The influence of magnesium on morphine-induced stimulation of the reward system

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Abstract. The present study was designed to assess the influence of magnesium (Mg) as MgCl₂ (10 or 40 mg/kg b.wt/day i.p.) and of its interaction with morphine on the reward system (RS) in Wistar rats. For this purpose, we evaluated conditioning place preference on a 12 day experiment schedule. Our data show that MgCl₂ (10 mg/kg b.wt/day) has moderate but significant effects on stimulating RS (increasing the time spent in associated conditioned compartment) (327.75 ± 11 s in the Mg (10 mg/kg b.wt) group vs 295.2 ± 8 s in the control (saline) group, p < 0.05) but not at higher Mg doses (40 mg/kg b.wt/day). We tested the influence of MgCl₂ (10 mg/kg b.wt./day i.p.) upon naloxone (2 mg/kg b.wt/ i.p.-induced place aversion. Administrated alone, naloxone has an aversive effect on place preference. MgCl₂ (10 mg/kg b.wt/day i.p.) has a significantly decreased aversive effect of naloxone (280.7 ± 37 s in naloxone + MgCl₂ (10 mg/kg b.wt) group vs 189 ± 21 s in naloxone group, p < 0.05). MgCl₂ at both tested doses, added to morphine (3 mg/kg b.wt/day i.p.), decreased the acquisition of morphine-induced place preference (262.2 ± 17 s) in morphine + MgCl₂ (40 mg/kg b.wt) group vs 462.15 ± 28 s in morphine group, p < 0.05). MgCl₂, 10 mg/kg b.wt/day i.p. decreased both morphine-induced place preference and naloxone-induced place aversion.

Key words: reward system, magnesium, naloxone, morphine, conditioning place preference

The reward system is a complex network that includes as key structures: orbital prefrontal cortex, anterior cingulated cortex, amygdale, hippocampus, pedunculopontine nucleus, raphe nucleus, locus coeruleus, nuclei accumbens, basal ganglia [1]. This system is involved in the control of motivated behavior [2]. Perturbation in the activity of this system has been observed in some psychiatric diseases, including addiction [3]. Magnesium (Mg) and other bivalent cations are found in all brain cells and play important roles at this level [4]. Mg is a cofactor for many enzymes from neurons and glial cells. In this way, Mg may influence neuronal activity and synaptic plasticity [5]. Mg is involved in many physiological processes in the brain (e.g. sleep [6]) and in some neurologic and psychiatric diseases such as brain trauma [7, 8], major depression [9, 10], Alzheimer’s disease [11] and others. Mg depletion is involved in age-related neurodegenerative disease [12]. Mg deficiency increases brain hyperexcitability. All substances that determine dependence produce a strong and prolonged RS stimulation [13]. Some substances, like ethanol and cocaine- strong stimulators of the reward system, change plasmatic and intracellular concentrations of Mg [7, 14]. An important number of natural factors stimulate RS (food, sexual activity, etc.). RS stimulation emerges after repeated administration but also after a single
administration of an addictive substance. The aim of this study was to follow the Mg effect upon RS and to assess how Mg influences morphine and naloxone-induced effects on RS in rats.

**Materials and method**

Adult, male Wistar rats bred in normal laboratory conditions and fed with regular chow pellets (food and water *ad libitum*), 8 animals in each group, were used for the experiments. Animals were exposed to a cycle 12 h light/12 h dark. In order to investigate the influence of Mg on RS we used conditioned place preference (CPP). The protocol was approved by the Ethical committee of University of Medicine and Pharmacy “Gr.T. Popa” Iasi.

The study was performed in a 3 compartment box (Panlab shuttle- compartments: 300 (W) x 300 (D) x 340 (H) mm (one with black walls and the other with white walls); Corridor: 80 (W) x 100 (D) x 340 (H) mm, after a classical conditioning procedure used to assess the rewarding properties of morphine and other drugs of abuse. The conditioning box was connected to a computer and the position of the animal was determined automatically. The time spent in each compartment was recorded. The conditioning procedure was conducted in 3 phases [15].

- Pre-conditioning: for preconditioning, rats were placed in the neutral area and allowed free access to all three chambers for 15 min. The natural preference of each animal for a compartment was measured.

- Conditioning: according to natural preference, rats were assigned to either the morphine/saline conditioning group or the saline/saline control group. The second phase consisted of four conditioning procedures (two morphine/ two saline pairings). On conditioning days 1, 3, 5 and 7, rats were injected with morphine (3 mg/kg b.wt. i.p., respectively MgCl₂ (within 2 hours before the morphine) and morphine and immediately confined to one chamber for 40 min. On alternate days, rats were injected with saline and immediately confined to the opposite chamber for 40 min. Control rats received saline in both chambers on all days.

- Post-conditioning: on the testing day, rats were placed into the neutral chamber and then allowed 15 min with free access to all three chambers in a drug-free state.

