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Research article

Effects of norepinephrine on spontaneous firing activity of cerebellar Purkinje cells in vivo in mice

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HIGHLIGHTS

- NE decreased spontaneous SS firing rate of PCs and increased spontaneous firing of MLIs.
- Blocking $GABA_A$ receptors activity revealed the NE-induced increase in SS firing of PCs.
- Blocking AMPA receptors enhanced the NE-induced inhibition in SS firing of PCs.

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ABSTRACT

Norepinephrine (NE), from the locus coeruleus (LC), has been supported to affect GABAergic system and parallel fiber (PF)-Purkinje cell (PC) synaptic transmission via adrenoceptor in cerebellum cortex. However, the effects of NE on the spontaneous spike activity of cerebellar PCs in living mouse have not yet been fully understood. We here examined the effects of NE on the spontaneous activity of PC in urethane-anesthetized mice by electrophysiological and pharmacological methods. Cerebellar surface application of NE (2.5–25 μM) reduced the PC simple spike (SS) firing rate in a dose-dependent manner. The half-inhibitory concentration (IC_{50}) was 5.97 μM. In contrast, NE significantly increased the spontaneous firing rate of molecular layer interneuron (MLI). Application of $GABA_A$ receptor antagonist, gabazine (SR95531, 20 μM) not only blocked the NE-induced inhibition of PC SS firing but also revealed NE-induced excitation of cerebellar PC. Blocking AMPA receptors activity enhanced NE-induced inhibition of PC spontaneous activity. Moreover, the effects of NE on PC spontaneous activity were abolished by simultaneously blocking $GABA_A$ and AMPA receptors activity. These results indicated that NE bidirectional modulated the spontaneous activity of PCs via enhancing both inhibitory inputs from MLIs and excitatory inputs of parallel fibers, but NE-induced enhance of inhibitory inputs overwhelmed the excitatory inputs under in vivo conditions.

1. Introduction

The mammalian cerebellar cortex mainly consists of Purkinje cells (PC), molecular interneurons (MLIs), granule cells and Golgi cell. The PC is the focus of computation in the cerebellar cortex, receiving converging projections from all other cortical neurons and providing the sole output from the cerebellar cortex to the deep cerebellar nuclei [1]. MLIs have historically been divided into basket and stellate cells, receiving excitatory input from parallel fibers and inhibitory input from other interneurons, and exerting GABAergic inhibition to PCs [1–4]. Stellate-type MILs provide dendritic inhibition to PCs, which may specifically counterbalance parallel fiber excitation in local regions of PC dendrites [5]. Basket-type MLIs offer powerful and rapid somatic inhibition to PCs, resulting in a direct influence on PC spiking output by the inhibition of the soma and initial segment of PCs [4,6,7]. We previously found that air-puff stimulation on ipsilateral whisker-pad evoked spike firing in MLIs resulting in a GABAergic inhibition of PCs, suggesting that MLIs play a critical role in the sensory information processing in cerebellar cortex [8].

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The cerebellum receives a rich norepinephrine (NE) innervation that originates in the locus coeruleus (LC) and distributes to all parts of the cerebellar cortex [9–11]. It has been reported that iontophoretical application of NE to cerebellar cortex or activation of the locus coeruleus induced a decrease in spontaneous simple spike firing activity of PCs and potentiation of GABAergic transmission at MLIs-PC inhibitory synapses, which indicated that NE modulated inhibitory synaptic transmission in cerebellar cortex [12–15]. On the other hand, it has been argued that NE modulated the parallel fiber (PF) excitatory synaptic transmission in cerebellar cortex. Although several reports demonstrated that NE did not significantly affect PF-PC synaptic transmission, there were studies illustrated that an inhibitory action of NE, mediated by α_2-adrenergic receptor (ARs) expressed on the field potential evoked by PF stimulation [14,17,18]. In addition, it has recently reported that NE bidirectional regulated the PF stimulation-evoked excitatory postsynaptic currents of PCs, which suggested that NE might regulate PF-PC synaptic transmission via bidirectional mechanisms [19]. Collectively, NE innervates the cerebellar cortical PCs activity by modulating inhibitory and excitatory inputs onto PCs. However, the effects of NE on the spontaneous spike activity of cerebellar PCs in living mouse are currently unclear. Therefore, we here examined the effects of NE on spontaneous spike firing of cerebellar PCs in vivo in mice.

2. Materials and methods

2.1. Anesthesia and surgical procedures

Experimental procedures were approved by the Animal Care and Use Committee of Jilin University and performed in accordance with the animal welfare guide lines of the National Institutes of Health (Permit number: SYXK [Ji] 2007-0011). Anesthesia and surgical procedures have been described previously [20]. In brief, 48 adult (6–8 weeks old) ICR mice were anesthetized with urethane (1.3 g/kg body weight, intraperitoneal injection). After a water tight chamber was created, a 1–1.5 mm craniotomy was drilled to expose the cerebellar surface corresponding to vermis and Crus II. The brain surface was constantly superfused with oxygenated artificial cerebrospinal fluid (ACSF: 125 mM NaCl, 3 mM KCl, 1 mM $MgSO_4$, 2 mM $CaCl_2$, 1 mM NaH_2PO_4, 25 mM $NaHCO^3$, and 10 mM D-glucose) with a peristaltic pump (Gilson Minipulse3; Villiers, LeBel, France). The rectal temperature was monitored, and maintained at 37.0 ± 0.2 °C.

