Pharmacology, Biochemistry and Behavior 103 (2012) 299–307

Contents lists available at SciVerse ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Antagonism of orexin-1 receptors attenuates swim- and restraint stress-induced antinociceptive behaviors in formalin test

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ARTICLE INFO

Article history:
Received 14 June 2012
Received in revised form 12 August 2012
Accepted 13 August 2012
Available online 19 August 2012

Keywords:
Stress-induced analgesia
Swimming stress test
Restraint stress test
Formalin test
SB-334867
Naloxone
Rat

ABSTRACT

Orexin (ORX) plays an important role in pain modulation. ORX receptors have been found in many brain structures and are known to be involved in pain processing. It is well-established that the acute and chronic forms of stress could induce hormonal and neuronal changes that affect both pain threshold and nociceptive behaviors. The role of OX1R receptors in stress-induced analgesia (SIA) has not been fully elucidated. In the present study, using the formalin test, attempts were made to evaluate the effects of acute immobilization restraint stress and swimming stress on pain behavioral responses following OX1R antagonist administration in rats. Animals received OX1R antagonist (SB-334867), vehicle, or naloxone before exposure to acute restraint stress (30 min) or swimming stress test (6 min, 20±1 °C), and immediately submitted to hind paw formalin injection (50 μl, 2%). Acute 30-min exposure to restraint stress as well as 6-min exposure to swim stress could significantly reduce the formalin-induced nociceptive behaviors in rats. This antinociceptive effect with either restraint stress or swim stress was fully prevented by OX1R antagonist (SB-334867), while the SB-334867 alone had no effect. However, the opioid receptor antagonist naloxone could not totally reverse the antinociception effect with either form of stress. It is suggested that OX1R might be involved in antinociception behaviors induced by these two forms of stress. These data highlight the significant role of OX1R as a novel target for treatment of stress-related disorders.

1. Introduction

Stress is shown to activate multiple neural and neurotransmitter systems involved in suppression of pain sensation (Madden et al., 1977; Guillemin et al., 1977; Bodnar et al., 1980a). This phenomenon, known as stress-induced analgesia (SIA), is activated by endogenous pain inhibitory systems (Madden et al., 1977; Guillemin et al., 1977; Bodnar et al., 1980a). Regarding the stress, the opioid and non-opioid forms of SIA have been elicited in rodents (Madden et al., 1977; Bodnar et al., 1980a). Acute stress initiates a cascade of neuronal and hormonal changes and induces analgesia in animals and humans (Amit and Galina, 1986; Ford and Finn, 2008). It has been reported that blocking the endogenous opioid system with naloxone or naltrexone attenuates the nociceptive behavioral responses following exposure to stress, indicating that the endogenous opioid system is involved in SIA (Amit and Galina, 1986). Systemic or intracerebroventricular (i.c.v.) injection of μ-, κ-, or δ-opioid receptor antagonists is demonstrated to prevent the SIA or fear-conditioned analgesia in rat (Akil et al., 1986; Fanselow et al., 1989; Butler and Finn, 2009).

Stress was shown to affect the brain activity and promote the long-term changes in multiple neural systems (McEwen, 2000; Imbe et al., 2004). Considering the features of stressor (such as duration, intensity, and temporal aspects of the same stressor) applied, the nature of analgesic response might be different (Watkins and Mayer, 1986; Amit and Galina, 1986). In some stressful situations, the blockade of endogenous opioid system is reported to lack the potential to completely reverse the SIA and this supports the idea that the activation of endogenous neurotransmitters and neuropeptides, as non-opioid mechanisms, may be involved in SIA (Lewis et al., 1980; Watkins and Mayer, 1982). There are evidences that OX1R is involved in responsiveness to both pain and stressful stimuli and, therefore, it may be involved in SIA (Watanabe et al., 2005; Sofi-Abadi et al., 2011). For example, ORX knockout mice were reported to present greater degree of hyperalgesia and lower SIA caused by peripheral inflammation than that observed in wild type mice (Watanabe et al., 2005). It has been shown that administration of orexin-A (ORXA) produces naloxone-insensitive analgesic effects by acting on spinal and/or supraspinal OX1R (Jeong and Holden, 2009). Also, it is documented that the intrathecal administration of

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http://dx.doi.org/10.1016/j.pbb.2012.08.007

ORXA inhibits pain perception in behavioral tests (Bingham et al., 2001; Yamamoto et al., 2002).

Furthermore, the data from a previously published study have confirmed that ORXA administration produces the analgesic effects in the hotplate, tail-flick, and formalin tests in mice, although these effects were not reversed by naloxone (Mobarakeh et al., 2005). Therefore, it was hypothesized that ORX possibly mediates the non-opioid SIA through OX1R activation. The present study was designed to investigate the possible role of OX1R on stress-induced antinociception. In this study, attempts were made to examine the effect of ICV injection of OX1R antagonist (SB-334867) on behavioral manifestations of animal pain during formalin test in rat as an animal model of tonic pain after acute exposure to either restraint or swim stress.

2. Materials and methods

2.1. Subjects

Adult Sprague–Dawley rats (220–300 g) were purchased from Razi institute (Karaj, Iran). Animals were housed in groups of three rats per cage at temperature controlled room, under a 12 h light–dark cycle with lights on from 7:00 to 19:00. Food and water were provided. All experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23, revised 1996) and were approved by the Research and Ethics Committee of Qazvin University of Medical Sciences, Qazvin, Iran.

