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Intra-Periaqueductal Gray Matter Microinjection of Orexin-A Decreases Formalin-Induced Nociceptive Behaviors in Adult Male Rats

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Abstract: **Intracerebroventricular injection of orexin-A (hypocretin-1) has been shown to elicit the analgesic responses. However, the locations of central sites that may mediate these effects have not been clearly elucidated. This study was performed using male Sprague Dawley rats to investigate the antinociceptive effects of intra-periaqueductal gray matter (PAG) administration of orexin-A, 5 minutes prior to formalin (50 μL of 2%) injection. Orexin-A had no effect on tail-flick test as thermal and acute model. In the formalin test, intra-PAG injection of orexin-A (10 nM) decreased the formalin-induced nociceptive behaviors in the interphase and phase 2, but not in phase 1, indicating an antinociceptive role of exogenous orexin-A in the PAG. While Orexin-A failed to produce a dose-dependent decrease in formalin-evoked behaviors in phase 1, it may have induced a dose-dependent decrease in formalin-evoked behaviors in early phase 2. Control injections of orexin-A into the sites near the PAG resulted in less or no reduction in pain, indicating that the analgesia observed is probably due to a site of action within the PAG rather than at surrounding neural structures. The antinociceptive effect of orexin-A was compared with intra-PAG administration of morphine (.5 μL of 20 mM, 5 minutes before the formalin injection). Morphine decreased the formalin-induced nociceptive behaviors in all phases. To investigate whether the orexin has a special action on the early part of the second phase, or its delayed effects are related to its pharmacokinetics, the orexin-A was injected into the PAG, 10 minutes before the formalin injection. No difference was observed between 5 and 10 minutes injection of orexin-A prior to formalin injection. The antinociceptive effect of orexin was blocked by intra-PAG injection of SB-334867, a putative type 1 orexin receptor antagonist, suggesting the involvement of orexin receptor type 1 in antinociception produced with intra-PAG injection of orexin-A. The results showed that the orexin-A plays an antinociceptive role in PAG in the interphase and the late phase of formalin test through type 1 orexin receptor dependent mechanism.**

Perspective: ***Orexin is produced exclusively in the lateral hypothalamus, where it is known to modulate the pain processing through PAG. The antinociceptive effect of orexin in PAG may provide a role for this neurotransmitter in the up-down modulating pain system and further support the development of orexin-1 agonists for pain treatment.***

Key words: *Orexin-A, periaqueductal gray matter, orexin receptor type 1, SB-334867, formalin test.*

The neuropeptides orexin-A and -B (also called hypocretin-1 and -2) are expressed in neurons of the lateral hypothalamus (LH), with a few orexin cells extending into the dorsomedial hypothalamic nucleus (DMH).[21] Orexin-A is a 33 amino acid peptide and orexin-B a 28 amino acid peptide.[21] Sakurai et al[21] described 2 orexin receptors coupled to G proteins. Orexin-A binds equally to both orexin receptor type 1 and 2, while orexin-B has a preferential affinity for orexin receptor type 2. The broad projections of the orexinergic system have led to its implication in a variety of functions,

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including feeding, sleep-wake cycle, cardiovascular function, hormone secretion,[8,20,22,23] and, more recently, the modulation of nociceptive processing.[4,5,18,24,26,27] Initial support for this idea came from a study in which intracerebroventricular (ICV) Orexin-A (3–30 μg) produced a dose-related analgesia in the hotplate test in rats.[4] Systemic (iv) administration of orexin-A also proved to be effective in this rat model and also in the equivalent mouse paradigm at doses between 3 and 30 mg/kg.[4] In other mouse models, orexin-A inhibited the visceral nociception (abdominal constriction; 10 and 30 mg/kg) and thermal hyperalgesia (in intraplantar carrageenan model; 3–30 mg/kg), with an efficacy equivalent to the opioid analgesic morphine.[4] Morphological studies have established that the orexin-containing neurons and fibers as well as orexin receptors (orexin receptor type 1 and 2) are distributed along all parts of pain circuitry, including the periaqueductal gray matter (PAG) region which is considered to be important for pain modulation.[13,19,25] Stimulation of the lateral hypothalamus elicits antinociception via relays to the PAG and the rostral ventromedial medulla (RVM), which ultimately triggers the activation of descending noradrenergic pathways.[3] Electrical stimulation applied in the PAG produced antinociception analogous to 10 mg/kg morphine.[28] Like electrical stimulation, opioid administration into the ventrolateral parts of PAG also produces robust behavioral signs of antinociception.[16] Although orexin-A exhibits analgesic effects when administered via ICV or intrathecal microinjection,[4,17,26] the effect of orexin-A in the PAG is unclear. In the present study, the effect of orexin microinjected into the PAG region on the tail-flick test and formalin test was investigated. The tail-flick test was used to evaluate the acute nociceptive transmission, and the formalin test to reflect an inflammatory pain condition to measure its therapeutic potential. Portions of these data were presented as abstracts at Neuro2010.[1]

