Interaction between the dopaminergic and opioidergic systems in dorsal hippocampus in modulation of formalin-induced orofacial pain in rats

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A B S T R A C T

The hippocampus is a region of the brain that serves several functions. The dopaminergic system acts through D1- and D2-like receptors to interfere in pain modulation and the opioid receptors play major roles in analgesic processes and there are obvious overlaps between these two systems. The present study investigated the interaction between the opioidergic and dopaminergic systems in the dorsal hippocampus (CA1) region for formalin-induced orofacial pain. Two guide cannulae were stereotaxically implanted in the CA1 region and morphine (0.5, 1, 2 and 4 μg/0.5 μl saline) and naloxone (0.3, 1 and 3 μg/0.5 μl saline) were used as the opioid receptor agonist and antagonist, respectively. SKF-38393 (1 μg/0.5 μl saline) was used as a D1-like receptor agonist, quinpirole (2 μg/0.5 μl saline) as a D2-like receptor agonist, SCH-23390 (0.5 μg/0.5 μl saline) as a D1-like receptor antagonist and sulpiride (3 μg/0.5 μl DMSO) as a D2-like receptor antagonist. To induce orofacial pain, 50 μl of 1% formalin was subcutaneously injected into the left side of the upper lip. Our results showed that different doses of morphine significantly reduced orofacial pain in both phases induced by formalin. Naloxone (1 and 3 μg) reversed morphine induced analgesia in CA1. SKF-38393 and quinpirole with naloxone (1 μg) significantly decreased formalin-induced orofacial pain in both phases. SCH-23390 had no effect on the antinociceptive response of morphine in both phases of orofacial pain. Sulpiride reversed the antinociceptive effects of morphine only in the first phase, but this result was not significant. Our findings suggest that there is cross-talk between the opioidergic and dopaminergic systems. Opioidergic neurons also exerted antinociceptive effects by modulation of the dopaminergic system in the CA1 region of the brain.

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1. Introduction

The hippocampus contributes to several major functions of the brain, including learning and memory, energy-intake regulation, reward-related mechanisms, and pain. (Davidson et al., 2007; Karami et al., 2002; Kenney and Gould, 2008; Khalilzadeh et al., 2010; Khanna et al., 2004). This region of the brain receives dopamine innervations from the ventral tegmental area (VTA) and substantia nigra (Scatton et al., 1980), and also includes high aggregation of opioidergic neurons and receptors (Drake and Milner, 1999). Dopamine is a catecholamine neurotransmitter which is involved in different activities through the D1- and D2-like receptors found in the dorsal region (CA1) of the hippocampus (Missale et al., 1998). The dopaminergic system has an antinociceptive effect with innervations on the different parts of the brain (Ansah et al., 2007; Meyer et al., 2009) that participate in pain modulation and transmission (Altier and Stewart, 1998; Burkey et al., 1999; Treister et al., 2009; Wood, 2008). The depletion of dopamine represses tonic pain in the nucleus accumbens (Altier and Stewart, 1998). D2 receptors have inhibitory effects on persistent pain (Morgan and Franklin, 1991) and also have a considerable role in tonic and dynamic modulations of pain in humans (Hagelberg et al., 2002). Rooney showed that D2 specific agonist can produce analgesia, but D1 agonist cannot (Rooney and Sewell, 1989). Patients who suffer from chronic orofacial pain have elevated D2/D3 receptor binding potential in their putamen in comparison with healthy control groups. Dang et al. suggest that the regulatory role of dopaminergic receptors in pain (Altier and Stewart, 1998; Hagelberg et al., 2003a,b) and that the dopaminergic system regulates persistent inflammatory pain. (Dang et al., 2011). Both D1- and D2-like receptors located in the dorsal hippocampus have antinociceptive effects on orofacial pain induced by subcutaneous injection of formalin (Shamsizadeh et al., 2013).

