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Zinc Defers the Initiation of a Phasic Burst-type Firing of Rat Thalamic Relay Neurons

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ABSTRACT

A visualized whole-cell voltage clamping of thalamic slices was used to examine the possibility of a Zn^{2+}-mediated alteration of Ca^{2+}-dependent burst firing of a central element in thalamocortical oscillation. We found that Zn^{2+} reversibly delayed the initiation of low-threshold spike (LTS), a base of the burst firing, only when the membrane potentials of a thalamic neuron were more positive than -65 mV. A hyperpolarization-activated cationic current (I_h), one of the central ionic conductances in determining LTS generation, was decreased by Zn^{2+}. However, the effective membrane potential range of the zinc action was below -75 mV so that the Zn^{2+} effect on I_h should not account for the LTS delay. We thus propose here that a transient low threshold Ca^{2+} current and/or a transient K^+ current must be main determinants for the LTS delay by Zn^{2+}.

Key words: H-current, oscillation, slice recording, thalamocortical pathway

INTRODUCTION

Zinc ion (Zn^{2+}), a heavy-metal divalent cation, is well known to involve in a myriad of cellular processes. In the CNS, Zn^{2+} localizes to several regions, such as the corticostriatal and thalamocortical (TC) pathways (Haug, 1973; Perez-Clausell and Danscher, 1986; Gibbs et al., 2000). This Zn^{2+} can be released in concentrations as high as 100~300 mM during intense neuronal firing, which may contribute to the generation of seizure activity (Assaf and Chung, 1984). Despite continuing studies for decades, however, the exact role of Zn^{2+} in the CNS in general remains unknown.

Recently it was reported that Zn^{2+} modulates spontaneous epileptic TC oscillations differentially upon the types of TC oscillation (Gibbs et al., 2000); Zn^{2+} exacerbates a simple TC burst complex (sTBC), which resembles spike-wave discharge activity, whereas Zn^{2+} blocks a complex TC burst complex (cTBC), which resembles generalized tonic clonic seizure activity. This differential effect of Zn^{2+} on TC oscillations is proposed to be owing to the effects on GABA receptors (Gibbs et al., 2000). Besides GABA receptors, variety of ionic conductances, including N-methyl-D-aspartate receptor currents and transient K^+ currents are known to interact with Zn^{2+}. Accordingly, much information about the effect of Zn^{2+} on all ionic conductances in a TC system should be needed to have the precise determination of physiological role of Zn^{2+}. However, we still do not even know whether or not directly Zn^{2+} alters the burst firings of a thalamic relay neuron (TRN) that are one of the most impor-

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tant determinants for either sTBC or cTBC.

In this study, employing a visualized whole-cell patch recording method for thalamic slices, we therefore sought to determine whether Zn^{2+} modified the thalamic burst firing. Also we have examined the effect of Zn^{2+} on a hyperpolarization-activated cationic conductance (I_h) that is one of the important ionic currents for the generation of the burst firing (McCormick and Pape, 1990).

MATERIALS AND METHODS

Slice preparation and maintenance

Young *Sprague*-Dawley rats (postnatal days 15～23) were anesthetized with isoflurane inhalation. After decapitation, the brain was drawn out of the opened skull and quickly placed in an ice cold (～4°C) oxygenated sucrose slicing solution, containing (in mM): sucrose, 200; PIPES, 20; KCl, 2.5; NaH_2PO_4, 1.25; $MgSO_4$, 10; $CaCl_2$, 0.5; D-glucose, 10; pH 7.3 with NaOH. The brain was incubated at least for 5 min to be cooled adequately. The cooled brain was trimmed leaving a small piece of brain including ventrobasal nucleus. The trimmed brain was fixed on the slicing chamber of a vibratome (Ted Pella, USA) using cyanoacrylate glue, sucrose slicing solution was then poured to the slicing chamber as quickly as possible, and coronal slices (250μm thick) were obtained. The slices were incubated, at least for three hours before recording, in a preheated (to 33～34°C) artificial cerebrospinal fluid (ACSF) containing (in mM); NaCl, 124; KCl, 3; NaH_2PO_4, 1.25; $MgSO_4$, 1.3; $CaCl_2$, 2.4; $NaHCO_3$, 26 and D-glucose, 10; pH 7.4 with continuous supply of 95% O_2/5% CO_2 gas.

