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Different effects of GABAergic receptors located in the ventral tegmental area on the expression of morphine-induced conditioned place preference in rat

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Abstract

In the present study, an unbiased conditioned place preference paradigm was used to study the effects of intra-ventral tegmental area injections of Gama-amino-butyric acid (GABA)-A and B ($GABA_A$ and $GABA_B$) receptor agonists and antagonists on the expression of morphine-induced conditioned place preference (CPP) in rats. Subcutaneous (s.c.) injections of morphine sulfate (5 mg/kg) induced CPP. Intra-ventral tegmental area administration of the $GABA_A$ receptor agonist, muscimol (6 μg/rat) reduced the expression of morphine-induced CPP. Muscimol (25 μg/rat) increased the expression of CPP induced by morphine. A reduction of the expression of morphine-induced CPP was observed on intra-ventral tegmental area injection of $GABA_A$ receptor antagonist bicuculline (25 μg/rat). Bicuculline (10 μg/rat) increased the expression of CPP induced by morphine. Baclofen (12 μg/rat) increased where as (19 and 25 μg/rat) reduced the expression of morphine-induced CPP. Injection of CGP38345 (10, 19, 25 and 50 μg/rat) into the ventral tegmental area significantly reduced the expression of CPP induced by morphine.

It is concluded that $GABA_A$ and $GABA_B$ receptor subtypes within the ventral tegmental area may have different effects on the expression of morphine-induced CPP.

Keywords: GABA receptor; Conditioned place preference; Morphine; Ventral tegmental area; (Rat)

1. Introduction

The role of the mesolimbic dopaminergic system in opioid reward is well known (Spanagel and Welss, 1999). Many studies have suggested that the mesolimbic dopaminergic system that projects from ventral tegmental area to the nucleus accumbens is critical for initiation of opioid reinforcement (Koob, 1992; Wise, 1998; Hyman and Malenka, 2001; Robinson and Berridge, 2003). For example, administration of μ-opioid receptor agonists into the ventral tegmental area induces conditioning place preference (CPP) (Phillips and Lepiane, 1980) and self-administration (Devine and Wise, 1994) in rats. Moreover, μ-opioid receptor knock out mice do not show place preference for an environment paired with morphine (Mattes et al., 1996).

It is well known that morphine activates ventral tegmental area dopamine neurons and enhances dopamine release in the nucleus accumbens via inhibition of Gama-amino-butyric acid (GABA) neurons (Johnson and North, 1992). Several lines of experimental evidence support this idea. First, systemic or microinjection application of morphine into the ventral

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tegmental area increased the firing rate of dopamine neurons and inhibits the firing rate of GABAergic neurons (Johnson and North, 1992; Koob, 1992). Second, micodialysis studies demonstrated an increase of nucleus accumbens dopamine release following morphine administration (Di Chiara and Imperato, 1988; Pontieri et al., 1995). In addition, anatomical evidence suggested that opiate μ-receptors in the ventral tegmental area are predominantly located on GABAergic neurons (Mansour et al., 1998), and systemic administration of morphine inhibited GABA release in the midbrain (Renno et al., 1992).

The effects of $GABA_B$ receptors on the acquisition (development) of morphine CPP (Tsuji et al., 1996; Kaplan et al., 2003) and locomotor activity (Leite-Morris et al., 2002) are well documented.

Although the role of GABA receptors within the ventral tegmental area is well defined in development of morphine reinforcement, the role of these receptors on the expression of morphine reinforcement is not so clear. In the present study, we attempt to further investigate the role of the $GABA_A$ and $GABA_B$ receptor subtypes within the ventral tegmental area on the expression of morphine-induced CPP. The CPP paradigm has been widely used as a model for studying the reinforcing effects of drugs of dependence and addiction (McBride et al., 1999; Tzschentke, 1998).

2. Materials and methods

2.1. Animals

Experiments were carried out on male Wistar rats ($n=190$ rats) weighing 200–300 g (Razi institute, Tehran, Iran). The animals were group housed (4 per cage) at a constant temperature of 22–24 °C on a 12–12 h-light/dark cycle (light period 0700–1900). Standard laboratory rat chow and water were available at all times except during experimentation. All experiments were conducted in accordance with standard ethical guidelines and approved by the local ethical committee (The Baqiyatallah University of Medical Committee on the Use and Care of Animals, 79/123, Dec. 21, 2000). Each animal used only once.

