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Central orexin inhibits reflex swallowing elicited by the superior laryngeal nerve via caudal brainstem in the rat

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HIGHLIGHTS

- Fourth ventricular administration of orexin-A decreased freq. of reflex swallowing.
- Microinjection of orexin-A into the medial DVC also suppressed reflex swallowing.
- The lesion of the commissural NTS abolished the orexin-response.
- Orexin-1 receptor antagonist suppressed orexin-response.
- Orexin-A inhibits reflex swallowing via orexin-1 receptors in the commissural NTS.

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ABSTRACT

We examined the effects of orexins on the reflex swallowing using anesthetized rats. Orexins were administered into the fourth ventricle. Swallowing was induced by repeated electrical stimulation of the central cut end of the superior laryngeal nerve (SLN) and was identified by the electromyogram lead penetrated the mylohyoid muscle through bipolar electrodes. The frequency of swallowing during the electrical stimulation of the SLN decreased after the administration of orexin-A in a dose-dependent manner. The latency of the first swallowing tended to be extended after the administration of orexin-A. The administration of orexin-B did not affect swallowing frequency. Pre-administration of SB334867, an orexin-1 receptor antagonist, attenuated the degree of inhibition of swallowing frequency induced by the administration of orexin-A. To identify the effective site of orexin-A, the effect of a microinjection of orexin-A into the dorsal vagal complex (DVC) was evaluated. Orexin-A was injected into one of the lateral DVC, the intermediate DVC, or the medial DVC. Microinjection of orexin-A into the medial DVC but not the other two sites decreased swallowing frequency. Pre-injection of SB334867 into the medial DVC disrupted the inhibitory response induced by fourth ventricular administration of orexin-A. The electrical lesion of the commissural part of the NTS, but not ablation of the AP, abolished the inhibition of reflex swallowing induced by fourth ventricular administration of orexin-A. These results suggest that orexin-A inhibits reflex swallowing via orexin-1 receptors situated in the commissural part of the NTS and/or its vicinity.

1. Introduction

Orexins/hypocretins (orexin-A or -B/hypocretin-1 or -2) are neuropeptides synthesized mainly in the lateral hypothalamic area (LHA) and are implicated in the regulation of food intake and arousal [1–4]. Orexins are involved in the regulation of various motor functions associated with feeding, such as gastric accommodation [5], gastric contractility [5,6], gastric acid secretion [7] and pancreatic secretion [8]. Recently, it has been reported that orexins also regulate mastication [9]. Thus, orexins participate in the regulation of various digestive functions of the digestive system [10,11]. Swallowing is an early step in feeding behavior to propel food into the stomach and is caused at the time between mastication and gastric accommodation. It is therefore easily inferred that orexigenic peptides, which enhance food intake, affect swallowing as well as other motor functions. Our previous study revealed that fourth ventricular administration of ghrelin, which has appetite-enhancing action [12,13], inhibited reflex swallowing [14]. In addition, our scant research revealed that fourth ventricular administration of orexin-A also induced the inhibition of reflex swallowing [14]. No systematic study, however, has reported the orexin-inducing inhibition of reflex swallowing.

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Two orexin receptors, namely orexin-1 receptor (OX1R) and orexin-2 receptors (OX2R), have been identified [4], and have differential expression patterns throughout the brain [15]. These receptors have different affinities for the two orexin peptides [4]: OX1R is selective for orexin-A, whereas OX2R is non-selective for orexin-A and orexin-B [4]. Different roles and/or effectiveness are therefore possible. For example, intracisternal injection of orexin-A but not orexin-B stimulates gastric acid secretion [7], suggesting the participation of OX1R [16]. Intracerebroventricular administration of orexins increased water intake and the effect of orexin-A was more potent than that of orexin-B [17]. Orexin-A and orexin-B, injected into the NTS, have a similar pressor effect suggesting specific actions at the OX2R [18]; thus, the two orexin receptor subtypes have different roles. It is therefore important to identify which orexin receptor subtype is involved in inhibiting reflex swallowing.

The dorsal vagal complex (DVC), which involves the nucleus of the solitary tract (NTS), dorsal motor nucleus of the vagus (DMV) and the area postrema (AP), is situated in the middle of the caudal part of the dorsal medulla. The DVC is related to ingestive behavior and various autonomic functions, and has dense projections from the periphery and higher center [19], including visceral sensory information, such as gastrointestinal, cardiovascular, and respiratory afferents. Since nerve endings that transmit visceral information compose complex topographic distribution in the DVC, site specificity is considered. Orexin-containing neurons in the hypothalamus also project throughout the DVC [20]. Immunohistochemical study revealed that fourth ventricular administration of orexin-A induced significant Fos-expression throughout the DVC [20]. Thus, orexin fiber terminals and orexin receptors are distributed widely in the DVC. The afferent fibers in the superior laryngeal nerve (SLN) respond to mechanical and chemical stimulation of the larynx and epiglottis [21,22]. The SLN convey sensory information to the lateral region of the DVC [23]. This region contains the swallowing pattern generator neurons, namely the dorsal swallowing group (DSG). The DSG receives both supramedullary inputs and peripheral afferents, such as the SLN, to elicit swallowing [24]; therefore, it is important to determine where orexins act in the DVC to regulate reflex swallowing.

