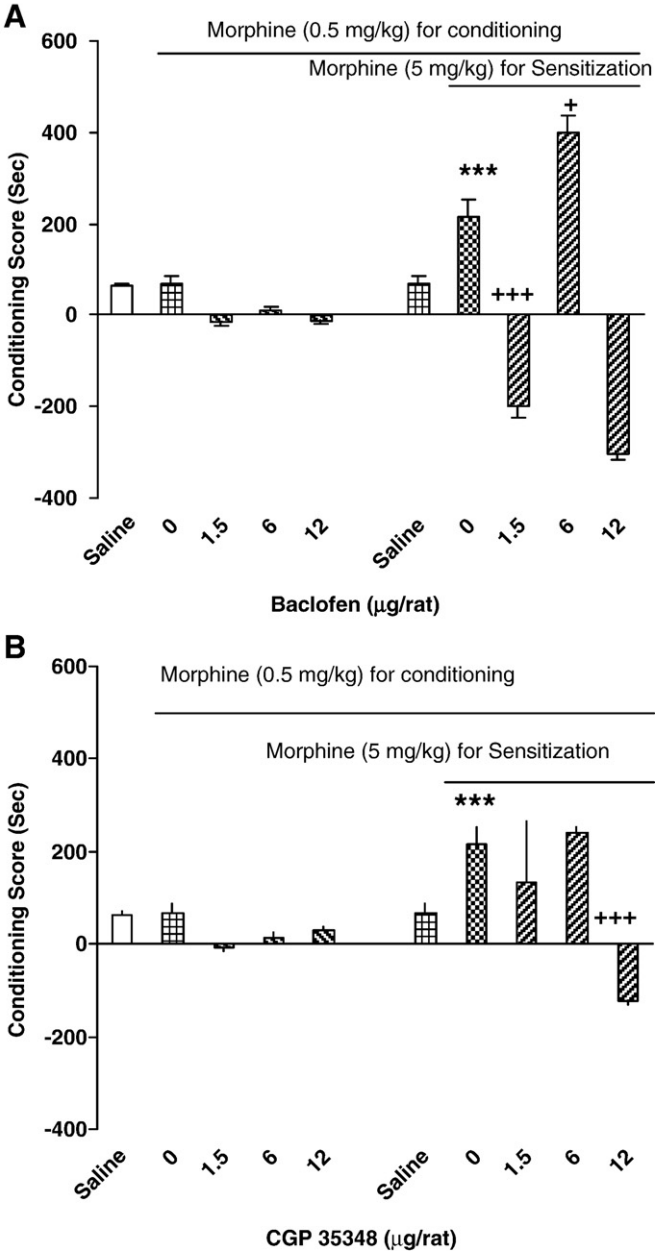

Fig. 3. (A) Effects of different doses of baclofen on the expression of morphine-induced sensitization. Animals received baclofen (1.5, 6 and 12 μg/rat) 5 min before the beginning of the test in the 8th day of experiments. Each point shows the mean ± S.E.M. for 7–8 rats. $^{***}P<0.001$, $^{+}P<0.05$ and $^{+++}P<0.001$ different from the respective control groups. (B) Effects of the intra-VTA administration of CGP35348 on the expression of morphine-induced sensitization. Animals received CGP35348 (1.5, 6 and 12 μg/rat) 5 min before the test. Each point shows the mean ± S.E.M. for 6–9 rats, $^{***}P<0.001$ and $^{+++}P<0.001$ from the respective control groups.