2.2. Apparatus

The place conditioning apparatus, which was constructed in our lab, was similar to that described by De Fonseca et al. (1995) with minor modification. It consists of three interconnected rectangular boxes of 30×30×45 cm, situated at 120° angles from each other. In the middle there is a triangular area with a smooth glass floor, from which any of the three compartments was accessible. Each compartment was equipped with a set of different sensory stimuli that make them unique. Compartment A was equipped with a sand floor, plain walls and a small container with a drop of 10% acetic acid. Compartment B contained a soft plastic floor and walls painted with white dot circles and a small container with a drop of anise extract. Compartment C had a cork floor, alternating white strips (5 cm wide) painted on the walls and no odor (water). The apparatus was placed in an isolated dimly illuminated room (100 lx).

2.3. Behavioral testing

Experiments were performed between 09:00 and 18:00 h. Each place conditioning experiment consisted of a 6-day schedule, with three phases: preconditioning, conditioning and testing.

2.3.1. Preconditioning phase

This phase lasts for two days. On day 1 of the preconditioning phase, animals were placed in the middle of the apparatus and they were allowed to freely explore the three compartments for 45 min in order to explorer the entire apparatus. On day 2 of the preconditioning phase each animal was placed in the middle of the apparatus and allowed to move freely to the three compartments for 10 min, this section was conducted for determining the preference of the animals. During this period the time spent in each compartment was computed and those animals exhibiting unconditioned aversion (<10% of the session) or preferences (>60% of the session) for any compartment were discarded for conditioning sessions. The two compartments, which exhibited the most similar time of preference, were chosen for each animal for the conditioning sessions. In one of these compartments, randomly chosen, the animals received drug and in the other saline was administered. This selection allowed us to avoid interference of the natural preference of the animals with the conditioning drugs.

2.3.2. Conditioning phase

This phase consisted of a 3-day schedule of double conditioning sessions. The first day involved a morning session (9:00–12:00 h) in which animals received a single dose of morphine and were immediately placed in one of the compartments chosen as described above for 30 min. This compartment had been isolated from the others using removable panels. In the evening session (16:00–18:00) the animals received a single injection of saline and were placed for 30 min in the other compartment chosen for conditioning experiments. On the second day of conditioning the animals received the saline injection in the morning session and morphine administration in the evening session. The third day of conditioning had the same schedule as the first one.

2.3.3. Testing phase

On the 6th day of the schedule, which in the pilot study it was shown that morphine preference had developed, the animals were allowed again to freely explore the three compartments for 10 min, exactly as in the preconditioning phase. The time spent in each compartment was computed. We defined the change in preference 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 preconditioning session. This variable was chosen as an index of drug-induced place preference, as previously described (Hand et al., 1989). In order

to minimize the effects of time, this phase of the experiments was done between 10:00–16:00 h.

To examine the effects of GABAergic drugs on the expression of morphine-induced CPP, animals which had been conditioned to morphine in the conditioning phase of the experiments, received different doses of GABAergic receptor agonists or antagonists 20 min before testing, on the test day (day 6).

2.3.4. Locomotion

Locomotor activity was measured by an open field method, for 10 min on the test day, during CPP recording. The doses of drugs, which were used in these experiments, did not alter locomotor activity.

2.4. Surgical and infusion procedures

Rats were anesthetized with sodium pentobarbital (45 mg/kg, i.p.) and two stainless steel cannulas (23-gauge) were placed stereotaxically (Stolting instruments, USA) into the ventral tegmental area (bilaterally). Stereotaxic coordinates according to Paxinos and Watson (1987) were: incisor bar (−3.3 mm), 4.8 mm posterior to the bregma, ±0.9 mm lateral to the sugittal suture and 8.5 mm from top of the skull. Cannula were secured to anchor jewelers' screws with dental acrylic. After completing the surgery, dummy inner cannula was inserted into the guide cannula, and left in place until injections were made. The length of dummy cannula matched that of the guide cannula. Animals were allowed one week to recover from surgery and anesthesia. For drug infusion, the animals were gently restrained by hand; the stylets were removed from the guide cannulas and replaced by 30-gauge (0.3 mm duter diameter) injection needles (1.5 mm below the tip of the guide cannula). The solutions were slowly administered in a total volume of 0.5 μl/rat (0.25 μl in each side) over a period of 60 s. Injection needles were left in place for an additional 60 s to facilitate diffusion of the drugs.

