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Intracellular Ca^{2+} regulates spike encoding at cortical GABAergic neurons and cerebellar Purkinje cells differently

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ABSTRACT

Spike encoding at GABAergic neurons plays an important role in maintaining the homeostasis of brain functions for well-organized behaviors. The rise of intracellular Ca^{2+} in GABAergic neurons causes synaptic plasticity. It is not clear how intracellular Ca^{2+} influences their spike encoding. We have investigated this issue at GFP-labeled GABAergic cortical neurons and cerebellar Purkinje cells by whole-cell recording in mouse brain slices. Our results show that an elevation of intracellular Ca^{2+} by infusing adenophostin-A lowers spike encoding at GABAergic cortical neurons and enhances encoding ability at cerebellar Purkinje cells. These differential effects of cytoplasmic Ca^{2+} on spike encoding are mechanistically associated with Ca^{2+}-induced changes in the refractory periods and threshold potentials of sequential spikes, as well as with various expression ratios of CaM-KII to calcineurin in GABAergic cortical neurons and cerebellar Purkinje cells.

Introduction

Sequential action potentials at the neurons are one type of essential neural codes, and the different spike patterns encode various messages to control the well-organized behaviors and cognition [1–6]. It is not clear whether neuronal spike patterns undergo the plasticity under the physiological conditions, similar to the plasticity of synaptic transmission that underlies learning and memory [1,7–12]. If it is a case, do the mechanisms underlying synaptic plasticity, such as intracellular Ca^{2+} level [1,6,13,14], lead to the plasticity of spike patterns?

GABAergic inhibitory interneurons, despite a small population in the brain, play an important role in maintaining the functional homeostasis of the brain [15–19]. There are two kinds of morphologically different GABAergic neurons in the brain, interneurons in cerebral cortex and Purkinje cells in cerebellum. The former displays enriched axonal arbors and less dendrite, whereas the latter shows enriched dendritical branches and a single axon [20,21]. Additionally, spike patterns in these two kinds of neurons are different [15,20,21]. Do the signaling pathways regulate spike encoding differently? We investigated these questions in cortical GABAergic neurons, which are genetically labeled with GFP, and cerebellar Purkinje neurons by whole-cell recordings and immunocytochemistry in mouse brain slices.

Methods and materials

Brain slices and neurons. Cortical and cerebellar slices (400 μm) were made from FVB-Tg(Gad GFP)4570Swn/J mice (Jackson Lab., Bar Harbor, ME 04609, USA) in postnatal day 17–22. Mice were anesthetized by inhaling isoflurane and decapitated with a guillotine. Cortical slices were cut with a Vibratome in oxygenated (95% O_2 and 5% CO_2) artificial cerebrospinal fluid (ACSF) in the concentrations (mM) of 124 NaCl, 3 KCl, 1.2 NaH_2PO_4, 26 $NaHCO_3$, 0.5 $CaCl_2$, 4 $MgSO_4$, 10 dextrose, and 5 HEPES, pH 7.35 at 4 °C. The slices were held in (95% O_2 and 5% CO_2) ACSF (124 NaCl, 3 KCl, 1.2 NaH_2PO_4, 26 $NaHCO_3$, 2.4 $CaCl_2$, 1.3 $MgSO_4$, 10 dextrose, and 5 HEPES, pH 7.35) at 25 °C for 2 h. A slice was then transferred to a submersion chamber (Warner RC-26G) that was perfused with ACSF oxygenated at 31 °C for whole-cell recording [17,18,22–24]. Chemical reagents were from Sigma. The entire procedures were approved by IACUC in Anhui, China.

The cortical GABAergic neurons for whole-cell recording in layer II and III of sensory cortex were selected based on GFP-labeled cells under fluorescent microscope (Nikon, FN-E600), in which an excitation wavelength was 488 nm. GABAergic Purkinje cells in cerebellum were selected based on their morphology under DIC microscope (Nikon, FN-E600). These neurons demonstrated

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the typical properties of interneurons, such as fast-spiking and less adaptation in spike amplitude and frequency [15,17,18,20,21,23,24].

