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Effects of CNQX and MPEP on sensitization to the rewarding effects of morphine

Carmen Manzanedo, María A. Aguilar, José Miñarro, Marta Rodríguez-Arias *

Unidad de Investigación Psicobiología de las Drogodependencias, Departamento de Psicobiología, Facultad de Psicología, Universitat de València, Av. Blasco Ibañez, 21, 46010 Valencia, Spain

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ABSTRACT

The present study was conducted to evaluate the influence of the glutamatergic receptors α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and metabotropic glutamate 5 (mGlu5) receptors on sensitization to the rewarding effects of morphine. The effects of pre-treatment with saline or 20 mg/kg morphine plus the AMPA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (5 or 10 mg/kg) or the metabotropic Glu5 receptor antagonist 6-methyl-2-(phenylethynyl)-pyridine (MPEP) (5 or 10 mg/kg) on the place conditioning induced by a low dose of morphine (2 mg/kg) were assessed. The 2 mg/kg dose of morphine was ineffective in animals pre-treated with saline but induced a clear conditioned place preference (CPP) in mice pre-treated with morphine alone and morphine plus any of the MPEP doses or the lowest dose of CNQX. Conversely, animals pre-treated with morphine plus 10 mg/kg of CNQX did not acquire CPP. Our results suggest that AMPA glutamate receptors are involved in the development of sensitization to the conditioned rewarding effects of morphine.

1. Introduction

Chronic exposure to drugs of abuse can cause changes in their behavioral effects. One example is sensitization, a process in which repeated intermittent administration of a drug such as morphine produces an increased responsiveness to its rewarding and motor effects. Several studies employing the CPP paradigm have shown that the rewarding effects of opiates are enhanced in rats (Lett, 1989; Shippenberg et al., 2009, 1996, 1998; Zarrindast et al., 2007) and in mice (Manzanedo et al., 2004, 2005 2009; Aguilar et al., 2009) with a prior history of opiate exposure.

There is limited information regarding the involvement of glutamatergic mechanisms in the expression of opiate sensitization. The role of N-methyl-D-aspartate (NMDA) glutamatergic receptors in morphine behavioral sensitization is controversial, since NMDA antagonists have been reported to prevent sensitization to the motor effects of morphine in some studies (Jeziorski et al., 1994; Mendez and Trujillo, 2008) but not in others (Atalla and Kuschinsky, 2006; Tzschentke and Schmidt, 1996). We have recently used the CPP paradigm to demonstrate the role of the NMDA receptor antagonist memantine in sensitization to the motor and rewarding effects of morphine (Aguilar et al., 2009).

On the other hand, chronic morphine administration is known to induce increases of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor glutamate receptor 1 (GluR1) subunit in the VTA (Lane et al., 2008). In line with these results, microinjection of the AMPA/kainite antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) into the VTA has been shown to block the CPP induced by morphine (Harris et al., 2004). In a more recent study, Shabat-Simon et al. (2008) demonstrated that CNQX blocked the rewarding effects of opiates in both the CPP and self-administration paradigms when injected into the anterior VTA and blocked locomotor sensitization when administered into the posterior VTA. These findings point to a critical role for glutamate receptors in opiate reward and suggest a behavioral and anatomical dissociation between the rewarding and psychomotor effects of opiates.

Recently, metabotropic glutamate receptors (mGluR) have become the focus of research related to addiction. MPEP (6-methyl-2-(phenylethynyl)-pyridine), a potent selective and systemically active metabotropic glutamate 5 (mGlu5) noncompetitive antagonist (Gasparini et al., 1999), attenuates the occurrence and severity of some symptoms of morphine withdrawal (Rasmussen et al., 2008). However, the effect of MPEP on morphine CPP is controversial, with

* Corresponding author. Departamento de Psicobiología, Facultad de Psicología, Universitat de Valencia, Avda. Blasco Ibañez, 21, 46010, Valencia, Spain. Tel.: +34 96 386 4637; fax: +34 96 386 46 68.
E-mail address: marta.rodriguez@uv.es (M. Rodríguez-Arias).