2.5. Experimental design

In a pilot study, the effects of subcutaneous (s.c.) administration of various doses of morphine (0.5, 1, 2.5, 5 and 10 mg/kg) on the conditioned place preference behavior were investigated.

To examine the effects of GABAergic drugs on the expression of morphine-induced CPP, those animals which conditioned to morphine in the conditioning phase of experiments, received different doses of GABAergic receptor agonists or antagonists 20 min before testing, on the test day (day 6 of the experiments). Since the GABAergic drugs were used acutely on the test day, it was not necessary to examine their effects on CPP paradigm behavior.

2.6. Drugs

The following drugs were used in these experiments: morphine sulfate (TEMAD, Iran), muscimol and (+)bicuculline (Sigma chemical Co, USA), baclofen and CGP35348 (Novartis Basel, Switzerland). The drugs were dissolved in saline except for bicuculline, which was dissolved in a small drop of acetic acid and then diluted, with saline. Morphine was injected subcutaneously in a volume of 1 ml/kg and other drugs given intra-ventral tegmental area in a volume of 0.5 μl/rat and was prepared before use. The control groups received saline or acetic acid solution.

2.7. Histology

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

2.8. Data analysis

Change in preference, representing the time that animals spent in the drug compartment in the test day minus the time that the animal spent in this compartment in the preconditioning day were calculated and were expressed as mean±S.E.M. Data were analyzed using one-way analysis of variance (ANOVA)

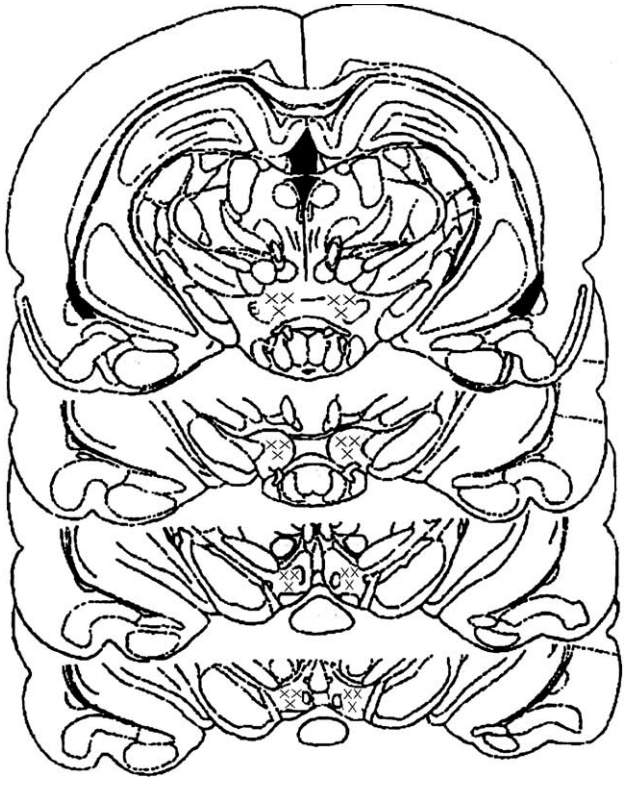

Fig. 1. Location of cannula tips in the ventral tegmental area of animals used in dose–response studies and experiments involving GABAergic agents. Symbols (X) indicate where the cannula tips are placed.

