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ARTICLE

Blockade of prelimbic glutamate receptor reduces the reinforcing effect of morphine

Fateme Aboutalebi, Hojjatallah Alaei, Shahrbanoo Oryan, and Maryam Radahmadi

Abstract: The prelimbic cortex (PrL) as a part of the medial prefrontal cortex (mPFC) plays a crucial role in drug addiction. Previous studies have shown that glutamatergic transmission through the NMDA and AMPA receptors plays an important role in morphine rewarding properties. In this study, we evaluated the effect of glutamate receptors blockade within the PrL on morphine self-administration. Male Wistar rats were randomly selected and divided into 7 groups. Trained rats were placed in self-administration apparatus, where they pressed an active lever for receiving morphine (5 mg/mL) in test groups and saline in saline group during 11 consecutive days for 2 h per session. The effects of intra-prelimbic AMPA receptor antagonist (CNQX; 0.5 and 2.5 μg/0.5 μL) and the NMDA antagonist (AP5; 0.1 and 1 μg/0.5 μL) on self-administration were tested. Our results demonstrated that intra-prelimbic injection of different doses of CNQX and AP5, and co-administration of these 2 drugs before self-administration significantly decreased active lever pressing compared with morphine group ($p < 0.001$). Also, the number of self-infusion significantly decreased in test groups compared with morphine group ($p < 0.001$). These findings suggest that a reduction in PrL glutamatergic output can modulate morphine reinforcement.

Key words: morphine, prelimbic cortex, glutamate receptors, reinforcing effect.

Résumé : Le cortex prélimbique (PrL) en tant que secteur du cortex préfrontal médian (CPFm) joue un rôle central dans la dépendance aux drogues. Des études antérieures ont montré que la transmission glutamatergique par l'intermédiaire des récepteurs NMDA et AMPA joue un rôle important dans les propriétés de récompense de la morphine. Dans cette étude, nous avons évalué l'effet de l'inhibition des récepteurs du glutamate dans le PrL sur l'auto-administration de morphine. Nous avons sélectionné aléatoirement des rats Wistar mâles, que nous avons répartis dans sept groupes. Nous avons placé les rats conditionnés dans un appareil d'auto-administration où ils devaient appuyer sur une manette active pour recevoir de la morphine (5 mg/mL) dans les groupes test et une solution saline dans le groupe saline au cours de séances de 2 h pendant 11 jours consécutifs. Nous avons étudié les effets d'un antagoniste des récepteurs AMPA intralimbiques (CNQX; 0,5 et 2,5 μg/0,5 μL), ainsi que d'un antagoniste des récepteurs NMDA (AP5; 0,1 et 1 μg/0,5 μL) sur l'auto-administration. Nos résultats ont montré que l'injection intralimbique de différentes doses de CNQX et d'AP5, ainsi que l'administration concomitante de ces deux médicaments avant l'auto-administration entraînaient une diminution plus marquée de l'actionnement de la manette que dans le groupe morphine ($p < 0{,}001$). Par ailleurs, le nombre d'autoperfusions diminuait nettement plus dans les groupes test que dans le groupe morphine ($p < 0{,}001$). Ces observations laissent entrevoir qu'une diminution de la production glutamatergique dans le PrL peut participer à la modulation du renforcement de l'utilisation de la morphine. [Traduit par la Rédaction]

Mots-clés : morphine, cortex prélimbique, récepteurs du glutamate, effet de renforcement.

Introduction

Drug addiction is a chronic relapsing disorder, characterized by compulsive drug seeking and periods of repeated drug use. It has been evidenced that the medial prefrontal cortex (mPFC) plays a critical role in learning and acquisition of drug self-administration (Gass and Chandler 2013), through modulation of dopamine in dopaminergic system and involvement in higher-order executive functions (for example, self-control, salience attribution, and awareness) (Goldstein and Volkow 2011). There is an important relationship between dopamine system and mPFC area that induced morphine addiction.

The mPFC consists of 4 main divisions: agranular medial, anterior cingulate, prelimbic cortex (PrL), and infralimbic cortex. The various subdivisions of the mPFC serve different and distinct functions (Kargari et al. 2012). Morphine self-administration induces alterations in neuronal circuit's organization of the PrL of the mPFC (Ballesteros-Yáñez et al. 2007). Previous studies on neurobiological substrates, underlying drug seeking and addiction, have focused on glutamate transmission (Gass and Olive 2008; Kalivas et al. 2009). Moreover, glutamatergic neuroadaptations induced by drug abuse, have revealed new promising treatment for addiction (Kalivas et al. 2009).

Animal studies have shown that ionotropic glutamate receptor antagonists have a significant role in rewarding and reinforcing effects of all drug abuse (Olive 2009). There are high expression levels of NMDA receptor within the mPFC (Bishop et al. 2010). In addition, the NMDA receptor agonists can decrease dopamine release and increase extracellular concentrations of gamma-amino butyric acid, directly within the mPFC (Bishop et al. 2010).

