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Administration of orexin receptor 1 antagonist into the rostral ventromedial medulla increased swim stress-induced antinociception in rat

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ABSTRACT

Objective(s): Intracerebroventricular injection of orexin-A (hypocretin-1) antagonist has been shown to inhibit stress-induced analgesia. However the locations of central sites that may mediate these effects have not been totally demonstrated. This study was performed to investigate the role of rostral ventromedial medulla (RVM) orexin receptor 1 in stress-induced analgesia (SIA).

Materials and Methods: Forced swim stress in water was employed to adult male rats (200-250 g). Nociceptive responses were measured by formalin test (50 µl injection of formalin 2% subcutaneously into hind paw) and, pain related behaviors were monitored for 90 min following intra-microinjection of SB-334867 (orexin receptor 1 antagonist) into RVM.

Results: Exposure to swimming stress test after administration of SB-334867 into RVM significantly reduces the formalin-induced nociceptive behaviors in phase1, interphase, and phase 2 in rats.

Conclusion: The result demonstrated the involvement of OXR1 in antinociceptive behaviors induced by swim stress in RVM.

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Introduction

Pain inhibition reaction that happens after exposure to a stressful situation called stress-induced analgesia SIA (1). Experimental animal models of this phenomena help to find potential therapeutic agent for disorders associated to pain and stress (1). Stress is shown to activate a neural and hormonal cascade that induced analgesia in animals and humans (2, 3). Analgesic responses to stress are mediated by opioid and non-opioid mechanisms (1, 4). It has been shown that blocking of endogens opioid system induced SIA, supporting that endogenous opioid system is involved in SIA. Some experiments have been shown that blockade of endogens opioid system cannot reverse SIA, it may be concluded that non-opioid mechanisms might be involved in SIA regulation (1, 5, 6). Consist with this, it has been shown that hypocretins 1 and 2 (orexin A and B) is synthesized by neurons in lateral hypothalamus and this neuropeptide represent a significant role in pain transmission (1, 7, 8). orexinergic has broad anatomical projections within the central nervous system which suggest widespread functions for orexin such as feeding, sleep-wake cycle, cardiovascular function, hormone secretion(8-11) and recently pain modulation and tolerance and dependence to morphine (12-19). Evidences supports that orexin A and B are involved in nociceptive processing hence orexin distribution in PAG (periaqueductal gray matter), raphe nuclei, locus coeruleus and superficial and deep layers of spinal and trigeminal dorsal horn(20, 21).

Rostral ventromedial medulla (RVM) (includes the raphe nuclei and adjacent reticular formation) can be viewed as the brainstem output for pain modulation system (PAG-RVM) (22, 23). In addition, several studies indicated that non opioid neuropeptide (orexin) in RVM has some roles in pain modulations (24, 25). In this study, we were interested to investigate the role of RVM OX1 receptors in modulation of SIA.

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Materials and Methods

Subjects

Adult male Wistar rats (200-250 g) were housed in temperature controlled cages, under a 12 hr Light/dark cycle with light on at 7 am to 7 pm and given ad libitum food and water. All behavioral tests started at approximately the same time each day. We did all experimental procedures followed the guidelines of the committee for Research and Ethical Issues of the International Association for the Study of Pain. In addition, experimental protocols were approved by animal ethic committee of Rafsanjan University of Medical Sciences.

Drugs

Two percent formalin (37% formaldehyde, Temad, Iran) was diluted in sterile physiological saline solution (Soha, Iran). SB-334867 (N- (Methyl-6-benzoxazolyl-N"-1,5- naphthyridin-4-yl urea; molecular weight = 356, Tocris) as an orexin1 receptor (OX1R) antagonist, 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 solution). Two different doses of SB-334867 (0.1 mmol and 1 mmol) were used in this study.

General procedure

Initially rats were anaesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg) and then animal, s head was shaved and cleaned after that rat was placed in surgery apparatus, skin was cut and connective tissues removed. For direct Intra-RVM administration, we used standard stereotaxic equipment, stainless and cannula (23 Gauge) was implanted according to coordinates from the atlas of Paxinos and Watson (anteroposterior -11.0 mm from bregma, lateral 0 from midline, dorsoventral -8.5 mm from the cranium) (26). The guide cannula was anchored to two screws in skull by dental cement.

