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Research Report

Decreased HCN2 expression in STN contributes to abnormal high-voltage spindles in the cortex and globus pallidus of freely moving rats

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ABSTRACT

Abnormal oscillation in the cortical-basal ganglia loop is involved in the pathophysiology of parkinsonism. High-voltage spindles (HVSs), one of the main type abnormal oscillations in Parkinson's disease, are regulated by dopamine D2-like receptors but not D1-like receptors. However, little is known about how dopamine D2-like receptors regulate HVSs and the role of hyperpolarization-activated cyclic nucleotide-gated2 (HCN2) in HVSs regulation. We simultaneously recorded the local field potential (LFP) in globus pallidus (GP) and electrocorticogram (ECoG) in primary motor cortex (M1) in freely moving 6-hydroxydopamine (6-OHDA) lesioned or control rats. The expression of HCN2 and dopamine D2 receptor in the subthalamic nucleus (STN) was examined by immunochemical staining and Western blotting. We also tested the role of HCN2 in HVSs regulation by using pharmacological and shRNA methodology. We found that dopamine D2-like receptor agonists suppressed the increased HVSs in 6-OHDA lesioned rats. HCN2 was co-expressed with dopamine D2 receptor in the STN, and dopamine depletion decreased the expression of HCN2 as well as dopamine D2 receptor which contribute to the regulation of HVSs. HCN2 was down regulated by HCN2 shRNA, which thereby led to an increase in the HVSs in naïve rats while HCN2 agonist reduced the HVSs in 6-OHDA lesioned rats. These results suggest that HCN2 in the STN is involved in abnormal oscillation regulation between M1 cortex and GP.

Abbreviations: BG, basal ganglia; ECoG, electrocorticogram; GP, globuspallidus; HCN, hyperpolarization-activated and cyclic nucleotide-gated; HVSs, high-voltage spindles; LFP, local-field potential; M1 cortex, primary motor cortex; 6-OHDA, 6-hydroxydopamin; PSD, power spectral density; STN, subthalamic nucleus

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1. Introduction

Parkinson's disease (PD) is a common neurodegenerative disorder characterized by motor symptoms including bradykinesia, rigidity and resting tremor. Evidence suggests that abnormally synchronized oscillatory activity in the cortical-basal ganglia (BG) loop is associated with PD motor deficiencies (Alonso-Frech et al., 2006; Brown et al., 2001; Garcia et al., 2005; Hammond et al., 2007; Meissner et al., 2005; Quiroga-Varela et al., 2013; Rivlin-Etzion et al., 2006; Weinberger et al., 2006). Additionally therapeutic strategies such as dopaminergic therapies (Alonso-Frech et al., 2006; Weinberger et al., 2006), deep-brain stimulation (DBS) (McConnell et al., 2012; Meissner et al., 2005) and ablative surgery, which ameliorate motor impairment in patients with PD, suppress abnormal synchronization (Hammond et al., 2007). The role of oscillations and synchrony of the BG in the pathophysiology of parkinsonism is increasingly recognized since Brown et al. reported local field potentials (LFPs) recorded from implanted DBS macro-electrodes in the subthalamic nucleus (STN) of PD patients (Brown et al., 2001; Quiroga-Varela et al., 2013). According to the oscillatory frequency, there are two main types of abnormal oscillations in PD. The first includes "beta-band activity" in the 14–30 Hz range. The second involves HVSs which exhibit a characteristic spike-and-wave pattern and an oscillation frequency ranging between 5 and 13 Hz (Buzsaki and Silva, 2012; Dejean et al., 2007, 2008, 2012; Ge et al., 2012). However, the precise neuronal mechanisms underlying abnormal activity, especially the HVSs, in pathological oscillations in the parkinsonian brain remain unclear.

Hyperpolarization-activated and cyclic nucleotide-gated (HCN) channels which generate I_h, first reported in 1976 in heart cells (Noma and Irisawa, 1976), are cation channels that open when the membrane potential is hyper-polarized. HCN channels are widely expressed in various parts of the brain such as the cortex, hippocampus, and thalamus, which are involved in a variety of neural functions both in healthy and diseased conditions (Notomi and Shigemoto, 2004). Previous studies showed that HCN2 and HCN1 channels govern the regularity of autonomous pacemaking and synaptic resetting in globus pallidus (GP) neurons (Chan et al., 2004, 2011). Furthermore, a recent study of HCN channel function in PD showed that a rodent model of PD, where dopamine depletion induced the downregulation of HCN channel expression, revealed a loss of the characteristic autonomous pacemaking in GPe neurons. Viral delivery of an HCN2 subunit expression construct restored pacemaking and reduced pathological rhythmic burst spiking of GPe neurons of PD models (Chan et al., 2004, 2011). However, little is known whether HCN2 also undergoes changes in the STN after dopamine depletion.