Lesions of the prefrontal cortex also prevent the development of sensitization in rats exposed to chronic cocaine and amphetamine (Schroeder et al. 2000). Moreover, it has been reported that chronic opiate exposure may alter the signaling of AMPA receptor

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within the mPFC (De Jaeger et al. 2013). Blocking the NMDA and AMPA receptors, specifically within the PrL, causes a strong potentiation of the rewarding effects of either systemic or intraventral tegmental area (intra-VTA), in morphine administration (Bishop et al. 2010; De Jaeger et al. 2013).

To understand the neural circuits controlling of opiate addiction, and the role of glutamate's receptors, AMPA and NMDA within the circuits, involved in morphine addiction, we used the blockade of these 2 receptors, to examine the role of PrL glutamate receptors, for the reinforcing effect of morphine, during self-administration.

Materials and methods

Animals and their housing

In this experiment, male Wistar rats weighing 270–320 g were used (Pasture Institute, Karaj, Iran). Before the surgery, the rats were housed 5 per cage, under standard conditions (temperature 22 ± 1 °C) at a 12 h dark – 12 h light cycle (lights on at 0700). They had ad libitum access to fresh tap water and food pellets, but during the training phase and the first 5 days of the experimental period, they had food restriction in their cages. After the surgery, the animals were placed in individual home cages and allowed to recover from the operation for 5 days, before starting the experiments. The day–night cycle was reversed for 3 days before tests, and the animals were recorded during the dark phase of the cycle. All experiments were conducted between 0700 and 1900 (Sahraei et al. 2004). All the animal experiments were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* (1996, published by National Academy Press, 2101 Constitution Ave. NW, Washington, DC 20055, USA) and approved by the University of Isfahan Animal Research Committee.

Drugs

The drugs used in the present study were morphine sulfate (Temad, Tehran, Iran), CNQX (6-cyano–7-nitroquinoxaline–2, 3-dione), AP-5 (2-amino–5-phosphonovaleric acid), and chloral hydrate, purchased from Merck (USA). All drugs were dissolved in sterile 0.9% saline, except CNQX, which required a 1% DMSO vehicle (Park et al. 2002).

Self-administration apparatus

Briefly, to aid in the acquisition of drug self-administration, rats were initially trained to press a lever, using food as reinforcement, before being surgically implanted with a chronic intravenous (i.v.) jugular catheter. Training and testing were performed in standard operant conditioning cages (21 cm × 21 cm × 28 cm), placed in a sound-attenuated room ventilated with fans, and based on a previously applied method (Sahraei et al. 2004; Alaei et al. 2005) with minor modifications. The apparatus was equipped with active and passive levers, 2 cm above the floor, with a red light located 4 cm above the active lever. The i.v. cannula was connected to an infusion pump via a swivel, allowing the animal to move relatively freely. Pressing the active lever (reinforcement lever), marked by a red light, results in a 10 s infusion of 0.1 mL fluid, through an infusion pump. The fluid was saline in the saline group, and morphine (5 mg/mL) in other groups. The red light disappeared during the infusion, and pressing the active lever during this time (10 s) did not affect the infusion of the drug, but PC computer calculated numbers of lever pressing. Pressing of the passive lever (nonreinforcement lever) had no programmed consequences. In this study, the number of lever pressing and number of infusions were considered, as a measure of the reinforcing action of the drug, whereas responding on the passive lever may be, in most cases, more regarded as a reflection of nonspecific behavior (Alaei et al. 2002, 2005; Sahraei et al. 2004).

Experimental design

Male rats were randomly selected and divided into 7 groups: (I) Saline group, receiving 0.1 mL saline in the self-administration sessions; (II) Morphine group, receiving 0.1 mL morphine (5 mg/mL) during the self-administration sessions; (III) and (IV) AP5 groups, receiving both minimum (0.1 μg/0.5 μL) and maximum (1 μg/0.5 μL) doses, 10 min before each session and morphine in the self-administration sessions, respectively; (V) and (VI) CNQX groups, receiving both minimum (0.5 μg/0.5 μL) and maximum (2.5 μg/0.5 μL) doses, 10 min before each session and morphine self-administration sessions, respectively; (VII) Co-administration group, receiving both CNQX (2.5 μg/0.5 μL) and AP5 (1 μg/0.5 μL), 10 min before each session and morphine in the self-administration sessions.

Training phase

The training procedure has been described in detail elsewhere (Hubner and Koob 1990). Briefly, to aid in acquisition of drug self-administration, rats were initially trained to press a lever, using food as reinforcement, before being surgically implanted with a chronic intravenous jugular catheter. Following 24 h of food restriction, rats were placed in the operant chambers, where a lever filled with food pellets was available. Lever pressing resulted in the delivery of a 100 mg pellet on a fixed ratio (FR) 1 schedule. Each rat was allowed to self-train and press for 40 pellets, before being returned to ad libitum food. Following acquisition of lever pressing behavior, rats were returned to ad libitum food, and allowed to gain their weight in 3 days, before performing the surgery (Sahraei et al. 2004; Brown et al. 2009).