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some authors reporting a blockade (Popik and Wrobel, 2002) and others observing no effect (McGeehan and Olive, 2003). Moreover, recent reports suggest that MPEP has positive reinforcing and rewarding effects (Van der Kam et al., 2009a,b; Rutten et al., 2011).

To our knowledge, no previous studies have evaluated the involvement of the AMPA or metabotropic glutamatergic receptors in sensitization to the rewarding effects of morphine. Thus, the aim of the present study was to employ the CPP paradigm to assess the influence of CNQX and MPEP in the conditioned rewarding properties of morphine after previous exposure to this drug. We investigated whether pre-exposure to the AMPA receptor antagonist CNQX or to the mGlu5 antagonist MPEP, alone or with morphine, affected the development of sensitization to morphine-induced CPP.

2. Material and methods

2.1. Subjects

Male mice of the OF1 strain were acquired commercially from Charles River (Barcelona, Spain). The animals arrived at our laboratory at 42 days of age and were housed in plastic cages (28 cm length × 28 cm width × 14.5 cm height) in groups of four for 10 days prior to initiation of the experiments, under the following conditions: constant temperature (21 ± 2 °C), a reversed light schedule (white lights on: 19.30–07.30 h), and food and water available ad libitum. Procedures involving mice and their care were conducted in compliance with national, regional and local laws and regulations, which are in accordance with the European Communities Council Directives (86/609/EEC, 24 November 1986). Animals were handled briefly on the 2 days preceding initiation of the experiments.

2.2. Drugs

During pre-treatment, animals were injected ip. with 5 or 10 mg/kg of CNQX (Laboratorios Sigma-Aldrich Química, Madrid, Spain), 5 or 10 mg/kg of MPEP (Research Biochemicals International, Natick, USA), 20 mg/kg of morphine (Laboratorios Alcaliber, Madrid, Spain) or physiological saline in a volume of 0.01 ml/g. All drugs were dissolved in physiological saline (NaCl 0.9%). During conditioning all animals were injected with saline and 2 mg/kg of morphine.

2.3. Apparatus

The apparatus consisted of four identical Plexiglas place-conditioning boxes. Each box is comprised of two equally sized compartments (30.7 cm. length × 31.5 cm. width × 34.5 cm. height) separated by a gray central area (13.8 cm. length × 31.5 cm. width × 34.5 cm. height). The compartments have different colored walls (black vs. white) and distinct floor textures (smooth in the black compartment and rough in the white one). Four infrared light beams in each compartment of the box and six in the central area allowed the position of the animal and its crossings from one compartment to the other to be recorded. The equipment was controlled by an IBM PC computer using MONPRE 2Z software (CIBERTEC, SA, Spain).

2.4. Procedure of sensitization

To study whether CNQX or MPEP block morphine-induced sensitization to the rewarding effects of morphine, all the animals were pre-treated before initiating the place conditioning procedure. This pre-exposure phase consisted of a daily injection of the corresponding drugs, which were administered in the home cage on five consecutive days. Three days after the last pre-treatment injection, the procedure of place conditioning began with three days of pre-conditioning without any treatment. In this way, there was a 6-day interval without injections.

2.4.1. Experiment 1: effects of CNQX on the sensitization effects of morphine

Animals were divided into five groups according to the treatment received in the pre-exposure phase: S + S, pre-treated with two injections of physiological saline (n = 10); S + CNQX10, pre-treated with saline plus 10 mg/kg of CNQX (n = 11); M20 + S pre-treated with 20 mg/kg of morphine plus saline (n = 10); and M20 + CNQX5 and M20 + CNQX10, which received 20 mg/kg of morphine plus 5 or 10 mg/kg of CNQX respectively (n = 11, and n = 12).

2.4.2. Experiment 2: effects of MPEP on the sensitizing effects of morphine

Animals were divided into five groups according to the treatment received in the pre-exposure phase: S + S, pre-treated with two injections of physiological saline (n = 11); S + MPEP10, pre-treated with saline plus 10 mg/kg of MPEP (n = 11); M20 + S pre-treated with 20 mg/kg of morphine plus physiological saline (n = 12); and M20 + MPEP5 and M20 + MPEP10, which received 20 mg/kg of morphine plus 5 or 10 mg/kg of MPEP respectively (n = 12, and n = 11).