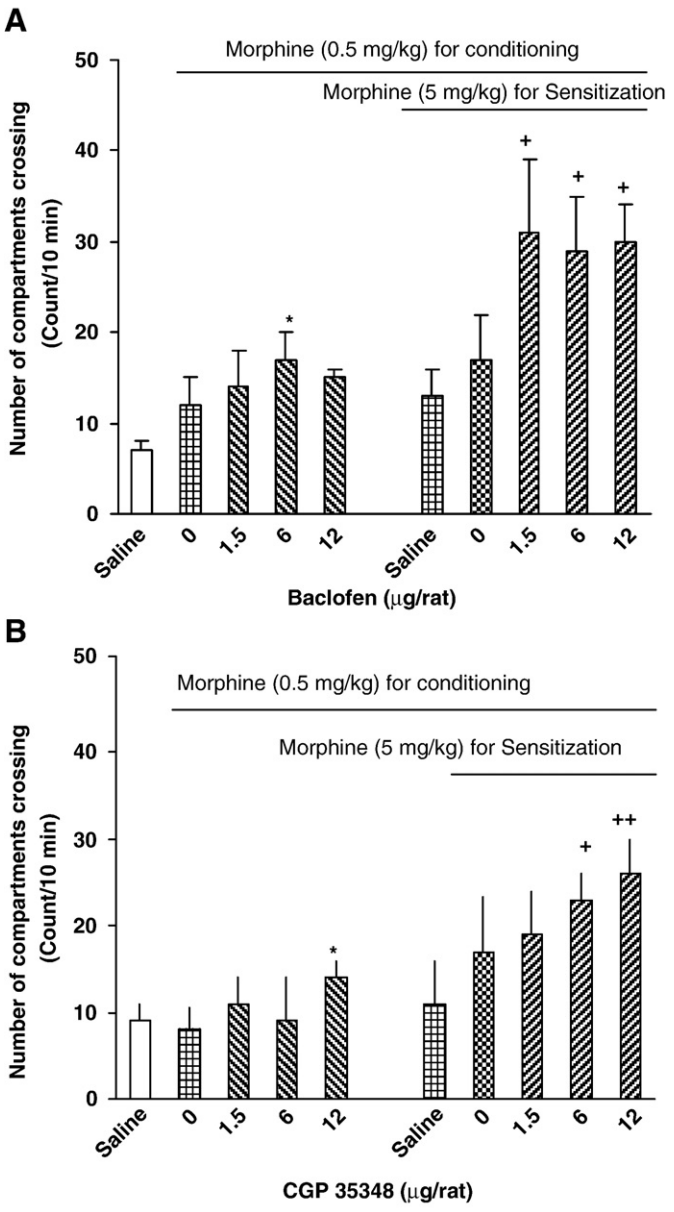

Fig. 4. (A) Changes of animals' activity as measured during the test. Animals received baclofen (1.5, 6 and 12 μg/rat) 5 min before the beginning of the test in the 8th day of experiments. Each point shows the mean±S.E.M. for 7–8 rats. *$P<0.05$ and+$P<0.05$ different from the respective control groups. (B) Effects of the intra-VTA administration of CGP35348 on the animals' activity during the test of the expression of morphine-induced sensitization. Animals received CGP35348 (1.5, 6 and 12 μg/rat) 5 min before the test. Each point shows the mean±S.E.M. for 6–9 rats, *$P<0.05$, +$P<0.05$ and ++$P<0.01$ from the respective control groups.

administration of CGP35348 (1.5, 6 and 12 μg/rat) decreased the acquisition of morphine CPP in morphine-sensitized rats [within-group comparison: CGP35348 effect: $F(9, 68)=3.45$, $P<0.001$, morphine effect: $F(1, 73)=7.4$, $P<0.001$, CGP35348×morphine: $F(9, 72)=6.17$, $P<0.001$] (Table 1B). However, the responses of both drugs were not dose-dependent. Animals' activities were decreased in all doses of CGP35348 [Two-way ANOVA; within-group comparison: CGP35348 effect: $F(9, 72)=1.02$, $P>0.05$, morphine effect: $F(1, 72)=1.07$, $P>0.05$, CGP5348×morphine: $F(9, 72)=9.12$, $P<0.0001$] (Table 2B).

4. Discussion

Despite several investigations regarding the role of $GABA_B$ receptors on morphine positive reinforcing properties (Sahraei et al., 2005; Kaplan et al., 2003; Tsuji et al., 1996; for rev see: Tzschentke, 1998), and morphine-induced behavioral sensitization (Leite-Morris et al., 2002; 2004; Woo et al., 2001), there is limited information on the role of $GABA_B$ receptors within the VTA on the morphine CPP in morphine-sensitized animals.

An important finding of the present study is that stimulation of $GABA_B$ receptors in the VTA resulted in a biphasic effect on the expression but not acquisition of morphine CPP in morphine-sensitized rats. The results of $GABA_B$ receptor inhibition showed a reduction of both expression and acquisition of morphine CPP. The effects of baclofen (a $GABA_B$ receptor agonist) on the expression but not acquisition of morphine CPP were dose-dependent while for CGP35348 (a $GABA_B$ receptor antagonist) were dose-independent on both. In addition, administration of baclofen and CGP35348 lead to an increase in the number of compartment entering. These results indicate that $GABA_B$ receptors may be involved in morphine sensitization as shown by the CPP paradigm.