Surgical procedures

The animals were anesthetized with 10% (450 mg/kg) chloral hydrate (Shahidani et al. 2012) and placed in a stereotaxic apparatus. A guide cannula (22 G) was implanted and secured with dental cement held on the skull, with small screws. Coordinates for the cannula implantation, according to the atlas of Paxinos and Watson were as follows: for PrL, anteroposterior, +3.2; mediolateral, +0.8; and dorsoventral, –3.6; relative to bregma and the skull surface (Paxinos and Watson 2005).

Immediately following the stereotaxic surgery, a cannula was inserted into the right jugular vein. The catheter was guided subcutaneously up to the skull, fixed to a metal tube, secured to the skull with small screws, and fixed with dental acrylic cement. After the cement was completely dry and hardened, 1 stainless steel stylet was used to occlude the catheter during the recovery period. Animals were individually housed and allowed to recover for 5 days before the experiments (Kim et al. 2005). To prevent infection, gentamycin (40 mg/mL, i.p.) was administered during the recovery period of rats.

Microinjection procedures

Initially, the rats were kept in hand and the injection needle, connected to the Hamilton syringe, placed in a short polyethylene tube (PE20) in the cannula. The microinjections were performed unilaterally, through lowering a stainless steel injector cannula with a length of 1 mm longer than the guide cannula within the PrL. The injector cannula was connected to a Hamilton syringe by polyethylene tube. Next, different doses of CNQX (0.5 and 2.5 μg/0.5 μL) (De Jaeger et al. 2013) and AP5 (0.1 and 1 μg/0.5 μL) (Bishop et al. 2010) were injected with a rate of 2 μL/min into the PrL, 10 min before the self-administration phase. The injection cannula was left in the guide cannula for an additional 60 s, to facilitate diffusion of the drug (Park et al. 2002).

Self-administration phase

Five days after recovery and following 30 h of the food restriction, rats were placed into the operant chambers where a lever filled with food pellets was available. In the first 5 days, every day

Fig. 1. Photograph scan of a coronal section of rat brain. Histological representation of cannula placement into the prelimbic cortex and site of antagonist microinjection in the rat's brain. [Colour online.]

6 h of starvation was declined, until they were no longer hungry in sixth day. Each active lever pressing resulted in the delivery of a 100 mg pellet. The jugular cannula of rats was connected to an infusion pump, and the animals were placed in the self-administration apparatus for 2 h each day, during 11 consecutive days on an FR-1 schedule (Sahraei et al. 2004). The trained animals were allowed to press active and passive levers freely. By pressing the active lever, the rats received 0.1 mL of morphine (5 mg/mL morphine sulfate in saline or 0.5 mg/kg per infusion) and small pellets in the first 5 days and morphine without pellets in the final 6 days of the experiment. The dose of 0.5 mg/kg was selected, according to previous studies (Alaei et al. 2002; Lee et al. 2015). Immediately following morphine delivery, there was a 10 s time-out period to prevent overdose (Alaei et al. 2005). Pressing the passive lever did not deliver fluid or food. In the first self-administration period (the first 5 days), the availability of food was restricted to reduce body weight by 15%, facilitating the initiation of intravenous self-administration (Brown et al. 2009). The changes less than 15% in the number of injections, in the last 3 days were considered as the baseline. For the next 6 days, the animals had ad libitum access to food. Catheters were flushed daily with 0.1 mL saline, containing heparin sulfate (50 U/mL), during the recovery period, and before and after the self-administration sessions. All operant sessions were conducted, during the animals' dark cycle. Catheter potency was tested by the injection of 0.1 mL of sodium pentobarbital solution (10 mg/mL) into the catheter and observation of animal behavior. Animals with patent catheters exhibit prominent signs of anesthesia (loss of muscle tone) a few seconds after the administration (Brown et al. 2009).

Histology

After completion of all experiments, the rats were sacrificed with an overdose of chloral hydrate, and transcardially perfused with 0.9% normal saline, followed by 10% buffered formalin. Brains were removed and placed in 10% formalin for 72 h. To evaluate the place of the antagonist injection and cannula, the PrL sections were checked (Fig. 1).

Data analysis

Data were presented as means ± SEM. The number of active and passive lever pressings, and also the number of self-infusion were compared, between different groups, using repeated-measures one-way analysis of variance (ANOVA), and Tukey's post hoc. The mean number of active lever pressings of the final 6 days and first 5 days, among different groups and the number of active and passive lever pressing every session, between different groups (summed over 11 sessions) were compared, using one-way ANOVA and Tukey's post hoc. The criterion for statistical significance was $p < 0.05$.