In the present study, we first confirmed that fourth ventricular administration of orexin-A inhibits reflex swallowing elicited by electrical stimulation of the SLN using anesthetized rats. We also determined the orexin receptor subtype inhibiting reflex swallowing. Finally, we identified the site of orexin in the DVC responsible for inhibiting reflex swallowing.

2. Materials and methods

2.1. Animals and surgery

Seventy-eight male Sprague–Dawley rats (280–320 g) were used. Each animal was anesthetized with an intraperitoneal injection of urethane–chloralose (urethane, 0.8 g/kg; chloralose, 65 mg/kg body wt.). Subsequent anesthesia was administered through silastic tubing (OD: 1.0 mm, ID: 0.5 mm) inserted into the right jugular vein. Each animal had a tracheal cannula made from polyethylene tubing (OD: 2.07 mm). The sternohyoid and omohyoid muscles were retracted. The SLNs were isolated from the surrounding tissue and bilaterally sectioned near the thyroid cartilage. After removing the digastric muscle, the mylohyoid muscle was exposed. A commercial bipolar recoding electrode, insulated except at the tip (UI2-513; Unique Medical Co., Osaka, Japan) penetrated the mylohyoid muscle to record the electromyographic (EMG) activities associated with swallowing. The electrode is made of the two stainless steel wires of 0.4 mm in diameter and anode–cathode distance is 1 mm. 0.2 mm of the tip is not insulated. Each animal was mounted on a stereotaxic apparatus. Then, the neck muscles were removed and the ligaments between the occipital bone and the atlas were carefully removed. A small hole was made through the dura mater to administer the drugs. When the drugs were injected into the DVC using a micro glass pipette, the occipital bone and dura mater were removed to expose the surface of the brainstem. These methods are similar to those used in our previous research [5,14,25–27]. Animals were placed in the supine position during the course of the experiments except when test solutions were administered. Body temperature was maintained at 36 °C using a heating pad placed under the body (ATB-1100; Nihon Kohden, Tokyo, Japan). Animal care was in accordance with the guidelines of the Physiological Society of Japan. The experimental protocols were approved by Okayama University Animal Use Committee (approval number: OKU-2012287).

2.2. Electrical stimulation and recording

Swallowing was triggered by stimulation of the central cut end of the SLN with repeat electric pulses (20 Hz, 0.2 ms in duration, 0.2 mA in intensity) for 20 s in the supine position. The parameter of stimulation is similar with others to induce reflex swallowing in anesthetized rats [14,28–30]. About 10 to 20 swallowing movements were induced during stimulation. Principally, a set of electrical pulse trains sustained for 20 s was delivered every 5 min. Before drug administration, the electric pulse train was delivered three times or more to confirm the stability of the responses in the supine position. The drug was administered into the fourth ventricle or into the dorsal medulla in the prone position. The animal was then returned to the supine position to deliver the electrical pulses to elicit swallowing. The EMGs through electrodes associated with swallowing were amplified through a biophysical amplifier with the aid of a high-cut filter (100 Hz) and low-cut filter (5 Hz), and then stored on a personal computer using LabChart software of the PowerLab system for later analysis (AD Instruments Japan Inc., Nagoya, Japan). Swallowing movements were identified under visual guidance and corresponded with EMG activities.

2.3. Fourth ventricular administration of drugs

The effect of fourth ventricular administration of orexins on reflex swallowing was studied. Peptides were dissolved in Ringer solution. Three different doses of orexin-A (0.3, 1, 3 nmol), orexin-B (3 nmol) and vehicle (Ringer solution) were used. Each rat received a single dose of orexins or vehicle (3 μl). To determine whether the orexin response was caused via OX1R or OX2R, the effects of SB334867 (an OX1R antagonist) were tested. Orexin-A (1 nmol, 3 μl) was administered 20 min after the administration of SB334867 (10 nmol, 3 μl). Orexins was purchased from the Peptide Institute (Osaka, Japan). SB334867 was purchased from Tocris Bioscience (Bristol, U.K.)

2.4. Local administration of drugs

Commercial glass micropipettes (30 μm in tip diameter; World Precision Instruments Co.) connected to a 50 μl Hamilton syringe were installed in a micro injector (XF-320 J; Nihon Kohden, Tokyo, Japan) to inject drugs into the dorsal medulla ipsilateral to the electrically stimulated SLN. Each glass pipette was first filled with paraffin fluid and the test solution was drawn up from the tip of a pipette.

Each rat received a single injection of orexin-A into one of the following three sites of the dorsal medulla: 1) the lateral region of the NTS around the solitary tract and its vicinity (lateral DVC). The tip of the pipette was oriented into the solitary tract at co-ordinates 0.5 mm anterior to the obex, 1.1 mm lateral to the midline, and 0.8 mm ventral from the surface of the brainstem. This region is the so-called dorsal swallowing group (DSG) where a central pattern generator is present [24] and is the region where SLN afferents terminate [23]. 2) The intermediate region of the NTS and its vicinity (intermediate DVC). The tip of the pipette was oriented to the boundary between the NTS and DMV. The co-ordinates were 0.5 mm anterior to the obex, 0.5 mm lateral to the midline, and 0.4 mm ventral from the surface of the brainstem. This is the region where abdominal visceral afferents terminate [31]. 3) The medial region of the DVC, which includes the AP, subpostrema region, the commissural part of the NTS (commissural NTS) and its