Available online at www.sciencedirect.com
SCIENCE DIRECT®
Neuroscience Research 51 (2005) 55–65
Neuroscience Research
www.elsevier.com/locate/neures

Orexins cause depolarization via nonselective cationic and K^+ channels in isolated locus coeruleus neurons

Yoshinaka Murai*, Tadashi Akaike

Department of Oral Functional Science (Physiology), Graduate School of Dental Medicine, Hokkaido University, Kita 13 Nishi 7, Kita-ku, Sapporo 060-8586, Japan

Received 3 April 2004; accepted 28 September 2004
Available online 6 November 2004

Abstract

The locus coeruleus (LC) contains noradrenergic neurons that are innervated by orexin (ORX)-like immunoreactive axons and express both orexin receptor-1 and -2. We studied effects of ORX-A and -B (ORX-A/B) on dissociated LC neurons by using whole-cell patch clamp techniques. In current-clamp mode, LC neurons were depolarized by application of ORX-A (10^{-7} M) [53% of neurons tested; 9.0 ± 0.2 mV ($n = 5$)], or ORX-B (10^{-7} M) [38% of neurons tested; 4.0 ± 0.1 mV ($n = 5$)]. Firing frequencies of action potentials increased during application [1.1 ± 0.2 Hz ($n = 5$) in ORX-A; 0.8 ± 0.2 Hz ($n = 5$) in ORX-B] and returned to the control level [0.2 ± 0.1 Hz ($n = 5$)] after removal. The ORX-A/B-induced depolarization was well maintained in the presence of TTX (3×10^{-7} M), CNQX (10^{-6} M) and AP5 (10^{-5} M). In voltage-clamp mode, removal of external Na^+ suppressed both ORX-A/B-induced currents and shifted their reversal potentials from approximately -45 mV to -60 mV. In addition, ORX-A/B inhibited sustained K^+ currents. These results suggest that ORX-A/B increase the firing frequency of LC neurons through the depolarization probably produced by both augmentation of the nonselective cationic conductance and inhibition of the sustained K^+ conductance.

Keywords: Mechanically dissociated neuron; Orexin (hypocretin); Locus coeruleus; Noradrenergic neuron; Whole-cell patch clamp; NSCC; Sustained K^+ current

1. Introduction

Orexin (ORX; hypocretin) is a neuropeptide, which is primarily discovered as a ligand for G-protein coupled orphan receptors. ORXs are produced from preprohypocretin, which are subdivided into two active neuropeptides, orexin-A (ORX-A; hypocretin-1) and orexin-B (ORX-B; hypocretin-2) (de Lecea et al., 1998; Sakurai et al., 1998). Neurons in the lateral, posterior, and perifornical hypothalamus express preprohypocretin. The efferent fibers of these neurons show ORX-like immunoreactivity, which widely spread throughout the posterior hypothalamus, and project to multiple targets in the brain (Peyron et al., 1998; Date et al., 1999; van den Pol, 1999). ORX-like immunoreactivity is also reported in the enteric nervous system and pancreas (Kirchgessner and Liu, 1999). Expression of ORX mRNA has also been found in the testes (Sakurai et al., 1998).

The locus coeruleus (LC) neurons are most densely innervated by ORX-imunoreactive fibers (Horvath et al., 1999). LC neurons express G-protein-coupled receptors (Luppi et al., 1995) and also mainly express orexin receptor-1 (OX_1R) while orexin receptor-2 (OX_2R) is less expressed (Trivedi et al., 1998; Greco and Shiromani, 2001; Marcus et al., 2001). The OX_1R shows a selective affinity (10–100 times greater) for ORX-A over ORX-B (Sakurai et al., 1998). The OX_2R, however, binds ORX-A and -B with equal affinities. Hypocretin (Orexin) deficiency appears to be the primary cause of narcolepsy, a disease characterized by excessive day time sleepiness accompanied by slow arousal, unusual REM sleep patterns, cataplexy and hypnogogic hallucinations (Nishino et al., 2000; Peyron et al., 2000; Thannickal et al., 2000). Intraventricular application of ORX-A enhances arousal (Hagan et al., 1999), and local administration of ORX-A to the LC suppresses REM sleep

* Corresponding author. Tel.: +81 11 706 4229; fax: +81 11 706 4227.
E-mail address: murai@den.hokudai.ac.jp (Y. Murai).

