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

Neda Soliemani 1, Ali Reza Moslem 2, Ali Shamsizadeh 1, Hassan Azhdari-Zarmehri 3*

1 Physiology-Pharmacology Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran
2 Faculty of Medicine, Sabzevar University of Medical Sciences, Sabzevar, Iran
3 Department of Basic Science and Neuroscience Research Center, Torbat Heydariyeh University of Medical Sciences, Torbat Heydariyeh, Iran

**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 phase 1, interphase, and phase 2 in rats.

**Conclusion:** The result demonstrated the involvement of OX1R in antinociceptive behaviors induced by swim stress in RVM.

**Please cite this article as:**

Soliemani N, Moslem AR, Shamsizadeh A, Azhdari-Zarmeh H. Administration of orexin receptor 1 antagonist into the rostral ventromedial medulla increased swim stress-induced antinociception in rat. Iran J Basic Med Sci 2016; 19:542-549.

**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.

*Corresponding author: Hassan Azhdari-Zarmehri, Department of Basic Science and Neuroscience Research Center, Torbat Heydariyeh University of Medical Sciences, Torbat Heydariyeh, Iran. Tel/Fax: +98 51-522 4697; email: hasan.azhdari@gmail.com
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-benzoazolyl-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 -1.10 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 °C 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 45° 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).
Soliemani et al

**Rostral ventromedial medulla and stress-induced analgesia**

Iran J Basic Med Sci, Vol. 19, No.5, May 2016

**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 phase 1 and phase 2A (all P<0.05) and in interphase (P<0.01) of formalin test. However, in phase 2B, Appyling 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 phase 1 (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.
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 phase 1, 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 behaviour 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.
There are limited evidences that orexinergic system might be connected with induction of analgesia in stress. For example Geraschenko 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 OX1 receptor 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 show no 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 agonists 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 intracerebroventricular (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 OXR1 might be involved in stress-induced analgesia phenomenon within RVM, hence blocking of OXR1 in RVM led to exaggerate SIA.

Acknowledgement
The authors acknowledge Physiology-Pharmacology Research Centre in Rafsanjan University of Medical Sciences for the financial support for this study. This article was extracted...
from the thesis prepared by Neda Soleimani to fulfill the requirements for the Master degree in physiology.