Testing began 7 days following surgery and rats were transferred to experimental room 60 min before drug injection to get used to new situation. Stainless steel cannula (30 Gauge, 0.3 mm outer diameter) as injection cannula that connected through a polyethylene tube to Hamilton syringe, inserted through the guide cannula and extended 2 mm beyond the tip of the guide cannula to reach the RVM. Microinjection was in a volume of 0.5 µl while the rat was gently restrained by hand over a period of 60 sec. Injection cannula removed 1 min later.

Testing was followed by stress procedures (6 min forced swim stress) and drying animals and then (not later of 10 min) formalin was injected into planter surface of right hand paw to induce nociception.

Swim stress test procedure

Firstly rats were transferred to experimental room to habituate and then swim stress occurred immediately after drug or vehicle injection. For swim stress, rats were forced to swim in a plastic pool containing 50 cm of water at 20 ºC during 6 min. Immediately rats were dried to follow the testing (27).

Formalin test

Before the commencement of this part of testing, to minimize environmental stress, rats were acclimatized for 30 min in formalin box. During the test, temperature was maintained at 24-26 °C. Then formalin 2٪ was injected subcutaneously into the planter surface of the right hind paw. Formalin injected was in volume of 50 µl with a 30 Gauge needle. Immediately rats returned to Plexiglas formalin box that is measured 34×34×34 and a mirror below the floor of box with angle 45º to permit observing of rats nociceptive responses. The formalin test was carried out according to weighed scores or rating scale method. Nociceptive were rated as followed: 0, the injected paw was not favoured; 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 licking or biting of the injected paw. The score obtained from nociceptive behaviours for each 3 min interval was calculated as the weighted average of the number of seconds spent in each behaviour, from the beginning of the experiment. In each group, the behavioural responses of each rat was evaluated during 90 min that divided to the first phase (1-7 min), inter-phase (8-14 min) and the second phases (15-90 min) (28, 29).

Experimental protocols

Three sets of experiments in this study were considered: 1) Rats were just given formalin injection as control group and in sham group, animals (after stereotaxic surgery) were given formalin 6 min after exposing to swim stress 2) Rats were RVM microinjected drugs (SB-334867 / saline) followed by formalin injection. 3) Rats first had RVM microinjected drugs (SB-334867 /saline) then performed forced swim stress (for 6min) and then received formalin.

Histology

At the end of the experiment rats were deeply anaesthetized with overdose of ketamine followed by injecting a volume of 0.5 µl of pontamine sky blue (0.2%) into the cannula site. Later, rats were transcardially perfused with 100 ml of 4% formalin solution and then animals sacrificed. The brains were removed and sectioned. Only those rats whose microinjection and diffusion site were located within the RVM were included in the results (29).

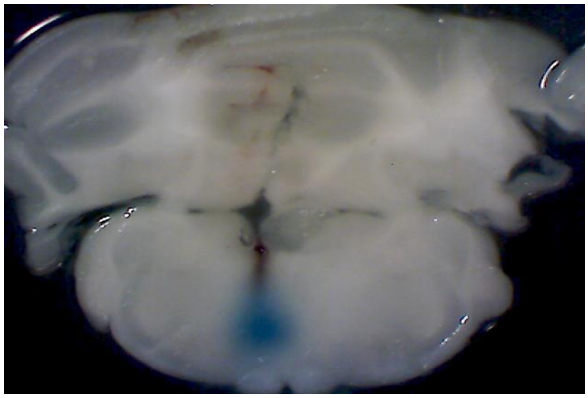

Figure 1. Histological verification of RVM (Rostral Ventromedial Medulla) cannula placement

Statistics

Data were presented as mean±SEM. The formalin pain score in all groups were analyzed by one-way analysis of variance followed by Dunnett's test for multiple comparisons as needed. The first phase (1–7 min), interphase (8–14 min), and second phase (15-90 min) of the formalin test were analyzed separately while using one time point for each phase: a time course of 7 min for phase 1 and interphase, and a 75 min-duration for phase 2. The defined level for statistical significance was (P<0.05).

Result

Nociceptive behaviors following injection of formalin into rats' hind paw

Injection of formalin produced typical biphasic pain responses that were monitored for 90 min (Figure 2). The first and second phases were separated by brief interphase with little nociceptive behavior. The second phase was further subdivided into two sub phases (phase 2a and phase 2b).

The effect of SB-334867 (0.1 and 1 mmol) microinjected into RVM on formalin-induced nociceptive behaviors

To evaluate the effect of SB-334867 on formalin test, we microinjected 2 different doses of SB-334867 (0.1 mmol and 1 mmol) into RVM and followed by hind paw formalin injection (Figure 1). Both doses of SB-334867 failed to produce significant effect on formalin induced nociceptive behaviors in the phase 1, interphase, phase 2A and phase 2B (P>0.5).

