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Ventral hippocampal nicotinic acetylcholine receptors mediate stress-induced analgesia in mice

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ABSTRACT

Evidence suggests that various stressful procedures induce an analgesic effect in laboratory animals commonly referred to as stress-induced analgesia (SIA). The aim of the present study was to assess the role of ventral hippocampal (VH) nicotinic acetylcholine receptors (nAChRs) in SIA in adult male NMRI mice. The VHs of animals were bilaterally cannulated and nociceptive threshold was measured using infrared source in a tail-flick apparatus. Acute stress was evoked by placing the animals on an elevated platform for 10, 20 and 30 min. The results showed that exposure to 20 and 30 min acute stress produced analgesia, while exposure to 10 min stress had no effect on the pain response. Intra-VH microinjection of nicotine (0.001–0.1 μg/mouse), 5 min before an ineffective stress (10 min stress), induced analgesia, suggesting the potentiative effect of nicotine on SIA. It is important to note that bilateral intra-VH microinjections of the same doses of nicotine without stress had no effect on the tail-flick test. On the other hand, intra-VH microinjection of mecamylamine (0.5–1 μg/mouse) 5 min before 20-min stress inhibited SIA. However, bilateral intra-VH microinjections of the same doses of mecamylamine without stress had no effect on the tail-flick response. In addition, the microinjection of mecamylamine into the VH reversed the potentiative effect of nicotine on SIA. Taken together, it can be concluded that exposure to acute stress induces SIA in a time-dependent manner and the ventral hippocampal cholinergic system may be involved in SIA via nAChRs.

1. Introduction

Stress, induced via various sources, is an inseparable part of our life. Acute, sequential, episodic, chronic, intermittent, sustained, and anticipated (Sapolsky et al., 2000) are some examples of the kinds of stressors that impair our bodily homeostasis (for a review see Chrousos, 2009). Stressors trigger physiological and psychological responses from the body (Armario et al., 2004; Kimura et al., 2007). Several attempts have been made to show that stress responses can be affected by several factors such as the intensity, duration and type of the stressor (Greenberg et al., 2002). Exposure to stress can lead to the activation of the hypothalamic–pituitary–adrenal (HPA) axis and the release of corticosteroids (De Kloet et al., 2005). Effects of corticosteroids are mediated via the mineralocorticoid (MR) and glucocorticoid (GR) receptors (Groeneweg et al., 2012) which activate appropriate hormone-responsive genes (Datson et al., 2001).

Ample evidence suggests that acute stress increases the pain threshold and induces a type of analgesia called stress-induced analgesia (SIA; for a review see Butler and Finn, 2009). Endogenous opioid peptides such as endorphins modulate the HPA axis and mediate SIA (for a review see McEwen and Kalia, 2010). In view of the fact that systemic or intra-cerebral administration of opioid receptor antagonists inhibits SIA (Rizzi et al., 2001), it seems that endogenous opioid peptides and their corresponding receptors in different brain regions play a more significant role in the expression of SIA. Considering that SIA depends on sensitivity to opiate antagonists, it can be categorized into two types: opioid and non-opioid mediated SIA. It has been suggested that acute stressors can produce opioid and non-opioid SIA, respectively (Lafrance et al., 2010; Hough et al., 2014).

The critical involvement of the cholinergic system in the modulation of pain has been previously identified (Jones and Dunlop, 2007). Nicotinic acetylcholine receptors (nAChRs) have an important role in analgesic response and nociception (Wang et al., 2005). The activity of the HPA axis and the increase of corticosterone level have been determined in nicotine-induced analgesia (Yamamoto et al., 2011) which can be prevented by the blockade of nAChRs (Bugajski et al., 2002). The blockade of hippocampal cholinergic system has been

Abbreviations: Ach, acetylcholine; ACTH, adrenocorticotropic hormone; ANOVA, analysis of variance; AUC, areas under curves; CRH, corticotropin-releasing hormone; GR, glucocorticoid receptor; HPA, hypothalamic–pituitary–adrenal; MR, mineralocorticoid receptor; %MPE, percentage of maximal possible effect; nAChRs, nicotinic acetylcholine receptors; SEM, standard error of mean; SIA, stress-induced analgesia; TFLs, tail-flick latencies; VH, ventral hippocampus.

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found to potentiate stress induced-HPA activity (Bhatnagar et al., 1997). According to the high expression of hippocampal GR and MR receptors (Han et al., 2005), it seems that the hippocampus can be affected by stress (Hirata et al., 2009). Moreover, exposure to restraint stress leads to the increase of ACh release in the hippocampus (Degroot et al., 2004). In view of (1) the involvement of nicotinic cholinergic systems in modulating pain, (2) the high expression of nAChRs in the ventral hippocampus (VH; Huang and Winzer-Serhan, 2006), and (3) the role of the VH in both pain (Al Amin et al., 2004) and stress (Ergang et al., 2014), the aim of the present study was to investigate the effects of intra-VH microinjections of nAChR agonist and antagonist on SIA, and also to assess the role of the VH nAChRs in the relationship between stress and pain.