The opioidergic system plays a major role in analgesic processes that occur in the nervous system. Administration of morphine through the nucleus raphe magnus has an analgesic effect on orofacial pain (Duale et al., 2007). There is a fundamental overlap between opioidergic and dopaminergic effects (Nestler, 1996; Wood, 1983). Dopamine release could be facilitated in the nucleus accumbens (NAc) by the activity of

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opioidergic neurons in the VTA or NAc (Koob and Volkow, 2010). Both opioid and dopamine agonists modify cAMP embellishment and modulate GABA consignment (Self, 2004; Vaughan and Christie, 1997). Orofacial region sensory information is transmitted by the trigeminal nerve to higher regions like the trigeminal sensory nucleus, periaqueductal gray (PAG) and the hippocampus (Takemura et al., 2006). Orofacial pain stimulation was first performed by Clavelou (Clavelou et al., 1989, 1995), who injected 2% to 10% formalin subcutaneously through the upper lip of rats to induce orofacial pain. Face rubbing with the ipsi lateral forepaw was considered to be the response to formalin injection. Two acute and chronic phases of pain were measured in their tests (Dallel et al., 1995; Raboisson and Dallel, 2004). Studies have shown that intra-CA1 administration of D1- and D2-like receptor agonists can reduce orofacial pain-related behavior in rats (Shamsizadeh et al., 2013). The present study demonstrates the interaction between the opioidergic and dopaminergic systems located in CA1 region to formalin-induced orofacial pain.

2. Materials & methods

2.1. Animal

Adult male albino Wistar rats (Pasteur Institute, Tehran, Iran) weighing 230–280 g were used in these experiments. Animals were housed in groups of three per cage in a 12/12 h light/dark cycle (light on between 7:00 a.m. and 7:00 p.m.) with access to chow and tap water. The animals were randomly allocated to different experimental groups. Each animal was used only once. Rats were habituated to their new environment and handled for 1 week before the experimental procedure started. All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 80–23, revised 1996) and were approved by the Research and Ethics Committee of Shahid Beheshti and Rafsanjan Universities of Medical Sciences.

2.2. Drugs

In our study the following drugs were used: morphine sulfate (Temad, Tehran Iran), naloxone hydrochloride (Sigma), (+)-1-Phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol hydrochloride, SKF-38393, as a D1-like receptor agonist, R(+)−7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride, SCH-23390, as a D1-like receptor antagonist, 4aR,8aR)-5-propyl-4,4a,5,6,7,8,8a,9-octahydro-1H-pyrazolo[3,4-g]quinoline, quinpirole, as a D2-like receptor agonist, (±)-5-(aminosulfonyl)-N-(1-ethylpyrrolidin-2-yl)[methyl]-2-methoxybenzamide and sulpiride, as a D2-like receptor antagonist. Control animals received normal saline or 10% dimethyl sulfoxide, DMSO (Sigma-Aldrich, Germany) as a vehicle.

2.3. Stereotoxic surgery

Rats were anesthetized by intraperitoneal (i.p.) injection of Xylazine (10 mg/kg) and Ketamine (100 mg/kg), and were placed in the stereotoxic device (Stoelting, USA). Lidocaine with epinephrine (0.2 ml) was injected in several locations around the area that the incision was to be made. Our incisions were made along the midline, and the area surrounding the bregma was cleaned and dried following the retraction of the scalp. Stainless steel guide cannulae were stereotaxically implanted bilaterally into the CA1 region of the hippocampus. The coordinates for this region were determined by the rat brain atlas (Paxinos and Watson, 2007), AP = 2.8 mm caudal to bregma, Lat = ±1.5 mm lateral to midline, DV = 2.8 mm ventral from the skull surface (cannula 23-gauge, 11 mm in length, guide cannulae were 1 mm above the appropriate injection place). Jeweler’s screws and dental acrylic cement were applied for securing the cannulae. After the cement was completely dried and hardened, two stainless steel stylets were used to occlude the guide cannulae during recovery period. Penicillin-G 200,000 IU/ml (0.2–0.3 ml/rat, single dose, intramuscular) was administered immediately after surgery. Animals were individually housed and allowed to recover for 5–7 days before the experiments.

Microinjections were performed by 30-gauge injector cannulae (1 mm below the tip of the guide cannulae). Polyethylene tubing (PE-20) was used to attach injector cannula to the 1-μl Hamilton syringe. The animals were gently restrained by hand during the microinjection of the drug; the stylets were removed from the guide cannulae and replaced by 30-gauge injector cannulae. All drug (Morphine, Naloxone, SKF-38393, SCH-23390, Quinpirole and Sulpiride) solutions were administered slowly in a total volume of 0.5 μl over a period of 60 s. Injection needles were left in place for an additional 60 s to prevent the backflow of drugs.