The effect of swim stress on nociceptive behaviors in formalin test

To evaluate the effect of swim stress on formalin induced nociceptive responses, swim stress was applied for 6 minutes before formalin injection. Results demonstrated that swim stress could attenuated nociceptive behaviors in the phase1 and phase 2A (all P<0.05) and in interphase (P<0.01) of formalin test. However, in phase 2B, Appling swim stress potentiated nociceptive responses in formalin test (P<0.01) (Figure 3 and 4).

The effect of 0.1 mM SB-334867 microinjection into RVM on antinociceptive behaviors induced by swim stress in formalin test

Intra-RVM microinjection of SB-334867 (0.1 mmol) before exposing the animals to swim stress could attenuate formalin induced nociceptive behaviors in phase1 (P<0.05), phase 2A (P<0.01) and phase 2B (P<0.05) compared to the group that received saline and then exposed to swim stress (swim stress group). However, nociceptive behaviors were not affected by SB-334867 (0.1 mmol) in the interphase of formalin test (P>0.05) (Figure 3).

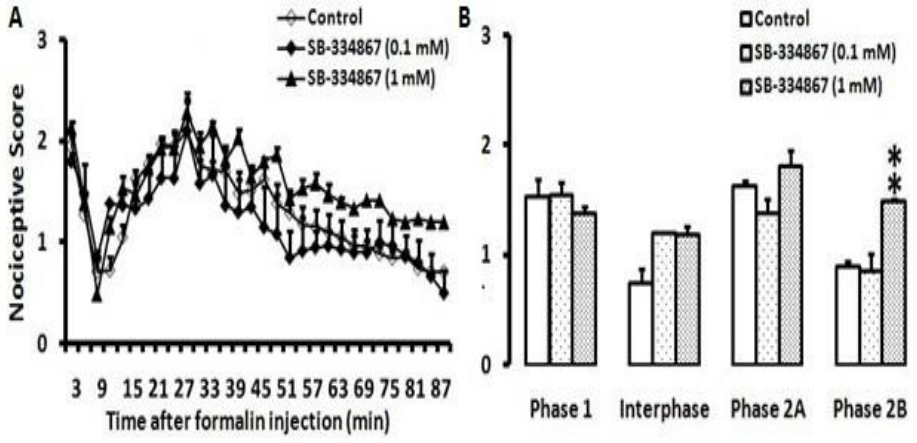

Figure 2. (A) Time-dependent curves of nociceptive behaviors induced by formalin (mean±SEM. of 9-10 rats per group) measured every 3 min for 90 min. (B) Bar chart for formalin nociceptive behaviors was obtained from the time-response curves shown in A. The columns represent the mean of nociceptive score in each phase: phase 1 (minutes 1–7), interphase (minutes 8–14), phase 2A (minutes 15–60) and phase 2B (minutes 61–90). Control group just received formalin injection in the right hind paw SB-334867 groups received different dose of SB-334867 before formalin injection

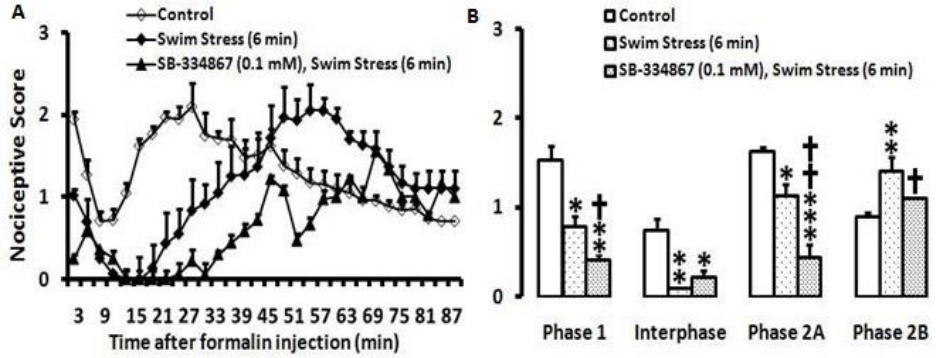

Figure 3. (A) Time-dependant curves of nociceptive behaviors induced by formalin representing the effect of intra-RVM administration of OX1R antagonist, SB-334867 (0.1 mmol) and swim stress on these behaviors. B) Bar chart for formalin nociceptive behaviors was obtained from the time-response curves shown in A. The columns represent the mean of nociceptive score in each phase: phase 1 (minutes 1–7), interphase (minutes 8–14), phase 2A (minutes 15–60) and phase 2B (minutes 61–90). Data are represented as mean±SEM for 10 rats.* P<0.05, ** P<0.01 and *** P<0.001 compared to control group. † P<0.05 and †† P<0.01compared to swim stress group