2.5. Place conditioning

This procedure, unbiased in terms of initial spontaneous preference, was performed as described previously (Manzanedo et al., 2001). To summarize, in the first phase, referred to as pre-conditioning (Pre-C), mice were allowed access to both compartments of the apparatus for 15 min (900 s) per day on 2 consecutive days. On day 3, the time spent in each compartment over a 900-s period was recorded. Six animals in Experiment 1 and ten animals in Experiment 2 showed a strong unconditioned aversion (less than 27% of the session time, i.e., 250 s) or preference (more than 73%, i.e., 650 s) for one compartment and were eliminated from the rest of the study. In each group, half the animals received the drug or vehicle in one compartment and the other half in the other compartment. After assigning the compartments, an analysis of variance (ANOVA) revealed no significant differences between the time spent in the drug-paired and vehicle-paired compartments during the pre-conditioning phase [$F(1, 106) = 0.101$, $P < 0.7514$] in Experiment 1 and [$F(1, 112) = 0.286$, $P < 0.5942$] Experiment 2. This is an important step in the experimental procedure that rules out any preference bias prior to conditioning. In the second phase (conditioning), which lasted 4 days, animals received an injection of physiological saline before being confined to the vehicle-paired compartment for 1 h, and after a further interval of 4 h received 2 mg/kg of morphine immediately before being confined to the drug-paired compartment for 1 h. During the third phase, known as post-conditioning (Post-C), the guillotine door separating the two compartments was removed (day 8) and the time spent by the untreated mice in each compartment was recorded during a 900-s observation period. The difference in seconds between the time spent in the drug-paired compartment in the Post-C test and in the Pre-C phase is a measure of the degree of conditioning induced by the drug. If this difference is positive, then the drug has induced a preference for the drug-paired compartment, while the opposite indicates an aversion.

2.6. Statistical analysis

To evaluate the effect of pre-treatment on the acquisition of morphine-induced CPP, data relating to the time spent in the drug-paired compartment were analyzed using a mixed ANOVA with two "between" subject variables – "morphine", with two levels (saline and 20), and "CNQX", with three levels (0, 5 and 10 mg/kg), for the first experiment, and "MPEP", with three levels (0, 5 and 10 mg/kg), for the second experiment – and a "within" subject variable — "days", with two levels (Pre-C, and Post-C). Bonferroni adjustment was employed for post hoc comparisons.

3. Results

3.1. Experiment 1

The ANOVA for the effect of CNQX (Fig. 1) showed a significant effect of the variable Days [$F(1,49) = 10.247$; $P<0.002$], the interaction days × morphine [$F(1,49) = 4.951$; $P<0.031$] and the interaction days × morphine × CNQX [$F(1,49) = 8.033$; $P<0.007$]. Post hoc comparisons revealed that the groups M20 + S and M20 + CNQX5 spent more time in the drug-paired compartment in the Post- than in the Pre-C test ($P<0.001$ and $P<0.02$, respectively).

3.2. Experiment 2

The ANOVA for the MPEP effect (Fig. 2) showed a significant effect of the variable days [$F(1,52) = 12.026$; $P<0.001$], as more time was spent in the drug-paired compartment in the Post- than in the Pre-C test ($P<0.001$), and the interaction days × morphine [$F(1,52) = 7.088$; $P<0.010$]. Animals pre-treated with morphine spent more time in the drug-paired compartment than those pre-treated with saline ($P<0.001$).

4. Discussion

The present work demonstrates the involvement of AMPA glutamatergic receptors in the acquisition of sensitization to the conditioned rewarding effects of morphine. However, interrupting glutamatergic neurotransmission by blocking mGlu5 did not affect the development of morphine sensitization.