The method is summarized in *table 1*. Groups were as follow:

- group I: saline (Sicomed®, Bucuresti, Romania) 1 mL/kg b.wt, i.p.;
- group II and III: Mg²⁺ as MgCl₂, respectively 10 mg MgCl₂ and 40 mg MgCl₂/kg b.wt/day i.p.;
- group IV: morphine (M) (as morphine hydrochloride) (Zentiva®, Prague, Czech Republic) 3 mg/kg b.wt/day, i.p.;
- group V and VI: morphine (M), 3 mg/kg b.wt/ day i.p. + Mg²⁺ as MgCl₂, respectively 10 mg and 40 mg MgCl₂/kg b.wt /day (2 h before morphine);
- group VII: naloxone (Nlx) (Fluka®, Buchs, Switzerland) s.c. 2 mg/kg b.wt;
- group VIII: Nlx 2 mg/kg b.wt, s.c. + Mg²⁺ as MgCl₂, 10 mg and 40 mg MgCl₂/kg b.wt/day, respectively (1 h before morphine).

Naloxone and MgCl₂ and morphine were dissolved in saline.

Date were statistically interpreted with the t test (pretest vs. post-test) and with ANOVA with one variable (treatment in post-conditioning) plus Bonferroni as a post-hoc test (where difference after ANOVA was statistically significant). The thresholds for statistically significance were p < 0.05. We used GraphPad Prism 5 soft (San Diego, CA, USA).

**Table 1.** Summary presentation of place preference protocol.

<table>
<thead>
<tr>
<th>Day</th>
<th>Description</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3</td>
<td>Accommodation - Animal handling</td>
<td>5 min</td>
</tr>
<tr>
<td>4</td>
<td>Pretest - Determination of natural preferences for box compartments in a “free exploration” model - Repartition of animals in groups - Choosing conditioning compartment</td>
<td>15 min</td>
</tr>
<tr>
<td>5, 7, 9, 11</td>
<td>Conditioning - Administration of tested substance (M/MgCl₂/naloxone) associated with a specific conditioning compartment - Animal is confined in this compartment</td>
<td>40 min</td>
</tr>
<tr>
<td>6, 8, 10, 12</td>
<td>Administration of saline in opposite compartment - Animal is confined in this compartment</td>
<td>40 min</td>
</tr>
<tr>
<td>13</td>
<td>Post-test - Determination of preference for box compartments in a “free exploration” model</td>
<td>15 min</td>
</tr>
</tbody>
</table>
Results

The influence of MgCl₂, 10 mg and 40 mg/kg b.wt/day on morphine-induced place preference is presented in figure 1. Morphine had a strong effect of conditioning place preference. Mg prevented acquisition of morphine-induced CPP at both doses, in a dose-variable manner (e.g. at 40 mg/kg b.wt (312.1 ± 37 s in morphine + MgCl₂ (40 mg/kg b.wt) group vs 462.15 ± 28.69 s in morphine group, p < 0.05) (figure 2).

Naloxone administrated alone decreased place preference (aversive effect). Associating naloxone with Mg influenced Mg-induced place preference, decreasing the rewarding effect of Mg.

MgCl₂, 10 mg /kg b.wt/day, reduced the naloxone-induced aversive effect on CPP (figure 3).

Discussion

Morphine is a strong stimulant of the RS. It induced conditioned place preference (CPP) in rats. In our experiment, morphine 3 mg/kg b.wt significantly raised the time spent in the conditioned compartment. This strong effect is produced mainly by stimulation of μ opioid receptors in neurons from RS and especially in the hippocampus. After stimulation of μ opioid receptors there are at least 3 different pathways for morphine-induced CPP:

- by stimulation of dopamine release and increasing dopaminergic mediators at the RS level;
- by stimulation of pre-synaptic release of glutamate and action of this excitatory aminoacid at the level of an NMDA receptor [16];
- other pathways (e.g. by stimulation of nitric oxide synthetase (NOS) in the brain [17].

Dopaminergic mezolimbic and nigrostriat systems are essential for reward [18] and the midbrain dopaminergic system is involved in the function of RS and behavior [19]. Beside dopamine, glutamate is another important neuromediator involved in reward [20]. Glutamatergic transmission plays an important role in the function of nucleus accumbens and RS [21]. Dopamine modulates presynaptic glutamate release [22]. Morphine stimulates dopamine and glutamate release. Mg decreases glutamate release in the hippocampus and other CNS areas [23] and glutamate-induced activation of NMDA receptors by antagonizing Ca²⁺ entrance through calcium channels coupled with NMDA receptors. In this way Mg²⁺ may decrease morphine-induced stimulation of RS. Mg also decreases morphine-induced RS stimulation by...
**Figure 2.** MgCl₂ influence on the morphine effect regarding the time spent in conditioned compartment. *p < 0.01 vs pre-test; **p < 0.05 vs pre-test; *p < 0.01 Morphine (M) + Mg vs M post-test; ** Mg10 = MgCl₂ 10mg/kg b.wt Mg40 = MgCl₂ 40mg/kg b.wt.