The STN is one of the most effective targets for DBS in treating PD (Garcia et al., 2005). The STN is a part of rhythm generation of the basal ganglia in both healthy and diseased. Previous studies have shown the HCN channels in STN are related to the rhythmic activities of the STN (Gillies and Willshaw, 2004; Kase et al., 2012). Our previous study (Yang et al., 2013) showed that dopamine depletion significantly increased the power and coherence of HVSs in the GP and motor cortex and that dopamine D2-like receptors, but not dopamine D1-like receptors, were involved in HVSs regulation. However, little is known about how dopamine D2-like receptors regulate HVSs or whether the HCN channel changes in PD and their roles in HVSs regulation.

In this study, we investigated the role of the HCN2 channel in STN for HVSs regulation after dopamine depletion by employing local-field potential (LFP) and electrocorticogram (ECoG) methodologies to simultaneously record the oscillatory activities in the GP and primary motor cortex (M1) in freely moving rats. We found that the decreased expression of HCN2 in STN after dopamine depletion is important for HVSs regulation both in the cortex and GP.

2. Results

2.1. Locomotor activity changes

To investigate the drugs' effect on behavioral changes, the locomotor activity was measured. The locomotor activity was significantly decreased in 6-OHDA rats compare to the control rats ($p<0.001$; Fig. 1A) and partially reversed by systemic injection of ropinirole ($p<0.001$). In the STN local microinjection study in 6-OHDA lesion rats, intra-STN injection of KA, which blocked the STN neuron activity, significantly increased the locomotor activity compared with saline injection ($t(14)=6.786$, $p<0.001$; Fig. 1B), however the injection of LTG had no significant effect on locomotor activity ($t(14)=0.935$, $p=0.366$; Fig. 1D). Intra-STN microinjection of ZD7288 in naïve rats reduced the locomotor activity significantly compared with saline injection ($t(14)=8.43$, $p<0.001$; Fig. 1C). Furthermore, the locomotor activity was also significant decreased when the expression of HCN2 in the STN was down regulated by HCN2-shRNA compared with control-shRNA ($t(14)=5.76$, $p<0.001$; Fig. 5C).

2.2. Systemic injection of ropinirole suppressed HVSs activity in 6-OHDA lesioned rats

To test whether dopaminergic treatment is involved in HVSs regulation, dopamine D2-like receptor agonist, ropinirole, was systemically injected in 6-OHDA lesioned rats. The number of HVSs in the saline-treated 6-OHDA lesioned rats was 66 ± 14.18, while in the ropinirole treated 6-OHDA lesioned rats, the number was significantly reduced ($t(14)=5.009$, $p<0.001$; Fig. 2A), to a number of 37.75 ± 7.30. The mean duration of HVSs in ropinirole-treated rats was significantly shorter than that in saline-treated rats ($t(14)=4.637$, $p<0.001$; Fig. 2B). The mean duration of HVSs was 2.735 ± 0.455 s after ropinirole injection and was 3.648 ± 0.321 s in saline treated rats. Meanwhile, the relative power of the peak in the frequency bands associated with HVS oscillations significantly decreased in ropinirole condition compared with saline-treated rats both in M1 cortex ($t(14)=4.398$, $p=0.001$; Fig. 2C) and GP ($t(14)=5.922$, $p<0.001$). The relative power in ropinirole condition was $7.283\pm1.233\%$ and $9.948\pm1.057\%$ in M1 cortex and GP, respectively, whereas in saline-treated rats it was $11.553\pm2.453\%$ and $14.307\pm1.793\%$, respectively. In addition, the coherence value relating to the HVSs between the M1 cortex and the GP in ropinirole condition was 0.606 ± 0.131, which was significantly smaller than that in saline-treated rats with the value of 0.756 ± 0.077($t(14)=2.785$, $p=0.015$; Fig. 2D).