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Acidosis leads to neurological disorders through overexciting cortical pyramidal neurons

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ABSTRACT

The patients suffering from acidosis usually sign psychological deficits. The cerebral dysfunction is reportedly caused by an acid-induced functional impairment of GABAergic neurons; however, the role of pyramidal neurons in this process remains unclear. By using electrophysiological method and changing extracellular pH, we investigated the influence of acidic environment on pyramidal neurons in the cortical slices, such as their ability of firing spikes and response to synaptic inputs. A low pH of artificial cerebral spinal fluid elevates the responses of pyramidal neurons to excitatory synaptic inputs and their ability of encoding digital spikes, as well as reduces the signal transmission at GABAergic synapses. The elevated ability of neuronal spiking is associated with the decreases of refractory periods and threshold potentials. Therefore, acidosis deteriorates brain functions through making the activities between cortical pyramidal neurons and GABAergic neurons imbalanced toward the overexcitation of neural networks, a process similar to neural excitotoxicity.

1. Introduction

The neurons integrate synaptic signals and produce action potentials as brain codes for controlling well-organized behaviors and cognitions [1–4]. Pathogens, such as ischemia and acidosis, impair brain functions including seizure [5], anxiety [6,7] and mental illness [8–11]. Ischemic cerebral dysfunctions are owing to the impairment of the encoding processes at the neurons and synapses [12–17]. How acidosis impairs neuronal and synaptic dysfunctions for psychological deficits needs to be systemically documented.

Acidosis in severe disorders of metabolism, kidney and respiration impairs brain function [6,7], which make the nervous system to be unable to regulate these organs well. Protecting brain functions is one of key strategies to block this negative loop. To fulfill this goal, the mechanisms underlying acidosis-induced cerebral deficits need to be studied. In terms of cellular pathology for acidic neural overexcitation, a current report shows that acidosis impairs the function of GABAergic cells [18]. The coordination between GABAergic cells and principal neurons maintains the balanced neural network [19–24]. How does acidosis influence the function of principal neurons? We investigated the effects of acidosis on the functions of pyramidal cells including their intrinsic properties and synaptic transmission by whole-cell patch-clamp in the cortical slices.

2. Materials and methods

2.1. Brain slices and neurons

The entire procedures were approved by IACUC in Harbin Heilongjiang, China. Cortical slices (400 μm) were made from FVB-Tg(Gad GFP)4570Swn/J mice (Jackson Lab, Bar Harbor, ME 04609, USA) during postnatal day 17–22. Mice were anesthetized by inhaling isoflurane and decapitated by guillotine. 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 transferred to a submersion chamber (Warner RC-26G) that was perfused with ACSF oxygenated at 31 °C for whole-cell recording [25–27]. Chemical reagents were from Sigma.

Cortical pyramidal neurons for whole-cell recording in sensory cortex were selected based on the neuronal shape in pyramidal with no labeling GFP under a DIC-fluorescent microscope (Nikon, FN-E600, Japan), in which excitation wavelength was 488 nm.

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The spike firing of these neurons was characterized as adaptation in spike amplitude and frequency, not likely typical properties of fast-spiking interneurons [4,28–31].

2.2. Whole-cell recording and neuronal functions

The cortical pyramidal neurons were recorded by an Axo-Patch-200B amplifier under current-clamp for studying their intrinsic properties, and under voltage-clamp for analyzing their responses to excitatory and inhibitory synaptic inputs. Electrical signals were inputted into pClamp 10 (Axon Instrument Inc., Foster CA, USA) for the data acquisition and analyses. Output bandwidth in this amplifier was 3 kHz. The pipettes for whole-cell recording were filled with the solution including (mM) 150 K-gluconate, 5 NaCl, 5 HEPES, 0.4 EGTA, 4 Mg–ATP, 0.5 Tris–GTP, and 5 phosphocreatine (pH 7.35 adjusted by 2 M KOH; [32,33]. The fresh pipette solution was filtered with centrifuge filters (0.1 μm). Its osmolarity was 295–305 mOsmol, and pipette resistance was 5–6 MΩ.

The functions of pyramidal neurons were estimated based on their intrinsic properties and responses to synaptic inputs. Spike patterns at these neurons were induced by injecting depolarization pulses, whose intensity and duration were based on the aim of specific experiments. Inter-spike intervals (ISI) were used to measure spike capacity [25], in which the sequential spikes were induced by the depolarization pulses with a duration of 200 ms and an intensity for a threshold pulse (10 ms) of inducing a single spike at each cell. Their intrinsic properties in our studies included threshold potentials (Vts) of firing spikes and absolute refractory period (ARP) of following spikes. Spike Vts were voltages of firing spikes [4,34–36], and ARPs were measured by injecting paired-depolarization currents (3 ms) into these neurons after each spike (Fig. 3). By changing inter-pulse intervals, we defined spike ARPs as the durations from a complete spike to its subsequent spike at 50% probability [25,37].

