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6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX) increases $GABA_A$ receptor-mediated spontaneous postsynaptic currents in the dentate granule cells of rat hippocampal slices

Yoshinori Hashimoto, Hiroyoshi Miyakawa, Yoshihisa Kudo, Masashi Inoue*

Laboratory of Cellular Neurobiology, Tokyo University of Pharmacy and Life Science, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan

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Abstract

6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX) is widely used as an antagonist on non-NMDA glutamate receptors. However, several studies have shown that CNQX increases the spontaneous inhibitory postsynaptic current frequency at hippocampal pyramidal neurons and cerebellar granule cells. Dentate granule cells are known to be another distinctive type of principal neurons in hippocampus, and receive dense synaptic input from hilar interneurons. Thus, we examined the effects of CNQX on the dentate granule cells and hilar interneurons with whole-cell recording. CNQX increased the frequency of GABAergic spontaneous postsynaptic currents (sPSCs) on the granule cells, and increased the resting potential and the action potential frequency of the hilar interneurons. These increases were not observed with other glutamate receptor antagonists. The increases in sPSC frequency may be caused by the depolarization and the action potentials of the interneurons.

Keywords: Dentate gyrus; Hilus; Interneuron; GYKI52466; NS-102; Inhibitory postsynaptic current

A commonly used α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate receptor antagonist [1], 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), has been reported to increase GABAergic input of hippocampal pyramidal neurons and cerebellar granule cells [2,5,6] with a mechanism that is unrelated to glutamate receptor blocks. In these studies researchers observed that direct generation of firing in interneurons lead to increases in frequency of spontaneous inhibitory postsynaptic currents on postsynaptic targets. Granule cells in the dentate gyrus are known to be another distinctive type of principal neurons in hippocampal formation. The granule cells receive dense synaptic input from interneurons in dentate gyrus and hilus [8]. Thus, in the present study, we examined the effect of CNQX on the spontaneous activities of dentate granule cells and hilar interneurons with whole-cell recording.

Hippocampal slices (400 μm) were prepared from the brain of 6–8-week-old male Wistar rats, which were decapitated after ether anesthesia. Slices were immersed in oxygenated artificial cerebrospinal fluid (ACSF) at room temperature (23–25 °C) containing (in mM)124 NaCl, 26 $NaHCO_3$, 10 glucose, 2.5 KCl, 1.25 NaH_2PO_4, 2.5 $CaCl_2$, and 1.5 $MgCl_2$. The granule cells or the interneurons were chosen by visual identification with infrared differential interference constant (IR-DIC) videomicroscopy. Whole-cell recordings were amplified with an AXOCLAMP-2B or AXOPATCH-1D amplifier (Axon Instruments, Foster City, CA). All responses were collected using pClamp 8.0 software (Axon Instruments). Responses were sampled at 33.3 kHz and stored in a PC. For the current clamp recordings from interneurons, patch pipettes were filled with internal solutions containing (in mM) 120 K-gluconate, 20 KCl, 4.0 NaCl, 5.0 EGTA, 10 HEPES, 2.0 Mg_2-ATP, and 10 biocytin (pH adjusted to 7.3 with KOH). For the voltage clamp recordings of granule cells, patch pipettes were filled with internal solutions containing (in mM) 140 CsCl, 4.0 NaCl, 5.0 EGTA, 10 HEPES, and 2.0 Mg_2-ATP (pH adjusted to 7.3 with CsOH). Patch electrode resistance was 5–8 MΩ. Drugs were diluted in standard aCFS to their final concentrations. All drugs were obtained from Sigma–Aldrich (Tokyo, Japan).

* Corresponding author. Tel.: +81-426-76-8831; fax: +81-426-76-8841.
E-mail address: inou@ls.toyaku.ac.jp (M. Inoue).