2.4. Noceptive testing (orofacial formalin test)

Orofacial formalin test was performed according to the method described by Raboisson and Dallel (2004). A plexiglass observation chamber (30 × 30 × 30 cm) was prepared with a mirror based at 45° below the floor to allocate an unobstructed view of the experimental region. Rats were held about 30 min in chambers for adaptation, and then 50 μl of 1% diluted formalin injected into the left side of the upper lip subcutaneously just lateral to the nose. The rat returned into the observation chamber right after the formalin injection. Index of noiception was considered as a time each animal spent face rubbing with the ipsilateral forepaw. This time was recorded with a stopwatch in a repeated 3-min block over a period of 45 min. Two definite distinct phases have been induced in formalin injection. In this study, data obtained between 0 and 3 min post-formalin injection were considered as the first phase (early or acute phase) and data obtained between 15 and 33 min after the injection of formalin were epitomized as the second phase (late or chronic phase). All the observers were blind to the protocol of the study.

2.5. Experiment design

2.5.1. Effect of intra-hippocampal morphine on orofacial pain-related behaviors

To investigate the effect of intra-hippocampal morphine on orofacial pain, animals received four different doses of morphine (0.5, 1, 2 and 4 μg/0.5 μl saline) while the control group received normal saline in the dorsal hippocampus. Then, orofacial formalin test was performed and data was collected in two phases of this protocol. To demonstrate the effect of morphine through the μ-opioid receptor, animals received different doses (0.3, 1 and 3 μg/0.5 μl saline) of naloxone as a μ-opioid receptor antagonist 5 min post-administration of an effective dose of morphine. Formalin test was performed, subsequently.

2.5.2. Effect of intra-CA1 administration of D1/D2-like receptor antagonists on antinoceptive responses of morphine

In this set of experiments, to find out the role of D1- and D2-like dopamine receptors located in the CA1 on the antinoceptive response of morphine, effective doses of SCH-23390 (0.5 μg/0.5 μl saline) as a D1-like receptor antagonist or sulpiride (3 μg/0.5 μl DMSO) as a D2-like receptor antagonist were separately injected into the CA1, 5 min prior to the intra-CA1 administration of effective dose of morphine (2 μg). Acute and chronic phases of orofacial pain were measured by the formalin test. Animals received saline (0.5 μl/rat) instead of SCH-23390 or 10% DMSO (0.5 μl/rat) instead of sulpiride in the CA1 region in saline control group and morphine receiving group.

2.5.3. Effects of intra-CA1 administration of naloxone on antinoceptive responses induced by D1/D2-like receptor agonists

To determine a possible interaction between D1- and D2-like dopamine and opioid receptors in the CA1 during orofacial pain modulation, at first, effective dose of SKF-38393 (1 μg/0.5 μl saline) as a D1-like
receptor agonist or effective dose of quinpirole (2 μg/0.5 μl saline) as a D2-like receptor agonist was separately injected into the CA1, and acute and chronic phases of pain-related behavior (face rubbing) were measured by the orofacial formalin test. In the vehicle group, animals received saline into the CA1 region. In the next step, the effective dose of naloxone (1 μg) was microinjected into the CA1 region, 5 min prior to administration of SKF-38393 (1 μg/0.5 μl saline) or quinpirole (2 μg/0.5 μl saline), and then orofacial formalin test was performed and data was collected for the two phases of pain.

2.6. Histological verifications

After the tests were performed, the animals were deeply anesthetized with Ketamine and Xylazine, followed by transcardiac perfusion with 0.9% saline and 10% formalin solution. The brains were removed, blocked and sliced coronally in 50 μm sections through the cannula placements. The neuroanatomical location of cannulae tip placements were confirmed using rat brain atlas (Paxinos and Watson, 2007). Only the animals with correct cannulae placements were included in the data analysis.

2.7. Statistics

The face rubbing time in each block is expressed as mean ± SEM (standard error of mean). Data were processed by commercially available software GraphPad Prism® 5.0. The mean face rubbing time values in the early (0–3 min) and/or late (15–33 min) phases of orofacial pain responses in all groups were subjected to the one-way analysis of variance (ANOVA) followed by protected Newman–Keuls test for multiple comparisons. *P*-values less than 0.05 were considered to be statistically significant.

3. Results

3.1. Nociceptive behavior

Subcutaneous injection of normal saline in the upper lip of the rat produced slight pain in the first 3 min interval time. To induce orofacial pain, an in accordance to our previous study (Shamsizadeh et al., 2013), we injected diluted formalin subcutaneously in the upper lip and the typical pattern of face rubbing was observed to measure induced pain behaviors. Repeated measures ANOVA followed by Newman–Keuls multiple comparison test [F(14,104) = 16.96, *P < 0.0001] revealed that there is a significant difference in the face rubbing time (sec) among the 3rd, 18th–33rd min time set intervals compared to the other time blocks of the experiment after the injection of formalin; so there is a biphasic time course in the formalin-induced nociceptive behavior (Shamsizadeh et al., 2013), in the next experiments, we recorded the face rubbing time in the first 3-min period as phase 1, and the 15–33 min time interval as phase 2.