2.2. General procedure

Rats were initially anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg) and later, a 23-gauge, 3 mm-long stainless steel guide cannula was stereotaxically (Narishige, Japan) lowered 2 mm above the lateral ventricle by applying coordinates from the atlas of Paxinos and Watson (2005): incisor bar −3.3 mm, 0.9 mm posterior to the bregma, 1.8 mm lateral to the sagittal suture and 3.8 mm down from top of the skull. Direct ICV administration of drug or respective vehicle was performed by implanting the guide cannula 7 days before the experiments. The cannula was anchored with dental cement to stainless steel screws in the skull. Immediately after waking up from surgery, rats were returned to their home cages. On the day of experiment, rats were transferred to individual experimental room and allowed to acclimatize for 60 min before drug injection. Direct ICV administration of drug or respective vehicle was conducted with a stainless steel cannulae (30-gauge; 0.3 mm outer diameter) connected through a polyethylene tube to a Hamilton syringe, inserted through the guide cannula and extended 2 mm beyond the tip of the guide cannula to reach the ventricle. A volume of 5 μl of drug or vehicle was injected into the ventricle over a period of 60 s and the injection cannula was gently removed 1 min later. After performing stress procedures (30 min in restraint stress and 6 min in swim stress) and drying of animals only in swim stress model, formalin was injected into the plantar surface of the right hind paw, using a disposable insulin syringe with a fixed 30-gauge needle.

2.3. Drugs

Two percent formalin (formaldehyde, Temad, Iran) was prepared in sterile physiological saline solution (Soha, Iran). Naloxone (Sigma-Aldrich) was dissolved in saline. An OX1R antagonist, SB-334867 (N-(2-Methyl-6-benzoxazolyl)-N″-1,5-naphthyridin-4-yl urea; molecular weight = 356, Tocris), was dissolved in dimethyl sulfoxide (DMSO) and diluted in saline on the day of experiment (DMSO was diluted 1/100 in 0.9% w/v saline solution).

2.4. Restraint stress and swim stress test procedure

Rats were habituated to animal house and experimental room used for stress protocol and formalin test on a daily basis. In the group that received restraint stress, after drug or vehicle injection, rats were placed into restrainer for 30 min, and then formalin was injected into the plantar surface of the right hind paw. In the group that received swim stress, after drug or vehicle injection, rats were placed in a plastic pool (50 cm high) filled with water and maintained at 20 ± 1 °C for 6 min. The water was clear and 2 rats were simultaneously used for swim stress paradigm. Animals were thoroughly dried within 10 min, and then formalin was injected into the plantar surface of the right hind paw. All experiments were carried out during working hours (8 AM to 2 PM). Restraint stress was performed by placing the animals into 25 cm × 6 cm Plexiglas tubes adjusted with a piston to minimize rat movement.

2.5. Formalin test

Rats were moved to the test room at least 1 h before starting the experiment. Formalin tests were performed in clear plastic boxes (30 × 30 × 30 cm) with a mirror placed underneath at a 45° angle to allow an unimpeded view of the animals' paws. In the present study, rats were first acclimatized for 30 min in an acrylic observation chamber and then formalin (50 μl; 2%) was injected subcutaneously into the plantar surface of the right hind paw with a 30 gauge needle. To ensure stable scores from formalin, it was necessary to make sure that the needle was inserted through the skin and run for 5 mm under the skin. Subsequently, each rat was immediately returned to the observation box, and behavioral recording commenced. Pain behaviors were scored as follows: 0, the injected paw was not favored; 1, the injected paw had little or no weight placed on it; 2, the injected paw was elevated and not in contact with any surface; and 3, the injected paw was licked or bitten. Recording of nociceptive behaviors began immediately after formalin injection (time 0) and was continued for 60 min. The score obtained from nociceptive behaviors for each 3 min interval was calculated as the weighted average of the number of seconds spent in each behavior, from the start of the experiment (Azhdari Zarmehri et al., 2011). The scores were recorded in normal rats as well as in those received stress. In each group, the behavioral responses of each rat during the first (1–7 min), interphase (8–14 min), and the second phases (15–60 min) were separately evaluated. The SB-334867, as OX1R antagonist, naloxone, or respective vehicle was given 5 min before each stress test.

2.6. Experimental protocols

The formalin test was the experimental procedure used in our study to examine the effect of antagonizing OX1R on SIA. Three sets of experiments in the formalin test were considered: (1) Rats were given formalin injection following performing either of the stress procedures (restraint stress, 30 min or swim stress test, 6 min at 20 ± 1 °C), (2) Rats were ICV microinjected SB-334867 (0.1 mM/5 μl DMSO) or 1% DMSO followed by formalin injection after exposing the animals to either of the stress protocols (3) Rats were first given naloxone (3 mg/kg; i.p.) or saline (i.p.) followed by formalin injection shortly after exposing the animals to either of the stress protocols.

2.7. Histology

At the end of the experiments, rats were deeply anesthetized with overdose of ketamine and xylazine followed by injecting a volume of 0.5 μl pontamine sky blue (0.2%) into the site 10–20 min before sacrificing the animals. Later, rats were transcardially perfused with 100 ml of 4% formalin solution; the brain was removed and sectioned. Only those