Electrophysiological recording and data analysis

Whole-cell patch records were made with an Axoclamp 2B amplifier (Axon Instruments Inc., USA), and the pulse-generation and data acquisition were obtained and analyzed using pClamp8 software (Axon) through an analog-to-digital converter (Digidata 1322A, Axon) connected to a PC. Current clamp recording was performed under bridge mode and voltage clamp recording was under continuous single electrode voltage clamp mode. During voltage clamp recording, the series resistance was compensated up to 70% and a P/5 leak subtraction technique was used to eliminate leak and capacitive currents.

Recording pipettes (3.5～4.0 MΩ) were pulled on an electrode puller PP-83 (Narishige, Japan) from borosilicate glass capillaries (KG-33, Garner Glass, USA; 1.2 mm I.D., 1.5 mm O.D.). The surface of a recording pipette was coated with Sigmacote (Sigma), and the electrode was accessed to a TRN optically identified under an upright microscope with a DIC optic (Olympus, Japan) from the brain slices in a submerged recording chamber maintained at 33～34°C.

Recording solutions

Recording pipettes were filled with the following solution (in mM): K-gluconate, 125; KCl, 10; $CaCl_2$, 1; $MgCl_2$, 1; N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), 10; ethyleneglycol-bis-(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), 11 and Na_2 ATP, 4; pH 7.25 with KOH; 290 mmol/Kg Osm. Because 300μM $ZnCl_2$ produces precipitation easily in the conventional ACSF, we have used a modified ACSF where NaH_2PO_4 was omitted and $MgSO_4$ was replaced by $MgCl_2$ (Deisz, 1992). The external solution which osmolarity was adjusted to 305～310 mmol/kg was applied using a rapid exchange perfusion system (Chung et al., 1993).

RESULTS AND DISCUSSION

Thalamic relay neurons (TRNs) are well known to exhibit state-dependent responses according to their membrane potentials (Steriade and Deschenes, 1984; Steriade and Llinas, 1988). A positive current stimulation causes a rebound burst firing from a TRN which membrane potential is below -60 mV and a train of single spike discharges from the cell which membrane potential is above -55 mV. High expression of T-type Ca^{2+} channels with inactivation property is responsible for the state-dependent activity of TRN (Coulter et al., 1989). Fig. 1 shows the typical state-dependent activity of a TRN used in this study. A depolarizing current stimulation (0.05 nA) evoked a burst firing from a TRN which resting membrane potential was adjusted from -65 mV to -70 mV by injecting DC current (Fig. 1A), and another depolarizing current elicited a train of single spikes from the same cell held at -50 mV (Fig. 1B). A gradual injection of positive current, so

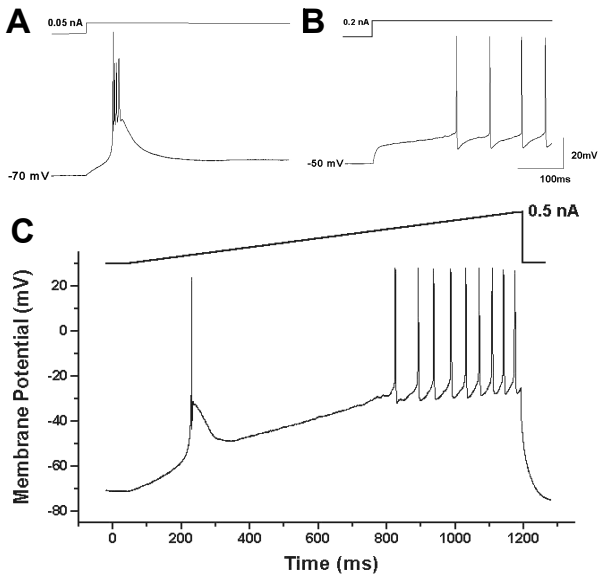

Fig. 1. State-dependent responses of a TRN. A depolarizing current pulse produced a high frequency spike burst from a TRN held at -70 mV (A) whereas another pulse produced repetitive impulses from the same cell held at -50 mV (B). A ramp stimulation protocol generated a phasic burst-type firing followed by a tonic repetitive firing from a TRN which membrane potential was initially adjusted to -70 mV by DC injection (C).