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We recorded the membrane currents from the dentate granule cells with whole-cell voltage clamp recording (Fig. 1). In this experiment, we used internal solutions with a high concentration of Cl^- ions to detect the $GABA_A$ receptor-mediated postsynaptic currents (PSCs). When a granule cell was held at −80 mV, spontaneous inward currents were observed under the control conditions. The currents were completely blocked by 10 μM bicuculline (Fig. 1B), indicating that spontaneous postsynaptic currents (sPSCs) were mostly $GABA_A$ receptor-mediated responses and the other postsynaptic currents including glutamate receptor-mediated currents were absent. Under these conditions, the reversal potential of sPSCs estimated from the I–V relationship was about 0 mV. Next, we applied 10 μM CNQX in ACSF to the hippocampal slice. To investigate whether CNQX affected the sPSCs, we examined the properties of the sPSCs and analyzed the kinetic properties of the sPSCs. These currents were also blocked by 10 μM bicuculline. We examined five parameters of sPSCs before and after the application of CNQX: amplitude (pA): 44.8 ± 1.4, 46.0 ± 1.6 (mean ± S.E.M.; control, CNQX); rise time (ms): 3.9 ± 0.1, 3.8 ± 0.1; decay time (ms): 5.8 ± 0.4, 6.2 ± 0.4; half width (ms): 2.2 ± 0.2, 2.3 ± 0.2; and area (pA ms): 280.0 ± 21.1, 296.2 ± 26.6 (control 232, CNQX 715 events observed in 1 min from one cell). The parameters of the sPSCs were not affected by the CNQX application. Fig. 1C shows traces of sPSCs before and after the application of CNQX. CNQX increased the number of events of the sPSCs and decreased the inter-event intervals of the sPSCs. The pooled data show that CNQX increased the mean sPSC frequency to 288.2 ± 31.6% of that of the control ($n = 12$).

CNQX is known as an AMPA and kainate receptor antagonist. To investigate whether the increase of the sPSC frequency was caused by a blockade of AMPA or/and kainate receptors, we examined the effects of the other glutamate receptor antagonists (Fig. 2 and Table 1). We applied 30 μM GYKI52466, specific AMPA receptor antagonist [1,3] to the hippocampal slice. The GYKI52466 did not increase the sPSCs frequency (102.3 ± 5.2% of the control, $n = 10$). Application of 5 μM NS-102, a kainate receptor competitive antagonist [1,9,10], did not increase the sPSC frequency (99.9 ± 3.9% of the control, $n = 6$). Application of 5 mM kynurenic acid, a broad-spectrum glutamate receptor antagonist, did not increase the sPSC frequency (35.2 ± 7.6% of the control, $n = 5$). These results were in agreement with a previous study on the cerebellar granule cells [2], indicating that the effect of CNQX is not due to a blockade of AMPA or/and kainate type glutamate receptors. Application of kynurenic acid decreased the sPSC frequency of the dentate granule cells. The causes of the decrease are unknown. However, we observed that further application of CNQX after kynurenic acid treatment increased sPSC frequency of the dentate granule cells (data not shown, $n = 3$). Therefore the effect of CNQX is an independent action of kynurenic acid.

How did CNQX increase the frequency of the GABA-ergic sPSCs for the dentate granule cells? Hilar interneurons

