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Modulation by orexin A of spontaneous excitatory and inhibitory transmission in adult rat spinal substantia gelatinosa neurons

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ABSTRACT

Hypothalamic neuropeptides, orexins A and B, differently inhibit nociceptive behavior. This difference is possibly due to a distinction between orexins A and B in modulating synaptic transmission in spinal substantia gelatinosa (SG) neurons that play a pivotal role in regulating nociceptive transmission. Although we previously reported a modulatory action of orexin B on synaptic transmission in adult rat SG neurons, it has not been fully examined how the transmission is affected by orexin A. The present study examined the effects of orexin A on spontaneous excitatory and inhibitory transmission in SG neurons of adult rat spinal cord slices by using the whole-cell patch-clamp technique. Like orexin B, orexin A produced an inward current at −70 mV and/or increased the frequency of spontaneous excitatory postsynaptic current without changing its amplitude. Half-maximal effective concentration values for their effects were 0.0045 and 0.030 μM, respectively; the former value was four-fold smaller than that of orexin B while the latter value was comparable to that of orexin B. Orexin A enhanced not only glycinergic but also GABAergic transmission, although only glycinergic transmission was facilitated by orexin B in the majority of neurons tested. Orexin A activities were inhibited by an orexin-1 receptor antagonist (SB334867) but not an orexin-2 receptor antagonist (JNJ10397049), as different from orexin B whose activation was depressed by JNJ10397049 but not SB334867. These results indicate that orexin A has a different action from orexin B in SG neurons in efficacy for inward current production and in GABAergic transmission enhancement, possibly owing to orexin-1 but not orexin-2 receptor activation. This difference could contribute to at least a part of the distinction between orexins A and B in antinociceptive effects.

1. Introduction

There is anatomical and behavioral evidence showing that hypothalamic neuropeptides, orexins A and B, inhibit nociceptive transmission in the spinal dorsal horn. Orexinergic fibers in the hypothalamus project to the spinal dorsal horn in rodents [1], orexins A and B exist in the rat spinal cord [2] and the rat spinal cord expresses orexin-1 and orexin-2 receptors [3,4] that are activated by orexins A and B [5]. Intrathecally-administrated orexins A and B produce antinociception in rodents [6,7].

In order to elucidate cellular mechanisms for antinociception produced by orexins A and B, their effects on glutamatergic excitatory transmission in spinal dorsal horn lamina II (substantia gelatinosa; SG) neurons have been examined in rats [8,9]. The SG neurons play a pivotal role in regulating nociceptive transmission from the periphery [10]. Orexin A produced a membrane depolarization and presynaptically enhanced spontaneous excitatory transmission, both of which actions were inhibited by orexin-1 and orexin-2 receptor antagonists, in young rat SG neurons [9]. Similar post- and presynaptic actions were produced by orexin B in young [8] and adult rat SG neurons [11]. Although the effects of orexin-receptor antagonists on orexin B activities were not tested in young rats [8], the activities in adult rat SG neurons were inhibited by orexin-2 but not orexin-1 receptor antagonist [11].

We previously reported that antinociception produced by another hypothalamic neuropeptide oxytocin may be attributed to GABAergic and glycinergic spontaneous inhibitory transmission enhancements in SG neurons [12]. This idea may be applied to

Abbreviations: EC_{50}, half-maximal effective concentration; sEPSC, spontaneous excitatory postsynaptic current; sIPSC, spontaneous inhibitory postsynaptic current; SG, substantia gelatinosa; TTX, tetrodotoxin; V_H, holding potential.

