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Monoaminergic modulation of GABAergic transmission onto cerebellar globular cells

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ABSTRACT

Cerebellar Purkinje cells (PCs) project their axon collaterals to underneath of the PC layer and make GABAergic synaptic contacts with globular cells, a subgroup of Lugaro cells. GABAergic transmission derived from the PC axon collaterals is so powerful that it could inhibit globular cells and regulate their firing patterns. However, the physiological properties and implications of the GABAergic synapses on globular cells remain unknown. Using whole-cell patch-clamp recordings from globular cells in the mouse cerebellum, we examined the monoaminergic modulation of GABAergic inputs to these cells. Application of either serotonin (5-HT) or noradrenaline (NA) excited globular cells, thereby leading to their firing. The 5-HT- and NA-induced firing was temporally confined and attenuated by GABAergic transmission, although 5-HT and NA exerted an inhibitory effect on the release of GABA from presynaptic terminals of PC axon collaterals. Agonists for 5-HT_{1B} receptors and α_2-adrenoceptors mimicked the 5-HT- and NA-induced suppression of GABAergic activity. Through their differential modulatory actions on the cerebellar inhibitory neural circuits, 5-HT facilitated PC firing, whereas NA suppressed it. These results indicate that 5-HT and NA regulate the membrane excitability of globular cells and PCs through their differential modulation of not only the membrane potential but also GABAergic synaptic circuits. Monoaminergic modulation of the neural connections between globular cells and PCs could play a role in cerebellar motor coordination.

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1. Introduction

Cerebellar Purkinje cells (PCs) are capable of firing at a high frequency and exhibiting high fidelity to action potential propagation into their myelinated axons (Eccles et al., 1967; Khalip and Raman, 2005; Monsivais et al., 2005; Hirono et al., 2015; Gründemann and Clark, 2015), as well as robustly inhibiting their main targets, such as neurons in the deep cerebellar nuclei (DCN) (Obata et al., 1967; Telgkamp and Raman, 2002; Pedroarena and Schwarz, 2003; Person and Raman, 2012). Moreover, PCs extend their axon collaterals through the granule cell layer to make synaptic contacts with Lugaro cells (Laine and Axelrad, 2002; Crook et al., 2007; Simat et al., 2007; Hirono et al., 2012). These cells are normally quiescent but show robust excitation in the presence of serotonin (5-HT) (Dieudonné and Dumoulin, 2000; Dumoulin et al., 2001; Dean et al., 2003), and receive extensive inhibitory GABAergic inputs (Hirono et al., 2012; Eyre and Nusser, 2016). Previously, we have shown that globular cells, a subgroup of Lugaro cells, are excited in response to mossy fiber inputs and monoaminergic inputs (not only serotonergic but also noradrenergic inputs) to fire action potentials (Hirono et al., 2012). Globular cells send output signals through their long transversal axons to form globular cell-PC circuit which allows clusters of PCs within different microzones to synchronize their firing activity (Strahlendorf et al., 1984). Furthermore, globular cells receive robust GABAergic inputs, most of which are derived from the PC axon collaterals. Therefore, it seems that globular cells constitute a new category of cerebellar cortical interneurons that serve a role in integrating the PC inhibitory inputs in concerted action of the excitatory mossy

Abbreviations: ACSF, artificial cerebrospinal fluid; GABA, γ-aminobutyric acid; 5-HT, serotonin; mIPSC, miniature inhibitory postsynaptic current; NA, noradrenaline; PC, Purkinje cell; sIPSC, spontaneous inhibitory postsynaptic current.

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fiber inputs and monoaminergic modulation. The properties of GABAergic transmission from PCs to globular cells, however, have yet to be characterized well. For example, what are the effects of monoaminergic inputs on the GABAergic synapses between axon collaterals of PCs and globular cells? This study, therefore, aimed to examine the modulatory actions of 5-HT and noradrenaline (NA) on the GABAergic transmission at PC axon collateral-globular cell synapses using whole-cell patch-clamp recordings from mouse cerebellar slices. Our data indicate that 5-HT reduced the frequency of miniature inhibitory postsynaptic currents (mIPSCs) recorded in globular cells via the activation of 5-HT_{1B} receptors, possibly located on presynaptic PC terminals, and increased the excitability of presynaptic PCs, resulting in their robust firing. Thus, 5-HT appears to bidirectionally modulate GABAergic transmission from PCs to globular cells, presumably under different physiological conditions. Moreover, NA inhibited the GABAergic transmission by causing a decrease in the mIPSC frequency via activation of α_2-adrenoceptors at presynaptic terminals and reduced the presynaptic PC firing. These results suggest that 5-HT and NA shape the firing patterns of globular cells by modulating the inhibitory GABAergic inputs from axon collaterals of PCs.

2. Materials and methods

2.1. Animals

The generation of GAD67-GFP (Δneo) mice has been described previously (Tamamaki et al., 2003), and the heterozygous mice used in the present study were termed GAD67$^{+/GFP}$ mice. We also used VGAT-Venus transgenic mice specifically expressing the Venus yellow fluorescent protein in inhibitory neurons (Wang et al., 2009). Both the transgenic lines were maintained with on a C57BL/6 genetic background at our animal facility. All the experimental procedures were in strict accordance with the *Guide for the Care and Use of Laboratory Animals* described by the National Institutes of Health and approved by the Animal Research Committees on the care and use of animals in experiments in the RIKEN BSI and the Doshisha University.