The results obtained in this study confirm that repeated administration of morphine produces sensitization to its positive rewarding properties. Prior exposure to morphine enhanced place conditioning, since a dose of 2 mg/kg, which was ineffective in animals pre-treated with saline, produced a significant place preference in those previously exposed to 20 mg/kg of morphine. Such findings are in accordance with those obtained in rats (Lett, 1989; Shippenberg et al., 1996, 1998) and confirm the results of our previous studies in mice (Manzanedo et al., 2004, 2005, 2009; Aguilar et al., 2009). It should be stressed that this experiment employed an 'unpaired' design in which animals are returned to their home cages after receiving the drug and are tested only at the end of the experiment. Thus, the resulting sensitization is

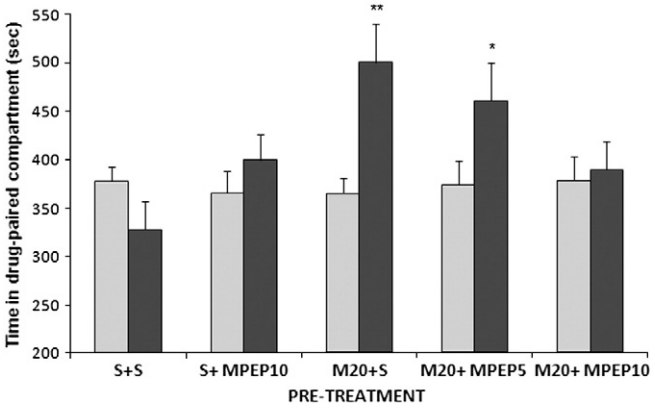

Fig. 1. Effects of CNQX on sensitization to the rewarding effects of morphine. Treatment groups during pre-exposure phase: S + S, animals receiving two injections of physiological saline; M + S, animals receiving an injection of 20 mg/kg of morphine plus an injection of saline; S + CNQX10, animals receiving an injection of saline plus an injection of 10 mg/kg of CNQX; M + CNQX5 and M + CNQX20, animals receiving an injection of 20 mg/kg of morphine plus an injection of 10 or 20 mg/kg of CNQX. These injections were administered on five consecutive days (pre-exposure phase), which was followed by an interval of 3 days before initiating the place conditioning procedure with 2 mg/kg of morphine. Bars represent the time in seconds spent in the drug-paired compartment in pre-conditioning (white bars) and post-conditioning (black bars) phases. ** $P<0.001$, * $P<0.02$, significant difference in the time spent in pre-conditioning vs. post-conditioning sessions.

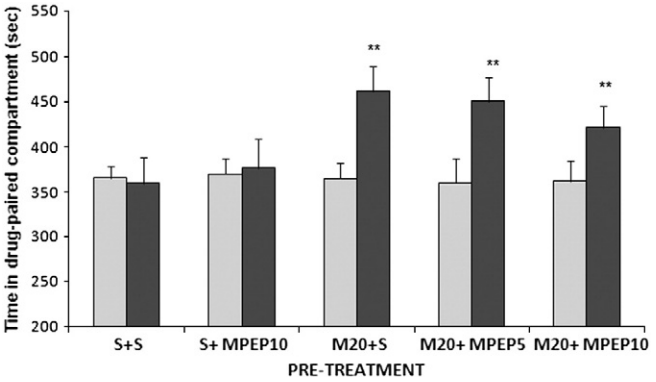

Fig. 2. Effects of MPEP on sensitization to the rewarding effects of morphine. Groups of treatment during pre-exposure phase: S + S, animals receiving two injections of physiological saline; M + S, animals receiving an injection of 20 mg/kg of morphine plus an injection of saline; S + MPEP10, animals receiving an injection of saline plus an injection of 10 mg/kg of MPEP; M + MPEP5 and M + MPEP 20, animals receiving an injection of 20 mg/kg of morphine plus an injection of 10 or 20 mg/kg of MPEP. These injections were administered on five consecutive days (pre-exposure phase) followed by an interval of 3 days before initiating the place conditioning procedure with 2 mg/kg of morphine. Bars represent the time in seconds spent in the drug-paired compartment in pre-conditioning (PRE) (white bars) and post-conditioning (POST) (black bars) phases. ** $P<0.001$, significant difference in the time spent in pre-conditioning vs. post-conditioning sessions.

context-independent, as contextual conditioning has been avoided (Tzschentke and Schmidt, 1998a). The increase in the effects of morphine on CPP after drug-pre-exposure could be due to both an increase in the rewarding effects of morphine and an enhancement of drug-seeking behavior. It has been argued that sensitization of the central reward mechanism potentiates the incentive properties of drugs, thus increasing the probability of addiction (Robinson and Berridge, 1993, 2003). On the other hand, enhancement of the conditioned rewarding effects of morphine produced by previous exposure may contribute to the conditioned drug-craving, as it is observed in former addicts when they are exposed to cues previously associated with the drug (Shippenberg et al., 1996).