Results

The number of self-infusion during morphine self-administration is presented in Fig. 2. Intra-PrL injection of the different doses of AP5 (0.1 and 1 μg/0.5 μL), CNQX (0.5 and 2.5 μg/0.5 μL), and co-administration of CNQX and AP5 (2.5 μg/0.5 μL + 1 μg/0.5 μL) significantly decreased the number of self-infusion in the last 6 days ($F_{[6,14]} = 29.93$, $p < 0.001$), in comparison with the morphine group (Figs. 2A, 2B, and 2C). This reduction was started on day 6, at which the restriction of food was removed. It is clear that this decrease was seen in all groups, except for the morphine group.

The number of active lever pressing were significantly ($F_{[6,14]} = 9.39$ $p < 0.01$) higher in morphine group than the saline group (Fig. 3), suggesting that the animals pressed the active lever for using the morphine. In comparison, the number of active lever pressing was significantly ($F_{[6,14]} = 9.39$ $p < 0.01$) decreased in the treatment groups (group III–VII), compared with the morphine group (Fig. 3A, 3B, and 3C). Furthermore, although the maximum doses of AP5 (1 μg/0.5 μL) and CNQX (2.5 μg/0.5 μL) are more effective than the minimum doses on the active lever pressing, there was no significant difference between these doses of AP5 or CNQX, on the active lever pressing (Figs. 3A, 3B, and 3C).

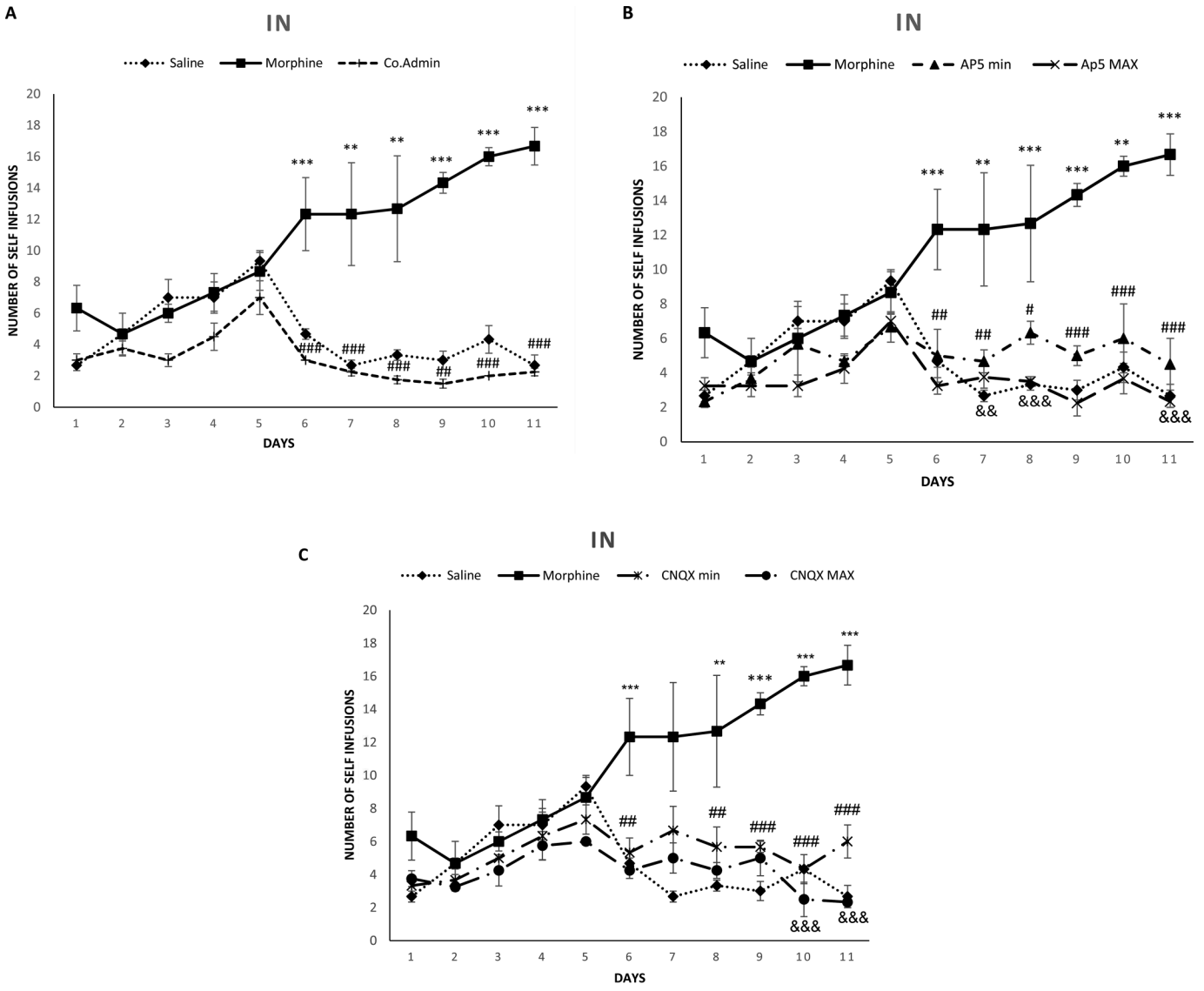
As Fig. 4 shows, there is no significant difference in the number of passive lever pressing between all groups. In addition, one-way ANOVA revealed that the mean number of active lever pressings of the first 5 days did not show any significant difference in morphine group, compared with the saline group. Meanwhile, the mean number of active lever pressings of the final 6 days significantly ($F_{[6,17]} = 21.85$ $p < 0.001$) increased in the morphine group, compared with the saline group (Fig. 5). This observation demonstrates that the increases in the active lever pressing of the final 6 days in the morphine group, is not related to the restriction of food and probably reflect the animal's tendency to get drug reinforcement.