2.2. Electrophysiology

Cerebellar slices from 24 GAD67$^{+/GFP}$ mice or 36 VGAT-Venus transgenic mice aged 18–25 days were made as described previously (Hirono et al., 2012). Adult cerebellar slices were made from 6 VGAT-Venus transgenic mice aged 5–6 weeks. The mice were treated with CO_2 and decapitated. Sagittal slices (250 μm thick) of the cerebellar vermis were obtained using a vibrating microtome (VT1200S; Leica) in an ice-cold extracellular solution containing (in mM) 252 sucrose, 3.35 KCl, 21 $NaHCO_3$, 0.6 NaH_2PO_4, 9.9 glucose, 1 $CaCl_2$, and 3 $MgCl_2$ and gassed with a mixture of 95% O_2 and 5% CO_2 (pH 7.4). The slices were maintained at 30 °C for 30 min in a holding chamber, where they were submerged in artificial cerebrospinal fluid (ACSF) containing (in mM) 138.6 NaCl, 3.35 KCl, 21 $NaHCO_3$, 0.6 NaH_2PO_4, 9.9 glucose, 2 $CaCl_2$, and 1 $MgCl_2$ (bubbled with 95% O_2 and 5% CO_2 to maintain the pH at 7.4). Thereafter, the slices were kept at room temperature.

Individual slices were transferred to a recording chamber attached to the stage of a microscope (BX51WI, Olympus) and superfused with oxygenated ACSF. Small inhibitory interneurons underneath the Purkinje cell layer were visually identified with GFP fluorescence in the cerebellar cortex of GAD67$^{+/GFP}$ mice (Tamamaki et al., 2003) and VGAT-Venus transgenic mice (Wang et al., 2009). Recordings were performed from neurons of lobules III-IX to limit the variability associated with the specialization of different regions of the cerebellar cortex (Hirono et al., 2012). Extracellular spike activity in globular cells and PCs was observed using loose cell-attached voltage-clamp recordings at a holding potential of 0 mV. Glass electrodes used for the cell-attached recordings were filled with ACSF. To isolate IPSCs, patch pipettes (2–4 MΩ) were filled with an intracellular solution containing (in mM) 140 CsCl, 0.1 $CaCl_2$, 1 K-EGTA, 10 Na-HEPES, 3 Mg-ATP, and 0.4 Na-GTP (pH 7.4), and the holding potential was set at −70 mV to detect IPSCs as larger inward current responses. A nonselective ionotropic glutamate receptor antagonist, kynurenic acid (1 mM) (or 10 μM 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[*f*]quinoxaline-7-sulfonamide disodium salt: NBQX and 30 μM DL-2-amino-5-phosphonovaleric acid: APV), was added to the ACSF throughout the IPSC recordings. Whole-cell capacitance was calculated by integrating the area under the transient following a 10 mV hyperpolarizing voltage step from the holding potential, −70 mV. Miniature IPSCs were recorded using a CsCl-based internal solution in the presence of tetrodotoxin (TTX, 0.5 μM). Evoked IPSCs were recorded using a cesium methanesulfonate-based internal solution containing (in mM) 140 $CsCH_3SO_3$, 5 CsCl, 0.1 $CaCl_2$, 1 K-EGTA, 10 Na-HEPES, 0.6 QX-314, 3 Mg-ATP, and 0.4 Na-GTP (pH 7.4) by applying focal stimulation (30–50 V, 0.1–0.2 ms) through a grass microelectrode containing ACSF. Series resistance (10–18 MΩ) was compensated by 70% and monitored using 2 mV hyperpolarizing voltage steps, and the experiments were discarded if the value changed by ~20%. Unless otherwise noted, the most experiments were performed at room temperature (24–26 °C). CGP55845, CP93129, ketanserin, EMD386088, RS79948, SB224289, SR95531, and WAY161503 were obtained from Tocris Bioscience. α-methyl 5-hydroxytryptamine (α-Me-5-HT) and TTX were obtained from Abcam and Wako, respectively. All the other chemicals were obtained from Sigma.

Membrane currents of globular cells were examined by whole-cell voltage-clamp recordings with patch pipettes (3–5 MΩ) filled with a CsCl-based internal solution. We easily observed spontaneous postsynaptic currents as one of the criteria for the identification of globular cells as described in our previous report (Hirono et al., 2012).

The membrane potentials and currents were recorded using the amplifier MultiClamp 700 B (Molecular Devices, Sunnyvale, CA) and pCLAMP 10.3 software (Molecular Devices), digitized, and stored on a computer disk for off-line analysis. All the signals were filtered at 2–4 kHz and sampled at 5–20 kHz, and synaptic events were analyzed with a threshold of 10 pA. The frequencies of synaptic events are shown by the number of synaptic events (for 30 s) divided by the time duration. Spike firing and synaptic events were analyzed using Mini analysis program 6.0 (Synaptosoft, Decatur, GA), pCLAMP 10.3 software, and Kyplot software 5.0 (KyensLab, Tokyo, Japan). The coefficient of variation (CV) of the inter-spike interval was calculated as CV = SD/M, where SD is the standard deviation and M is the mean. SD and M were calculated from firing events for 1 min after the onset of firing. All the data are expressed as the mean ± standard error of the mean (S.E.M.). The numbers in the parentheses represent the numbers of tested neurons. Unless otherwise stated, the level of significance was determined using the paired or unpaired Student's *t*-test between related or independent sample groups, respectively.

2.3. Morphological analyses

It was difficult to unequivocally distinguish the three types of small inhibitory interneurons under Nomarski optics. To visualize their shape, 40 μM Alexa Fluor 594 (Invitrogen) was added to the internal solution. After the whole-cell patch-clamp experiments, the patch pipette was removed from the cell. Fluorescent images were obtained by fluorescent microscopy (BX51WI, Olympus) with

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