2. Materials and methods

2.1. Animals

The experiments were conducted using adult male NMRI mice (Pasteur Institute, Iran) weighing 20–25 g. They were kept eight per plastic cages and maintained under standard laboratory conditions (temperature of 22 ± 2 °C,12/12-h light/dark cycle) and had ad libitum access to food and water at all times except during the experiments. The animals were adapted to laboratory conditions for 5 days prior to the surgery. Experiments were done between 8:00 a.m.–14:00 p.m. Each animal was used once only and eight animals were used in each experiment. The animals were acclimated to the testing room prior to the experiments. All procedures for the treatment of animals were approved by the Research and Ethics Committee of the School of Biology, University of Tehran and were done in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH publications no. 80–23). Moreover, all efforts were made to minimize the number of animals used and their suffering.

2.2. Surgery and microinjection procedures

Mice were anesthetized with intraperitoneal administration of ketamine hydrochloride (50 mg/kg) plus xylazine (5 mg/kg). The ventral hippocampi were bilaterally cannulated according to the atlas of Paxinos and Franklin (2001) from −2.8 mm AP, 3 mm lateral to midline, 3 mm ventral. The cannula tip was1 mm above the microinjection site and was secured to the skull with dental cement. After the surgery, the mice were placed into the cage and were allowed to recover from the surgery for 5 days, before testing.

The microinjections into the ventral hippocampus (intra-VH) were bilaterally performed with a 0.5-μl solution in each side. The solution was injected slowly (over 1 min) and the cannula was left in place for an additional 60 s to avoid the backflow of the solution. During the microinjections, the animals were held gently by hand. The movement of an air bubble inside the polyethylene tubing connecting the microsyringe (2-μl Hamilton microsyringe) to a dental needle confirmed the drug flow. The microinjections were performed in the test room.

2.3. Drugs

Nicotine hydrogen tartrate (Sigma, USA) and mecamylamine (a nicotinic receptor antagonist, Sigma, USA) were used in this study. Mecamylamine was dissolved in sterile 0.9% saline and nicotine was dissolved in sterile saline and then the pH of the solution was adjusted to 7.2–7.4 with NaOH (0.1 normal solution). The nicotine doses are expressed as the salt weight. Nicotine and mecamylamine were injected into the VH at a volume of 1 μl/mouse.

2.4. Apparatus

2.4.1. Tail-flick

Pain threshold was assessed using a tail-flick analgesia meter (Borj Sanat Company, Iran). The tail was divided to 4 segments and marked with marker. Each mouse was fixed with one hand and the tail was positioned in the apparatus. Radiant heat from a bulb was focused on each segment from the tail distal end. The time from the onset of the thermal stimulus to withdrawal of the tail from the heat source was recorded. A cut-off time of 10 s was used to reduce the probability of skin damage. Animals were tested every 15 min for a 60 min period. Antinociception measurements were made before and after stress and/or drug treatment. It is important to note that mice were gently restrained with a soft towel and placed on the tail-flick apparatus to get habituated to the procedure 5 days before testing. Tail-flick latencies (TFLs) are expressed as the percentage of maximal possible effect (%MPE) which can be calculated using the following formula: Post-drug latency (s) − Baseline latency (s) / Cut-off value (10 s) − Baseline latency (s) × 100. Linear trapezoidal method was used to calculate the area under the curve (AUC) of MPE% for each group of animals in the tail-flick test (Heinzen and Pollack, 2004).

2.4.2. Elevated platform

To induce acute stress, a circular elevated platform (100 cm high, 20 cm in diameter) in the middle of a brightly lit room (300 lx) was used. Animals were picked up and placed on an elevated platform for different times (10, 20 and 30 min). During this period, the animals showed the behavioral signs of stress such as immobility for up to 10 min, urination and defecation.

2.5. Experimental design

2.5.1. Experiment 1

In four groups of animals, acute stress was induced by placing the mouse on the elevated platform for 10, 20 and 30 min. 15 min after the stress, the analgesic response was assessed with a tail-flick apparatus every 15 min for a 60 min period. One group did not receive stress and acted as a control group (Fig. 2).

2.5.2. Experiment 2

Four groups of animals received intra-VH microinjections of different doses of nicotine (0, 0.001, 0.01 and 0.1 μg/mouse) and after 5 min they were placed on the elevated platform for 10 min. After stress exposure, TFL of each animal was tested every 15 min for a 60 min period (Fig. 3).

2.5.3. Experiment 3

Different doses of nicotine (0, 0.001, 0.01 and 0.1 μg/mouse) were injected into the VH in all animals in four groups of animals. After drug treatment, TFL of each animal was tested every 15 min for a 60 min period (Fig. 4).

2.5.4. Experiment 4

Four groups of animals received intra-VH microinjections of different doses of a non-subtype selective nAChR antagonist, mecamylamine (0, 0.5, 0.7 and 1 μg/mouse) and after 5 min they were placed on the elevated platform for 20 min. After stress exposure, TFL of each animal was tested every 15 min for a 60 min period (Fig. 5).

2.5.5. Experiment 5

Four groups of animals received intra-VH microinjection of mecamylamine (0, 0.5, 0.7 and 1 μg/mouse). After drug treatment, TFL of each animal was tested on every 15 min for a 60 min period (Fig. 6).