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Alterations of Gating Kinetics of High-threshold Ca^{2+} Currents by Aspirin in Rat Cortical Neurons

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ABSTRACT

Aspirin, a well-known anti-inflammatory agent, is recently found to prevent Zn^{2+}-mediated neuronal death by interfering with voltage-gated Ca^{2+} channels (VGCC). Here we employed a whole-cell voltage-clamp technique for cortical neurons acutely isolated from rat pups, in order to elucidate the mechanism by which aspirin modulates VGCC. Aspirin reduced high-threshold Ca^{2+} currents (HTCC) in a concentration-dependent manner. Aspirin did not affect voltage-dependency of either HTCC activation or inactivation. However, the gating kinetics of HTCC was altered by aspirin: time constants for activation were increased by aspirin whereas time constants for inactivation were decreased. Therefore, the reduction of HTCC by aspirin seems to result from slower activation and faster inactivation.

Key words: acetyl salicylic acid, cortex, voltage-clamp, voltage-gated Ca^{2+} channels, whole cell recording

INTRODUCTION

In addition to its well-known role as analgesic-antipyretic and anti-inflammatory agent, aspirin was found to protect neurons against excess NMDA or a neurotoxin like 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (Grilli et al., 1996; Aubin et al., 1998; Kim et al., 2001). The protective effect of aspirin is suggested to result from the blockade of NF-κB, c-Jun N-terminal kinase, or oxidative stress (Kopp and Ghosh, 1994; Frantz and O'Neill, 1995; Grilli et al., 1996; Aubin et al., 1998; Ko et al., 1998). We also previously observed that aspirin protects cortical neurons from Zn^{2+}-induced neurotoxicity (Kim et al., 2001); this neuroprotective effect of aspirin has been attributed to prevent intracellular Ca^{2+} concentration from being elevated by Zn^{2+} via voltage-gated Ca^{2+} channels (VGCC). The fact that aspirin reduces the activity of VGCC strongly suggests that aspirin directly affect the information processing of nervous system as VGCC are one of the important intrinsic properties for neuronal excitability. Accordingly, the molecular mechanism by which aspirin modifies the activity of voltage-gated ion channels should be helpful to determine the role of aspirin on neuronal information processing.

Here, employing a whole-cell voltage clamp configuration for cortical neurons acutely isolated from rat pups, we examined the molecular mechanism underlying the effect of aspirin on VGCC and found that aspirin reduces high-threshold Ca^{2+} currents

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(HTCC) in a concentration-dependent manner by altering the gating kinetics of VGCC.

MATERIALS AND METHODS

Rat cortical cell dissociation

Freshly dissociated cortical neurons were prepared from young rat (Sprague-Dawley) aged 5～12 days. Rats were decapitated and the brain was removed rapidly and submerged in an ice-cold oxygenated HEPES-buffered solution (HBS) containing (mM): 135 NaCl, 5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 25 D-glucose, 10 HEPES, and pH 7.3 with NaOH. Coronal slices (300～400μm thick) were obtained using a Vibratome (Ted Pella, USA). Cortical tissues dissected out of the slices were incubated in HBS containing protease (0.8～1.0 mg/ml; Type I, Sigma, USA) at 36°C for 60 min. The tissue segments were then rinsed and mildly triturated with fire-polished Pasteur pipettes of decreasing tip diameter. Dissociated cells were plated onto 35-mm Petri-dishes.

Electrophysiological recording and data analysis

Whole-cell voltage-clamp records were made with an Axopatch 200A amplifier (Axon Instruments Inc., USA) at room temperature (22～24°C) and the data were analyzed using pClamp6 software (Axon). Recording pipettes (2.5～2.7 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 electrode series resistance compensation (>50%) was routinely applied. Currents were recorded at room temperature, filtered at 5 KHz, and digitized at 20～200μs intervals. P/4 pulse protocols were used to remove leak and capacitive current interference. The best fit of a theoretical curve to decay of tail currents, for activation of high-threshold Ca^{2+} current, was determined using the curve fitting feature of the Clampfit program of the pClamp6 software and analysis of variance (ANOVA) of Prism software was used to examine differences between groups of data.

Recording solutions

Recording pipettes were filled with the following solution (in mM): 2 $MgCl_2$, 130 CsCl, 20 HEPES-CsOH, 10 ethylene-glycol-bis(b-aminoethly ether)-N, N,N',N'-tetraacetic acid (EGTA), and pH 7.4. Dissociated cells were placed in HBS. After formation of a gigaohm seal gaining intracellular access, the external solution was switched to the following (in mM): 20 $BaCl_2$, 125 tetraethylammonium chloride (TEA-Cl), 10 HEPES-$BaOH_2$, and pH 7.4. Aspirins were obtained from Sigma. The external solution was applied using a rapid exchange perfusion system (Chung et al., 1993).