Whole-cell recording. Electrical signals were recorded by using an Axoclamp-2B amplifier under current-clamp, and were inputted into pClamp 9 (Axon Instrument Inc., Foster CA, USA) for data acquisition and analysis. The output bandwidth in the amplifier was 3 kHz. Spike patterns were evoked by depolarization current pulses, in which the amplitude and duration were based on the aim of experiments. Pipettes for whole-cell recordings were filled with the standard solution that contained (mM) 150 K-gluconate, 5 NaCl, 5 HEPES, 0.4 EGTA, 4Mg-ATP, 0.5 Tris–GTP, and 5 phosphocreatine (pH 7.35 adjusted by 2 M KOH). Fresh pipette solution was filtered with centrifuge filters (0.1 μm) before the use, and osmolarity was 295–305 mOsmol. Pipette resistance was 5–6 MΩ.

The intrinsic properties of GABAergic neurons in our studies include the threshold potentials (Vts) of firing spikes and absolute refractory periods (ARP) following each spike. Vts are a start voltage of spike rising phase [15,22,25]. The ARP of sequential spikes are measured by injecting multiple depolarization-current pulses (3 ms) into GABAergic neurons after each of spikes (Fig. 2). By changing inter-pulse intervals, we define ARP as the duration from a complete spike to its subsequent spike at 50% probability [22]. Spike programming (capacity and timing precision) is represented as inter-spike interval (ISI) and standard deviation of spike timing (SDST), respectively.

Data were analyzed if the recorded neurons had the resting membrane potentials negatively more than −60 mV. The criteria for the acceptation of each experiment also included less than 5% changes in resting membrane potential, spike magnitude, and input resistance throughout each of experiments. Input resistance was monitored by measuring cell responses to the hyperpolarization pulses at the same values as the depolarization that evoked spikes. Vts, ARP and ISI are presented as mean ± SE. The comparisons before and after applying adenophostin-A are done by *t*-test.

In the immunocytochemical study of calcineurin (CaN) and CaM-KII, FVB-Tg (GadGFP)4570Swn/J mice during postnatal days (PND) 20–24 were perfused with 4% paraformaldehyde in PBS through left ventricle and aorta until their body is hard. The brain was isolated, and further fixed in 4% paraformaldehyde PBS for 48 h. Brain tissue was sliced by a Vobratom at 40 μm. The slices were incubated in monoclone anti-CaN and polyclone anti-CaMKII antibodies (Sigma, USA) at 4 °C for 48 h, and then were incubated in FTIC-coupled anti-mouse and red-fluorescent-coupled anti-rabbit antibodies (Sigma). The images of CaM-KII (red) and CaN (green) in cortical and cerebellar neurons were taken under confocal microscope (Olympus FV-1000, Japan), in which the parameters of laser beam and PMT are fixed for all experiments.

Results

Intracellular Ca^{2+} regulates spike encoding at cortical and cerebellar GABAergic neurons differently

Whole-cell current-clamp recordings were conducted at GABAergic neurons in cortical slices and Purkinje cells in cerebellar slices. Cytoplasmic Ca^{2+} was raised by infusing adenophostin-A, an agonist of IP3R, intracellularly via whole-cell pipettes [1]. Inter-spike intervals (ISI, an index of spike capacity) were measured by evoking action potentials with depolarization currents (100 ms).

Fig. 1A and B illustrates the effect of adenophostin-A on sequential spikes at cortical GABAergic neurons. The elevation of intracellular Ca^{2+} appears to lower the number of spikes in a given time (Fig. 1A). The ISI values of spikes 1–2 to 4–5 are 7.47 ± 0.35, 8.01 ± 0.34, 9.28 ± 0.37 and 10.51 ± 0.41 ms under the control (blue symbols in Fig. 1B); and ISIs' values are 8.9 ± 0.2, 9.94 ± 0.33, 11.94 ± 0.57 and 14.21 ± 0.86 ms by infusing adenophostin-A (red symbols, $n = 15$). ISI values for corresponding spikes under these two conditions are statistically different ($p < 0.01$). Thus, intracellular Ca^{2+} attenuates spike capacity at cortical GABAergic neurons.