3.2. Effects of intra-hippocampal morphine on orofacial pain-related behaviors

In order to determine the effect of morphine on orofacial pain, animals received four different doses of morphine (0.5, 1, 2 and 4 μg/0.5 μl saline) while the control group received normal saline. One-way ANOVA followed by Newman–Keuls multiple comparison tests showed that 1, 2 and 4 μg doses of morphine significantly reduced the formalin-induced orofacial pain in the first phase [F(4,39) = 4.822, *P < 0.0033; Fig. 1, left panel] but in the second phase, 2 and 4 μg doses of morphine significantly reduced the formalin-induced orofacial pain [F(4,39) = 4.16, *P < 0.0073; Fig. 1, right panel]. There was no significant difference between the 0.5 μg dose of morphine and saline-treated groups. To clarify the effect of morphine through μ-opioid receptor, animals received different doses of naloxone (0.3, 1 and 3 μg/0.5 μl saline), as a μ-opioid receptor antagonist, post-administration of effective dose (2 μg) of morphine. One-way ANOVA followed by Newman–Keuls multiple comparison tests showed that 1 and 3 μg doses of naloxone significantly reduced the antinociceptive responses of morphine on orofacial pain in the first phase [F(4,39) = 4.1, *P < 0.0079; Fig. 2, left panel] and the second phase [F(4,39) = 6.091, **P < 0.0008; Fig. 2, right panel].

3.3. Effects of intra-CA1 administration of D1/D2-like receptor antagonists on antinociceptive responses of morphine

In this set of experiments, in order to find the role of D1- and D2-like dopamine receptors located in the CA1 on the antinociceptive responses of morphine, effective doses of SCH-23390 (0.5 μg/0.5 μl...
saline) or sulpiride (3 μg/0.5 μl DMSO) were separately injected into the CA1 region 5 min prior to the administration of morphine in CA1 (2 μg/0.5 μl). One-way ANOVA followed by Newman–Keuls multiple comparison tests showed that 0.5 μg dose of SCH-23390 and 3 μg dose of sulpiride have not had a significant effect on the antinociceptive responses of morphine on orofacial pain in the first phase [F (3, 31) = 2.721, P > 0.05; Fig. 3, left panel] and also in the second phase [F (3, 31) = 7.148, P < 0.001; Fig. 3, right panel].

3.4. Effects of intra-CA1 administration of naloxone on antinociceptive responses induced by D1/D2-like receptor agonists

In this set of experiment, the interaction between D1- and D2-like dopamine and opioid receptors in the CA1 on orofacial pain modulation was investigated. As shown in Fig. 4, the effective dose of SKF-38393 (1 μg/0.5 μl saline) and quinpirole (2 μg/0.5 μl saline) as a D1-like and D2-like receptor agonists significantly reduced the formalin-induced orofacial pain in the first phase [left panel] and the second phase [right panel]. For the next step, the effective dose of naloxone (1 μg) was microinjected into the CA1, 5 min prior to the administration of SKF-38393 (1 μg/0.5 μl saline) or quinpirole (2 μg/0.5 μl saline). One-way ANOVA followed by Newman–Keuls multiple comparison tests showed that 1 μg dose of naloxone significantly reduced the antinociceptive responses of SKF-38393 and quinpirole on orofacial pain in the first phase [F(4, 39) = 4.857, P < 0.0032; Fig. 4, left panel] and the second phase [F(4, 39) = 8.355, P < 0.0001; Fig. 4, right panel].

4. Discussion

The present study investigated possible interactions between the dopaminergic and opioidergic systems at the level of the dorsal hippocampus (CA1 region) on orofacial pain induced by subcutaneous injection of formalin. The major findings were: (a) Analgesic effects were observed after morphine administration in the CA1 region of the hippocampus; (b) Naloxone treatment reversed all analgesic effects of morphine in both phases; (c) None of the SCH-23390 and sulpiride as D1- and D2-like antagonists respectively reversed the analgesic effects of morphine significantly; (d) SKF-38393 and quinpirole as D1- and D2-like receptor agonists respectively produced analgesia and (e) Naloxone quashed the analgesic effects of SKF-38393 and quinpirole in both phases of pain. There was significant difference between the saline- and morphine-treated groups. Morphine administration produced analgesia in the acute phase of pain, demonstrating the involvement of opioid receptors in pain modulation in this region of the brain. Naloxone as an opioid receptor antagonist reversed analgesic effects of morphine which specified the role of the opioid receptor in the observed analgesic effects. There was no significant difference between morphine- and naloxone-treated groups. The analgesic effects observed in the orofacial test in the subnucleus oral for the administration of morphine were reversed with naloxone treatment (Luccarini et al., 1995). Rosenfeld and Stocco (1981) observed that significant analgesic effects from the administration of morphine through the nucleus reticular are paragigantocellular in the orofacial thermal test.

