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The Subthalamic Neurons are Activated by Both Orexin-A and Orexin-B

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Abstract—The subthalamic nucleus is an important nucleus in the indirect pathway of the basal ganglia circuit and therefore is involved in motor control under both normal and pathological conditions. Morphological studies reveal that the subthalamic nucleus receives relatively dense orexinergic projections originating from the hypothalamus. Both orexin-1 (OX_1) and orexin-2 (OX_2) receptors are expressed in the subthalamic nucleus. To explore the functions of orexinergic system in the subthalamic nucleus, extracellular electrophysiological recordings and behavioral tests were performed in the present study. Exogenous application of orexin-A significantly increased the spontaneous firing rate from 5.70 ± 0.66 Hz to 9.87 ± 1.18 Hz in 64.00% subthalamic neurons recorded. OX_1 receptors are involved in orexin-A-induced excitation. Application of orexin-B increased the firing rate from 7.47 ± 0.92 Hz to 11.85 ± 1.39 Hz in 80.95% subthalamic neurons recorded, entirely through OX_2 receptors. Both OX_1 and OX_2 receptor antagonists decreased the firing rate in 43.75% and 62.50% subthalamic neurons recorded respectively, suggesting the involvement of endogenous orexinergic system in the control of spontaneous firing activity. Further elevated body swing test revealed that microinjection of orexins and the receptor antagonists into the subthalamic nucleus induced contralateral-biased swing and ipsilateral-biased swing, respectively. Taken together, the present study suggests that orexins play important roles in the subthalamic nucleus which may provide further evidence for the involvement of subthalamic orexinergic tone in Parkinson's disease.

Significance: **Previous morphological studies indicate that the subthalamic nucleus receives orexinergic innervation and expresses both OX_1 and OX_2 receptors. Using *in vivo* multibarrel electrophysiological recordings, the present study revealed that exogenous application of orexin-A and orexin-B increased the spontaneous firing rate of the subthalamic neurons through OX_1 and OX_2 receptors. Endogenous orexinergic system was involved in the control of spontaneous firing of the subthalamic neurons. Further behavioral test revealed that intrasubthalamic application of orexins and the receptor antagonists induced biased swing behavior. The present study may provide further evidence for the involvement of subthalamic orexinergic tone in Parkinson's disease.**

Key words: subthalamic nucleus, orexin-A, orexin-B, single unit recording, elevated body swing test.

INTRODUCTION

The subthalamic nucleus is an important structure in the indirect pathway of the basal ganglia circuit (Tepper et al., 2007). In terms of anatomy, the subthalamic nucleus receives its main inhibitory GABAergic input from the globus pallidus and excitatory glutamatergic inputs from the cerebral cortex (Bevan et al., 1995, 2002; Yasoshima et al., 2005). The principal neurons of the subthalamic nucleus are glutamatergic neurons, which send efferent axons to multi-targets including the substantia nigra, the globus pallidus (Smith et al., 1998; Bolam et al., 2000). The subthalamic neurons generate action potentials spontaneously, with abnormal oscillatory and synchronous firing activity recorded from parkinsonian patients and animal models (Rodriguez-Oroz et al., 2001). Nowadays, the most common surgical therapeutic approach of Parkinson's disease involves deep brain stimulation of the subthalamic nucleus (Kocabicak et al., 2012; Wichmann and DeLong, 2016).

Orexins, generally known as hypocretins, include orexin-A and orexin-B which are derived from the same prepro-orexin precursor in the lateral hypothalamus (de Lecea et al., 1998). Although orexinergic cell bodies are located restrictedly within the lateral hypothalamus, their ascending and descending projections are widely distributed in central nervous system (Peyron et al., 1998). There are two types of orexin receptors, orexin-1 (OX_1)

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Abbreviations: EBST, elevated body swing test; OX_1, orexin-1; OX_2, orexin-2.

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and orexin-2 (OX_2) receptors, which belong to G-protein-coupled receptors. Orexin-A and orexin-B bind to both OX_1 and OX_2 receptors. However, orexin-B shows 10-fold affinity for OX_2 receptors, while orexin-A shows similar affinity at both receptors (Marcus et al., 2001; Langmead et al., 2004). Central orexinergic system plays important roles in many physiological processes (Li et al., 2014; Sakurai, 2014), including regulation of sleep and wakefulness (Sutcliffe and de Lecea, 2002; Ohno and Sakurai, 2008; Tsujino and Sakurai, 2013), energy metabolism, feeding behavior (Ganjavi and Shapiro, 2007; Girault et al., 2012), modulation of nociceptive behavior (Mobarakeh et al., 2005; Mohammad Ahmadi Soleimani et al., 2015; Razavi and Hosseinzadeh, 2017), drug withdrawal (Ahmadi-Soleimani et al., 2014, 2017), neuroendocrine, reward processes (Sadeghi et al., 2016) and motor regulation (Hara et al., 2001; Zhang et al., 2011; Hu et al., 2015).