The effect of 1 mmol SB-334867 microinjection into RVM on antinociceptive behaviors induced by swim stress in formalin test

Intra-RVM microinjection of SB-334867 (1 mmol) before exposing the animals to swim stress also could attenuate formalin induced nociceptive behaviors in phase1, phase 2A and phase 2B (all P<0.05) compared to the group that received saline and then exposed to swim stress (swim stress group). However, nociceptive behaviors were not affected by SB-334867 (1 mmol) in the interphase of formalin test (P>0.05) (Figure 4).

Discussion

In this study we investigated the role of Orexin-A receptor in the RVM (Rostral Ventromedial Medulla) in stress-induced analgesia. Our results demonstrated that antinociceptive responses which induced by swim stress were significantly potentiated by a prior microinjection of OX1R antagonist (SB-334867) in phase 1, 2a and 2b of formalin test.

Also we clearly demonstrated that application of forced swim stress during 6 min as acute stress reduced the formalin-evoked nociceptive behaviors in phase 1, inter phase and initial part of phase2. Abundant studies have shown that acute exposure of animals to stressful situation produce antinociception measured by different pain models (1,30, 31). Formalin injection induces nociceptive bahaviour in phase I and II, with a quiescent phase between them. While active inhibitory mechanisms are proposed to be responsible for initiation of interphase termination of the nociceptive response in phase 2 (32). However, some stress parameters could also give rise to raise pain sensitivity, called stress-induced hyperalgesia (33). Several studies have indicated that SIA mediated by top-down inhibitory pain pathway to spinal cord (1). Although opioid system appears to be pivotal mediators in antinociception response in SIA (1, 4, 34), numerous studies now support that inhibition of pain in SIA don't depend on the endogenous opiates (i.e. it is not reversed by the opioid antagonist and is not produced a change in morphine-tolerance animals) (30). Orexin-A microinjection into the lateral ventricle produced tolerance to the anti-nociceptive effect of this peptide (35). Since the administration of OX1R antagonist into RVM led to increase antinociception response in SIA we hypothesized that OX1R might mediate non-opioid inhibitory pain role in RVM in SIA.

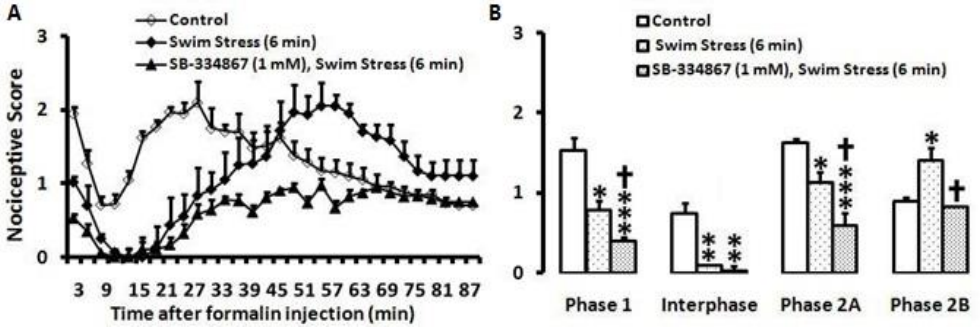

Figure 4. (A) Time-dependent curves of nociceptive behaviors induced by formalin representing the effect of intra-RVM administration of OX1R antagonist, SB-334867 (0.1 mmol) and swim stress on these behaviors. B) Bar chart for formalin nociceptive behaviors was obtained from the time-response curves shown in A. The columns represent the mean of nociceptive score in each phase: phase 1 (minutes 1–7), interphase (minutes 8–14), phase 2A (minutes 15–60) and phase 2B (minutes 61–90). Data are represented as mean ± SEM for 10 rats.* P<0.05, ** P<0.01 and *** P<0.001 compared to control group. † P<0.05 compared to swim stress group

