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Research Report

N-methyl-D-aspartate inhibits cerebellar Purkinje cell activity *via* the excitation of molecular layer interneurons under *in vivo* conditions in mice

Heng Liu[a,b,1], *Sheng-Nan Zhao*[a,b,1], *Guo-Yan Zhao*[a,b,1], *Lei Sun*[a,b], *Chun-Ping Chu*[a,b,*], *De-Lai Qiu*[a,b,*]

[a]*Cellular Function Research Center, Yanbian University, 977, GongYuan Road, Yanji City, Jilin Province, China*
[b]*Department of Physiology and Pathophysiology, College of Medicine, Yanbian University, Yanji City, Jilin Province, China*

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ABSTRACT

N-methyl-D-aspartate (NMDA) receptors play a key role in synaptic transmission, and are widely expressed on the membrane of granule cells, parallel fibers, and molecular layer interneurons (MLIs) in the cerebellar cortex of mammals. In cerebellar slices, activation of NMDA receptors increases inhibitory postsynaptic currents (IPSCs) of Purkinje cells (PCs). However, the effects of NMDA on the cerebellar network under *in vivo* conditions are currently unclear. In the present study, we examined the effects of NMDA on the spontaneous activity of PCs and MLIs in urethane-anesthetized mice by electrophysiological, pharmacological, and juxtacellular labeling methods. Our results revealed that cerebellar surface application of NMDA (5–200 μM) reduced the PC simple spike (SS) firing rate in a dose-dependent manner. Application of $GABA_A$ receptor antagonist, SR95531 (20 μM) abolished NMDA-induced inhibition of PCs spontaneous activity, and revealed NMDA-induced excitation of cerebellar PCs. NMDA receptor antagonist, DAP-V (250 μM) did not affect the mean frequency of SS firing, but the SS firing rate of PCs became more regular than the control. In addition, NMDA increased the spike firing of both basket-type and stellate-type MLIs. Overall, these results indicated that NMDA-induced excitation of MLIs at the cerebellar surface may inhibit PC activity. Thus, NMDA receptors of MLIs may play a key role in regulating the spontaneous activity of PCs, and in information transmission and integration in cerebellar cortex.

Abbreviations: ACSF, artificial cerebrospinal fluid; NMDA, N-methyl-D-aspartate; MLI, molecular layer interneuron; PC, Purkinje cell; GABA, γ-aminobutyric acid

*Corresponding authors at: Cellular Function Research Center, YanBian University, 977, GongYuan Road, Yanji City, Jilin Province, China. Fax: +86 433 243 5051.
E-mail addresses: cpchu@ybu.edu.cn (C.-P. Chu), dlqiu@ybu.edu.cn, dlqiu_neuron@hotmail.com (D.-L. Qiu).
[1]These authors contributed equally to this manuscript.

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1. Introduction

N-methyl-D-aspartate (NMDA) receptors are glutamate ion channels involved in the postsynaptic neuronal response and the regulation of presynaptic neurotransmitter release, which plays a key role in the modulation of synaptic transmission in the central nervous system.

NMDA receptors are composed of three subunit families, NR1, NR2, and NR3. NR1 subunits are expressed in all cerebellar neurons of young and adult rodents, whereas the expression of NR2A/B subunits is dependent on the cell type and the degree of animal development and maturation (Akazawa et al., 1994; Wee et al., 2008). Purkinje cells (PCs) in immature rodents express NR1 and NR2 (Dupont et al., 1987; Rosenmund et al., 1992; Cull-Candy et al., 1998). However, reports on the expression of functional NMDA receptors in adult PCs are contradictory. Electrophysiological recordings have concluded that functional NMDA-Rs no longer express after the first postnatal week (Konnerth et al., 1990; Llano et al., 1991; Farrant et al., 1994). On the other hand, NMDA evokes responses have been recorded in cerebellar PCs of the adult rat and mouse (Dupont et al., 1987; Billard and Pumain, 1989), as well as in the adult guinea pig under *in vitro* conditions (Sekiguchi et al., 1987). NR2B subunits are prominent in PCs of adult rats and mice, whereas PCs-containing NR2A is found in only adult mice (Thompson et al., 2000). However, PC-containing NR1 is abundant throughout adulthood (Nakagawa et al., 1996; Hafidi and Hillman, 1997). Postsynaptic NMDA receptors are prominent in synapses of climbing fiber-PCs in mice, which are barely detectable until one month after birth, and reach mature expression at approximately 2 months of age (Piochon et al., 2007; Renzi et al., 2007). Functional NMDA receptors in PCs of 2–3-month-old rats have also been demonstrated in climbing fiber-evoked excitatory postsynaptic currents (Bidoret et al., 2009).