**Figure 3.** MgCl₂ influence on naloxone (Nlx) effect regarding the time spent in conditioned compartment. *p < 0.01 vs pre-test; **p < 0.05 vs pre-test; #p < 0.01 Nlx vs Mg10+Nlx post-test; & p < 0.05 Mg10 vs Mg10+Nlx post-test; Mg10 = MgCl₂ 10 mg/kg b.wt; Mg40= MgCl₂ 40 mg/kg b.wt.
decreasing the pre-synaptic release of dopamine. On the other side, Mg increases the inhibitory effects of adenosine on endogenous dopamine release in rat brain [24, 25]. Tegmental pedunculopontin nucleus is critical for mediating the acute rewarding action of morphine and other opiates [26]. Stimulation of NMDA receptors in this area determines reward. Different antagonists of NMDA receptors (as AP-7) blocked morphine rewards (measured by CPP). The effects of other substances too on RS, are mediated by NMDA receptors [27]. Another pathway for Mg decreasing morphine-induced CPP is its influence on NO synthesis. Mg decreases nitrergic neuron activity [28] and inhibits the NOS activity of neurons [29]. Morphine stimulates NOS in the brain [17]. NO synthesis is involved in the rewarding effects of morphine and NOS inhibition decreased morphine-induced CPP [17]. It is to be remarked that the rewarding effect of opioids mainly implies stimulation of μ-opioid receptors [30] and to a lesser extent of presynaptic δ opioid receptors. The involvement of these 2 types of receptors is different. Morphine-induced stimulation of μ receptors increased glutamate release and CPP. On the other hand, stimulation of presynaptic δ opioid receptors by a selective agonist such as D-Pen(2)D-Pen(5) enkephalin produced a significant inhibition of glutamate release in central glutamate synapses and decreased conditioned place preference [31].

Karami and Zarrindats [32] have shown that morphine, 0.5-10 mg/kg b.wt/s.c produced a significant place preference in Wistar rats, in agreement with our data. Our data show that MgCl₂ (at both doses) associated with morphine significantly decreased morphine-induced place preference, using a medium morphine dose (3 mg/kg b.wt) compared with other authors.

Our data show that in naïve rats, Mg alone (10 mg/kg b.wt) increased moderately but statistically significantly the time spent in the conditioned compartment, indicating Mg induced its own action upon RS.

Naloxone at doses above 3 mg/kg b.wt is a competitive non-selective antagonist on μ, δ, κ receptors [33]. At lower doses it is a selective antagonist on the μ receptor [34, 35]. Naloxone failed to produce conditioned place aversion in μ-opioid receptor knockout mice [36]. A δ selective antagonist, naltrindole (10-30 mg/kg b.wt s.c.) failed to produce conditioned place aversion [36]. This means that despite naloxone activity (at higher doses) on opioid κ and δ receptors, only the blocking μ receptors are involved in naloxone-induced conditioned place aversion. At doses of 0.1-10 mg/kg b.wt day, naloxone might behave as an inverse agonist on μ receptors [37]. Regarding the aversive effect of naloxone, our data are in agreement with Veraksits et al., [38] and Solecki et al. [30], who showed a dose-dependent place aversion in mice at doses of 1-10 mg/kg b.wt i.p. Naloxone-MgCl₂ association does not change MgCl₂ induced place preference at 10 mg/kg b.wt i.p. (figure 3) despite μ receptors being blocked by naloxone. Our data regarding the aversive effect of naloxone 2 mg/kg b.wt in rats are in agreement with Solecki et al., [30]. This effect is explained by naloxone blocking of μ receptors and consequently suppressing the action of brain endorphins. This demonstrates the involvement of endogenous opioids in place preference and RS [39]. The doses of naloxone that we used during place preference experiments are similar to those used by other authors in place preference studies [32]. These might suggest that μ receptors are not involved in mediation of Mg (at 10 mg/kg b.wt MgCl₂)-induced place preference.

Glutamate enhances release and synthesis of dopamine [40]. Mg inhibits dopamine release [41] and decreases stimulation of morphine-induced stimulation of RS [42]. At the same time Mg has a calcium antagonistic effect on calcium channels linked with NMDA receptors [43]. A calcium channel blocker-nifedipine attenuates cocaine-induced place preference [44]. Mg, which also blocks Ca²⁺ channels coupled with NMDA receptors, also proved to decrease cocaine-induced place preference [45].

Mg is not an addictive substance, but stimulates RS. Our data show that only MgCl₂ 10 mg/kg b.wt i.p., increases CPP significantly. This effect on CPP was not observed at a MgCl₂ dose of 40 mg/kg b.wt i.p. This difference might be explained by the depressing effect upon the central nervous system and motor behavior of high Mg doses (40 MgCl₂ mg/kg b.wt) [46]. Associating naloxone with (MgCl₂ 10 mg/kg b.wt i.p.) decreased the naloxone-induced aversive effect.

In conclusion, MgCl₂ 10 mg/kg b.wt increased CPP in naïve animals, decreased the morphine-induced place preference but also the aversive effect of naloxone.

Disclosure

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References


