Neuroscience Letters 520 (2012) 92–97

Contents lists available at SciVerse ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Orexin-A excites pyramidal neurons in layer 2/3 of the rat prefrontal cortex

Jie Yan, Chao He, Jian-Xia Xia, Dan Zhang, Zhi-An Hu *

Department of Physiology, Third Military Medical University, Chongqing, PR China

HIGHLIGHTS

- Orexin-A directly excited the layer 2/3 pyramidal neurons in the rat mPFC.
- This excitatory effect was mediated via OX1R.
- The ionic mechanisms involved K^+ channels and nonselective cation channels.

ARTICLE INFO

Article history:
Received 29 February 2012
Received in revised form 23 April 2012
Accepted 10 May 2012

Keywords:
Orexin-A
Prefrontal cortex
Layer 2/3

ABSTRACT

The arousal peptides, orexins, play an important role in regulating the function of the prefrontal cortex (PFC). Although orexins have been shown to increase the excitability of deep-layer neurons in the medial prefrontal cortex (mPFC), little is known about their effect on layer 2/3, the main intracortical processing layer. In this study, we investigated the effect of orexin-A on pyramidal neurons in layer 2/3 of the mPFC using whole-cell recordings in rat brain slices. We observed that orexin-A reversibly depolarized layer 2/3 pyramidal neurons through a postsynaptic action. This depolarization was concentration-dependent and mediated via orexin receptor 1. In voltage-clamp recordings, the orexin-A-induced current was reduced by the replacement of internal K^+ with Cs^+, removal of external Na^+, or an application of flufenamic acid (an inhibitor of nonselective cation channels). A blocker of Na^+/Ca^{2+} exchangers (SN-6) did not influence the excitatory effect of orexin-A. Moreover, the current induced by orexin-A reversed near E_k when the external solution contained low levels of Na^+. When recording with Cs^+-containing pipettes in normal external solution, the reversal potential of the current was approximately −25 mV. These data suggest an involvement of both K^+ channels and nonselective cation channels in the effect of orexin-A. The direct excitatory action of orexin-A on layer 2/3 mPFC neurons may contribute to the modulation of PFC activity, and play a role in cognitive arousal.

1. Introduction

Orexins (hypocretins) have been recognized as a critical regulator of arousal, as disturbances of the orexin system are associated with the sleep-wake disorder, narcolepsy [24]. Moreover, several lines of evidence indicate that orexins may also participate in the regulation of a variety of affective and cognitive processes during wakefulness. For example, narcoleptic patients often show psychiatric-like symptoms and have deficits in attentive processing [7,23].

The prefrontal cortex (PFC) shows high levels of activity during arousal and has been implicated in working memory, attention, and emotional behavior. Both types of orexin receptor, orexin receptor 1 (OX1R) and orexin receptor 2 (OX2R), as well as orexin-containing nerve terminals, are distributed extensively throughout the PFC [3,6,11]. In addition, infusions of orexin-B into the rat medial prefrontal cortex (mPFC), an area homologous to the primate PFC, can improve the attention of rats [16]. These results suggest that the hypothalamic orexin system may regulate PFC activity through direct innervation of PFC neurons. Indeed, our previous studies have revealed a postsynaptic excitatory effect of orexin-A on PFC neurons in layers 5 and 6, due to inhibition of K^+ channels and hyperpolarization-activated/cyclic nucleotide-gated channels, as well as activation of an extracellular Ca^{2+} influx [17,26,28,29]. Nevertheless, all these studies have focused on neurons in the deep layers of the PFC, which are the main source of cortico-subcortical connections. It has yet to be determined whether orexins directly act on neurons in layer 2/3, the layer which mediates communication across the cortex [9]. A study in the primary somatosensory

Abbreviations: ACSF, artificial cerebrospinal fluid; FFA, flfemaic acid; I–V, current–voltage; mPFC, medial prefrontal cortex; NMDG, N-methyl-D-glucamine; OX1R, orexin receptor 1; OX2R, orexin receptor 2; PFC, prefrontal cortex; TTX, tetrodotoxin.

* Corresponding author at: Department of Physiology, Third Military Medical University, Chongqing 400038, PR China. Tel.: +86 023 68752254; fax: +86 023 68752254.
E-mail address: zhianhu@yahoo.com.cn (Z.-A. Hu).

0304-3940/$ – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neulet.2012.05.038