RESULTS AND DISCUSSION

The low-threshold Ca^{2+} currents of cortical neurons are known to be fully inactivated at 60 mV (Sayer et al., 1990). Accordingly, the high-threshold Ca^{2+} currents (HTCC) were selectively activated by step pulses (20 ms) from cultured cortical cells held at 60 mV and tail currents were obtained by repolarization to 40 mV (Brown et al., 1993). Ba^{2+} ions instead of Ca^{2+} ions were used in this study as a charge carrier to minimize Ca^{2+}-dependent inactivation. The evoked Ba^{2+} current was blocked by 10 mM aspirin (Fig. 1A). The reduction of Ba^{2+} currents was dependent on the concentrations of aspirin; 3 mM aspirin decreased the current by 19 ±5% (n=4; mean±SEM) and 10 mM aspirin decreased it by 46±5% (n=4; Fig. 1B). Possible explanations for the reduction of HTCC by aspirin include changes in either voltage dependence of activation or inactivation, kinetics, a decrease in the number of channels, or a decrease in single-channel conductance.

First, we examined a possibility of the shift in the voltage dependence either of steady-state inactivation to the hyperpolarizing direction or of activation to the depolarizing direction. The voltage dependence of activation was determined by measuring tail current amplitudes that were expected to reflect the fraction of Ca^{2+} channels opened during the preceding depolarization (Fig. 2A) (Brown et al., 1993). In each cell, the amplitudes of tail currents were normalized to the maximal value and then the ratios fit to the following Boltzmann relation with a power factor N=2:

$$I/I_{max} = [1/\{1+\exp((V_h-V_m)/k)\}]^N$$

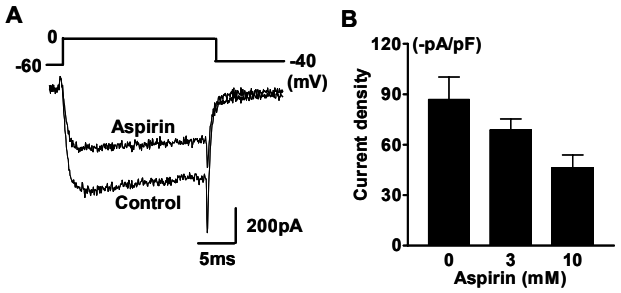

Fig. 1. Reduction of high-threshold Ba^{2+} currents by aspirin. (A) Inward Ba^{2+} currents in the absence (control; lower trace) and the presence of 10 mM aspirin (upper trace) were elicited for 20 ms by depolarization to the test potential (0 mV) from the holding potential (-60 mV) and tail currents evoked by repolarization to -40 mV (top trace; stimulation pulse). (B) Aspirin reduced high-threshold Ba^{2+} currents in a concentration-dependent manner (n=4).

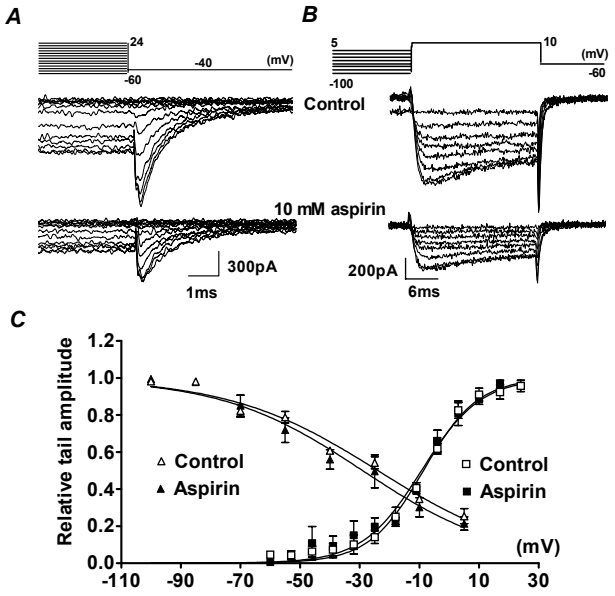

Fig. 2. Effect of aspirin on the voltage dependence of steady-state inactivation and activation of high-threshold Ba^{2+} currents. (A) Tail currents were measured at 40 mV after series of prepulses incremented in 7 mV steps from -60 mV of holding potential (the top trace, a stimulation protocol). The middle and bottom traces indicate tail currents before and after the application of aspirin, respectively. (B) A 20 ms-test pulse to 10 mV was preceded by 8 pre-pulses (each duration, 2 s) varied from 100 mV in 15 mV increments (top trace). The middle and bottom traces indicate HTCC currents before and after the application of aspirin, respectively. (C) Voltage-dependence of activation and inactivation. The right side curves plotted by normalizing tail currents show the voltage dependence of activation (□, control; ■, aspirin). The left side curves indicate the voltage dependence of steady-state inactivation (△, control; ▲, aspirin).