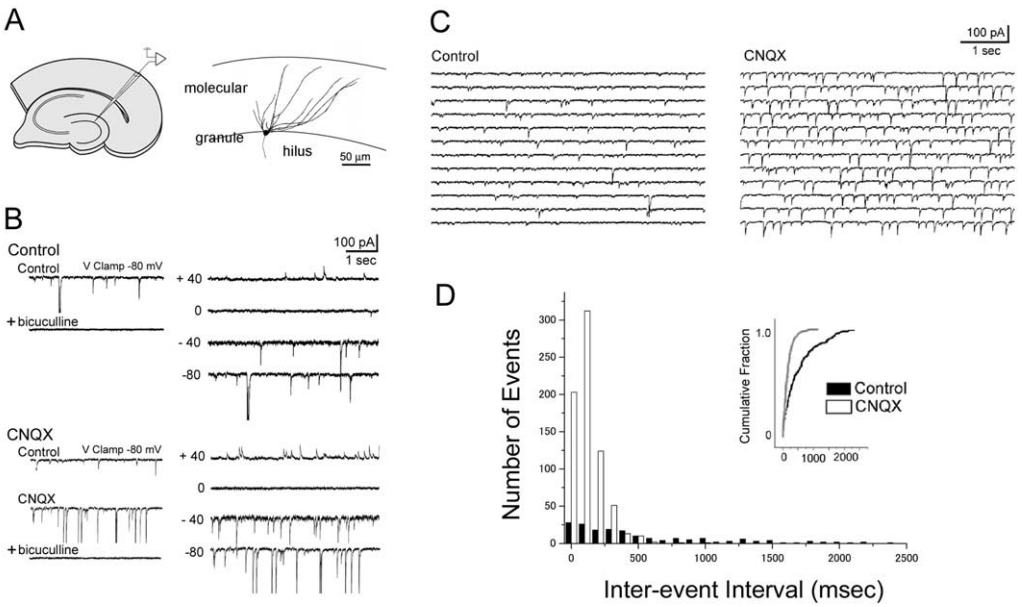

Fig. 1. CNQX increases the frequency of sPSCs mediated by $GABA_A$ receptors in the granule cells. (A) Anatomical reconstruction of dentate granule cell in rat hippocampal slice with a biocytin method (molecular = molecular layer; granule = granule cell layer). (B) Top left: traces of sPSCs observed before (Control) and after the application of 10 μM bicuculline (+bicuculline). Top right: traces of sPSCs recorded at each holding potential. Bottom left: traces of sPSCs observed after the application of 10 μM CNQX (CNQX) and further application of 10 μM bicuculline (+bicuculline). Bottom right: traces of sPSCs recorded at each holding potential during CNQX application. (C) Traces of sPSCs observed before (Control) and after the application of 10 μM CNQX (CNQX). Application of CNQX increases the number of sPSCs (308.2% of the control). (D) Number of events for inter-event interval of sPSCs in every 100 ms at C. Inset: cumulative fraction for inter-event interval of sPSCs.

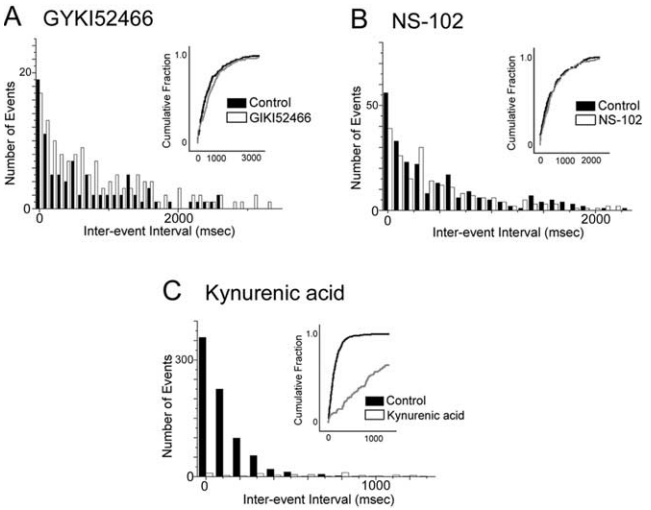

Fig. 2. Increase in the frequency of sPSCs was not observed with the other glutamate receptor antagonists. (A) GYKI52466, a selective AMPA receptor antagonist. Number of events for inter-event interval of the sPSCs in every 100 ms. Inset: cumulative fraction for inter-event interval. The number of the sPSCs was 112.3% of the control. (B) NS-102 (5 μM), the selective kainate receptor antagonist. The number of sPSCs was 105.6% of the control. (C) Kynurenic acid (5 mM), the broad-spectrum glutamate receptor antagonist. The number of sPSCs was 11.7% of the control.

make synaptic contact to the dentate granule cells. If the hilar interneuron produces action potentials, the neuron will release GABA, and will induce postsynaptic currents in the granule cells. Thus, we examined firing activity in the interneurons with whole-cell current clamp recording (Fig. 3 and Table 2). We categorized two types of interneurons by the form of the cell with biocytin staining, bipolar interneurons and multipolar interneurons (Fig. 3A). The bipolar interneurons were fusiform in shape with two thick primary dendrites emerging from the two poles of the soma. They had properties that a maximum spike rate by the current injection of 117.9 ± 3.9 s^{-1}, a membrane time constant (τ_m) of 29.3 ± 1.9 ms, and an input resistance (R_N) of 308.4 ± 21.6 MΩ. The multipolar interneurons had 3–6 primary dendrites, maximum spike rate of 63.8 ± 6.6 s^{-1}, τ_m of 8.0 ± 1.7 ms, and R_N of 316.5 ± 11.9 MΩ. Both types of interneurons, which we recorded, are included in the aspiny hilar interneuron in previous studies [4,7], and have been found to make synapses with granule cells [7,8]. The recorded interneurons spontaneously generate action potentials under control conditions (bipolar, 5 of 7 cells;