2.2. Electrophysiological recordings, stimulation and drug application

Cell-attached recordings from PCs and whole-cell recording from MLIs were performed with an Axopatch-200B amplifier (Molecular Devices, Foster City, CA). The potentials were acquired through a Digidata 1440 series analog-to-digital interface on a personal computer using Clampex 10.3 software. Recording electrodes were filled with ACSF and with resistances of 3–5 MΩ. NE, NBQX and SR95531 (all from Sigma-Aldrich, Shanghai, China) were applied to the cerebellar surface (in ACSF at 0.4 ml/min). PCs were identified by the presence of both simple and complex spikes under cell-attached recording conditions. All recorded MLIs were finally identified by biocytin hostochamistry [8]. Recording pipettes were filled a solution consisting of 120 mM potassium gluconate, 10 mM HEPES, 1 mM EGTA, 5 mM KCl, 3.5 mM $MgCl_2$, 4 mM NaCl, 8 mM biocytin, 4 mM Na_2ATP and 0.2 mM Na_2GTP. The pipette resistances were 5–7 MΩ in the bath. After electrophysiological recording, the whole brain was removed and fixed in 4% paraformaldehyde in 0.1 phosphate buffer (PB). Biocytin was detected using 3,39-diaminobenzidine tetrahydrochloride histochemistry. Spontaneous activity was calculated from a train of interspike intervals recorded for 100 s.

2.3. Statistical analysis

All data are expressed as the mean ± S.E.M. Differences between the mean values recorded under control and test conditions were evaluated with the Student's paired *t*-test and ANOVA using SPSS (Chicago, IL) software. *P* values below 0.05 were considered to indicate a statistically significant difference between experimental groups.

3. Results

3.1. Cerebellar surface application of NE decreased spontaneous SS firing rate of PCs and increased spontaneous firing rate of MLIs

Under cell-attached recording conditions, a total of 58 PCs were identified by SS firing and the presence of complex spikes (CS). These PCs expressed regular SS firing at mean rate of 36.4 ± 2.9 Hz. Cerebellar surface perfusion of NE (7.5 μM) induced a significant decrease in spontaneous simple spike firing rate to 57.42 ± 6.23% of baseline (99.17 ± 1.8%; $P < 0.01$; n = 6; Fig. 1A, B) and the mean instant frequency of PC SS firing was decreased from 23.6 ± 3.5 Hz to 13 ± 2.3 Hz (Fig. 1; $P < 0.01$). NE depressed the PC SS spike firing in a dose-dependent manner. Fifty percent inhibiting concentration (IC_{50}) was 5.97 μM. These results indicated that cerebellar surface perfusion of NE inhibited the spontaneous SS firing, suggesting that NE enhanced inhibitory inputs of PCs.

Since NE has been demonstrate to enhance the GABAergic transmission at MLI-PC inhibitory synapses under in vitro conditions [14–16]. Therefore, we examined the effect of NE on the spontaneous spike firing activation of MLIs by in vivo whole-cell recording technique. As shown in Fig. 2, application of NE (25 μM) induced an increase in spike firing. The frequency of spike firing increased to 137.71 ± 2.85% of baseline (ACSF: 100.05 ± 0.83%; $P < 0.01$; n = 6). NE induced excitation of MLI was concentration-dependent (Fig. 2A, Lower). These results indicated that cerebellar surface perfusion of NE induced excitation of cerebellar MLIs, suggesting that NE induced inhibition of PC spontaneous activity via activation of MLIs under in vivo conditions.

3.2. Blocking $GABA_A$ receptors activity revealed the NE-induced increase in SS firing of PCs

MLIs receive excitatory inputs from parallel fibers and inhibitory inputs from other interneurons, and exerting GABAergic inhibition to PCs [1–3]. Our results showed that cerebellar surface application of NE induced inhibition of PC spontaneous activity, which suggested that NE-induced inhibition of PC SS firing activity via $GABA_A$ receptor. Therefore, we used a $GABA_A$ receptor specific antagonist, gabazine (SR95531) to examine whether NE-induced inhibition of spontaneous activity of PCs via $GABA_A$ receptor. Application of NE (25 μM) decreased SS firing of PCs to 48.48 ± 4.64% of baseline (ACSF: 100.22 ± 4.02%; $P < 0.01$; n = 6; Fig. 3). However, the additional application of SR95531 (20 μM) not only blocked the NE-induced inhibition of SS firing, but also revealed that NE-induced an increase in PC SS firing in the absence of $GABA_A$ receptors activity. A mixture of NE (25 μM) and SR95531 increased the frequency of PC SS firing to 118.57 ± 3.63% of baseline (ACSF: 100.22 ± 4.02%; n = 6; $P < 0.05$). Consistent with previous studies [20], application of SR95531 alone failed to affect the PC SS frequency (101.8 ± 2.54% of baseline; $P > 0.05$; n = 6). These data indicated that NE decreased PC SS firing via enhance of MLI-PC GABAergic transmission, while