Methods

Subjects

All experiments involving the animals were conducted according to the policy of Iranian Convention for the Protection of Vertebrate Animals used for experiments and the protocol was approved by the Ethics Committee of the School of Medical Sciences, Tarbiat Modares University (TMU), Tehran, Iran. Efforts were made throughout the experiments to minimize the animal discomfort and to reduce the number of animals used. Adult male, Sprague-Dawley rats (220–300 g) purchased from Razi Institute (Hesarak Karj, Iran), were housed in groups of 3 in a temperature controlled room, under a 12 hour light/dark cycle with lights on at 0700 to 1900. Food and water were provided ad libitum. During the experiments, attention was strictly paid to the regulations of local authorities for handling laboratory animals.

Surgical Preparation for Intra-PAG and ICV Microinjections

To perform direct intra-PAG and ICV administrations of drugs or the respective vehicle (saline), rats were anaesthetized with Ketamine (100 mg/kg)/Xylazine (10 mg/kg). The rats were placed in a stereotaxic apparatus (Narishige, Japan), holes were drilled in the skull over the PAG, and the dura was removed to allow the placement of a guide cannula. 23-gauge, 5 mm-long stainless steel guide cannula was stereotaxically lowered until its tip was 2 mm above the PAG by applying coordinates from the atlas of Paxinos and Watson[10] (for PAG: A, −7.8 mm from bregma; L, .5 mm; V, 5.7 mm below the bregma and for ICV A, −.9 mm from bregma; L, .1.8 mm; V, 3.8 mm below the bregma). The cannula was anchored with dental cement to a stainless steel screw in the skull. The guide cannula for intra-PAG microinjection was implanted 7 days before the experiment for pain formalin or tail-flick tests in awake rats. Immediately after waking from surgery, rats were returned to their home cages to await the formalin test procedure. Direct intra-PAG administration of drugs, or respective vehicle, was conducted with a stainless steel cannula (30 gauge) connected via 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 PAG. At day 7, while the animals were habituated, volumes of .5 μL of drug solutions or vehicles were injected into the PAG over a period of 60 seconds or 5 μL orexin-A 10 nM or vehicles injected into the ICV over a period of 100 seconds and the injection cannula was gently removed later. After 5 minutes, formalin was injected into the plantar surface of the right hind paw using a disposable insulin syringe with a fixed 30-gauge needle. A separate group of animals were used for conducting tail-flick test.

Tail-Flick Test

Latency to tail-flick against heat was used as a measure of nociceptive responsiveness. At day 7, rats were kept in a glass restrainer and the dorsal surface of the tail between 4 and 6 cm from the tip of the tail was exposed to a beam of light generated from an automated analgesia meter (Harvard Tail-flick Analgesia Meter, Holliston, MA). The timer stopped when the animal flicked its tail away from the beam of light. Tail-flick latency was measured at 5-minute intervals until a stable baseline was obtained over 3 consecutive trials. The latency was measured 10 minutes after intra-PAG orexin-A injection up to 60 minutes. Orexin-A (.01–10 nM) and saline were given intra-PAG, in experimental and vehicle groups, respectively. Latencies were determined twice at 10-minutes interval after the administration of orexin-A or vehicle, and the average of 2 frequent readings was taken as the predrug latency. To avoid tissue damage, a 10-second cut-off was used.

Formalin-Induced Nociceptive Behavior

Formalin-induced nociceptive behavior is a widely used animal model of persistent pain.[2,7] Formalin-induced nociceptive behavior was used because it employs an adequate painful stimulus; the animals show a spontaneous response and it is sensitive to the commonly used analgesics. Moreover, the pain stimulus