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Paliperidone increases spontaneous and evoked firing of mesocortical dopaminergic neurons by activating a hyperpolarization-activated inward current

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ABSTRACT

Mesocortical dopaminergic (DA) subtype neurons specifically project to the prefrontal cortex, which is closely related with schizophrenia. Mesocortical DA neurons have unique physiological characteristics that are different from those of mesostriatal and mesolimbic DA neurons. Paliperidone, an atypical antipsychotic, is currently used to treat schizophrenia and has better therapeutic effects than typical antipsychotics. However, the underlying physiological mechanism remains unclear. To explore the effects of paliperidone on mesocortical DA neuron activity, here, we retrogradely labeled these cells with fluorescent microsphere retrobeads, and the electrophysiological changes were recorded in whole-cell recordings in rat midbrain slices with or without paliperidone. The data showed that paliperidone (20 μmol/L) increased the spontaneous firing rates of labeled mesocortical neurons ($P < 0.05$). Moreover, paliperidone also increased the frequency of evoked action potentials by current injection stimulation ($P < 0.05$), whereas the accompanying amplitude decreased. Furthermore, to explore the mechanisms of paliperidone's effect, I_h currents were detected, and the results showed that hyperpolarizing voltage pulses evoked instantaneous I_h inward currents and paliperidone increased the maximum I_h current. In addition, paliperidone decreased the spontaneous inhibitory postsynaptic currents. Thus, paliperidone increased the spontaneous and evoked firing of mesocortical neurons, possibly by activating the I_h inward current and reducing the inhibitory synaptic transmission, which provides an underlying mechanism of paliperidone's application in schizophrenia.

1. Introduction

Mesencephalon dopaminergic (DA) neurons are mainly distributed in the substantia nigra (SN) and ventral tegmental area (VTA). These neurons project to the striatum, prefrontal cortex and nucleus accumbens based on their anatomical locations and accordingly are subdivided into mesostriatal, mesocortical and mesolimbic DA subtype neurons (Hu et al., 2004). Mesocortical and mesolimbic DA neurons are mainly located in the VTA, while mesostriatal DA neurons are located in the SN. Clinical studies have indicated that DA neurons in the SN selectively degenerate in Parkinson's disease, whereas adjacent VTA DA neurons are rarely affected (Datla et al., 2001; Grimm et al., 2004). This implied different characteristics of these three subtype DA neurons. Mesocortical DA neurons project to the prefrontal cortex and secrete dopamine, which is a key neurotransmitter that affects the cognitive function of the brain (Lessard et al., 2009; El-Rawas et al., 2009). Moreover, an increasing number of studies have also demonstrated that the mesocortical projection is associated with psychiatric disorders and dysfunctional activity; for example, cell excitability defects of mesocortical neurons may cause schizophrenia (Lammel et al., 2008; Carr and Sesack, 2000). Therefore, understanding the biological characteristics of mesocortical subtype neurons is critical for exploring their contributions to psychiatric diseases.

Considerable evidence suggests that an abnormal level of dopamine in the prefrontal cortex is associated with neuronal synaptic plasticity and cognitive dysfunction (Selemon and Zecevic, 2015). Accordingly, the most of prescribed drugs for treating schizophrenia target the dopamine pathway. Paliperidone is a new type of atypical antipsychotic and is an active metabolite of risperidone (Corena-McLeod, 2015). Previous studies have demonstrated that paliperidone exhibits effective therapeutic efficacy for schizophrenia, especially for the negative symptoms and cognitive disorders (Meyer et al., 2005). It was reported that paliperidone binds dopamine D_2 receptors and also antagonizes the adrenergic and histamine receptors (Muly et al., 2012). The cell

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excitability of mesocortical neurons determines the neurotransmitter secretion and then regulates the physiological functions. However, we knew relatively little about paliperidone's effects on neuronal activity of mesocortical DA neurons and, thereby, schizophrenia.

Hyperpolarization-activated (I_h) current is carried by Na^+ and K^+ ions (Rateau and Ropert, 2006). In midbrain slices, DA neurons can be identified by the electrophysiological characteristics of the presence of I_h (Chu and Zhen, 2010). Additionally, it has been reported that nearly 98% of VTA neurons exhibiting I_h are also tyrosine hydroxylase positive (TH), which suggests a high correlation between I_h and DA neurons (Wanat et al., 2008). Importantly, if I_h is blocked or inhibited, the spontaneous firing activity in a subtype of DA neurons is obviously reduced (Neuhoff et al., 2002). Thus, the neuronal excitability can be regulated by I_h channel activity. Due to the importance of I_h current activity in neuronal physiological properties and its role in synaptic transmission learning and memory (Brager et al., 2013), it is plausible that the alteration of I_h channel activity in DA neurons may cause functional changes associated with psychiatric disorders. It has previously been demonstrated that GABA synaptic receptors, which are located at synapses of postsynaptic neurons, generate the inhibitory postsynaptic currents (IPSCs) (Pavlov et al., 2009). These GABA receptor-mediated currents reduce neuronal excitability. It has also been suggested that paliperidone prolongs the cortical silent period, possibly due to GABA receptor-mediated neurotransmission (Prikryl et al., 2009). Whether paliperidone affects the cellular excitability of the mesocortical subtype of dopaminergic neurons and, if so, what the underlying mechanisms are need to be determined.