Molecular layer interneurons (MLIs) are divided into stellate-type MILs or basket-type MILs. Both types receive excitatory input from parallel fibers and inhibitory input from other interneurons, and induce gamma-aminobutyric acid (GABA) ergic inhibition of PCs (Palay and Chan-Palay, 1974; Häusser and Clark, 1997; Mittmann et al., 2005, Bower, 2010). Inhibition of PCs by stellate-type MILs is thought to specifically counterbalance the excitation of parallel fibers in local regions of PC dendrites (Jaeger and Bower, 1999). In contrast, basket-type MLIs induce powerful and rapid somatic inhibition of PCs, resulting in the direct influence on PC spiking output by inhibition of the soma and initial segment of PCs (Bower, 2010; Santamaria et al., 2007; Huang et al., 2007, Chu et al., 2012). NMDA receptor subunits have been found in cultured MLIs (Duguid and Smart, 2004) and on the axonal pinceau of basket-type MLIs (Petralia et al., 1994). Bath application of NMDA to rat cerebellar slices increases the frequency of spontaneous inhibitory synaptic currents (IPSCs) in PCs, which have been commonly assumed to result from an increase in presynaptic neuronal firing after activation of somatodendritic NMDA receptors (Farrant and Cull-Candy, 1991; Llano et al., 1991). Bath application of NMDA induces an inward current in both types of MLIs, shown as a small depolarization under current-clamp conditions (Glitsch and Marty, 1999). Strong increases in the frequency of miniature inhibitory postsynaptic currents (mIPSCs) by extracellular NMDA have been shown to be partly mediated by presynaptic NMDA receptors (Glitsch and Marty, 1999). The activation of presynaptic NMDA receptors in MLIs indirectly leads to the release of GABA, which is further enhanced upon secondary depolarization of synaptic terminals (Christie et al., 2011; Bouhours et al., 2011). NMDA elicits Ca^{2+} transients at basket cell-PC terminals, and presynaptic NMDA receptors act as local high-gain glutamate detectors in the cerebellar MLIs (Rossi and Collin, 2013). However, iontophoretic application of aspartate to basket cell axons does not affect the amplitude of IPSCs in PCs nor calcium responses in the axons of basket-type MLIs, suggesting that NMDA receptors are not expressed on the axons of basket-type MLIs (Christie et al., 2011).

The functional role of NMDA receptors has been extensively studied *in vitro*; however, it is less known in the cerebellar cortex under *in vivo* conditions. Therefore, in the present study, we explored the mechanisms of NMDA-mediated spontaneous activity of cerebellar PCs and MLIs in urethane-anesthetized mice by electrophysiological and pharmacological methods. Our results showed that cerebellar surface application of NMDA failed to excite PCs but increased spike firing of MLIs, resulting in a decrease in spontaneous simple spike firing (SS) firing rate of PCs via $GABA_A$ receptor activation. These findings suggest that postsynaptic NMDA receptors of MLIs in the cerebellar cortex of mice play a key role in the regulation of spontaneous activity of PCs, and in information transmission and integration.

2. Results

2.1. *Cerebellar surface application of NMDA decreased SS firing of PCs*

Cell-attached recording configurations were used on a total of 32 PCs. Recordings were identified by SS firing and the presence of complex spikes (Fig. 2), according to Chu et al. (2011). PCs induced regular SS firing, continuously at mean rates of 35.6±3.4 Hz (n=38 cells in 25 mice). The highest and lowest rates of SS firing were 67 Hz and 12 Hz, respectively. Cerebellar surface perfusion of NMDA (50 μM) time-dependently decreased the SS firing rate (Fig. 1 A). The spike firing rate was reduced approximately 4 min after the application of NMDA, and significantly (P<0.01) reached a maximum decrease at 57.6±6.9% from baseline (Fig. 1B). Administration of 100 μM NMDA strongly (P<0.01) inhibited SS firing of PCs by 88.3±8.4% of baseline (Fig. 1C). NMDA also reduced PC spike firing in a concentration-dependent manner (Fig. 2). SS firing of PCs was significantly (P<0.05) reduced by 4.8±2.3% of control (ACSF) with 5 μM NMDA (n=6) (Fig. 2), and its IC_{50} was 50.3 μM. SS firing of PCs was significantly (P<0.001) reduced to 96.5±2.1% from baseline with 200 μM NMDA (n=5) (Fig. 2). These data indicated that cerebellar surface perfusion of NMDA reduced PC SS firing in a concentration-dependent manner, suggesting that NMDA may enhance the activation of $GABA_A$ receptors.