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Research article

Orexin exerts excitatory effects on reticulospinal neurons in the rat gigantocellular reticular nucleus through the activation of postsynaptic orexin-1 and orexin-2 receptors

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HIGHLIGHTS

- Orexin dose-dependently evokes inward currents in Gi reticulospinal neurons.
- The orexin-induced responses are mediated by co-activation of orexin-1 and orexin-2 receptors in postsynaptic neurons.
- Orexin does not influence the frequency and amplitude of mEPSCs/mIPSCs in Gi reticulospinal neurons.
- Orexin may participate in Gi-mediated motor inhibition.

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ABSTRACT

Previous studies have revealed that orexin may actively participate in central motor control. The gigantocellular reticular nucleus (Gi) is a key element of the brainstem motor inhibitory system. The descending orexinergic projections also reach Gi region, and microinjection of orexin into Gi causes robust muscle tone inhibition. However, the modulation effects of orexin on Gi neurons remain unclear. In the present study, using whole-cell patch-clamp recordings, we initially observed that orexin elicited an inward current in Gi neurons at a holding potential of −70 mV in a concentration-dependent manner. By combining electrophysiology with neuropharmacological methods, we further determined that the orexin-induced inward current was directly mediated by the activation of postsynaptic orexin-1 and orexin-2 receptors. Moreover, orexin did not affect the frequency and amplitude of miniature excitatory and inhibitory postsynaptic currents in Gi neurons, which suggests that orexin had no effects on neurotransmission to these neurons. Therefore, the direct excitatory effect of orexin on an inhibitory motor structure, the Gi, was reported in the present study. This modulation may be integrated into the role of orexin in central motor control.

1. Introduction

The neuropeptide orexin (or hypocretin), which is exclusively synthesized by a specific group of neurons in the lateral hypothalamus/perifornical area, has been implicated in the regulation of diverse brain functions, such as reward processes, sleep/wakefulness cycles, and energy homeostasis [1]. Accumulating evidence has also revealed that the orexinergic neurons may actively participate in central motor control through its direct connections with nearly all levels of motor control structures, including the motor cortex, brainstem motor nuclei, spinal cord motor circuitry, basal ganglia, and cerebellum [2]. Generally speaking, coordinated motor excitation and inhibition mediated by these structures are important for central motor control [3,4]. A specific connection between orexin and motor inhibition has been proposed, since cataplexy, a motor disorder that is characterized by sudden attacks of muscle weakness, is caused by the loss of orexin-

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ergic neurons [5]. However, the direct modulation effects of orexin on neurons in motor structures related to motor inhibition are still largely unknown.

It is generally accepted that the gigantocellular reticular nucleus (Gi) in brainstem plays an important role in motor inhibition. Electrical or chemical stimulations of this region produce robust muscular inhibition in both decerebrate and intact animals [6–8]. This inhibition is largely mediated by the excitation of reticulospinal neurons within the Gi, which hyperpolarize motoneurons through descending projections into the spinal cord [6]. Morphological studies have revealed that orexinergic fibers are present in the Gi [9]. Furthermore, microinjections of orexin into Gi produce strong inhibition of muscle tone in decerebrate rats [4]. Therefore, the modulation effects of orexin on Gi reticulospinal neurons and the underlying mechanisms have aroused interest and need to be clarified.

In the present study, using whole-cell patch-clamp recordings, the modulation of orexin on Gi reticulospinal neurons, including the postsynaptic dynamic and neurotransmission, were investigated. By combining electrophysiology with neuropharmacological method, the receptor mechanism underlying these effects was also determined. Our results provided a direct observation of the excitatory effect of orexin on Gi reticulospinal neurons, which are key elements in central motor inhibition. Together with previous studies showing identical excitatory effects of orexin on neurons in multiple motor structures [2], our study has demonstrated that orexin may facilitate central motor control via coordinately regulating motor excitation and inhibition.

2. Materials and methods

2.1. Animal and brain slice preparation

Coronal brainstem slices (300–400 μm in thickness) containing the Gi region were obtained from Sprague-Dawley rats aged 9–14 days according to the rat brain atlas [10]. A previous study has shown that the electrical stimulation of Gi in young rats (7–10 days) can induce similar motor inhibition to that observed in adult rats [8]. Under sodium pentobarbital (40 mg/kg) anesthesia, rats were quickly decapitated, and the brainstem was rapidly removed and placed into 95% O_2 and 5% CO_2 oxygenated ice-cold cutting solution (composition in mM: 220 sucrose, 2.5 KCl, 1.25 NaH_2PO_4, 6 $MgCL_2$, 26 $NaHCO_3$, 1 $CaCl_2$, and 10 D-glucose). Coronal slices were cut with a VT1200 vibroslicer (Leica, Germany) and then incubated in oxygenated artificial cerebrospinal fluid (ACSF, composition in mM: 125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 1.3 $MgSO_4$, 26 $NaHCO_3$, 2 $CaCl_2$, and 20 D-glucose) at room temperature for at least 40 min. The slices were then transferred to a submerged chamber and continuously superfused with oxygenated ACSF at a rate of approximately 2 ml per minute at room temperature. All animal care and experimental procedures complied with the US National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication 80–23, revised 1996). All efforts were made to minimize the number of animals used and their suffering.