References

1. Butler RK, Finn DP. Stress-induced analgesia. Prog Neuropsychopharmacol Biol Psychiatry 2009; 33:184-202.
2. Bodnar RJ, Kelly DD, Brutus M, Glusman M. Stress-induced analgesia: neural and hormonal determinants. Neurosci Biobehav Rev 1980; 4:87-100.
3. Ford GK, Finn DP. Clinical correlates of stress-induced analgesia: evidence from pharmacological studies. Pain 2008; 140:3-7.
4. Akli H, Young E, Walker JM, Watson SJ. The many possible roles of opioids and related peptides in stress-induced analgesia. Ann N Y Acad Sci 1986; 467:140-153.
5. Lewis JW, Cannon JT, Liebeskind JC. Opioid and nonopioid mechanisms of stress analgesia. Science 1980; 208:623-625.
6. Watkins LR, Mayer DJ. Organization of endogenous opiate and nonopiate pain control systems. Science 1982; 216:1185-1192.
7. Ferguson AV, Samson WK. The orexin/hypocretin system: a critical regulator of neuroendocrine and autonomic function. Front Neuroendocrinol 2003; 24:141-150.
8. Sakurai T. Roles of orexin/hypocretin in regulation of sleep/wakefulness and energy homeostasis. Sleep Med Rev 2005; 9:231-241.
9. Samson WK, Taylor MM, Ferguson AV. Non-sleep effects of hypocretin/orexin. Sleep Med Rev 2005; 9:243-252.
10. Siegel JM. The narcoleptic borderland of Sleep Med 2003; 4:3-4.
11. Sofiabadi M, Nazemi S, Erami E, Azhdari-Zarnehri H. Role of orexinergic system in the effects of morphine on food and water intake in male rat. Koomesh 2014; 15:380-387.
12. Azhdari-Zarnehri H, Mohammad-Zadeh M, Shabani M. The role of hypocretin/orexin in stress-induced analgesia. Journal of Kerman University of Medical Sciences 2015; 22:205-217.
13. Azhdari-Zarnehri H, Semnian S, Fathollahi Y. Orexin A modulates rostral ventromedial medulla neuronal activity of rat in vitro. Abstracts of the 33rd Annual Meeting of the Japan Neuroscience Society (Neuro 2010)
14. Shabani M, Mohammad-Zadeh M, Azhdari-Zarnehri H. Orexin (hypocretin): A multi-functional hypothalamic peptide. Koomesh 2014; 15:275-281.
15. Azhdari-Zarnehri H, Semnian S, Fathollahi Y, Pakdel FG. Tail flick modification of orexin-A induced changes of electrophysiological parameters in the rostral ventromedial medulla. Cell J 2014; 16:131-140.
16. Nazemi S, Azhdari-Zarnehri H, Haghdoost-Yazdi H. Role of orexin in the tolerance and physical dependence to morphine. JBUMS 2014; 16:54-61.
17. Abadi MS, Oranjaghi NH, Ghasemi E, Esmaeili MH, Haghdoost-Yazdi H, Erami E, et al. Assesment of orexin receptor 1 in stress attenuated nociceptive behaviours in formalin test. Physiol and Pharmacol 2011; 15:395-402.
18. Azhdari-Zarnehri H, Semnian S, Fathollahi Y. Orexin-A microinjection into the rostral ventromedial medulla causes antinociception on formalin test. Pharmacol Biochem Behav 2014; 122:286-290.
19. Ghasemi E, Heidari-Oranjaghi N, Azhdari-Zarnehri H, Sadegh M. Repeated injections of orexin-A developed behavioral tolerance to its analgesic effects in rats. Iran J Basic Med Sci 2015; 18:1183-1188.
20. Peyron C, Tighe DK, van den Pol AN, de Leece L, Hefter HC, Sutcliffe JG, et al. Neurons containing hypocretin (orexin) project to multiple neuronal systems. J Neurosci 1998; 18:9996-10015.
21. Trivedi P, Yu H, MacNeil DJ, Van der Ploeg LH, Guan XM. Distribution of orexin receptor mRNA in the rat brain. FEBS Lett 1998; 438:71-75.
22. Heinricher MM, Barbaro NM, Fields HL. Putative nociceptive modulating neurons in the rostral ventromedial medulla of the rat: firing of on- and off-cells is related to nociceptive responsiveness. Somatosens Mot Res 1989; 6:427-439.
23. Kim SJ, Calejesan AA, Zhuo M. Activation of brainstem metabotropic glutamate receptors inhibits spinal nociception in adult rats. Pharmacol Biochem Behav 2002; 73:429-437.