The responses of cortical pyramidal neurons to excitatory synaptic inputs were evaluated by whole-cell voltage-clamp recording, in which excitatory postsynaptic currents (EPSCs) at these neurons were evoked by stimulating presynaptic axons [27,38–41]. It is noteworthy that EPSCs were blocked by washing 10 μM CNQX (6-cyano-7-nitroquinoxaline-2,3-(1*H*,4*H*)-dione, SIGMA) onto the slices in experiments to make sure the currents to be glutamatergic.

In addition, the responses of cortical pyramidal neurons to inhibitory synaptic inputs were evaluated by whole-cell voltage-clamp recording, in which the spontaneous inhibitory postsynaptic currents (sIPSC) from GABAergic synapses were recorded. Standard pipette solution contained (mM) 135 K-gluconate, 20 KCl, 4 NaCl, 10 HEPES, 0.5 EGTA, 4 Mg–ATP, and 0.5 Tris–GTP. The osmolarity of pipette solutions was 295–310 mOsmol, and the resistance of filled pipettes was 5–7 MΩ. Based on Nernst equation, the concentration of chloride ions in this pipette solution made reversal potentials around −43 mV. When we held membrane potential at −70 mV, GABAergic sIPSCs were inward (down-fluctuation). Series and input resistances for all of the neurons were monitored by injecting hyperpolarization pulses (5 mV/50 ms), and calculated by voltage pulses versus instantaneous and steady-state currents. 10 μM 6-cyano-7-nitroquinoxaline-2,3-(1*H*,4*H*)-dione and 40 μM D-amino-5-phosphonovanolenic acid were added into ACSF to block ionotropic receptor-channels in glutamatergic synapses [42,43]. These procedures isolated GABAergic IPSCs out. At the end of experiments, 10 μM bicuculline was washed onto the slices to test whether synaptic responses were purely mediated by $GABA_AR$, which did block sIPSCs recorded in our experiments.

2.3. In vitro acidosis

To simulate acidosis in internal environment for the brain cells, we changed the pH values of perfusion solutions from 7.35 to 6.75 [18,44], in which the difference between the solutions was only pH, but not other composites. This acidic internal environment, different from cellular acidosis [15], is more close a situation in clinical practice for acidosis. Under this condition, the changes of pH values can be executed precisely.

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

3. Results

The patients with acidosis show the disorder of brain functions [6,7]. In terms of cellular mechanisms underlying psychological deficits, it is found that acidosis leads to malfunction of GABAergic cells [18]. As a balance between GABAergic neurons and principal neurons is essential to fulfill the brain functions [22–24], here, we examined the influences of acidosis on the functions of cortical pyramidal neurons including their responses to synaptic inputs and ability to encoding action potentials.

3.1. Acidosis increases cortical pyramidal neurons in response to excitatory synaptic inputs

The responses of pyramidal neurons to excitatory synaptic inputs were studied under the condition of stimulating presynaptic axons, and excitatory postsynaptic currents (EPSCs) were recorded by whole-cell voltage-clamp. Acidosis in internal environment was simulated by perfusing artificial cerebral spinal fluid (ACSF) at pH 6.75 to cortical slices, compared with control ACSF (pH 7.35).

Fig. 1 illustrates the influence of acidosis on evoked synaptic transmission at cortical pyramidal cells. Compared with control condition (red trace in Fig. 1A), extracellular low pH (low $[pH]_o$) appears to raise excitatory synaptic responses (blue trace in 1A).

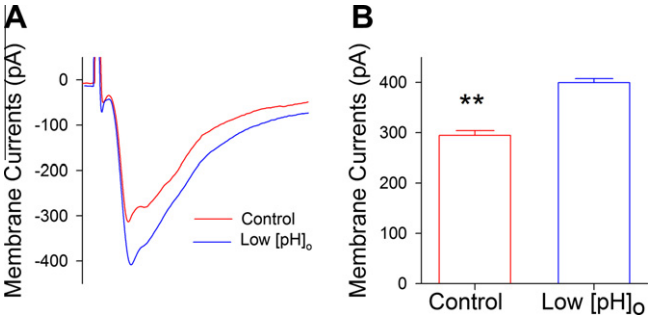

Fig. 1. Acidosis enhances the signal transmission at excitatory synapses of cortical pyramidal neurons. EPSCs were recorded by using whole-cell voltage-clamp and evoked by stimulating presynaptic axons. Acidosis was made by perfusing cortical slices with ACSF at pH 6.75 after control ACSF. Panel (A) shows the evoked EPSCs under the control (red trace) and subsequent extracellular low pH (blue trace), respectively. Panel (B) shows the values of evoked EPSCs under the conditions of control (pH at 7.35, red bar) and low $[pH]_o$ 6.75 (blue bar). Asterisks show $p < 0.01$ ($n = 11$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)