2.2. Whole-cell patch-clamp recording

Gi regions were verified with a BX51WI microscope (Olympus, Japan) with infrared differential interference contrast (IR-DIC) illumination in the slice according to the rat brain atlas [10]. According to previous studies, Gi neurons with a large soma diameter have been identified as reticulospinal neurons [11]. Therefore, in our study, patch-clamp recordings were specifically performed on Gi reticulospinal neurons with a soma diameter larger than 25 μm. Patch-clamp recordings were performed with borosilicate glass

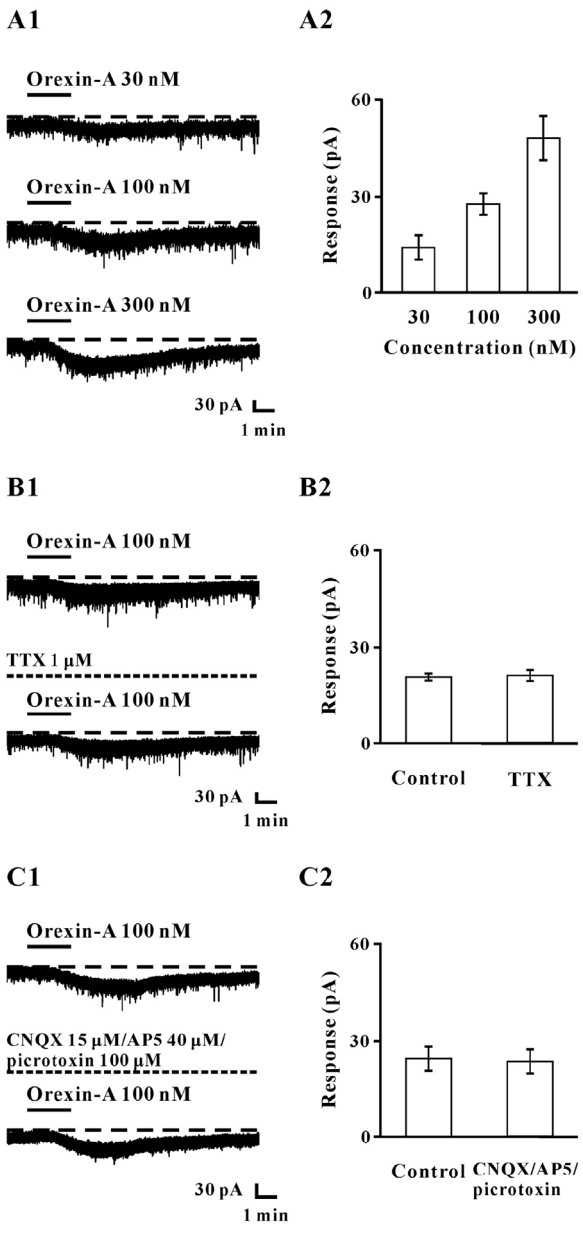

Fig. 1. Orexin evokes inward currents in Gi reticulospinal neurons through postsynaptic mechanisms. (A1) Orexin-A (30–300 nM) dose-dependently evokes inward currents in a tested neuron. (A2) Summary data showing the amplitude of inward currents evoked by orexin-A at concentrations of 30 (n = 6), 100 (n = 8), and 300 (n = 7) nM. (B1) The inward current evoked by orexin-A (100 nM) in the presence and absence of TTX (1 μM) in a tested neuron. (B2) Summary data showing the inward current is not affected by TTX (n = 8). (C1) The inward current evoked by orexin-A (100 nM) in the presence and absence of CNQX (15 μM), AP5 (40 μM) and picrotoxin (100 μM) in a tested neuron. (C2) Summary data showing the inward current also remain unaffected by CNQX, AP5 and picrotoxin (n = 8). In this figure and the following figures, bars represent the mean ± SEM.

pipettes (3–5 MΩ) filled with an internal solution (composition in mM: 130 K-gluconate, 5 KCl, 2 $MgCl_2$, 10 HEPES, 0.1 EGTA, 2 Na_2ATP, 2 Na_2-GTP, 4 Na_2-phosphocreatine, adjusted to pH 7.25 with 1 M KOH) and acquired with an Axopatch-700B amplifier (Axon Instruments, USA). The electrophysiological signals were digitized at 10 kHz using the Digidata-1440A interface (Axon Instruments, USA) and analyzed with Pclamp 10.0 software (Axon Instruments, USA). Neurons were held at a membrane potential of −70 mV and characterized by injection of a rectangular voltage pulse (5 mV, 50 ms) to monitor the whole-cell membrane capacitance, series resistance and membrane resistance. Neurons were excluded from the study