The data sets created and (or) analysed during the current study are available from the corresponding author on reasonable request.

Discussion

In our study, the effect of glutamate receptor blockade within the PrL on morphine self-administration were evaluated. These results showed that NMDA or AMPA antagonists reduced morphine craving in rats. In this experiment, the morphine group showed a growing trend in self-infusion related to the saline group. Animals in the morphine group showed an increase trend

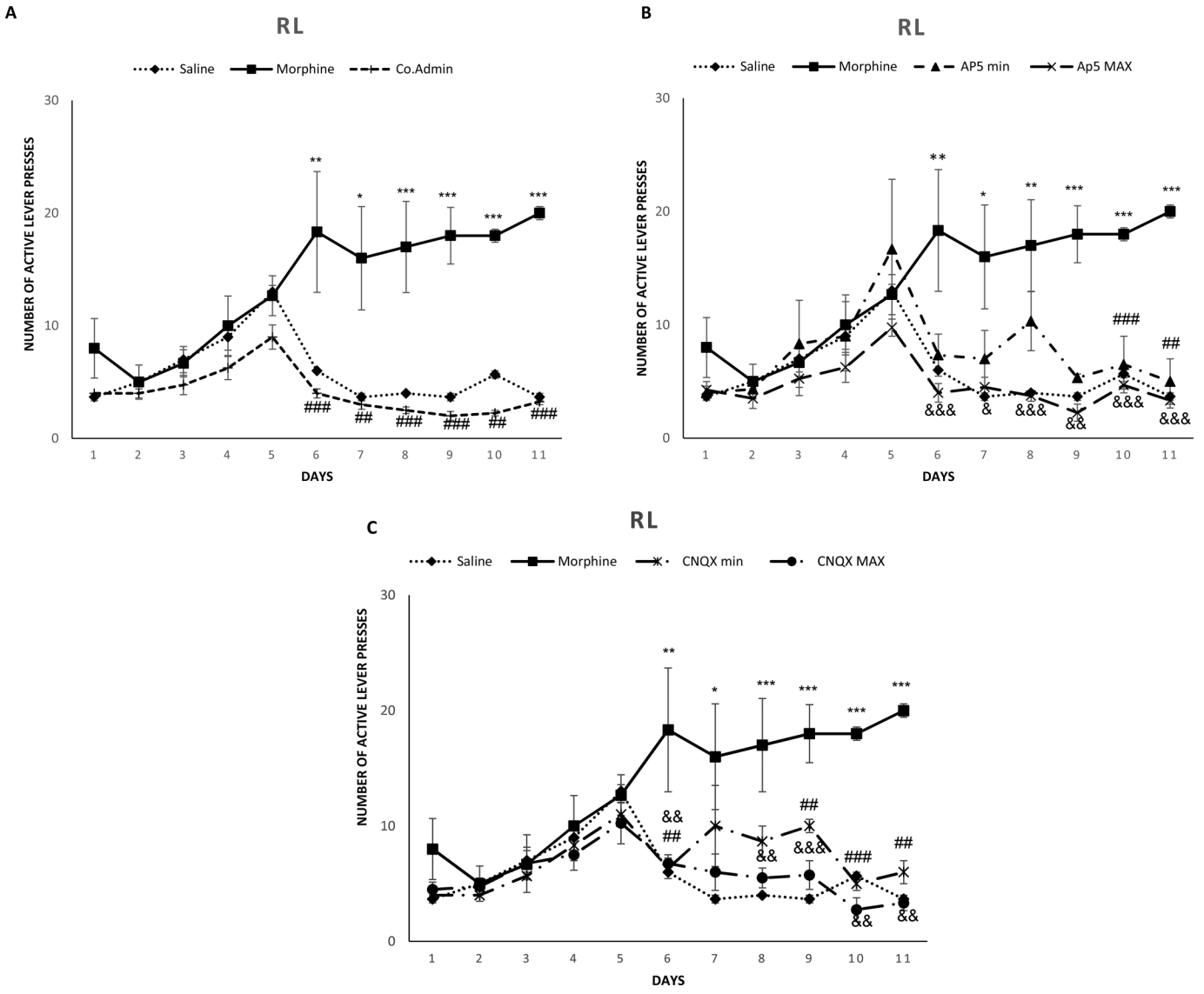
Fig. 2. Comparison of the number of self-infusion (IN) between groups on all days. Data are presented as mean ± SEM. Animals were tested during 11 consecutive daily 2-hour sessions. The mean number of self-infusions (SI) is plotted vs. the day of testing in all groups: saline and morphine groups received saline or morphine, respectively, in self-administration sessions; and Ap5 min, AP5 MAX, CNQX min, CNQX MAX, and Co. admin groups, which received both minimum or maximum dose of AP5 or CNQX or maximum dose of AP5 and CNQX before sessions and morphine in self-administration sessions. (A) The numbers of infusion significantly were more in morphine group than saline group but co-administration of CNQX and AP5 (2.5 μg/0.5 μL + 1 μg/0.5 μL) significantly reversed this effect of morphine. (B) Different doses of AP5 (0.1 and 1 μg/0.5 μL) significantly decreased SI compare with the morphine group. (C) Different doses of CNQX (0.5 and 2.5 μg/0.5 μL) significantly decreased SI compared with the morphine group. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ with respect to saline group. $^{\&,\#}p < 0.05$, $^{\&\&,\#\#}p < 0.01$, and $^{\&\&\&,\#\#\#}p < 0.001$ with respect to the morphine group.