SCH-23390 as a D1-like receptor antagonist reversed the analgesic effects of morphine in the first phase of pain, but this effect was not significant. There was no significant difference between morphine-treated and sulpiride pre-treated groups, indicating that D2-like receptors may not suppress opioid analgesic effects. The results suggest that dopamine may have a higher affinity for D1-like receptors in this region. Only D1-like receptors were stimulated in this region in response to the decrease in the release of dopamine caused by morphine administration.

Flores et al. (2004) showed that the analgesic effect of morphine was attenuated by D1-like receptor blockade, but not with D2-like receptors in periaqueductal gray (PAG) region, in the hot plate test. D1-like receptor blockade had a similar impact on the analgesic effects of morphine that was not significant. D1 receptors significantly mediated opioid-induced antinociception with D2 receptors in CA1 (Flores et al., 2004). The antinociceptive effects of apomorphine were reversed by D2-like antagonist eticlopride, but not by SCH-23390 as a D1-like antagonist (Meyer et al., 2009). The obtained results raise the possibility that the D2-like receptor could directly affect the dopamine-induced antinociception while D1-like receptors had indirect modulating effect through opioids. Morgan and Franklin (1991) found that morphine...
and amphetamine, when injected systemically, produced analgesia. This effect was diminished by pretreatment with a dopaminergic antagonist, whereas the blockade of dopamine receptors couldn’t reverse the analgesic effects of morphine. Locomotor activity stimulated by intra-VTA administration of μ-opioid receptor agonists was suppressed by the blockade of dopamine receptors in the intra-nucleus accumbens septi (NAS) (Kalivas et al., 1983; Stinus et al., 1980). Morphine-induced analgesia in the formalin test was attenuated by NAS D2-like receptor blockade (Altier and Stewart, 1998).

In another phase, SKF-38393 and quinpirole as D1- and D2-like receptor agonists were administered to examine the role of dopamine receptors on pain modulation. There was a significant difference between the saline- and agonist-treated groups. Both SKF-38393 and quinpirole produced analgesic effects in the acute and chronic phases of pain. These results showed that the analgesic effect of dopamine receptor agonists was significantly reversed with the administration of naloxone. This interesting data may indicate that dopamine has no effect during the blockade of opioid receptors.

Several lines of evidence point to the role of dopamine neurons in antinociception (Lin et al., 1989; Magnusson and Fisher, 2000). Antinociceptive effects induced by conditioning noxious stimulation were reversed by dopamine receptor blockade (Gear et al., 1999). Di Chiara and Imperato (1988) have demonstrated that morphine boosts dopamine metabolism in antinociception (Lin et al., 1989; Magnusson and Fisher, 2000). Dopamine barricades the electrical activity of pain-excitatory neurons and improves pain-inhibitory neurons in opioid dependent rats (Zhang et al., 2012).

In summary, there is a definite interaction between the opioidergic and dopaminergic systems in formalin-induced orofacial pain at the level of the dorsal hippocampus. D1- and D2-like receptors had no significant impact on morphine analgesia, but the dopamine analgesic effect through the D1- and D2-like receptors was significantly reversed by opioid receptor blockade. This suggests that opioids enhance dopamine activities while dopamine does not have the same effect on opioids.

D1-like and D2-like receptors have different functions, but this study produced similar consequences. It may relate about the site of these receptors’ neuron circuits. According to study performed by Taniguchi et al. (2011), dopamine antinociceptive is mediated by the actions of both pre- and post-synaptic sites. Also Gao et al. (2000, 2001) stated that intrathecral administration of both D1- and D2-like agonists produced antinociception. There are evidences which proclaimed that intrathecal administrations of DA or D2-like receptor agonists had diminished pain induced by thermal stimuli (Barasi and Duggal, 1985; Liu et al., 1992). Further investigation is needed to clarify the exact mechanisms involve in the modulation of orofacial pain in this region.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.pbb.2014.06.015.

References