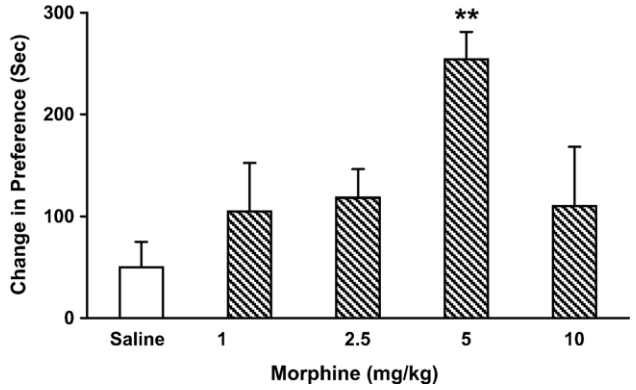

Fig. 2. Place conditioning induced by morphine. Animals received different doses of morphine (1, 2.5, 5 and 10 mg/kg, s.c.) on the conditioning days and were tested on the 6th day. Each point represents the mean±S.E.M. of 7–9 rats. **$P<0.01$ different from the saline control group.

following Student–Newman–Keuls test. $P<0.05$ was considered significant.

3. Results

3.1. Effects of morphine on CPP paradigm

The effects of morphine have been shown in Fig. 2. Injection of different doses of morphine sulfate (1, 2.5, 5 and 10 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 [$F(4,41)=6.25$, $P<0.01$]. Subcutaneous injection of saline to the animals (saline control group) in the conditioning compartments did not produce any preference or aversion for either place. Based on these data, the dose of 5.0 mg/kg of morphine was selected for the rest of the experiments. Since the test was done at different times on the test days, analyzing the data revealed that the effects of time (in morning or afternoon) on CPP were not significant.

3.2. Effects of intra-ventral tegmental area injections of GABAA receptor agents on the expression of morphine-induced CPP

To determine the effects of $GABA_A$ receptors on the expression of morphine-induced CPP in rats, muscimol (a $GABA_A$ receptor agonist) and bicuculline (a $GABA_A$ receptor antagonist) were administered before the beginning of the test on the 6th day of the experiments. Administration of muscimol (6 μg/rat) decreased the expression of morphine-induced CPP, while a dose of 25 μg/rat increased the expression of morphine-induced CPP [$F(5,41)=9.81$, $P<0.0001$] (Fig. 3A).

Bicuculline (10 μg/rat) increased the expression of morphine-induced CPP. However, bicuculline (25 μg/rat) decreased the

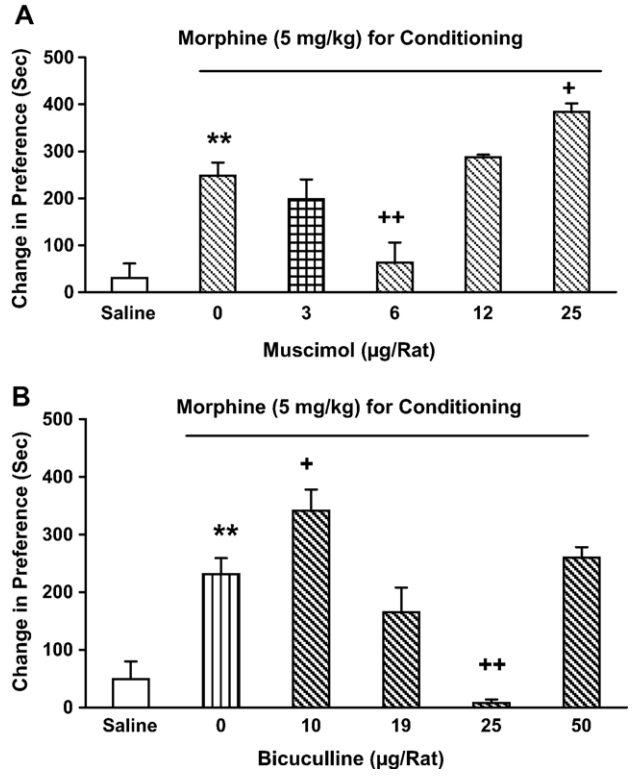

Fig. 3. Effects of intra-ventral tegmental area administration of muscimol (A) and bicuculline (B) on the expression of morphine-induced CPP. Muscimol (3, 6, 12 and 25 μg/kg) or bicuculline (10, 19, 25 and 50 μg/kg) were given on the test day, 20 min before the beginning of the test. Each point shows the mean±S.E.M. of 7–8 rats. **$P<0.01$ compared with saline control group. $^{+}P<0.05$, $^{++}P<0.01$ compared with morphine control group.