For a long time, dopamine was considered the main neurotransmitter involved in processes underlying addiction, although the importance of the role of glutamate has been demonstrated more recently (Kalivas and O'Brien, 2008; Kalivas et al., 2005; Tzschentke and Schmidt, 2003). Glutamatergic receptors play a crucial role in the CPP induced by morphine (Tzschentke and Schmidt, 1998b), although there is controversy regarding the role of NMDA receptors in opiate sensitization (Carlezon et al., 2000; Iijima et al., 1996; Jeziorski et al., 1994; Ranaldi et al., 2000; Tzschentke and Schmidt, 1996, 1998b; Tzschentke and Schmidt, 2000; Vanderschuren et al., 1997). Recently, Mendez and Trujillo (2008) reported that NMDA receptor antagonists inhibit the development of sensitization to the locomotor stimulant effect of morphine, and our group has also demonstrated that memantine blocks sensitization to the rewarding effects of morphine (Aguilar et al., 2009).

Few studies have been performed in order to evaluate the role of other glutamate receptors. Repeated intermittent drug administration alters the levels of glutamate-receptor subunits within the VTA. Acute and chronic morphine administration produces a significant increase in GluR1 particles, an AMPA receptor subunit, in the VTA (Lane et al., 2008). Elevated levels of GluR1 favor the formation of Ca2 + -permeable AMPA receptors composed entirely of this subunit (Hollmann and Heinemann, 1994; Carlezon et al., 1997; Neve et al., 1997), which, in theory, could trigger a cascade of molecular events that contribute to sensitization.

There is some controversy concerning the role of AMPA receptors in the development of morphine-induced CPP, although the majority of evidence suggests that these receptors are necessary. In an early report, the AMPA antagonist DNQX did not affect acquisition of morphine-induced CPP when administered in the nucleus accumbens

but did block its expression (Layer et al., 1993). More recently, administration of the AMPA antagonist CNQX to the VTA has been shown to efficiently block morphine-induced CPP (Harris et al., 2004). In another study, a cocktail of AMPA (CNQX) and NMDA (AP-5) antagonists injected into the ventral pallidum also blocked both the CPP induced by morphine and the motor changes induced by previous morphine administration (Dallimore et al., 2006). A more recent and comprehensive study has pointed to a critical role for AMPA/kainate receptors in the VTA in opiate reward, as well as a behavioral and anatomical dissociation between the rewarding and psychomotor effects of opiates (Shabat-Simon et al., 2008). Administration of CNQX to the anterior VTA blocked the rewarding effects of opiates in both the conditioned place preference and the self-administration paradigms without affecting the gradual increase of the psychomotor response to opiates. In contrast, administration of CNQX to the posterior VTA did not affect the rewarding properties of opiates; however, it did block the initial sedative effect of opiates and the gradual increase of the psychomotor response to the drug. In the study in question, the sensitization to the locomotor effects of morphine was context-dependent.

The effect of AMPA receptor antagonists on morphine-induced behavioral sensitization has been the subject of little study, and the little evidence available is controversial. Carlezon et al. (1999) reported that the AMPA receptor antagonist LY293558 blocked sensitization to the motor effects of morphine when administered during the acquisition phase but not in the expression phase. The noncompetitive AMPA receptor antagonist GYKI 52466 [1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5 H-2,3-benzodiazepine hydrochloride] was shown to selectively reduce food-induced conditioned activity and to block cross-sensitization to cocaine or morphine (Le Merrer and Stephens, 2006). A recent study by Sepehrizadeh et al. (2008) revealed that administration of a high dose of CNQX failed to reverse morphine-induced locomotor sensitization. However, Shabat-Simon et al. (2008) observed that injection of CNQX into the posterior VTA blocked morphine motor sensitization. In line with these findings, our results demonstrate that blockade of the AMPA receptors with CNQX impedes the development of sensitization to the rewarding effects of morphine. The group pre-treated with the highest dose of CNQX plus morphine failed to develop CPP after being conditioned with 2 mg/kg of morphine, while the groups pre-treated with morphine alone or plus the lowest dose of CNQX did develop CPP.

