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Acidosis and alkalosis impair brain functions through weakening spike encoding at cortical GABAergic neurons

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ABSTRACT

Acidosis and alkalosis, associated with metabolic disorders, lead to the pathological changes of cognition and behaviors in clinical practices of neurology and psychology. Cellular mechanisms for these functional disorders in the central nervous system remain unclear. We have investigated the influences of acidosis and alkalosis on the functions of cortical GABAergic neurons. Both acidosis and alkalosis impair the ability of encoding sequential spikes at these GABAergic neurons. The impairments of their spiking are associated with the increases of refractory periods, threshold potential and afterhyperpolarization. Our studies reveal that acidosis and alkalosis impair cortical GABAergic neurons and in turn deteriorate brain functions, in which their final targets may be voltage-gated channels of sodium and potassium.

1. Introduction

Action potentials at the neurons and their transmission at the synapses are essential codes in the brain to encode various messages of controlling well-organized behaviors and cognition [1–6]. The impairment of their encodings leads to the functional disorders and psychological deficits in neurological diseases, such as epilepsy [7], ischemia [8–10] and psychiatric disorders [11–14]. The evidence on how acidosis and/or alkalosis impair neuronal encoding to cause psychological deficits has not been documented.

The patients with acidosis and alkalosis, which are caused by the severe disorders in the metabolism, kidney and respiration, show pathological cognition and behavior, such as depression, anxiety, convulsion, confusion and even unconsciousness [15,16]. Although carbonic anhydrase plays a role in the acidosis of hippocampal pyramidal neurons [17], cellular mechanisms underlying psychological deficits induced by acidosis and alkalosis are not clear. As the rhythmic activities of GABAergic neurons coordinate the behaviors of principal neurons in their networks [18–24], we mainly investigated the influences of acidosis and alkalosis on spike encodings at the GABAergic neurons of cortical slices by whole-cell patch-clamp.

2. Materials and methods

2.1. Brain slices and neurons

Cortical slices (400 μm) were made from FVB-Tg(Gad GFP) 4570Swn/J mice (Jackson Lab, Bar Harbor, ME 04609, USA) in postnatal days 17–22. Mice were anesthetized by inhaling isoflurane and decapitated by 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 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. Entire procedures were approved by IACUC in Harbin Heilongjiang, China.

GABAergic neurons for whole-cell recording in layers II–III of the sensory cortex were selected based on GFP-labeled cells under a fluorescent microscope (Nikon, FN-E600), in which an excitation wavelength was 488 nm. These neurons demonstrated the typical properties of interneurons, such as fast-spiking and less adaptation in spike amplitudes and frequency [6,26–31].

2.2. Whole-cell recording

Electrical signals were recorded by using an AxoClamp-2B amplifier under current-clamp, and inputted into pClamp 9 (Axon Instrument

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Inc., Foster, CA, USA) for data acquisition and analysis. Output bandwidth in the amplifier was 3 kHz. Pipettes for whole-cell recordings were filled with the standard solution that contained (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). Fresh pipette solution was filtered with centrifuge filters (0.1 μm). Its osmolarity was 295–305 mOsm, and pipette resistance was 5–6 MΩ. Spike patterns were evoked by depolarization current pulses, whose amplitude and duration were based on the aim of experiments. Inter-spike intervals (ISI) were used to represent spike capacity [25], in which sequential spikes are induced by depolarization pulses with a duration of 200 ms and an intensity at a threshold pulse (10 ms) of inducing a single spike for each cell.

The intrinsic properties of GABAergic cells in our study include threshold potentials (Vts) of firing spikes as well as absolute refractory period (ARP) and afterhyperpolarization (AHP) following each spike. Vts are a voltage of firing spikes [25,28,32], and spike ARPs are measured by injecting depolarization-current pulses (3 ms) into GABAergic neurons following each spike (Fig. 4). By changing inter-pulse intervals, we define ARP as the time from a complete spike to its subsequent spike at 50% probability [25]. AHP values are measured based on the difference between threshold potential and hyperpolarization.

2.3. In vitro acidosis and alkalosis

In order to simulate cellular acidosis and alkalosis, we back-filled the recording pipettes with the solutions at either pH 6.5 [9,33] or 8.0, in which their tips were filled the standard pipette solution with a pH 7.35. The difference of the solutions between their tips and back was only pH, but not other compositions. With these pipettes for whole-cell recording, intracellular pH will be balanced with the pipettes within a few minutes. Under this condition, the recording in the beginning of a few minutes can be used as the control, an approach similar to infusing other reagents [6,9,27,34,35].

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 spikes. Vts, ARP and ISI were presented as mean ± SE. The comparisons before and after acidosis or alkalosis were done by t-test.