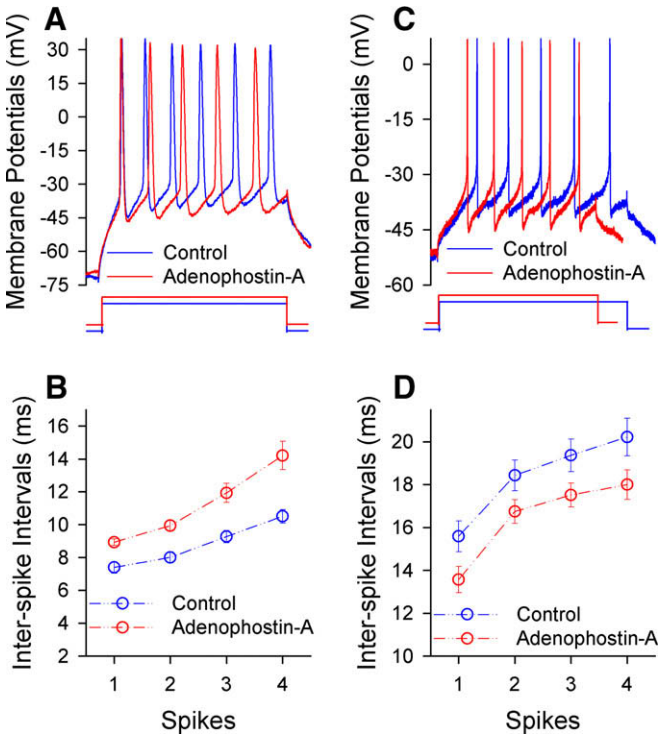

Fig. 1. Intracellular Ca^{2+} attenuates spike encoding at cortical GABAergic neurons and enhances spike encoding at cerebellar Purkinje cells in brain slices from FVB-Tg(Gad GFP)4570Swn/J mice. (A) The superimposed waveforms of sequential spikes are evoked by depolarization pulses (100 ms) during infusing adenophostin-A (red trace) vs. control (blue trace) at cortical GABAergic neurons. (B) Inter-spike intervals (ISI) of sequential spikes under control (blue symbols) and adenophostin-A infusion (red symbols) at cortical GABAergic neurons. (C) The superimposed waveforms of sequential spikes are evoked by depolarization pulses during infusing adenophostin-A (red trace) vs. control (blue trace) at cerebellar Purkinje cells. (B) Inter-spike intervals (ISI) of sequential spikes under control (blue symbols) and adenophostin-A infusion (red symbols) at cerebellar Purkinje cells. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

Fig. 1C and D shows the effect of adenophostin-A on sequential spikes at cerebellar GABAergic Purkinje cells. The elevation of intracellular Ca^{2+} appears to increase the number of spikes in a given time (Fig. 1C). The ISI values of spikes 1–2 to 4–5 are 15.59 ± 0.72, 18.44 ± 0.7, 19.37 ± 0.75 and 20.22 ± 0.9 ms under the control (blue symbols in Fig. 1D); and ISIs' values are 13.57 ± 0.6, 16.75 ± 0.55 17.52 ± 0.56 and 18 ± 0.7 ms in adenophostin-A (red symbols, $n = 14$). ISI values for corresponding spikes under these two conditions are statistically different ($p < 0.01$). Thus, intracellular Ca^{2+} enhances spike capacity at cerebellar Purkinje cells.

Mechanisms underlying the differential regulations of intracellular Ca^{2+} to spike encoding at cortical and cerebellar GABAergic neurons

As action potentials are navigated by refractory periods and threshold potentials [15,22], the differential regulations of Ca^{2+} to spike encoding at cerebellar and cortical GABAergic neurons are likely due to the influence of Ca^{2+} on ARP and Vts differently. The changes in ARPs were measured by injecting depolarization pulses (3 ms) into the recoding neurons after each of spikes. Threshold potentials are presented as the gap between the resting membrane potential (Vr) and threshold potential (Vts).