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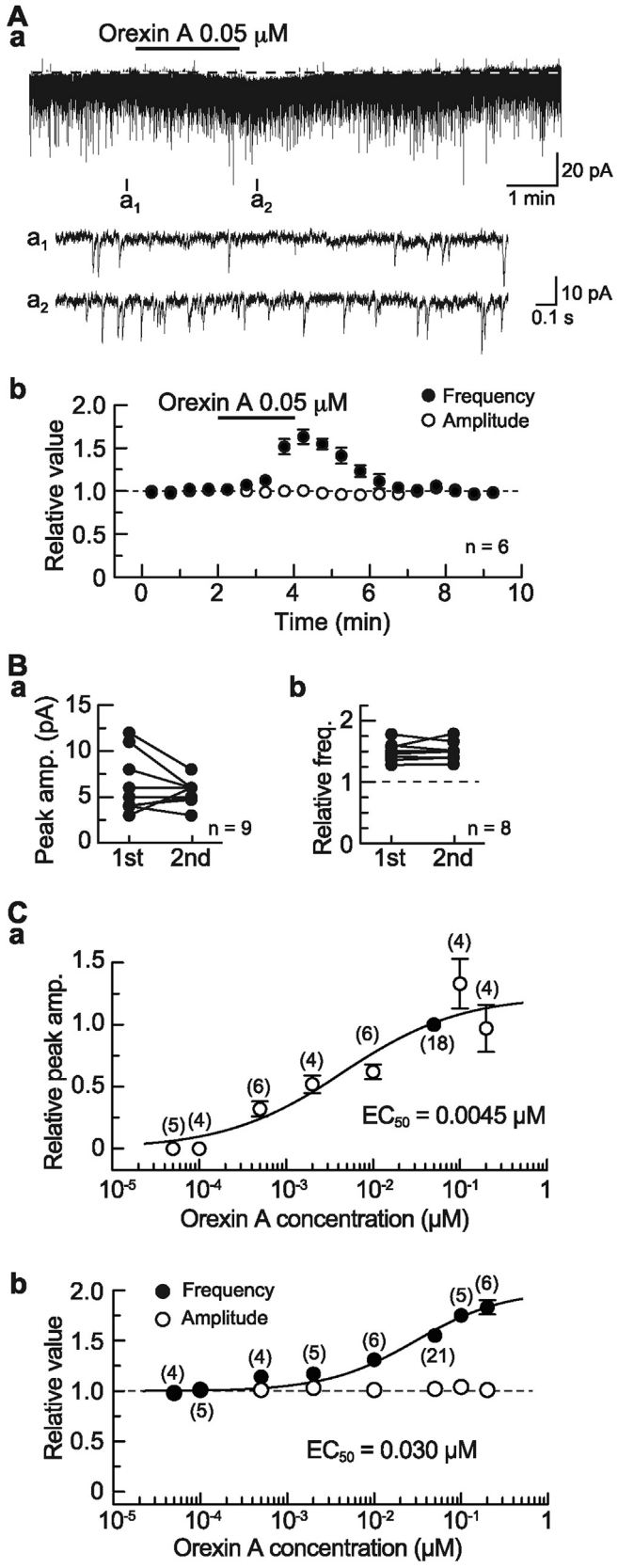