24. Da Silva LF, Desantana JM, Shuka KA. Activation of NMDA receptors in the brainstem, rostral ventromedial medulla, and nucleus reticularis gigantocellularis mediates mechanical hyperalgesia produced by repeated intramuscular injections of acidic saline in rats. J Pain 2009; 11:378-387.
25. Heinricher MM, Kaplan HJ. GABA-mediated inhibition in rostral ventromedial medulla: role in nociceptive modulation in the lightly anesthetized rat. Pain 1991; 47:105-113.
26. Paxinos G, Watson C. The rat brain in stereotaxic coordinates. New York: Academic Press. 2005.
27. Fereidoni M, Javan M, Semnian S, Ahmadiani A. Chronic forced swim stress inhibits ultra-low dose morphine-induced hyperalgesia in rats. Behav Pharmacol 2007; 18:67-672.
28. Heidari-Oranjaghi N, Azhdari-Zarnehri H, Erami E, Haghparast A. Antagonism of orexin-1 receptors attenuates swim- and restraint-stress-induced antinociceptive behaviors in formalin test. Pharmacol Biochem Behav 2012; 103:299-307.
29. Azhdari-Zarnehri H, Semnian S, Fathollahi Y, Erami E, Khlkpay A, Azizi H, et al. Intraperiaqueductal gray matter microinjection of orexin-A decreases formalin-induced nociceptive behaviors in adult male rats. J Pain 2011; 12:280-287.
30. Lafrance M, Roussy G, Belleville K, Maeno H, Beaudet N, Wada K, et al. Involvement of NTS2 receptors in stress-induced analgesia. Neuroscience 2010; 166:639-652.
31. Mohammad-Zadeh M, Azhdari-Zarnehri H, Mosavi F, Haghdoost-Yazdi H, Nazeri M, Shabani M. Modulation of different phases of formalin test by force swim stress. BCN 2014; 5:303-307.
32. Azhdari-Zarnehri H, Mohammad-Zadeh P, Feridoni M, Nazeri M. Termination of nociceptive behaviour at the end of phase 2 of formalin test is attributable to endogenous inhibitory mechanisms, but not by opioid receptors activation. BCN 2014; 5:48-54.
33. Satoh M, Kuraishi Y, Kawamura M. Effects of intrathecal antibodies to substance P, calcitonin gene-related peptide and galanin on repeated cold stress-induced hyperalgesia: comparison with carrageenan-induced hyperalgesia. Pain 1992; 49:273-278.
34. Amit Z, Galina ZH. Stress-induced analgesia: adaptive pain suppression. Physiol Rev 1986; 66:1091-1120.
35. Ghasemi E, Heidari-Oranjaghi N, Azhdari-Zarnehri H, Sadegh M. Repeated injections of orexin-A developed behavioral tolerance to its analgesic effects in rats. Iran J Basic Med Sci 2015; 18:1183-1188.
36. Gerassimou L, Horvath TL, Xie XS. Direct inhibition of hypocretin/orexin neurons in the lateral hypothalamus by nociceptin/orphanin FQ blocks stress-induced analgesia in rats. Neuropharmacology 2011; 60:543-549.
37. Watanabe S, Kuwaki T, Yanagisawa M, Fukuda Y, Shiomaya M. Persistent pain and stress activate pain-inhibitory orexin pathways. Neuropeptide 2005; 16:5-8.
38. Fields HL, Basbaum AI, Heinricher MM. Central nervous system mechanisms of pain modulation. In: McMahon S, Koltzenburg M, eds. Textbook of Pain. 5th ed. Burlington, Massachusetts, USA: Elsevier Health Sciences; 2005:125-142.
39. Fields HL, Bry J, Hentall I, Zorman G. The activity of neurons in the rostral medulla of the rat during withdrawal from noxious heat. J Neurosci 1983; 3:2545-2552.
40. Foo H, Helmstetter FJ. Hypoalgesia elicited by a conditioned stimulus is blocked by a mu, but not a delta or a kappa, opioid antagonist injected into the rostral ventromedial medulla. Pain 1999; 83:427-431.
41. Morgan MM, Whitney PK. Immobility accompanies the antinociception mediated by the rostral ventromedial medulla of the rat. Brain Res 2000; 872:276-281.
42. Bederson JB, Fields HL, Barbaro NM. Hyperalgesia during withdrawal-precipitated withdrawal from morphine is associated with increased on-cell activity in the rostral ventromedial medulla. Somatosens Mot Res 1990; 7:185-203.
43. Heinricher MM, Morgan MM, Fields HL. Direct and indirect actions of morphine on medullary neurons that modulate nociception. Neuroscience 1992; 48:533-543.