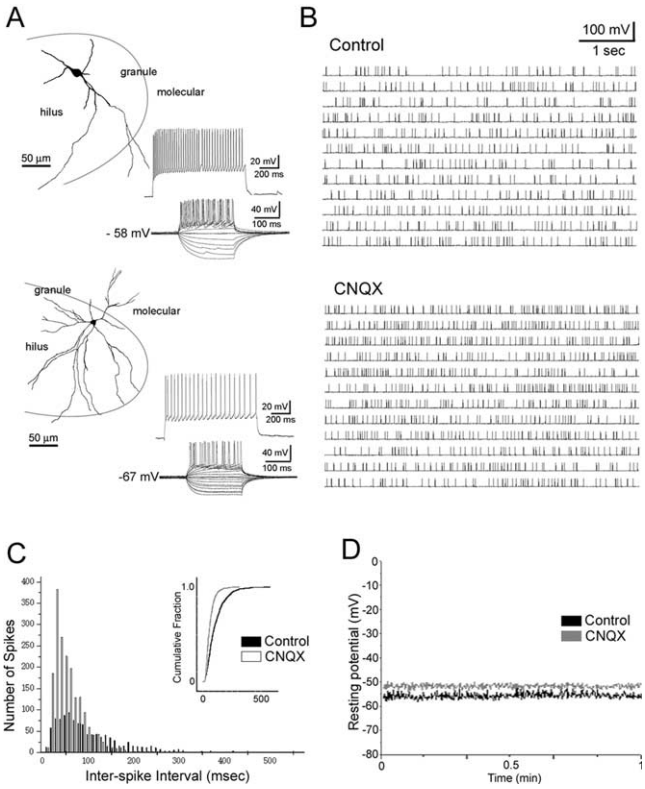

Fig. 3. CNQX increases the resting potentials and the frequency of spontaneous action potentials (sAPs) in the interneurons of the hilar region. (A) Top left: anatomical reconstruction of the bipolar interneurons in the hilar region with biocytin method. Top right; whole-cell recording from bipolar interneuron; responses evoked by a current step (200 ms, 1 s). Bottom left: anatomical reconstruction of the multipolar interneurons. Bottom right: whole-cell recording from multipolar interneuron. (B) Traces of sAPs observed before (Control) and after the application of 10 μM CNQX (CNQX). The number of sAPs increased to 174.5% of the control. (C) Number of spikes for inter-spike interval of action potentials in every 10 ms. Inset: cumulative fraction for inter-spike interval. (D) Time course of resting potential before and after the addition of CNQX.

multipolar, 4 cells). Application of CNQX increased spontaneous action potentials (sAPs) frequency of the interneurons (percent of the control: bipolar, 187.5 ± 23.7%; multipolar, 219.9 ± 30.1%; Fig. 3B,C). Moreover, CNQX increased the resting membrane potential of the interneurons by 6.8 ± 0.8 mV (Fig. 3D). The increase of the resting potentials was observed not only by the spontaneous spiking interneurons (9 of 11) but also non-spontaneous spiking interneurons (2 of 11). From the

Table 1
Frequency changes of postsynaptic current in dentate granule cells

	No. of cells	Event frequency (% of control)	Amplitude (pA)	Rise time (ms)	Decay time (ms)
CNQX	12	288.2 ± 31.6	41.0 ± 3.2	4.0 ± 0.5	5.6 ± 0.4
GYKI52466	10	102.3 ± 5.2	39.7 ± 4.4	3.9 ± 0.5	5.2 ± 0.7
NS-102	6	99.9 ± 3.9	37.0 ± 3.2	3.6 ± 0.4	7.5 ± 0.4
Kynurenic acid	5	35.2 ± 7.6	37.6 ± 6.1	3.6 ± 0.2	6.9 ± 0.3
Time control	3	98.7 ± 1.6	38.5 ± 2.3	3.8 ± 0.3	5.6 ± 0.3

Values are mean ± S.E.M.

Table 2
Frequency changes of action potential in hilar interneurons

		No. of cells	Action potential frequency		Resting potential (mV)
			(min^{-1})	(% of control)	
Bipolar interneurons	Control	7	347.8 ± 59.0[a]		−58.8 ± 2.1
	CNQX		649.0 ± 70.6[a]	187.5 ± 23.7[a]	−51.7 ± 1.9
Multipolar interneurons	Control	4	230.5 ± 59.0		−61.2 ± 3.2
	CNQX		500.8 ± 103.4	219.9 ± 30.1	−54.2 ± 3.7

Values are mean ± S.E.M.
[a] Action potential frequency was calculated from only spontaneous spiking interneurons ($n = 5$ cells).

results, the increase in APs frequency of the interneurons may have induced the increase of sPSC frequency in the granule cells. The increase rate of the sPSC frequency at the granule cells was larger than that in previous studies [2,5,6], suggesting that the granule cells receive more dense input from interneurons.

Our results indicate that CNQX increases $GABA_A$ receptor-mediated sPSCs in dentate granule cells as well as the pyramidal neurons in the hippocampus and the granule cells of the cerebellum. These increases in sPSC frequency may be caused by the depolarization and the action potentials of the interneurons.

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