Morphological studies reveal relatively dense orexinergic projections in the subthalamic nucleus (Peyron et al., 1998). Both OX_1 and OX_2 receptors are expressed in the subthalamic nucleus. Early *in situ* hybridization illustrates that OX_2 receptor mRNA is abundant in some brain regions including the subthalamic nucleus (Trivedi et al., 1998). Immunohistochemical studies also reveal that strong OX_2 receptor-like protein immunosignals are present in the subthalamic nucleus (Cluderay et al., 2002). In addition to OX_2 receptor, high levels of OX_1 receptor mRNA (Lu et al., 2000) and protein (Hervieu et al., 2001) are distributed in the subthalamic nucleus. Being a critical position in the basal ganglia circuits, the subthalamic nucleus plays important roles in movement control. Recently, the involvement of central orexinergic system in motor control receives more and more attention (Hu et al., 2015). Both orexinergic fibers (Peyron et al., 1998) and orexinergic receptors (Trivedi et al., 1998) are distributed in central motor control system. Orexin deficiency in motor cortex causes higher motor thresholds (Oliviero et al., 2005; Nardone et al., 2011). In addition, microinjection of orexins into the substantia nigra pars compacta significantly increases time spent for moving (Kotz et al., 2006). The above findings may suggest some association between subthalamic orexinergic tone and motor control. However, to date, the functions of orexinergic system in the subthalamic nucleus are unknown. To answer these questions, in the present study, *in vivo* multibarrel electrophysiological recordings were used to study the direct electrophysiological effects and receptor mechanisms of subthalamic orexinergic tone at single cell level. We also microinjected orexins directly into the subthalamic nucleus to observe the possible influence on motor behaviors. Our results revealed that both orexin-A and orexin-B increased the spontaneous firing rate of the subthalamic neurons. Blockade of OX_1 and OX_2 receptors decreased the firing rate of the subthalamic neurons, suggesting that endogenous orexinergic system modulates the spontaneous discharge of the subthalamic neurons. The subthalamic orexinergic tone may be involved in motor behavior through its modulation of spontaneous firing activity.

EXPERIMENTAL PROCEDURES

Animals

Adult male Wistar rats (220–320 g) were used in present electrophysiological and behavioral studies. Rats were obtained from Qingdao Experimental Animals Centre (Qingdao, China). The animals were housed in a temperature-regulated (23 ± 1 °C) room with a 12-h light/dark cycle. The rats were fed with standard pellet diet and water *ad libitum*. All the experiments were approved and the procedures were performed in accordance with institutional guidelines of the Animal Care and Use Committee at Qingdao University. The rats used for *in vivo* electrophysiological recordings and behavioral tests were randomly divided into various experimental groups. All efforts were taken to reduce rats' pain and sufferings.

In vivo electrophysiological recordings

Rats were deeply anesthetized with urethane (1 g/kg, i.p.). During the period of electrophysiological recordings, the anesthesia was maintained with supplemental doses of urethane (0.16 g/kg). The rats were gently placed in a stereotaxic apparatus (Narishige SN-3, Tokyo, Japan) with rectal temperature maintained at 36–38 °C. A craniotomy was performed at coordinates of 3.1–4.2 mm posterior and 2.0–3.0 mm lateral from the bregma according to the stereotaxic atlas (Paxinos and Watson, 1998). Three-barrel microelectrodes were prepared using a vertical pipette puller (Stoelting, IL, USA), with the tip diameter of 3–10 μm and the resistance of 10–20 MΩ. Then the microelectrodes were stereotaxically positioned into the subthalamic nucleus. One of the three-barrel microelectrodes was recording barrel which was filled with 0.5 M sodium acetate containing 2% pontamine sky blue dye. The other two barrels connecting to four-channel pressure injector (PM2000B, Micro Data Instrument, Inc., USA) were drug application barrels. Based on the stereotaxic location, as well as the discharge features of short (~1 ms duration) and biphasic waveform (Urbain et al., 2002), the spontaneous firing neurons were identified online as presumed subthalamic neurons. Drugs were ejected onto the surface of the neurons with gas pressure (1500 ms, 5.0–15.0 psi).

The electrical signals of spontaneous firing were amplified by a micro-electrode amplifier (MEZ-8201, Nihon Kohden, Tokyo, Japan). The recorded signals were displayed on a memory oscilloscope (VC-11, Nihon Kohden) and monitored by an audiomonitor. The amplified signals were passed through low pass filter (0.3 kHz) and high-pass filter (3 kHz) into a bioelectricity signal analyzer and computer. Micro 1401 and spike 2 software (Cambridge Electronic Design, UK) were used to analyze the spike data online and further offline. Usually after 10 min stable baseline recording, drug was ejected into the subthalamic nucleus. Drug application was performed only one time in the same track. The baseline spontaneous firing parameters, including firing rate, coefficient of variation (CV) of the interspike intervals (ISI) and Fano Factor (FF), were determined