in drug intake, during the 11 days, even after removal of food restriction. These observations demonstrate that increase in the active lever pressing of the final 6 days in the morphine group is not related to the restriction of food and probably reflect the animal's tendency to get drug reinforcement, suggesting that morphine acts as a positive reinforcement. This study has shown that the total respondent on the active lever in the morphine group is higher than passive lever, which is related to the reinforcing effects of the morphine (Alaei et al. 2002). In contrast to the saline group, the number of self-infusion and the number of active lever pressing in the NMDA and AMPA receptor-blockade groups decreased, compared with the morphine group, and attenuated the reinforcing effects of morphine (Figs. 2 and 3). Furthermore, there was no significant difference in the number of passive lever pressing, between the morphine group and other groups, proving that the animal behavior was directed to get the reward effects of morphine (Fig. 4) (Hosseini et al. 2009). It is possible that, glutamate receptors play an important role in reinforcement effect of morphine.

Evidence indicate the importance of the reinforcing properties of opiates in the development and maintenance of addiction. In addition, glutamate receptors are involved in the development, maintenance, and expression of opioid action. Thus, anti-addictive treatment should inhibit the reinforcing effect of opiates agents (Popik et al. 1998). Some studies suggested that ionotropic glutamate receptor antagonists decrease the reinforcing effect of all drug abuse (Popik et al. 1998; Olive 2009). Our results are consistent with findings of Popik and Danysz who showed NMDA receptor antagonist inhibits morphine self-administration in rodents (Popik and Danysz 1997). In addition, development of sensitiza-

Fig. 3. The number of active lever pressing (RL, reinforcement lever) in self-administration of all groups. Animals were tested during 11 consecutive daily 2-hour sessions. Data are presented as mean ± SEM. (A) The number of active lever pressing in the morphine group is more than that in the saline group. Co-administration of CNQX and AP5 (2.5 μg/0.5 μL + 1 μg/0.5 μL) significantly decreased active lever pressing. (B) The number of active lever pressing in AP5 groups (0.1 and 1 μg/0.5 μL) was less than that in the morphine group. (C) The number of active lever pressing in CNQX groups (0.5 and 2.5 μg/0.5 μL) was less than that in the morphine group. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ with respect to saline group. $^{\&,\#}p < 0.05$, $^{\&\&,\#\#}p < 0.01$, and $^{\&\&\&,\#\#\#}p < 0.001$ with respect to morphine group.



tion in rats exposed to chronic morphine was prevented by MK-801 (non-competitive NMDA antagonist) (Jeziorski et al. 1994). Although numerous studies have demonstrated that NMDA and AMPA receptor antagonists reduce both the expression and acquisition phases of morphine conditioned place preference, there is limited information about the role of glutamate in opiate-conditioned reinforcement (Zarrindast et al. 2007; Shabat-Simon et al. 2008; Heinmiller et al. 2009; Kao et al. 2011). Self-administration paradigm is the standard method for assessing conditioned reinforcement (Peters and De Vries 2012). In general, self-administration studies also support conditioned place preference observations. For example, it has been shown that NMDA receptor channel blockers reduce morphine self-administration (Peters and De Vries 2012). In line with these studies, it was found that D-cycloserine (a partial agonist of NMDA receptor) facilitates the formation of extinction memory (Davis et al. 2006). NMDA receptor blockade prevents the increase in sensitization in rats exposed to morphine. This finding was achieved by systematic injection of non-competitive antagonist MK-801 (Schroeder et al. 2000). Our results prove that AMPA and NMDA receptors in PrL play an important role in morphine addiction. Moreover, the NMDA receptor has shown a specific role in opioid addiction, because it persistently maintains the representation of opiate primary rewarding (e.g., opiate value) (Peters and De Vries 2012). Thus, NMDA receptor antagonists can be used in the treatment of opioid addiction (Peters and De Vries 2012). AMPA receptor blockade also inhibits drug seeking, but its severe side effects prevent using this antagonist (Peters and De Vries 2012).

