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The role of nicotinic acetylcholine and opioid systems of the ventral orbital cortex in modulation of formalin-induced orofacial pain in rats

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ABSTRACT

Nicotinic acetylcholine and opioid receptors are involved in modulation of pain. In the present study, we investigated the effects of microinjection of nicotinic acetylcholine and opioid compounds into the ventral orbital cortex (VOC) on the formalin-induced orofacial pain in rats. For this purpose, two guide cannulas were placed into the left and right sides of the VOC of the brain. Orofacial pain was induced by subcutaneous injection of a diluted formalin solution (50 μl, 1.5%) into the right vibrissa pad and face rubbing durations were recorded at 3-min blocks for 45 min. Formalin produced a marked biphasic pain response (first phase: 0–3 min and second phase: 15–33 min). Epibatidine (a nicotinic receptor agonist) at doses of 0.05, 0.1 and 0.2 μg/site, morphine (an opioid receptor agonist) at doses of 0.5, 1 and 2 μg/site and their sub-analgesic doses (0.025 μg/site epibatidine with 0.25 μg/site morphine) combination treatment suppressed the second phase of pain. The antinociceptive effect induced by 0.2 μg/site of epibatidine, but not morphine (2 μg/site), was prevented by 2 μg/site of mecamylamine (a nicotinic receptor antagonist). Naloxone (an opioid receptor antagonist) at a dose of 2 μg/site prevented the antinociceptive effects induced by 2 μg/site of morphine and 0.2 μg/site of epibatidine. No above-mentioned chemical compounds affected locomotor activity. These results showed that at the VOC level, epibatidine and morphine produced antinociception. In addition, opioid receptor might be involved in epibatidine-induced antinociception, but the antinociception induced by morphine was not mediated through nicotinic acetylcholine receptor.

1. Introduction

Anatomical and physiological studies in animals, as well as functional imaging studies in humans have shown that multiple cortical areas including primary and secondary somatosensory, prefrontal, cingulate, motor and orbital cortices are involved in central processing of pain (Treede et al., 1999; Xie et al., 2009; Quintero, 2013). The Orbital cortex (OC) extends mediolaterally across the ventral surface of the rostral pole of the prefrontal cortex and participates in mediating maternal aggression, lateralization, reversal learning, obsessive–compulsive disorder and drug addiction (Schilman et al., 2008; Leite-Almeida et al., 2014; de Almeida et al., 2014). In addition, the OC is a multisensory receiving area involved in processing the physical and affective dimensions of sensory stimuli (Dalley et al., 2004; Schoenbaum and Roesch, 2005). Ventral orbital cortex (VOC) is a subdivision of OC and has important role in pain modulation (Hoover and Vertes, 2011). In spared nerve injury model of neuropathic pain in rats, c-fos expression was observed in the VOC (Leite-Almeida et al., 2014). The VOC is also involved in aversive aspect and descending inhibitory pathway of pain (Lowe et al., 2007).

Brain acetylcholine system, through nicotinic receptors, is involved in processing of many functions including depression, cognition, addiction and pain processing (Hurst et al., 2013; Lendvai et al., 2013; Leslie et al., 2013; Umana et al., 2013). The opioid system in the brain, through opioid receptors, mediates pain and analgesia, depression and various behaviors (Bodnar, 2013, 2014; Lutz and Kieffer, 2013). Possible interactions between nicotinic acetylcholine and opioid systems have been reported at the brain areas and nuclei such as nucleus accumbens, striatum, ventral tegmental area and medial prefrontal cortex (Vezina et al., 1992; Vihavainen et al., 2008).

Abbreviations: OC, orbital cortex; VOC, ventral orbital cortex; VLOC, ventrolateral orbital cortex; PAG, periaqueductal gray; LC, locus coeruleus; DRN, dorsal raphe nucleus

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Although there are no reports showing the involvement of the VOC acetylcholine and opioid systems in modulation of orofacial pain, Zaborszky et al. (2015) reported that the OC receives a cholinergic input from basal forebrain. Moreover, OC contains considerable densities of opioid receptors (Mansour et al., 1987) and the distribution of nicotinic acetylcholine receptor in this area has been reported (Mendez et al., 2013). In addition, Miyashita et al. (1994) reported reciprocal connections between OC and vibrissa motor cortex. Therefore, the present study was aimed to investigate the effects of microinjection of nicotinic acetylcholine and opioid compounds into the VOC on the nociceptive behavior induced by subcutaneous injection of formalin into the vibrissa pad in rats. We also examined the effects of the above-mentioned compounds on locomotor activity using an open-field test.

2. Materials and method

2.1. Chemical compounds

Epibatidine, mecamylamine and naloxone were purchased from sigma-aldrich (Sigma-Aldrich, Chemical Co., St. Louis, MO, USA). Morphine sulfate was obtained from TEMAD (TEMAD, Tehran, Iran). All drugs were dissolved in sterile normal saline prior to intra-VOC microinjection.