Contrary results were obtained with the mGlu5 antagonist MPEP, which was not capable of blocking the effects of pre-treatment with morphine. The groups pre-treated with morphine plus either of the doses of MPEP presented CPP after being conditioned with 2 mg/kg of morphine, similarly to the group pre-treated only with morphine. The extent of the mGlu5 receptor's blockade of the behavioral effects of opiates is less clear than that of CNQX. MPEP attenuates intravenous self-administration of heroin in rats only at high doses (Van der Kam et al., 2007). In this context, several groups have reported that high doses of MPEP ($\geq$30 mg/kg) also attenuate morphine-induced CPP (Herzig and Schmidt, 2004; Herzig et al., 2005; Popik and Wrobel, 2002), while one group did not observe an effect with lower doses (McGeehan and Olive, 2003). Although the potential of mGlu5 receptor antagonists in the treatment of withdrawal from opiates and other drugs of abuse has been suggested (Rasmussen et al., 2008), recent reports have demonstrated that MPEP can have positive rewarding effects. A moderate dose of MPEP (10 mg/kg i.p.) potentiates rather than attenuates the rewarding effect of heroin in the CPP paradigm and is capable of reinstating the preference once extinction is achieved (van der Kam et al., 2009b). In addition, MPEP has been reported to maintain a self-administration behavior in rats that have learned to self-administer heroin, to induce self-administration itself and to produce a dose-dependent place preference (van der Kam et al., 2009a). In this way, a sensitized response to the locomotor effects of MPEP has also been observed (Herzig and Schmidt, 2004). It should be taken into consideration that MPEP was administered at doses that can induce target-off effects. MPEP has been reported to have electrophysiological effects on human NMDA1A/2B and kainate Glu6 receptor subtypes and on rat NMDA receptors (Olive et al., 2005); thus, it is not recommended for paradigms in which NMDA receptors play a significant role (Lea and Faden, 2006). The tendency for morphine sensitization to attenuate as the dose of MPEP increases could be due to its effects on the NMDA receptor.

However, the mGlu5 antagonist MTEP (2-methyl-1,3-thiazol-4-yl) ethynyl] pyridine), at doses that did not modify acute locomotor effects, inhibited the expression of sensitization to the locomotor effect of morphine in mice (Kotlinska and Bochenski, 2007). A recent study has found that MPEP does not attenuate the increase in locomotor activity induced by acute and repeated nicotine administration in animals habituated to the test environment on the day of testing, but that it significantly reduces the conditioned locomotor stimulation evoked by pairing nicotine with a specific environment (Tronci et al., 2010). These results suggest that MPEP preferentially undermines the conditioned response evoked by exposure to contextual cues previously paired with nicotine. The authors of the study in question suggest that the drug attenuates the influence of contextual cues on behavior in general rather than exerting a selective effect on specific behavioral responses related to reinforcement. This hypothesis can be applied to the results obtained in the present study, in which sensitization was context-independent. It is possible that, in other experimental designs in which the effects of a drug are associated with the contextual cues of the test arena (the drug-associated compartment of the apparatus), MPEP affected the sensitization response.

Sensitization is an important factor in the induction and maintenance of drug dependence in both animal and human organisms, and contributes to relapse into drug abuse after periods of abstinence. Our results support a role for AMPA glutamate receptors in the development of sensitization to the reinforcing effects of morphine, and suggest that this role is due to the blockade of AMPA receptors composed mainly of the GluR1 subunit. On the other hand, we observed no effect after administration of the mGlu5 antagonist MPEP. This could be because these receptors play no role in the sensitization process studied, or because we applied a sensitization procedure that did not involve contextual cues. In conclusion, the present work supports AMPA antagonists as a potential tool for the treatment of opiate dependence and relapse.

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