A possible mechanism that reduces the number of active lever pressing with a glutamate receptor blockade can be explained by anatomical evidence. The mesolimbic dopamine system has received considerable attention, as a major neurobiological substrate, involved in mediating the reinforcing actions of morphine, and many drug abuses have the common action of increasing

Fig. 4. The number of passive lever pressing (NRL, non-reinforcement lever) in self-administration of all groups. No significant difference in the number of passive lever pressing was observed between all groups.

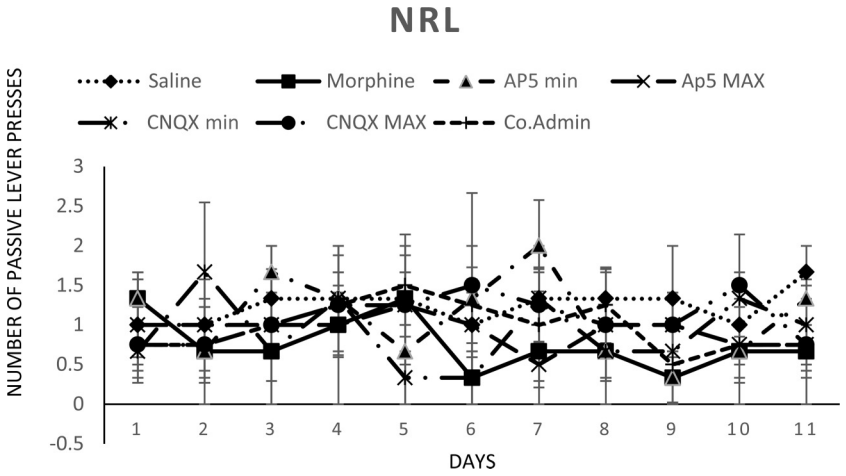
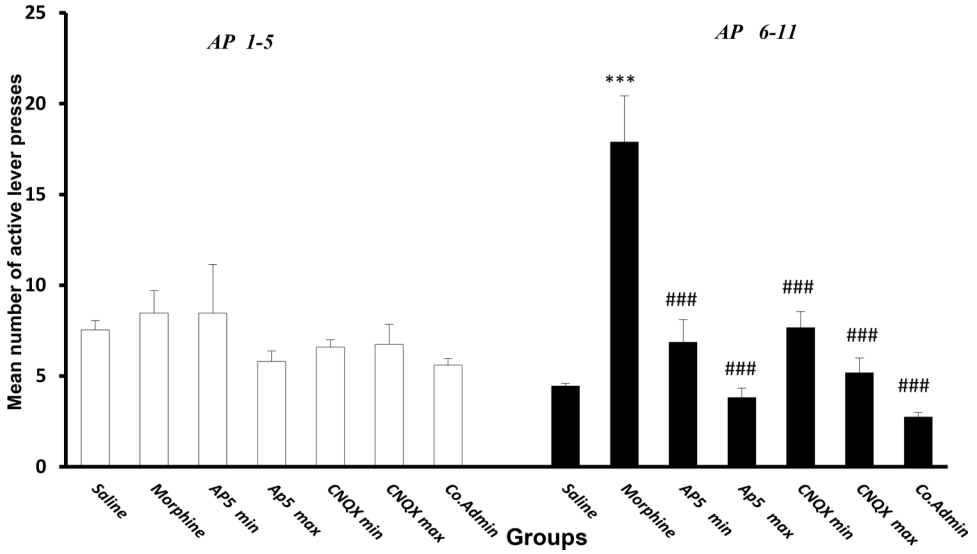


Fig. 5. The mean of active lever pressing during i.v. morphine self-administration of first 5 days and final 6 days between different groups. Data are presented as mean ± SEM. The mean number of active lever pressings of first 5 days did not show significant difference between all groups but the mean number of active lever pressings of final 6 days significantly ($p < 0.001$) decreased in test groups compare with the morphine group ($p < 0.001$). $^{***}p < 0.001$ with respect to the saline group. $^{###}p < 0.001$ with respect to the morphine group.