3. Results

3.1. Acidosis and alkalosis attenuate spike encoding at cortical GABAergic neurons

To study the influences of acidosis and alkalosis on the encoding of neuronal spikes that may cause psychological impairment, we made cellular acidosis or alkalosis by using whole-cell recording pipettes containing solutions with pH 6.5 or 8.0. As GABAergic neurons coordinate the activities of principal neurons in their network [22–24], we studied the influences of acidosis and alkalosis on the spike encoding of GABAergic neurons in cortical slices.

Fig. 1 illustrates the influences of acidosis and alkalosis on spike encodings of cortical GABAergic neurons. Compared with the control (red trace in the middle panel of Fig. 1A), both acidosis and alkalosis appear to impair the ability of encoding sequential spikes (blue trace in the top panel for pH 8.0 and green in the bottom panel for pH 6.5). Fig. 1B illustrates the values of spike frequency under the conditions of various intracellular pH, i.e., 80.62 ± 10.4 Hz at pH 8.0 (blue bar, n = 12), 101.95 ± 13.2 Hz at pH 7.35 (control, red bar, n = 27) and 85.62 ± 11.1 Hz at pH 6.5 (green bar, n = 15), respectively. Statistical analyses indicate that the spike frequency under the control versus acidosis or alkalosis is significantly different ($p<0.01$, showed by asterisks). Thus, acidosis and alkalosis attenuate the encoding ability of cortical GABAergic neurons.

3.2. Acidosis and alkalosis increase spike thresholds and refractory periods at cortical GABAergic neurons

Action potentials are navigated by refractory periods and threshold potentials mediated by voltage-gated sodium channels [25,28,32]. The attenuation of spike encoding at cortical GABAergic neurons by acidosis and alkalosis is likely due to the elevation of threshold potentials (Vts) and/or the prolongation of refractory periods (ARP). The changes in ARPs and Vts under these conditions were measured (Materials and methods). Figs. 2 and 3 illustrate the influences of acidosis and alkalosis on Vts and ARPs at cortical GABAergic neurons.

Fig. 2 illustrates the comparisons of threshold potentials under the control versus higher pH and lower pH. The values of threshold potentials are 35.6 ± 0.94 mV at pH 8.0 (blue bar, n = 12), 32.13 ± 0.92 mV at pH 7.35 (control, red, n = 27) and 36.59 ± 1.0 mV at pH 6.5 (green, n = 15), respectively. Statistical analyses indicate that threshold potentials under the control versus acidosis or alkalosis are significantly different ($p<0.01$, showed by asterisks). Thus, acidosis and alkalosis elevate the energetic barrier of firing sequential spikes at cortical GABAergic neurons.

Fig. 3 illustrates the comparisons of spike refractory periods under the control versus higher pH and lower pH. Both acidosis and alkalosis appear to prolong spike refractory periods (Fig. 3A–C). The values of refractory periods are 4.96 ± 0.12 ms at pH 8.0 (blue bar, n = 12), 4.39 ± 0.11 ms at pH 7.35 (control, red, n = 27) and 4.86 ± 0.15 ms at pH 6.5 (green, n = 15), respectively. Statistical analyses indicate that refractory periods under the control versus acidosis or alkalosis are significantly different ($p<0.01$, showed by asterisks). Therefore, acidosis and alkalosis prolong the refractory period of sequential spikes at cortical GABAergic neurons.

3.3. Acidosis and alkalosis increase spike afterhyperpolarization at cortical GABAergic neurons

The generation of action potentials is also influenced by afterhyperpolarization (AHP) mediated by voltage-gated potassium channels [32,36–39], in which a proper AHP facilitates spike initiation, and a larger AHP weakens subsequent spike generation. We proposed to examine whether the attenuation of spike encoding at cortical GABAergic neurons by acidosis and alkalosis is likely due to an increase in AHP. The changes in AHP under these conditions were measured (Materials and methods).

Fig. 4 illustrates the comparisons of afterhyperpolarization under the control versus higher pH and lower pH at cortical GABAergic neurons. The values of AHP are 15.56 ± 0.22 mV at pH 8.0 (blue bar, n = 12), 13.64 ± 0.2 mV at pH 7.35 (control, red, n = 27) and 16.23 ± 0.13 mV at pH 6.5 (green, n = 15). Statistical analyses indicate that AHP under the control versus acidosis or alkalosis is significantly different ($p<0.01$, showed by asterisks). It is noteworthy that AHP values under higher pH and lower pH are statistically different ($p = 0.047$). Therefore, acidosis and alkalosis increase afterhyperpolarization to lower the ability of firing sequential spikes at cortical GABAergic neurons, especially during acidosis.

4. Discussion

Our studies indicate that the lower and higher values of cellular pH attenuate the encoding ability of cortical GABAergic neurons (Fig. 1), which may cause psychological deficits in the acidosis and alkalosis patients. Thus, physiological pH is optimal for neuronal encoding. The mechanisms underlying acidosis- and alkalosis-induced impairment of neuronal encoding include the elevation of spike threshold