44. Barbaro NM, Heinricher MM, Fields HL. Putative pain modulating neurons in the rostral ventral medulla: reflex-related activity predicts effects of morphine. Brain Res 1986; 366:203-210.
45. Fields HL, Heinricher MM. Anatomy and physiology of a nociceptive modulatory system. Philos Trans R Soc Lond B Biol Sci 1995; 350:361-374.
46. Kincaid W, Neubert MJ, Xu M, Kim CJ, Heinricher MM. Role for medullary pain facilitating neurons in secondary thermal hyperalgesia. J Neurophysiol 2006; 95:33-41.
47. Azhdari-Zarnehri H, Semnani S, Fatollahi Y. Orexin-a modulates firing of rat rostral ventromedial medulla neurons: an in vitro study. Cell J 2015; 17:163-170.
48. Azhdari-Zarnehri H, Semnani S, Fatollahi Y, Pakdel FG. Tail flick modification of orexin-a induced changes of electrophysiological parameters in the rostral ventromedial medulla. Cell J 2014; 16:131-140.
49. Berridge CW, Espana RA, Vittoz NM. Hypocretin/orexin in arousal and stress. Brain Res 2010; 1314:91-102.
50. Bingham S, Davey PT, Babbs AJ, Irving EA, Sammons MJ, Wyles M, et al. Orexin-A, an hypothalamic peptide with analgesic properties. Pain 2001; 92:81-90.
51. Nambu T, Sakurai T, Mizukami K, Hosoya Y, Yanagisawa M, Goto K. Distribution of orexin neurons in the adult rat brain. Brain Res 1999; 9:243-260.
52. Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, et al. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. Cell 1998; 92:573-585.
53. Bartsch T, Levy MJ, Knight YE, Goadsby PJ. Differential modulation of nociceptive dural input to [hypocretin] orexin A and B receptor activation in the posterior hypothalamic area. Pain 2004; 109:367-378.
54. Azami J, Green DL, Roberts MH, Monhemius R. The behavioural importance of dynamically activated descending inhibition from the nucleus reticularis gigantocellularis pars alpha. Pain 2001; 92:53-62.
55. Saade NE, Al Amin HA, Barchini J, Tchagaghian S, Shamaa F, Jabbur SJ, et al. Brainstem injection of lidocaine releases the descending pain-inhibitory mechanisms in a rat model of mononeuropathy. Exp Neurol 2012; 237:180-190.
56. Cho HJ, Baasbaum AI. GABAergic circuitry in the rostral ventral medulla of the rat and its relationship to descending antinociceptive controls. J Comp Neurol 1991; 303:316-328.
57. Clark FM, Proudfit HK. Projections of neurons in the ventromedial medulla to pontine catecholamine cell groups involved in the modulation of nociception. Brain Res 1991; 540:105-115.
58. Kato G, Yasaka T, Katafuchi T, Furue H, Mizuno M, Iwamoto Y, et al. Direct GABAergic and glycineergic inhibition of the substantia gelatinosa from the rostral ventromedial medulla revealed by in vivo patch-clamp analysis in rats. J Neurosci 2006; 26:1787-1794.
59. Gilbert AK, Franklin KB. GABAergic modulation of descending inhibitory systems from the rostral ventromedial medulla (RVM). Dose-response analysis of nociception and neurological deficits. Pain 2001; 90:25-36.
60. Shamsizadeh A, Soliemani N, Mohammad-Zadeh M, Azhdari-Zarnehri H. Permanent lesion in rostral ventromedial medulla potentiates swim stress-induced analgesia in formalin test. Iranian Journal of Basic Medical Sciences 2014; 17:209-215.
61. Azhdari-Zarnehri H, Pazesh S, Rahmani A, Erami E, Emamjomeh MM. Assessing the effect of lidocaine injection into the nucleus paragigantocellularis-lateralis on formalin test and hot plate test induced nociceptive behaviors in rats. ZUMS Journal 2013; 21:10-29.
62. Azhdari Zarmehri H, Haidari-Oranj N, Soleimani N, Sofigabadi M. Effects of lidocaine injections into the rostral ventromedial medulla on nociceptive behaviours in hot-plate and formalin tests in rats. Koomesh 2013; 14:490-496.

63. Soleimani N, Erani E, Abbasnejad M, Shamsizadeh A, Azhdari-Zarmehri H. Effect of transient inactivation of rostral ventromedial medulla on swim stress-induced analgesia in formalin test in rats. Physiol and Pharmacol 2013; 17:116-124.