2.2. Animals

Healthy adult male Wistar rats (280–320 g) were used throughout the study. Rats were maintained in polyethylene cages with food and water ad libitum in a laboratory with controlled ambient temperature (22 ± 0.5 °C) and under a 12 h light-dark cycle (lights on at 07:00 h). All experiments were performed between 12:00 h and 16:00 h. The experimental protocol was approved by the Laboratory Animal Care and Use Center of the Faculty of Veterinary Medicine of Urmia University, and was performed in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

2.3. Surgical procedure

After a 15-day adaptation period, each rat was anesthetized with intraperitoneal injection of a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg), and then placed in a stereotaxic apparatus (Stoelting, Wood Dale, IL, USA). The scalp was incised, and the skull was leveled off around the bregma. Two 24 gauge, 13 mm stainless steel guide cannulas were bilaterally placed 1 mm over the right and left sides of VOC according to the following coordinates: 3.4 mm anterior to the bregma, 1.4 mm left and right sides of the midline and 5 mm below the top of the cortical surface (Paxinos and Watson, 2007). The cannulas were then fixed to the skull using three screws and dental acrylic (Acropars, Tehran, Iran). A 13 mm stylet was inserted to each cannula to keep them patent prior to microinjection. All animals were allowed 10 days to recover from surgery.

2.4. Intra-VOC microinjection

A 30-gauge, 14 mm injection needle attached to a 30 cm polyethylene tube fitted to a 1 μl Hamilton syringe was used for intra-VOC microinjections. The volume of the chemical compound solution to be injected into each VOC was 0.25 μl/site and the injection was slowly made over a period of 60 s. The injection needle was left in place for a further 60 s after the completion of the injection to facilitate the diffusion of the chemical compound. Normal saline (0.25 μl/site, control), epibatidine (a nicotinic receptor agonist) at doses of 0.025, 0.05, 0.1 and 0.2 μg/site and morphine (an opioid receptor agonist) at the doses of 0.25, 0.5, 1 and 2 μg/site were microinjected into the VOC. In addition, mecamylamine (a nicotinic receptor antagonist) and naloxone (an opioid receptor antagonist) at a same dose of 2 μg/site were also microinjected into the VOC. Co-microinjection of 0.025 μg/site epibatidine with 0.25 μg/site morphine was also performed. Prior microinjection of 2 μg/site mecamylamine and or naloxone was performed before 0.2 μg/site of epibatidine and 2 μg/site of morphine. Microinjections of antagonists (mecamylamine and naloxone) and agonists (epibatidine and morphine) were performed six and three min, respectively, before locomotor and or pain recordings. In the case of epibatidine plus morphine, a two min elapse time was considered between microinjections. The doses of chemical compounds and the treatment schedule used here were according to previous studies (Cucchiaro et al., 2005, 2006; Tamaddonfard et al., 2011).

2.5. Orofacial formalin test

Orofacial formalin test was performed according to the method described previously (Raboisson and Dallel, 2004). Each rat was placed in plexiglass observation chamber (30 cm × 30 cm × 30 cm) with a mirror mounted at 45° beneath the floor to allow an unobstructed view of the orofacial region. After a 30-min adaptation period, 50 μl of a diluted (1.5%) formalin solution was subcutaneously injected into the left vibrissa pad using a 29-gauge injection needle. Immediately following formalin injection, the rat was returned into the observation chamber. The time duration of face rubbing with ipsilateral forepaw was recorded (using a stopwatch), in consecutive 3-min blocks over a period of 45 min, and was considered as an index of nociception. A stereotyped response characterized by two well distinct phases was induced after subcutaneous injection of formalin in the orofacial region areas such as vibrissa pad and upper lip (Raboisson and Dallel, 2004; Erfanparast et al., 2010, 2014). In the present study, face rubbing durations between 0 and 3 min and between 15 and 33 min were considered as the first and second phases of pain, respectively (Erfanparast et al., 2010, 2014; Tamaddonfard et al., 2011; Donatti et al., 2014). All the observers were blinded to the protocol of the study.

2.6. Locomotor activity

Fifteen days after the end of pain study, locomotor activity was assessed using an open-field test as described previously (Markowska and Lukaszewska, 1981; Hamzeh-Gooshchi et al., 2015). The apparatus consisted of a wooden box measuring 120 cm × 120 cm × 50 cm. The floor of the arena was divided into 16 equal squares. To monitor the activity, animals were removed from the home cage and placed directly into one corner of the open field apparatus. The number of squares crossed with all paws (line-crossings) and the number of rearing were counted in a 5-min session.

2.7. Cannula verification

At the end of each experiment, anesthetized rats were intracardially perfused with physiological saline followed by 10% formalin solution. The brains were removed and placed in a formalin solution (10%). One week later, thin transverse sections (5–10 μm) were provided and stained with Hematoxylin and Eosin. The sections were viewed under a light microscope to localize the microinjection site according to the atlas of Paxinos and Watson (2007). The results obtained from seven rats with guide cannulas outside the VOC were eliminated from the data analysis.