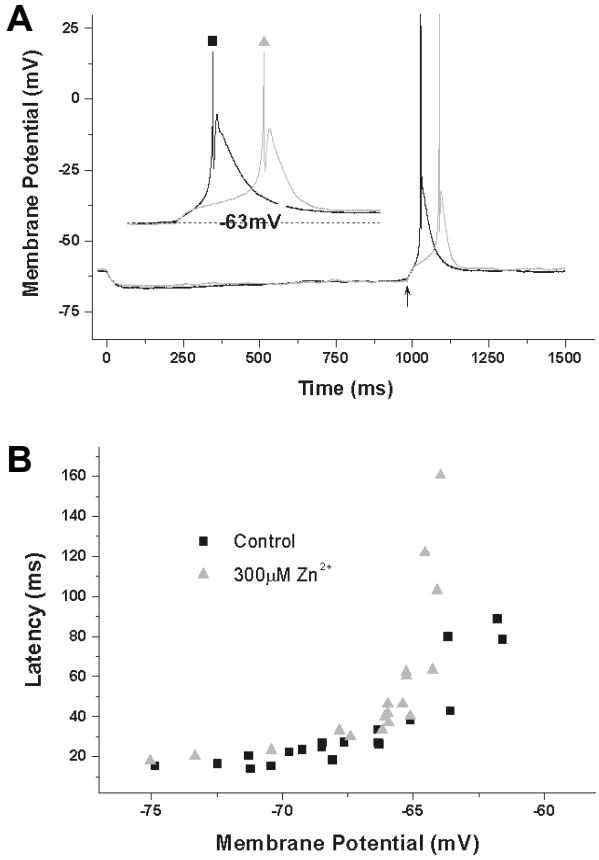

Fig. 2. Effect of Zn^{2+} on a burst firing. (A) The shift of the membrane potential from -65 mV to the resting potential of -60 mV by eliminating a hyperpolarizing current pulse evoked a rebound burst firing. Application of 300μM Zn^{2+} slowed down the generation of the rebound burst (gray-colored trace). The inset shows the spike in a magnified manner. (B) A plot of the latency varying with membrane potentials. The latency was determined as the period from the end of the hyperpolarizing current (the arrow of figure 2A) to the peak of the first sodium spike. Application of 300μM Zn^{2+} increased the latency in a voltage-dependent manner (■ Control; ▲, 300μM Zn^{2+}).

called a ramp, which elicits serially a burst firing and a tonic firing, was very often used to identify TRNs. Fig. 1C shows an example of a ramp protocol applied to a TRN which resting membrane potential was adjusted to -70 mV; a burst firing occurred at about -55mV, and a train of single spikes at about -30 mV.

Zn^{2+} appeared to slow down the initiation of burst firings elicited from a TRN (resting membrane potential, -60 mV) by anodal break excitation. The burst firing started from the end of a negative square pulse (indicated by the arrow in Fig. 2A), showing a typical low-threshold calcium spike and a burst of sodium spike discharges. Zn^{2+} significantly increased the latency, a period between the end of the negative stimulation pulse (indicated by the arrow) and the peak of the first spike, but in a voltage-dependent manner. That is to say, Zn^{2+} did not show any significant increment of the latency of a TRN which membrane potential was more negative than -65 mV (Fig. 2B). The burst firing is a high frequency burst of two to six Na^{+} action potentials riding on a low-threshold Ca^{2+} spike (LTS). Accordingly, Zn^{2+} seems like to affect the generation of LTS.