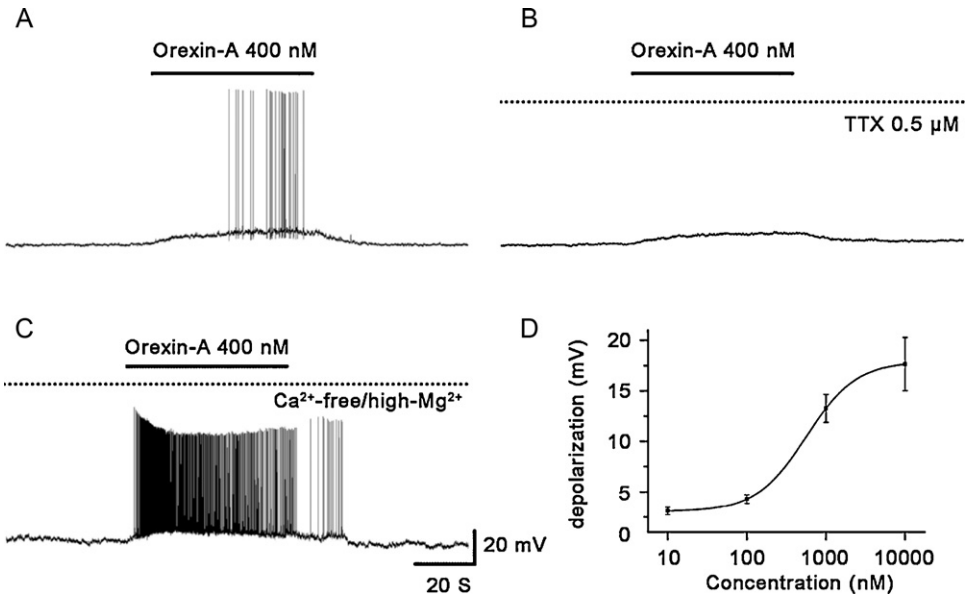

Fig. 1. Orexin-A excites layer 2/3 mPFC neurons in a reversible, postsynaptic, and concentration-dependent manner. (A) The excitatory response of a layer 2/3 mPFC neuron to orexin-A (400 nM) in normal ACSF. (B) The response to orexin-A in the presence of TTX at 0.5 μM. (C) The response to orexin-A in Ca^{2+}-free/high-Mg^{2+} ACSF. (D) Concentration-response curve of the depolarization induced by orexin-A.

and motor cortex has reported that orexins exclusively excite layer 6b neurons, with other layers showing little response [1]. However, histochemical studies showing the presence of a higher density of OX1R in the PFC compared with other cortical areas and the distribution of OX1R in the superficial layers of the mPFC, suggest that orexins may have a more extensive action in the mPFC [17,20]. In the present study, we tested the effect of orexin-A on layer 2/3 pyramidal neurons in rat mPFC slices, and examined the possible mechanisms involved.

2. Materials and methods

The experimental protocols used conformed to the China Animal Welfare Legislation and were approved by the Third Military Medical University Committee on Ethics in the Care and Use of Laboratory Animals. Coronal brain slices (350 μm thick) containing the mPFC were prepared from Sprague Dawley rats (14–18 days old) using methods as previously described [17]. The slices were incubated in oxygenated artificial cerebrospinal fluid (ACSF, composition in mM: NaCl 124, KCl 3, $NaHCO_3$ 26, $MgCl_2$ 2, NaH_2PO_4 1.25, $CaCl_2$ 2, glucose 10, aerated with 95% O_2 and 5% CO_2, with a pH of 7.3–7.4) at 35 °C for at least 1 h. During recording, the slices were transferred to a submerged chamber and continuously perfused (1–2 ml/min) with oxygenated ACSF at room temperature (23–25 °C). Whole-cell recordings were performed with pipettes (5–7 MΩ) filled with (in mM): K-gluconate 145, HEPES 10, EGTA 1, $MgCl_2$ 2, K_2-ATP 2, with a pH of 7.3. Layer 2/3 pyramidal neurons in the mPFC were visually identified using a Leica DMLFS

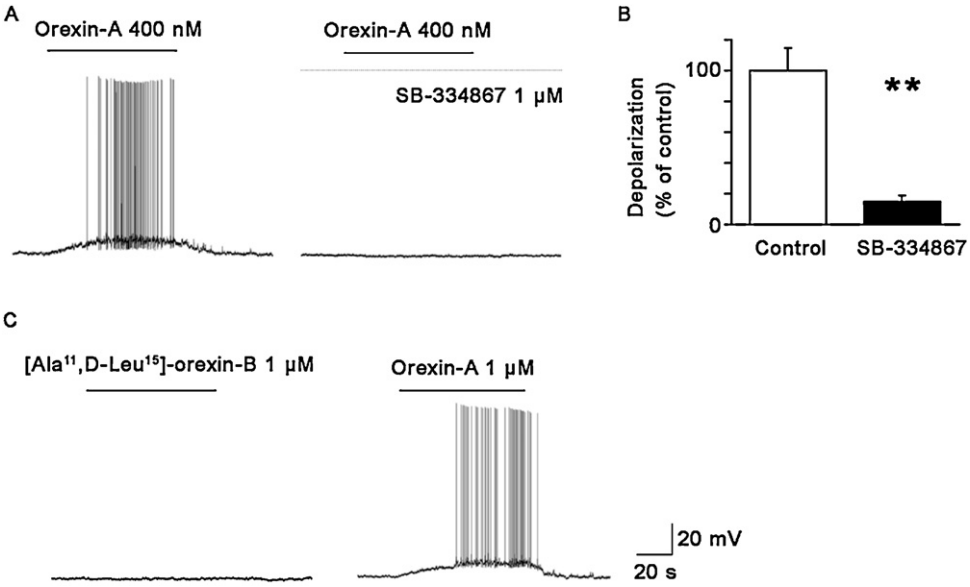

Fig. 2. The excitation induced by orexin-A is mediated through OX1R. (A) The selective OX1R antagonist SB-334867 (1 μM) blocked the excitation caused by orexin-A. (B) Bar graph showing the decrease in the orexin-A-induced depolarization in the presence of SB-334867 ($n = 5$). $^{**}p < 0.01$. (C) Example traces from a neuron that did not respond to the OX2R-selective agonist [Ala^{11}, D-Leu^{15}]-orexin-B at 1 μM, but was excited by orexin-A at the same concentration.