As previous studies showed, our data indicate that low doses of repeated morphine injections produce sensitization in rats comparing with normal rats (Carlezon et al., 1997; Shippenberg et al., 1996) and mice (Sahraei et al., 2006; Biala and Weglinska, 2004). Our results showed that morphine sensitization was not dose-dependent over the dose range. Based on the results of a dose of 2.5 mg/kg of morphine-induced CPP in morphine naive animals, we suggest that a wider range of doses of morphine should not be examined in future studies.

The exact cellular and molecular mechanisms have not been established. Some data have shown that dopamine; glutamate and μ-opioid receptor mechanisms in the VTA (Spanagel, 1995; Kalivas, 1995; Vanderschuren et al., 1999), $GABA_B$ receptors in the VTA (Leite-Morris

Table 1
Effects of intra-VTA injections of baclofen and intra-VTA administration of CGP35348 on the acquisition of morphine-induced sensitization

A: Effects of intra-VTA injections of baclofen on the acquisition of morphine-induced sensitization	
Baclofen (μg/rat)+saline	Conditioning score (mean±S.E.M.)
Saline	63±6.62
0	69±17
1.5	29.14±71
6	74.5±43
12	−34.28±45
Baclofen (μg/rat)+morphine (5 mg/kg) for sensitization	Conditioning score (mean±S.E.M.)
Saline	19±17
0	217±32.1***
1.5	−14.25±24+++
6	−53.12±18.6+++
12	−70.14±14.1+++
B: Effects of the intra-VTA administration of CGP35348 on the acquisition of morphine-induced sensitization	
CGP35348 (μg/rat)+saline	Conditioning score (mean±S.E.M.)
Saline	63±6.62
0	69±17
1.5	13.5.±41.2
6	−15±75
12	41±4.1
CGP35348 (μg/rat)+morphine (5 mg/kg) for sensitization	Conditioning score (mean±S.E.M.)
Saline	69±17
0	217±37.6***
1.5	30.42±33+++
6	11.42±70+++
12	32.5±76+++

Animals received baclofen (1.5, 6 and 12 μg/rat) 5 min before morphine injection during the induction of sensitization. Each point shows the mean±S.E.M. for 6–8 rats, ***$P<0.001$, +++$P<0.001$ different from the respective control groups.
Animals received CGP35348 (1.5, 6 and 12 μg/rat) 5 min before morphine injections on the sensitization phase. Each point shows the mean±S.E.M. for 6–9 rats, ***$P<0.001$, +$P<0.05$, ++$P<0.01$ from the respective control groups.

Table 2
Effects of intra-VTA injections of baclofen and CGP35348 on the total compartment crossing during the test of the acquisition of morphine-induced sensitization

A: Effects of intra-VTA injections of baclofen on the total compartment crossing during the test of the acquisition of morphine-induced sensitization	
Baclofen (μg/rat)+saline	Number of compartments crossing (count/10 min), (mean±S.E.M.)
Saline	7.9±1.01
0	9±1.3
1.5	9.12±1.48
6	6.18±2.03
12	7.86±2.1
Baclofen (μg/rat)+morphine (5 mg/kg) for sensitization	Number of entering to the compartments (count/10 min) (mean±S.E.M.)
Saline	7.15±1.3
0	16.34±2.14**
1.5	5.12±1.32++
6	6.45±2.33++
12	9.3±2.6++

B: Effects of intra-VTA injections of CGP35348 on the total compartment crossing during the test of the acquisition of morphine-induced sensitization	
CGP35348 (μg/rat)+saline	Number of compartments crossing (count/10 min) (mean±S.E.M.)
Saline	8.1±0.89
0	6.35±1.04
1.5	7.14±2.1
6	7.63±1.4
12	9.2±3.1
CGP35348 (μg/rat)+morphine (5 mg/kg) for sensitization	Number of entering to the compartments (count/10 min) (mean±S.E.M.)
Saline	6.9±1.04
0	17.2±2.43***
1.5	6.9±2.31++
6	8.46±2.51++
12	9.6±4.35++

Animals received baclofen (1.5, 6 and 12 μg/rat) 5 min before morphine injection during the induction of sensitization. Each point shows the mean±S.E.M. for 6–8 rats, $^{**}P<0.01$, $^{++}P<0.01$ different from the respective control groups.
Animals received CGP35348 (1.5, 6 and 12 μg/rat) 5 min before morphine injections on the sensitization phase. Each point shows the mean±S.E.M. for 6–9 rats, $^{***}P<0.001$ and $^{++}P<0.01$ from the respective control groups.

et al., 2004; 2002; Woo et al., 2001) have an important role in morphine sensitization. In addition, coupling of the $GABA_B$ receptors to their G-protein effectors within the VTA and nucleus accumbens was reduced during drug sensitization (Narita et al., 2003). Several data have also indicated similar changes for nicotine (Amantea et al., 2004), metamphetamine (Zhang et al., 2000) and cocaine (Xi et al., 2003) sensitization in rat nucleus accumbens, which may be implicated in the VTA as well.