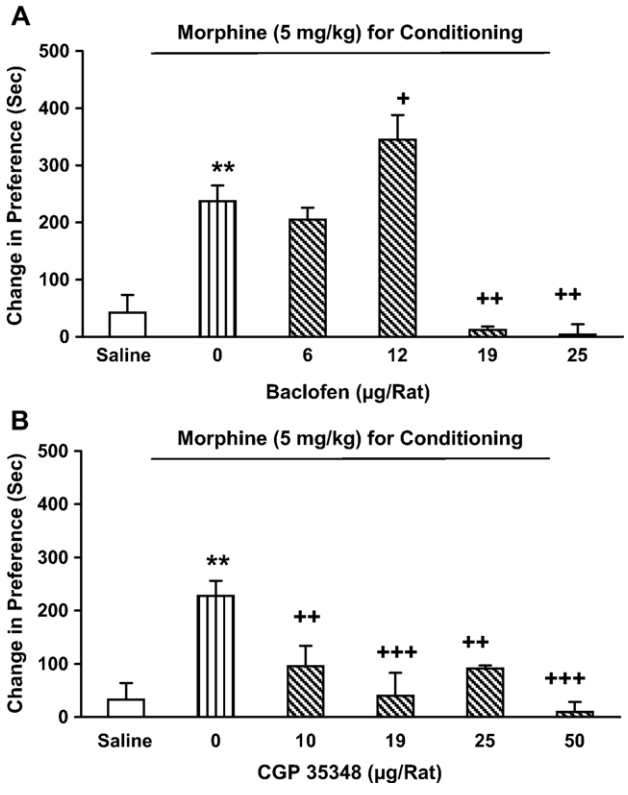

Fig. 4. Effects of intra-ventral tegmental area administration of baclofen (A) and CGP 35348 (B) on the expression of morphine-induced CPP. Baclofen (6, 12, 19 and 25 μg/kg) or CGP 35348 (10, 19, 25 and 50 μg/kg) was given on the test day, 20 min before the beginning of the test. Each point shows the mean±S.E.M. of 7–9 rats. **$P<0.01$ compared with saline control group. $^{+}P<0.05$, $^{++}P<0.01$ and $^{+++}P<0.001$ compared with morphine control group.

expression of morphine-induced CPP [$F(5,45)=8.2$, $P<0.007$]. However, post hoc analysis indicated that bicuculline at a dose of 50 μg/rat, slightly increased the expression of morphine CPP, but this increase was not statistically significant (Fig. 3B).

3.3. Effects of intra-ventral tegmental area injections of $GABA_B$ receptor agents on the expression of morphine-induced CPP

To determine the effects of $GABA_B$ receptor drugs on the expression of morphine-induced CPP in rat, baclofen (a $GABA_B$ receptor agonist) and CGP35348 (a $GABA_B$ receptor antagonist) were administered 20 min before the beginning of the test on the 6th day of experiments. Administration of baclofen (12 μg/rat) significantly increased the expression of morphine-induced CPP. On the other hand, baclofen in doses of 19 and 25 μg/rat, significantly attenuated the expression of morphine-induced CPP [$F(5,34)=27.25$, $P<0.0001$] (Fig. 4A), while, administration of CGP35348 (10, 19, 25 and 50 μg/rat), decreased the expression of morphine-induced CPP [$F(5,39)=7.47$, $P<0.0001$] (Fig. 4B).

4. Discussion

The effects of the GABAergic system in the ventral tegmental area on the expression of morphine-induced CPP are not well understood. The aim of the present study was a further evaluation of the effects of stimulation or block of $GABA_A$ and $GABA_B$ receptor subtypes in the ventral tegmental area on the expression of morphine-induced CPP. The present data indicate that administration of both $GABA_A$ and $GABA_B$ receptor agonists and antagonists into the ventral tegmental area may affect the expression of morphine-induced place conditioning. One can hypothesize from these data that both $GABA_A$ and $GABA_B$ receptor mechanism(s) in the ventral tegmental area are involved in the expression of morphine positive reinforcement as shown by the CPP paradigm, which is the important finding of this study.