dopamine transmission in the nucleus accumbens (NAc). Limbic cortical structures, such as the PrL, are the primary sources of information about conditioned reinforcers that processed within the NAc (Everitt et al. 2001). It is notable that descending glutamate inputs from PrL to VTA is projected to the neurons that dopamine input sends to PrL (Carr and Sesack 2000). Moreover, dopamine input from VTA to mPFC is mainly directed to mPFC pyramidal neurons, which are projected to NAc (Bishop et al. 2010). The dopamine projection from VTA to NAc is critical for reward-related behaviors (Taylor et al. 2014). Hence, it can be claimed that with a glutamate receptor blockade in PrL, rewarding signals from VTA dopaminergic neurons and mPFC glutamatergic neurons into NAc are weakened. However, further studies are needed to examine this behavior thoroughly. In addition to anatomical evidence, stimulation of the mPFC led to the increase in extracellular glutamate in the VTA, the activation of dopamine neurons of the VTA, and the elevation of dopamine release in the forebrain (Zheng et al. 2017). Morphine selectively promotes glutamate release from glutamatergic terminals of projection neurons from mPFC to dopamine neurons of VTA. Recently, Zheng et al. found that morphine induces glutamate release from glutamatergic terminals, in the mPFC to VTA dopamine neurons, using optogenetic strategy (Zheng et al. 2017). Morphine selectively promotes glutamate release from glutamatergic terminals of projection neurons from mPFC to dopamine neurons of VTA. Recently, Liu et al. reported that the microinjection of morphine into the mPFC cannot produce rewarding effects (Liu et al. 2015), because the site of action of morphine on mPFC-VTA glutamatergic projection neurons is at terminals, rather than in the cell body in the mPFC (Zheng et al. 2017). Morphine selectively promotes glutamate release from glutamatergic terminals of projection neurons from mPFC to dopamine neurons of VTA.

Studies in humans and numerous findings related to the PrL inactivation in rats emphasize that PrL acts as an “on switch” for drug seeking (Gass and Chandler 2013). In addition, activation of glutamate projections from mPFC to NAc, is important in expressing addictive behaviors, such as drug seeking or behavioral sensitization (Van den Oever et al. 2010). Thus, it can be suggested that by blocking glutamate receptor in PrL, glutamate transition in the NAc changes, leading to a reduction in the tendency to morphine

consumption. In general, pharmacological treatments that help reduce glutamatergic mPFC output to the NAc can cause a decrease in relapse rates in human addicts (Van den Oever et al. 2010).

Because mPFC plays a role in cognitive functions, such as learning, memory, decision-making, and temporal sequencing, the reduction tendency for consumption of morphine may not only be due to reductions in the rewarding effects of a given treatment, but also to impaired detection, calculation, and presentation of rewarding message (Schroeder et al. 2000). In other words, the deficit of drug self-administration after glutamate receptors antagonists might also be due to a learning deficit, which would also impair the process of conditioning (Tzschentke 2000). Various studies have reported that the administration of NMDA receptor antagonists, such as PCP or ketamine can strongly disrupt mPFC-dependent behaviors, likely related to a disruption of prefrontal cortical synchrony (Bishop et al. 2010). It is clear that the PrL is directly involved in reward-related mechanisms and in the mediation of the rewarding effects of opiates (Tzschentke 2000). It is, therefore, not possible to discriminate between these 2 interpretations (reinforcing effect and associative process), based on the present data alone. Further studies are clearly warranted to address this issue.

There is evidence that the molecular and cellular pathways of drug addiction and learning and memory, have converged (Everitt et al. 2001). It is well documented that the mPFC is clearly involved in associative processes, during the opiate addiction process. Several factors make it a likely site for being involved in conditioning effects of morphine. First, self-administration of morphine affects the structure and morphology of spine of pyramidal neurons, in the PrL (Ballesteros-Yáñez et al. 2007). Second, recent work identified a population of the layer 1 interneurons that mediate disinhibitory control over cortical processing and thereby enable associative learning (Pi et al. 2013). Third, the subpopulations of neurons within the PrL are involved in the acquisition and recall phases of morphine-related associative learning, and show strongly increased activity, specifically during the acquisition and recall of opiate-related reward memories (Tan et al. 2014). In addition, glutamate receptors have been implicated in normal learning processes in limbic structures, each of which could have rebounded effects on drug seeking and (or) taking behavior, as well as on cognitive decision-making. In addition, excitotoxic lesions of the mPFC disinhibit drug-seeking, but not food-seeking, behavior (Everitt et al. 2001). There is little known about how modulation of glutamate receptor signaling within the mPFC may regulate associative opiate reward learning and memory formation. However, it is generally agreed that NMDA receptor antagonists affect the acquisition of new information, but not the storage or recall of associations that are well established (Popik and Danysz 1997).

Conclusion

Microinjection of different doses of the CNQX (0.5 and 2.5 μg/0.5 μL) and AP5 (0.1 and 1 μg/0.5 μL) in PrL prevents the development of morphine dependence, using self-administration. One possible mechanism for this phenomenon is that the hypofunction of AMPA and NMDA receptors transmission in the PrL disrupts the pathway between the prefrontal cortex, VTA, and NAc that will change dopaminergic neural activity and function of the rewarding system. Moreover, the present data suggest that mPFC NMDA and APMA receptors play an important role in mediating conditioned reinforcement of morphine, and PrL is a key region for glutamate-receptor-dependent modulation of opiate reward processing.

Conflict of interest

The authors declare that there is no conflict of interest associated with this work.

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