From an anatomical view, in addition to the VTA, nucleus accumbens and other limbic regions of the brain are also involved in morphine sensitization (For rev see: Robinson &and Berridge, 2003).

Findings from the second part of our experiments showed that intra-VTA injections of the $GABA_B$ receptor agonist, baclofen, produced a biphasic effect on the expression of morphine-induced CPP in morphine-sensitized rats while Sahraei et al. (2005) found similar results in morphine-naive rats. The difference could be referred to the fact that the animals in the present study were sensitized to morphine prior to the conditioning or due to sex differences. Other investigators have shown that baclofen could inhibit morphine-induced behavioral sensitization (Leite-Morris et al., 2004), morphine- (Kaplan et al., 2003) and amphetamine (Li et al., 2001)-induced place preference, heroin (Xi and Stein, 1999), D-amphetamine (Brebner et al., 2005), cocaine (Shoaib et al., 1998) and nicotine self-administration (Corrigall et al., 2000) in rats. The drug also reduces cocaine- and heroin-induced seeking behavior in rats (Di Ciano and Everitt, 2003). It seems that activation of $GABA_B$ receptor subtypes could inhibit brain stimulation in rats (Macey et al., 2001) and has an important role in reducing the activity of drugs of abuse.

However, the puzzling data indicated that baclofen enhanced morphine CPP expression in dose 6 μg but it induced potent aversion in doses 1.5 and 12 μg. For resolving these startling observations, we recorded the number of compartment entering by each animal as an index of their activities during CPP testing. Data indicated — in all doses — an increase in number of compartment entering by the animals which may be due to an increase of animals' exploratory behavior. Some data indicated that the ability of baclofen in the reduction of behavioral sensitization was induced by morphine in male C57BL/6 (Leite-Morris et al., 2004) and ddY (Narita et al., 2003) mice. At first glance, it seems that these data are in contradiction with our observation but the problem may be better explained if we have a look at the first part of the results which indicated baclofen administration to the saline control group increases the number of compartment entering by the animals. As Leite-Morris et al. (2004) reported, a part of this increment could be related to baclofen ability in increasing animals' activities when administered repeatedly. Another explanation could be that baclofen administration may cause confusion in the animals, in which they can not find the right compartment and therefore they spend their time regardless of their previous experience on morphine receipt. This theory could be allied by our results that indicated that administration of CGP35348 also increased the number of compartment entering by the morphine sensitized and saline control animals as well. In contrast to the baclofen, however, CGP35348 administration only produces place aversion in dose 12 μg of the drug. However, to date no data exist on either rejection or acceptance of this hypothesis.

This result is well in agreement with the previous study that showed a reduction of morphine (Sahraei et al., 2005), a reduction of brain stimulation reward in rats (Macey et al., 2001), a reduction of morphine-induced motor sensitization in mice (Leite-Morris et al., 2004), and in reducing the acquisition (Tsuji et al., 1996) or expression (Sahraei et al., 2005). Based on these findings, it may appear that there are several $GABA_B$ receptor subtypes in the VTA.