In agreement with previous studies (De Fonseca et al., 1995; Tsuji et al., 1996; Kaplan et al., 2003; For review see: Tzschentke, 1998; McBride et al., 1999) our findings show that morphine injection during the conditioning phase of the experiments could induce a significant place conditioning in the rats. It is hypothesized that morphine acts on μ-opioid receptors within the ventral tegmental area, which are located on GABAergic neurons, and brings about its inhibitory influence on dopaminergic neurons (Gysling and Wang, 1983; Kalivas et al., 1990; Johnson and North, 1992; Koob, 1992; Renno et al., 1992; Wise, 1998; Spanagel and Welss, 1999; Hyman and Malenka, 2001). The consequence of this dis-inhibition phenomenon is stimulation of dopamine release in the nucleus accumbens (Pontieri et al., 1995; Di Chiara and Imperato, 1988). In this regard, it has been shown that morphine does not induce place conditioning in μ-opioid receptor gene knockout mice (Mattes et al., 1996). Similar results also were observed with heroin (Contarino et al., 2002). In addition, intra-ventral tegmental area administration of diallyl-nor-morphinium, a hydrophilic opiate receptor antagonist could inhibit heroin self-administration in rats (Britt and Wise, 1983). Moreover, microinjection of morphine in to the VTA had been shown to have reinforcing effects in rats (Phillips and Lepiane, 1980) and finally, rats readily self-administer morphine and other opiate peptides directly in to the VTA (Devine and Wise, 1994). This mechanism plays a key role in morphine-induced positive reinforcement (Wise, 1998; Robinson and Berridge, 2003).

Because previous studies did not reveal significant differences between the duration of the conditioning phases, we carried out the conditioning phase in three days (Tzschentke, 1998). Furthermore, in order to minimize the effects of the time, we decided to administer the morphine and saline doses interchangeably in either the morning or the afternoon (De Fonseca et al., 1995; Hand et al., 1989).

In the second part of the experiments, our findings showed that intra-ventral tegmental area injections of muscimol and bicuculline show biphasic and dose-dependent effects on the expression of morphine-induced CPP.

The effect of $GABA_A$ receptors on the brain reward systems is well documented. For example, intra-ventral tegmental area administration of $GABA_A$ receptor agonist, muscimol, increases locomotion (Kalivas et al., 1990), increases ventral pallidum self-stimulation (Paganis and Kastellakis, 2002), produces place conditioning (Laviolette and van der Kooy, 1991) and increases dopamine release in the nucleus accumbens (Xi and Stein, 1998). Moreover, it has been shown that the effects of direct microinjection of $GABA_A$ receptor antagonists, picrotoxin and bicuculline into the ventral tegmental area of rats (Ikemoto et al., 1997), were abolished by muscimol pre-administration. Our results are in agreement with results showing intra-ventral tegmental area muscimol reduced medial forebrain bundle (Willick and Kokkinidis, 1995) and ventral pallidum electrical self-stimulation (Paganis and Kastellakis, 2002) in the rats. The present results are also in agreement with the study reporting that intra-tegmental muscimol attenuated nicotine self-administration (Carrigall et al., 2000) and ethanol (Hodge et al., 1996) in the rats. Both pre- and post-synaptic $GABA_A$ receptors exist in the ventral tegmental area (For review see: Koob, 1992; McBride et al., 1999). Activation of pre-synaptic $GABA_A$ receptors leads to decreased GABA release from GABAergic interneurons and increased dopaminergic neurons activity subsequently. As a result, dopamine release in target sites of ventral tegmental area dopaminergic neurons, such as nucleus accumbens, increases (Kalivas et al., 1990). This effect is similar to the effect of morphine in the ventral tegmental area (Phillips and Lepiane, 1980; Johnson and North, 1992; Koob, 1992). The overall result of this function may be a decrease in the expression of morphine-induced CPP. On the other hand, activation of post-synaptic $GABA_A$ receptors may reduce the activity of dopaminergic neurons within the VTA. This function is similar to GABA activity and as a result, reduces dopamine release in target sites including nucleus accumbens. Reduced dopamine release (and perhaps dopaminergic activity) in the nucleus accumbens is considered as a sign of drug seeking behavior (Gerrits et al., 2002). This

phenomenon might be considered as the main cause of enhancement of the expression of morphine-induced CPP by muscimol and bicuculline.