Several currents have been identified that are involved in the generation of LTS. These currents include the transient low threshold Ca^{2+} current (I_T; Coulter et al., 1989), the rapidly inactivating and transient K^{+} current (I_A; Huguenard et al., 1991), and a hyperpolarization-activated, mixed cationic conductance with slow kinetics, I_h (McCormick and Pape, 1990). The interaction of I_T and I_A is supposed to play a major role in the Ca^{2+}-dependent burst firing (Huguenard et al, 1991). Zn^{2+} was first reported to decrease I_A in cultured rat sympathetic neurons (Constanti and Smart, 1988), and there-

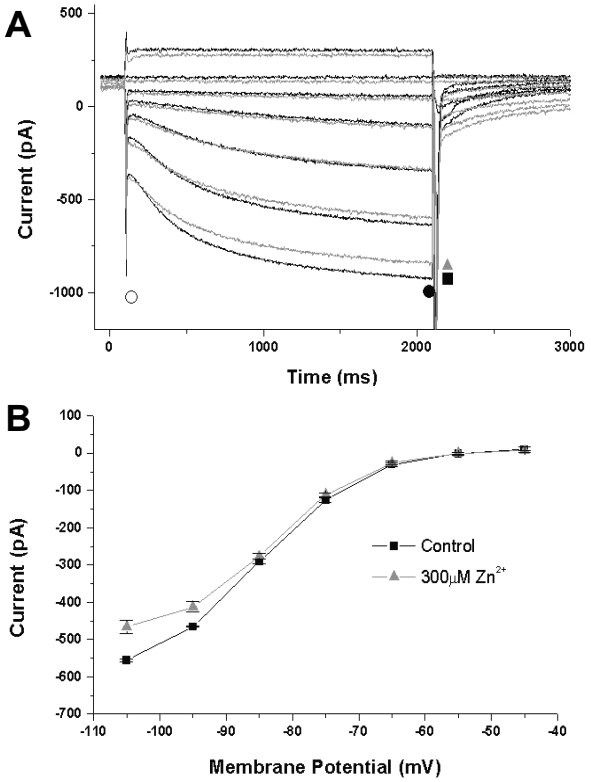

Fig. 3. Effect of Zn^{2+} on I_h. (A) Representative I_h traces elicited by 2-s voltage command from a holding potential of -55 mV to various potentials ranging from -45 mV to -105 mV in 10-mV steps (■, Control, ▲, 300μM Zn^{2+}). Application of 300μM Zn^{2+} reduced I_h in a voltage-dependent manner. (B) A plot of current *versus* membrane potential. Each current amplitude was determined by subtracting the minimum amplitude (reading on ○ of Fig. 3A) from the maximum value (read on ●). Significant reduction ($p < 0.05$, n=4) of I_h by 300μM Zn^{2+} was observed only when membrane potential was below -90 mV.

after other studies also showed its inhibitory role (Harrison et al., 1993a and 1993b). However, several mechanism studies strongly suggest that Zn^{2+} alters I_A depending upon membrane potentials (Huang et al., 1993; Bardoni and Belluzzi, 1994). Zn^{2+} would have accelerated the elicitation of LTS by increasing the ratio of I_T to I_A, if it just inhibited I_A. It is also noteworthy that Zn^{2+} decreases I_T reversibly (Noh and Chung, 2003). Accordingly, we propose here that Zn^{2+} may either decrease or increase I_A of a TRN depending on membrane potential. This suggestion is supported by our preliminary data showing that Zn^{2+} decreased I_A of a TRN which membrane potential was more negative than -75 mV and increased it when membrane potential was more positive than -75 mV (Noh et al., 2003). In addition to I_T and I_A, I_h must be involved in generating the LTS because it activates on hyperpolarization and generates a "pacemaker" potential for the generation of slow oscillation. We have thus examined the effect of Zn^{2+} on I_h using a continuous single electrode voltage-clamp mode, in order to determine whether or not Zn^{2+} reduces I_h.

Long hyperpolarizing voltage steps (2-s) to a TRN which membrane potentials were more negative than -65 mV elicited slow inward currents without showing any inactivation with time (Fig. 3A). Zn^{2+} reduced the I_h amplitude about 15% and 30% when membrane potential was -95 mV and -105 mV, respectively (Fig. 3B). However, no significant changes of I_h by Zn^{2+} were observed from TRNs which membrane potentials were within a physiological resting potential ranging from -65 to -85 mV, suggesting that the effect of Zn^{2+} on I_h seems not likely to have any contribution in deferring the elicitation of LTS.

Therefore, we tentatively conclude here that Zn^{2+} defers the LTS initiation mainly by the reduction of the ratio of I_T to I_A, probably by decreasing I_T and increasing I_A. Further systematic research for the dual role of Zn^{2+} on I_A should be needed to draw any conclusion.

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