The role of $GABA_B$ receptor subtypes on opioid reward is well recognized (For review see: Xi and Stein, 2002). Treatment with baclofen reduces the acquisition of morphine CPP in mice that is not biphasic (Kaplan et al., 2003). However, the effects of baclofen on the acquisition, but not the expression of morphine CPP were not biphasic and only a reduction of the acquisition of morphine CPP was observed (Tsuji et al., 1996; Sahraei et al., 2005). In a study by Panagis and Kastellakis (2002) it was found that intra VTA injection of baclofen was effective in increasing the threshold of ventral pallidum electrical self-stimulation in the rat. This effect was also not biphasic, which may be due to the different mechanisms involved in the research methods (McBride et al., 1999). The biphasic result obtained in the present study may further suggest that there is more than one type of $GABA_B$ receptor within the VTA. $GABA_B$ receptors are further subdivided into $GABA_{B(1a)}$, $GABA_{B(1b)}$ and $GABA_{B(2)}$ receptors (Bowery et al., 2002; Charles et al., 2003; Bettler et al., 2004). Radioligand binding and in situ hybridization studies suggested different locations for $GABA_B$ receptors. That is, $GABA_{B(1a)}$ receptors are located primarily pre-synaptically, $GABA_{B(1b)}$ receptors are located primarily post-synaptically, while $GABA_{B(2)}$ receptors are located at both pre and post synaptic sites (Bowery et al., 2002; Charles et al., 2003; Bettler et al., 2004). Pre-synaptic $GABA_B$ receptor activity leads to reduce neurotransmitter release while $GABA_B$ post-synaptic receptor activity leads to cell hyperpolarization and reduces cell activity. Based on our results, it seems that baclofen, in low doses, acts on post-synaptic $GABA_B$ receptors which are located on the cell body of dopaminergic neurons within the VTA in which their activation causes a decrease in dopamine neurons' activity and thereby, dopamine release in the targets of these neurons which in fact enhances drug seeking behavior and subsequently increases the time spent in the drug-paired side by the animals. Baclofen at higher doses may activate pre-synaptic $GABA_B$ receptors and decrease the GABAergic inhibitory interneuronal tone

which in turn, dis-inhibits dopaminergic neurons in the VTA and cause an increase in dopamine release in the target nuclei of these neurons, including the nucleus accumbens. This may be explained by a reduction of the time spent in the drug-paired side. So, it may be concluded that the effect of baclofen on the time spent in the drug-paired side may be due to an increase and/or decrease in dopamine levels in the nucleus accumbens.

Present results are in agreement with our previous findings (Sahraei et al., 2005), although the findings related to the doses of CGP35348 were different. The differences may be due to the fact that animals in the present study were sensitized to morphine prior to the conditioning. Another possible explanation is sex differences.

It may be anticipated that opposite effects to baclofen or no response would be obtained from CGP35348. However, since baclofen produced a biphasic response, the opposite concept could not be correct for the CGP35348 response. In this regard one may conclude that at least in some doses, baclofen and CGP35348 activate or inhibit different $GABA_B$ receptor subtypes respectively but the results are in the same direction. Since the administration of CGP35348 decreased the expression of morphine CPP, it could be suggested that the drug may inhibit the $GABA_B$ post-synaptic receptors and induce dopamine release in the nucleus accumbens (Spanagel and Weiss, 1999) the mechanism that is opposite to the function of baclofen at low doses.

In the next part of the experiments, intra-VTA administration of baclofen or CGP35348 reduced the acquisition of morphine CPP in morphine-sensitized rats significantly. The response was similar for both $GABA_B$ receptor agonist and antagonist. Our data supported previous study (Macey et al., 2001; Tsuji et al., 1996). Baclofen also reduced cocaine self-administration (Shoaib et al., 1998) and heroin self-administration (Xi and Stein, 1999) in rats. Surprisingly, all of these responses were not biphasic. The similarity between our finding and previous studies revealed that baclofen activates a group of $GABA_B$ receptor subtype located in the VTA as a function in both morphine naive and morphine-sensitized rats. Activation of these receptors probably prevents the effect of morphine for stimulation of dopamine release in the nucleus accumbens, which is considered as one of the major targets of dopaminergic projections originating from the VTA (Koob and Bloom, 1988; Koob, 1992) which reduces reward. The inhibition of dopaminergic neurons with in the VTA is opposite to the effects of morphine for inhibition of GABAergic interneurons located in the VTA, which is the main effect of morphine for induction of positive reinforcement, and in part, sensitization (DiChiara, 2002).

Overall, baclofen shows a clear biphasic effect on the expression of morphine CPP, which indicates that the drug may act on different receptor subtypes that are located pre- or post-synaptically when the drug dose changes. This fact is not true for the drug in the acquisition study and moreover, the drug may act mainly at only one receptor subtype, which is probably located pre-synaptically. The fact that CGP35348 inhibits both expression and acquisition of morphine CPP in morphine-sensitized rats indicates that the drug may act on only one-receptor subtype, which is located pre-and/or post-synaptically. For this reason, the biphasic response for CGP35348 was not achieved. In addition, we did not record other animals' behavior such as sniffing, rearing and locomotion during the test which could offer additional data on interaction between morphine and $GABA_B$ receptors.

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