There are limited evidences that orexinergic system might be connected with induction of analgesia in stress. For example Gerashchenko *et al* indicated that direct block orexin neurons in lateral hypothalamus by nociceptin/orphanin FQ prevents stress-induced analgesia development in rat (36). Consist with this OXR knockout mice show higher degree of hyperalgesia caused by peripheral inflammation and reduced degree of SIA than the wild type mice (37). Recently Azhdari-Zarmehri *et al* indicated that direct ICV administration of OX1R antagonist attenuates swim- and restraint stress-induced analgesia in formalin test (28). Taking our previous result together with current results, one can conclude that the effects of Orexin-1 in SIA might be depend on location of drug administration. So that administration of orexin-1 antagonist in cerebral ventricles reduce SIA while administration of this drug in RVM increase SIA .

The RVM consist of nucleus raphe magnus and adjacent reticular nuclei which play bidirectional role in top-down pain control system (1, 38-41).The neuronal population within the RVM is divided to three classes: on-cells, off-cells and neutral cells (33, 34). On-cells are characterized by a burst of activity during noxious reflexes and they are inhibited by μ-opioid agonists (42, 43). Conversely, Off-cells as pain-inhibiting neurons are identified by a pause of activity during noxious reflexes and their activity are increased with morphine (44, 45). The remaining cells, neutral-cells don't show any change in firing activity associated with nociception (46).

There are reports demonstrating that orexin-A can modulate on and off-cells in RVM. For on-cells, it was reported that both spontaneous and ongoing activity of these cells were inhibited by orexin-A following thermal analgesia.

For off-cells, it was reported that spontaneous activity of these cells were increased by orexin-A following thermal analgesia, whereas the neutral-cells were unaffected (47, 48).

The decrease in on-cell firing rate by Orexin-A and the increase in off-cell spontaneous firing rate might be involved in Orexin-A induced analgesia.

The reduction in the nociceptive behaviors during stress was not significant when orexin-A as agonist OXR1 were microinjected into RVM. Multiple lines of evidence indicate that a potential involvement of orexinergic system in pain modulation and in stress-related behaviors (28, 35, 36, 48). The role of orexin in generation of SIA was first indicated by Watanabe and his colleagues (37). More recent work suggests that during arousal and stress-like conditions, orexinergic system is activated (20, 49). Initial support for involvement of orexin in pain modulation came from an experiment in which intracerebroventicular (ICV) administration of Orexin-A caused a dose-related antinociceptive behaviors in the hot plate test in rats (50). In agreement with this, it was reported that the intra-PAG administration of Orexin-A attenuate the inter phase and phase 2 in formalin-induced nociceptive responses in rats without any significant effect on tail-flick latency (29).

SB-334867 as putative Orexin receptor antagonist just adheres to type1 Orexin receptors (28, 29). But, Orexin-A as agonist OXR have an affinity to type2 orexin receptors (51, 52). Some testing has shown OXR1 and OXR2 exhibited strikingly different distribution in rat brain. Within RVM(8, 29), OXR1 mRNA is more abundant than OXR2 mRNA (21). Although some experimental studies have demonstrated that OXR1 activation produces analgesic effect rather than OXR2. Even OXR2 activation elicits an excitatory effect on pain modulation (53). It is thus possible that application of OXR2 may be effective in our result. However, more experiments is needed to confirm this hypothesis.

More recently, some studies have demonstrated that descending pain-inhibitory system in RVM is more compatible than descending pain-facilitator system especially during abnormal situation such as chronic inflammation (54, 55). We could hypothesize SIA (stress induced analgesia) as abnormal situation could trigger pain-inhibitory system. Consist with this, several old studies have revealed that a majority of neuronal population of the RVM is made of GABAergic neurons that are involved the locally projecting (56, 57) or reticulospinal neurons (58). During normal situation intrinsic GABAergic neurons elicit inhibitory effect on the inhibitory descending control of dorsal horn from RVM (56, 59). On the other hand, in unusual situation leading to a block of GABAergic neurons and consequently decreasing effects on descending inhibitory system (54). In the previous studies we showed the role of the RVM in formalin-induced nociceptive behaviors and inactivation of RVM modulated stress-induced analgesia in formalin test (60-63).

A limitation of study described above is restricted doses of antagonist of OXR1 that we used. It will be of interest, to survefazhdy on-cells and off-cells in RVM novel methods such as optogenetics.

Conclusion

This study is suggested that OXR1might be involved in stress-induced analgesia phenomenon within RVM, hence blocking of OXR1 in RVM led to exaggerate SIA.

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