where V_h is the membrane potential for 50% inactivation, V_m is the membrane potential, k is the slope factor, and N is the power factor. No significant changes in kinetic parameters for activation were not observed in the presence of aspirin: the V_h for the control (□) and the aspirin-treated (■) were -8.66±0.858 and -9.30±0.97 mV, respectively; the slope factors, k, for the control and the aspirin-treated were 9.12±0.61 and 9.89±0.87 mV/e (n=4) (the right side of Fig. 2C). The voltage-dependence of the slow inactivation was also examined by measuring the peak Ba^{2+} currents obtained during a test potential to +10 mV following 2 s pre-pulses to various potentials. Fig. 2B shows samples of current records used to determine the voltage dependence of steady-state inactivation. In each cell, peak current values were normalized to the maximal current amplitude and then the ratios were fit to the Boltzmann relation with a power factor N=1. No significant alterations of inactivation parameters were observed: V_h=-23.98±1.60 mV (control, △) and -29.07±2.44 mV (aspirin, ▲); the slope factors, k=-24.93±1.66 (control) and -23.64±2.49 mV/e (aspirin) (the left side of Fig. 2C; n=4).

A next candidate mechanism for the HTCC reduction by aspirin might be an alteration of gating properties. To determine whether slowed activation or an increased rate of inactivation was responsible for the HTCC reduction by aspirin, we measured the time constants for activation (τ_m) and inactivation (τ_h) by fitting the individual current traces to an exponential function. Aspirin (10 mM) increased by 50% τ_m for the Ba^{2+} currents obtained from a cortical neuron held at -60 mV (the *upper* panel of Fig. 3A), and the increasing effect of aspirin on τ_m appeared to be voltage-dependent (the lower panel of Fig. 3A; n=4; $p<0.001$). The rate of inactivation was also affected by aspirin. Aspirin decreased τ_h for the slow inactivation evoked by a long stimulation (1.6 s) in a concentration dependent manner (Fig. 3B): 3 mM aspirin decreased τ_h by 68±2% and 10 mM aspirin decreased it by 72±5% (n=4; $p<0.05$). This represents that aspirin made activation become slower and inactivation faster, resulting in the reduction of Ca^{2+} currents.

Taken all together, therefore, we tentatively con-

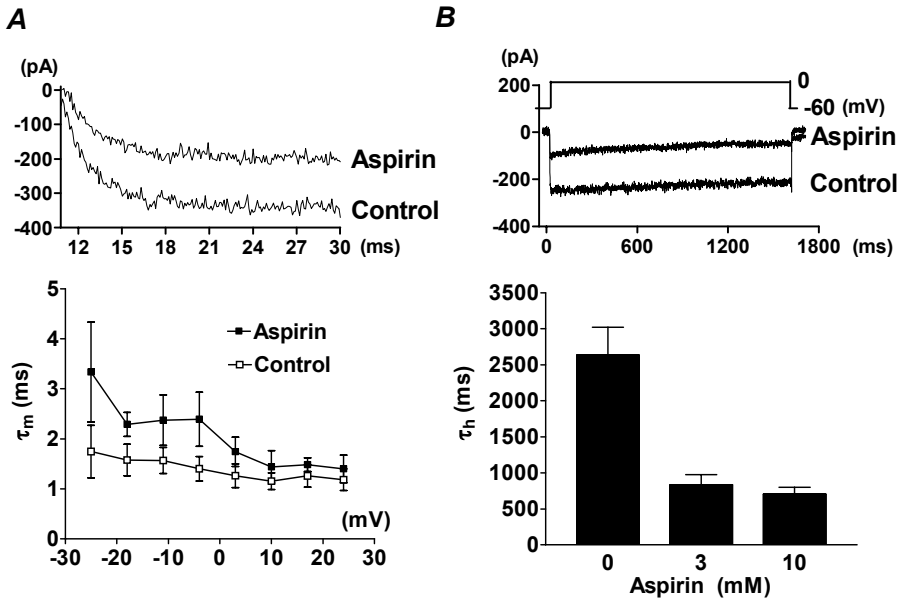

Fig. 3. Effect of aspirin on its gating kinetics of high-threshold Ba^{2+} currents. (A) The Ba^{2+} currents were evoked by depolarization to -11 mV from a holding potential of -60 mV in the presence (aspirin) and the absence (control) of aspirin, and aspirin increased their time constants for activation (upper panel). The lower panel shows the changes in the time constants for activation as a function of membrane potential, which was obtained by fitting the tail currents of Fig. 2A with an exponential function (n=4). (B) The current traces showed Ba^{2+} currents evoked by depolarization (top trace) to 0 mV from a holding potential of -60 mV for 1.6 s in the absence and the presence of aspirin (upper panel). The long stimulation (1.6 s) elicited slowly inactivating currents which time constants were decreased by aspirin in a concentration-dependent manner (n=4; lower panel).

clude that aspirin, which is well known to reduce the fever and relieve the aches produced by a variety of acute and shiver-provoking illness (Weissmann, 1991), can reduce the HTCC in rat cortical neuron by altering the properties of the gating kinetics without affecting the voltage dependence of VGCC.

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