Fig. 1. Orexin A produced inward current and presynaptic spontaneous excitatory transmission enhancement in a reversible and concentration-dependent manner in adult rat SG neurons. (Aa, b) Orexin A (0.05 μM) produced an inward current and increased the frequency of sEPSC without changing its amplitude. (Aa) Recordings showing inward current and excitatory transmission enhancement following orexin A superfusion for 2 min. (Ab) Average time courses of changes in sEPSC frequency and amplitude following exposure to orexin A for 2 min, relative to those before its application (control). (Ba, b) Peak amplitude (amp.) of inward current (Ba) and sEPSC frequency (freq.; Bb; measured for 30 s around 2 min after superfusion onset; relative to control) in response to orexin A in the first (1st) and second (2nd) applications. The interval between repeated applications was 20 min. In each of (Ba) and (Bb), results obtained from the same neuron are bound by a straight line. (Ca, b) Concentration dependencies for orexin A activities. (Ca) Peak amplitudes of inward currents elicited by orexin A at various concentrations, relative to current elicited at 0.05 μM (closed circle; 5.9 ± 0.4 pA, $n = 18$), are plotted against the logarithm of orexin A concentration. (Cb) Frequency and amplitude of sEPSCs in response to orexin A, relative to control, are plotted against the logarithm of orexin A concentration. Results were obtained from neurons where orexin A (0.05 μM) increased sEPSC frequency by more than 5% [control frequency and amplitude = 10.8 ± 1.2 Hz and 11.2 ± 0.8 pA ($n = 21$), respectively]. This effect of orexin A was measured for 30 s around 2 min after commencing superfusion. Continuous curves in (Ca) and (Cb) were drawn according to the Hill equation [half-maximal effective concentration (EC_{50}) and Hill coefficient in (Ca) and (Cb) = 0.0045 μM and 0.64, and 0.030 μM and 0.85, respectively]. In (Ab), (Ca) and (Cb), each point with vertical bars represents mean values and SEM; if the SEM of values is less than the size of the symbol, the vertical bar is not shown. In this and subsequent figures, the duration of drug superfusion is shown by a horizontal bar above the chart recording; traces of synaptic responses for periods indicated by short vertical bars designated as a-d, located below the chart recording, are shown in an expanded scale in time; value in parentheses indicates the number of neurons tested; holding current level in the control in the chart recording is indicated by a dotted line; and control level (1) in the graph is indicated by a dotted line. $V_H = -70$ mV.

antinociception produced by orexins, because orexin B enhances glycinergic spontaneous inhibitory transmission in young and adult SG neurons [8,13]. It has not been examined yet how orexin A affects spontaneous inhibitory transmission in rat SG neurons. Although orexin A is reportedly more effective in producing antinociception than orexin B [6,7], this difference may be due to a distinction between orexins A and B in modulating synaptic transmission in SG neurons. To address this issue, we examined the effect of orexin A on spontaneous excitatory and inhibitory synaptic transmission in SG neurons of adult rat spinal cord slices by using the whole-cell patch-clamp technique.

2. Materials and methods

All animal experiments were approved by the Animal Care and Use Committee of Saga University. Slice preparations from the adult rat spinal cord were prepared as described elsewhere [12]. Briefly, adult male Sprague-Dawley rats were anesthetized with urethane, and then a lumbosacral segment (L_1-S_3) of the spinal cord was extracted and placed in cold pre-oxygenated Krebs solution (2–4 °C) pre-equilibrated with 95% O_2 and 5% CO_2. After cutting all ventral and dorsal roots, the pia-arachnoid membrane was removed. The spinal cord was mounted on a microslicer, and then a 650 μm-thick transverse slice was cut. The slice was transferred to a recording chamber, and completely submerged and superfused at a rate of 12–15 ml/min with Krebs solution saturated with 95% O_2 and 5% CO_2 at 36 ± 1 °C. The composition of Krebs solution used was (in mM): NaCl, 117; KCl, 3.6; $CaCl_2$, 2.5; $MgCl_2$, 1.2; NaH_2PO_4, 1.2; $NaHCO_3$, 25; and glucose, 11 (pH = 7.4).

Whole-cell voltage-clamp recordings were made from SG neurons by using patch-pipettes fabricated from thin-walled, fiber-filled capillaries, as done previously [12]. The patch-pipette solutions used (in mM) to record spontaneous excitatory and inhibitory postsynaptic currents (sEPSCs and sIPSCs, respectively) contained: 135 K-gluconate; 5 KCl; 0.5 $CaCl_2$; 2 $MgCl_2$; 5 EGTA; 5 HEPES; 5 Mg-ATP; and 110 Cs_2SO_4; 0.5 $CaCl_2$; 2 $MgCl_2$; 5 EGTA; 5 HEPES; 5 Mg-ATP; 5 tetraethylammonium-Cl (pH = 7.2), respectively. The sEPSCs and sIPSCs were recorded at the holding potentials (V_Hs) of −70 and 0 mV, respectively. Signals were acquired using an Axopatch 200 B amplifier (Molecular Devices, Sunnyvale, CA, USA). Data were low-pass filtered at 5 kHz and digitized at 333 kHz with an A/D

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