In the final part of our experiments, administration of the $GABA_B$ receptor agonist, baclofen also showed biphasic effects on the expression of morphine-induced CPP. However, despite of investigations regarding the effects of baclofen on the acquisition of morphine (and other opioid) CPP (Tsuji et al., 1996; Kaplan et al., 2003), cocaine self-administration (Shoaib et al., 1998) and heroin self-administration (Xi and Stein, 1999), little is known about the effects of baclofen on the expression of morphine CPP. Our results showed that baclofen might interact with the mechanism(s) involved in the expression of morphine CPP in a dose-dependent manner. On the other hand, the $GABA_B$ receptor antagonist, CGP35348 reduced the expression of morphine CPP. The role of $GABA_B$ receptor subtypes in the ventral tegmental area 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). In addition, intra-ventral tegmental area baclofen administration reduces morphine-induced motor sensitization in mice (Leite-Morris et al., 2004). Baclofen is also effective to reduce the acquisition of morphine CPP in rats (Tsuji et al., 1996) when injected into the ventral tegmental area. In this study, the effect of baclofen is not biphasic as well and only reduction of the acquisition of morphine CPP was observed (Tsuji et al., 1996). In another study, Paganis and Kastellakis (2002) have shown that intra-ventral tegmental area injection of baclofen is effective to increase the threshold of ventral pallidum electrical self-stimulation in the rat. This effect is also not biphasic. The possible explanation for the controversy of the results obtained from the present study and other studies is that there are different mechanisms responsible for the acquisition and expression of morphine-induced CPP (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; Bettler et al., 2004). Radioligand binding and in situ hybridization studies suggest that $GABA_{B(1a)}$ 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; Bettler et al., 2004). Pre-synaptic $GABA_B$ receptor activity leads to reduced neurotransmitter release while $GABA_B$ post-synaptic receptor activity leads to cell hyperpolarization and reduced cell activity as a result. Based on our results, it seems that baclofen in low doses acts on post-synaptic $GABA_B$ receptors which locate on the cell body of dopaminergic neurons within the VTA and causes a decrease in dopamine release and subsequently increases the time spent in the drug-paired side by animals. Baclofen at higher doses, may activate pre-synaptic $GABA_B$ receptors and decrease the GABAergic inhibitory interneuron tone which in turn, dis-inhibits dopaminergic neurons in the VTA and causes an increase in dopamine release in the target nuclei of these neurons including nucleus accumbens. This may be explained by a reduction of the time spent in the drug-paired side. It is well known that during drug seeking behavior, the concentration of dopamine is reduced in the NAc (Gerrits et al., 2002). 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.

Administration of CGP35348 ($GABA_B$ receptor antagonist) reduces the expression of morphine CPP. The effect was not biphasic. 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 concept of opposite could not be correct for 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 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 (Gerrits et al., 2002), the mechanism that is opposite to the function of baclofen at low doses. In agreement with our findings, Macey et al. have shown that both $GABA_B$ receptor agonists and antagonists decreased brain stimulation reward in the rat. The authors presented similar suggestion for the controversy observed by the $GABA_B$ receptors agonist and antagonist (Macey et al., 2001).

Another possible mechanism which may explain the effects were observed from baclofen and CGP35348 is that it has been shown that administration of baclofen and CGP35348 induced the release of neurotransmitters such as glutamate and GABA via inhibition of Ca++ or activation of K+ channels in several regions of the central nervous system (For review see: Bowery et al., 2002; Bettler et al., 2004). Since the VTA has enriched glutamate inputs (for review see: Kalivas, 1993), it could be concluded that both drugs may interact with morphine effects by activation of the release of glutamate and/or GABA in the VTA. However, the exact mechanism(s) by which baclofen and CGP35348 inhibit the expression of morphine-induced CPP is not clear and need further studies.

Overall, the GABAergic drugs used in the present study except CGP35348 exhibit a biphasic effects on the expression of morphine-induced CPP. These findings show that muscimol, bicuculline and baclofen may act on the different receptor subtypes that are located pre- or post-synaptically when the dose of drug is changed. This fact is not true for CGP35348 and this drug may act mainly at only on one receptor subtype in the dosage range used and for this reason, biphasic activity did not occur for CGP35348.

In conclusion, our results support a role for ventral tegmental area $GABA_A$ and $GABA_B$ receptors in the expression of morphine-induced CPP.

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