To determine paliperidone's effects on mesocorical neurons, first, we retrogradely labeled mesocortical subtype neurons with microsphere retrobeads that were identified by tyrosine hydroxylase (TH) immunohistochemical staining. Second, in whole-cell recordings in rat brain slices, we detected the effects of paliperidone on spontaneous and evoked firing of mesocortical DA neurons. Furthermore, I_h inward currents and spontaneous inhibitory postsynaptic currents (sIPSCs) of labeled cells were examined to explore the underlying mechanisms of paliperidone's effects on mesocortical neurons. Studies of the regulatory mechanisms of paliperidone in mesocortical neurons may not only provide a new insight in physiological function but also offer a novel target of drug therapy for schizophrenia.

2. Experimental procedures

2.1. Animals and materials

Postnatal Wistar rats (P0) were used in this study. Protocols for the treatment of animals were in accordance with the procedures of the Animal Care and Use Committee of Shandong University, which were established based on the International Guiding Principles for Animal Research. Paliperidone was synthesized by Jinan Weidu Chemical Company (Shandong, China). Green retrobeads were purchased from Lumafluor Company, and other chemical reagents were provided by commercial sources.

2.2. Retrograde labeling

Neonatal rats were put on ice for 5 min during anesthesia and then placed in a small animal stereotaxic apparatus (Kopf Instruments, Tujunga, CA). According to the developing atlas of mouse brain (Paxinos et al., 2006), the stereotaxic coordinates with reference to bregma and skull were determined as follows: anterior 0.9 mm, lateral 0.7 mm and ventral 1.4 mm. Two hundred nanoliters of green microspheres (Lumafluor, Naples, FL) was stereotaxically injected into the region of prefrontal cortex using a glass pipette. The injected animals were bred for another 2 weeks to allow the transportation of fluorescent retrobeads to neuronal bodies.

2.3. Immunohistochemical staining

The injected animals were anesthetized with pentobarbital (50 mg/kg, intraperitoneal) and intracardially perfused with 15 ml of ice-cold phosphate-buffered saline (PBS, pH 7.4), followed with 15 ml of 4% paraformaldehyde (PFA). Immunohistochemical staining was performed as previously described (Carriere et al., 2014). In short, brains were removed and post-fixed overnight at 4 °C in 4% PFA, then cryoprotected in 30% sucrose for 24 h at 4 °C and cryosectioned at 20 μm in the coronal plane with a freezing microtome. The brain sections containing labeled cells were immunostained using a mouse anti-TH primary antibody (1:2000, Sigma) at 4 °C overnight and then incubated with TRITC-conjugated goat anti-mouse antibody (1:200, Zhongshan Goldenbridge Biotechnology Company, Beijing, China) for 6 h at room temperature. Finally, sections were stained with DAPI (4′6-diamidino-2-phenylindole, 300 nM, Sigma) for 5 min. Images were captured using a Zeiss LSM780 Axioscope microscope. The control sections were stained with the same reagents except for the primary antibodies.

2.4. Brain slices preparation

Two weeks after injecting fluorescent microspheres in the prefrontal cortex, coronal midbrain sections were prepared as previously described (Accorsi-Mendonça et al., 2015). Briefly, the brain tissue was quickly removed and fixed on the slot of a vibratome VT1000S (Leica, Germany) and then filled with pre-cooling of the cutting solution (in mmol/L): 112 NaCl, 3.1 KCl, 1.8 $CaCl_2$, 12 $MgSO_4$, 0.4 K_2HPO_4, 20 glucose, 26 $NaHCO_3$. Brain sections of the midbrain (300 μm) were cut and incubated at 34 °C for 35 min, then transferred onto a mesh platform in a recording chamber and perfused continuously with oxygenated incubating solution (in mmol/L): 120.5 NaCl, 3.1 KCl, 1.8 $CaCl_2$, 1.2 $MgSO_4$, 0.4 KH_2PO_4, 20 glucose, 26 $NaHCO_3$. The cutting solution and incubating solution were both saturated with 95% O_2 and 5% CO_2.

2.5. Brain slice patch clamp recording

Mesocortical DA neurons labeled by fluorescent microspheres in VTA were detected under a microscope BX51 (Olympus, Japan). Patch pipettes (5–7 MΩ) were pulled with P-97 microelectrode puller (Sutter Instruments, Novato, CA, USA) and filled with an internal solution containing (in mmol/L): 125 potassium gluconate, 6.7 KCl, 10 HEPES, 0.1 EGTA, 2 ATP Tris, 20 creatine phosphokinase (pH 7.4). Paliperidone (20 μM) was applied by bath-superfusion. Recordings were performed with the whole cell patch-in-the-slice method (Liu et al., 2003), and the spontaneous and evoked firings (current stimulation: 300 pA, 500 ms) of labeled DA neurons were monitored in current-clamp mode before and during drug application via an Axon MultiClamp 700B amplifier (Molecular Devices, CA, USA). During recording, the sections were kept at room temperature (22 ± 2 °C) and perfused constantly at 2–3 ml/min with the oxygenated incubating solution. Current steps and data acquisition were accomplished with Axon Clampfit 10.2 software (Molecular Devices, CA, USA). Data analysis was conducted using IgorPro (WaveMetrics) software.

2.6. Detection of I_h current

To further explore the effects of paliperidone, hyperpolarization-activated inward current (I_h) was assessed in mesocortical neurons. Labeled cells were identified in brain sections and were monitored by whole cell patch clamp. Standard extracellular and intracellular solutions were used during evaluation as described above. Using the voltage-clamp model, hyperpolarization pulses were given from −60 mV to −140 mV to activate the I_h currents following a previous report (Chabbert et al., 2001). Data were recorded with an