0168-0102/$ – see front matter
doi:10.1016/j.neures.2004.09.005

and increases wakefulness (Bourgin et al., 2000). These findings are comparable to the observations that the noradrenergic LC neurons play important roles in directed attention (Usher et al., 1999) and arousal/sleep modulation (Aston-Jones and Bloom, 1981; Hagan et al., 1999; Bourgin et al., 2000). The unique distribution of ORX-containing axons and functional activation of various neuronal circuits by ORXs suggest that ORXs within the central nervous system (CNS) may function as neurotransmitters (de Lecea et al., 1998; Hagan et al., 1999; Horvath et al., 1999; Bourgin et al., 2000). Administration of ORX-A depolarizes the membrane and increases the excitability of LC neurons in brain slice preparations (Soffin et al., 2002; van den Pol et al., 2002). However, it is rather difficult to delineate the site of ORX action in the brain slices, since the effects on presynaptic terminals, postsynaptic somata and glial cells are intermingled.

In the present study, we used mechanically dissociated LC neurons from rat brain slices and applied ORXs directly on the cell membrane. We studied effects of the ORXs on the LC neurons under the current- and voltage-clamp conditions. ORXs depolarized the membrane and increased the firing frequency, probably through both activation of the non-selective cationic conductance (NSCC) and suppression of the sustained K^+ conductance in the LC neurons.

2. Materials and methods

2.1. Preparation

The LC neurons were acutely dissociated from 10- to 14-day-old Wistar rats as described previously (Shirasaki et al., 1990). Briefly, the rats were anesthetized with pentobarbital sodium (50 mg kg^{-1} i.p.) and decapitated. The brain was quickly removed, cooled at 4 °C with chilled artificial cerebrospinal fluid (ACSF) for 5 min, and was cut into slices (400 μm) with a microslicer (DTK-3000W, Dosaka). The slices were pre-incubated in ACSF solution saturated with 95% O_2/5% CO_2 at room temperature for 30–60 min. Under the binocular microscope the LC were identified as an aggregate of neurons that located in a region adjacent to the medial side of the mesencephalic trigeminal nucleus (Vmes) and to the lateral side of the fourth ventricle at the level of the cerebellar peduncle. To dissociate the LC neuron from the slice preparation, a fire-polished glass pipette (tip diameter of 0.3 mm) was touched lightly onto the slice surface of the LC region and was vibrated horizontally at 30–40 Hz with a custom made device in the plastic culture dish (diameter 35 mm; Falcon). Among the mechanically dissociated LC neurons, a few were alive and adhered to the bottom of the dish within 10 min. The dissociated LC neurons were easily distinguished from the Vmes neurons morphologically; the former has an oval shaped cell body with a few short robust dendrites, while the latter has a round cell body without dendrites.

2.2. Solutions

The ACSF solution had the following composition (in mM): 124 NaCl, 1.8 KCl, 2.5 $CaCl_2$, 1.3 $MgCl_2$, 26 $NaHCO_3$, 1.2 KH_2PO_4, and 10 glucose. The standard external solution for patch-clamp recording contained (in mM): 150 NaCl, 5 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), and 10 glucose. The pH was adjusted to 7.4 with tris(hydroxymethyl) aminomethane (Tris-base). The internal solution of patch pipettes for whole-cell recording had the following ionic composition (in mM): 140 K-gluconate, 0.1 $CaCl_2$, 2 $MgCl_2$, 1.1 ethylene glycol-bis(β-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 2 ATP-Na_2, and 10 HEPES and was adjusted to pH 7.4 with KOH. A rapid application of external solution was performed with 'Y-tube' technique described previously (Nakagawa et al., 1990).

2.3. Whole-cell recordings

The membrane currents were measured with a patch-clamp amplifier (CEZ-2400, Nihon Kohden). Glass patch-pipettes were made with a micropipette puller (model P-97, Sutter instrument co.). The resistance of the patch-pipette was 4–6 MΩ when filled with the internal solution. The seal resistance was usually more than 5 GΩ. The series resistance was usually less than 15 MΩ, and recordings in which the series resistance was more than 20 MΩ were not included in the analysis. Input resistance was measured by the voltage responses to hyperpolarizing current pulses. Whole-cell currents were low-pass filtered at 5–10 kHz (3-pole Bessel filter), digitized at a sampling rate of 20 kHz and stored on a computer hard disc (pCLAMP 8, Axon instruments, Inc.). All experiments were performed at room temperature (21–24 °C).

2.4. Identification of the LC neurons

In addition to morphological features (see above) typical LC neurons show several electrophysiological properties (Henderson et al., 1982); the LC neurons fire action potentials (APs) repetitively in response to depolarizing current pulses and show linear current–voltage relation at low intensities of injected currents (Fig. 1A). While, the Vmes neurons generate one or a few APs with low intensities of depolarizing pulses, and show an accommodation; an initial burst of APs driven by strong depolarizing pulses are not sustained. The Vmes cells have time-dependent anomalous rectification with hyperpolarizing current pulses. In this study, the LC neurons were identified with these properties for each experiment.

2.5. Drugs

Drugs used in the present study were: 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), DL-2-amino-5-phos-