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Effects of lithium chloride on outward potassium currents in acutely isolated hippocampal CA1 pyramidal neurons

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Abstract Although lithium possesses neuroprotective functions, the molecular mechanism underlying its actions has not been fully elucidated. In the present paper, the effects of lithium chloride on voltage-dependent potassium currents in the CA1 pyramidal neurons acutely isolated from rat hippocampus were studied using the whole-cell patch-clamp technique. Depolarizing test pulses activated two components of outward potassium currents: a rapidly activating and inactivating component, I_A and a delayed component, I_K. Results showed that lithium chloride increased the amplitude of I_A in a concentration-dependent manner. Half enhancement concentration (EC_{50}) was 22.80±5.45 μmol·L^{-1}. Lithium chloride of 25 μmol·L^{-1} shifted the steady-state activation curve and inactivation curve of I_A to more negative potentials, but mainly affected the activation kinetics. The amplitude and the activation processes of I_K were not affected by lithium chloride. The effects of lithium chloride on potassium channel appear to possess neuroprotective properties by Ca^{2+}-lowing effects modulate neuronal excitability by activating I_A in rat hippocampal neurons.

Keywords: lithium chloride, CA1 neuron, potassium current, patch-clamp technique.

Lithium is best known for its therapeutic efficacy in the treatment of manic-depressive illness. Its clinical profile includes the antimanic and antidepressant actions as well as prophylaxis of both mania and depression. Despite its efficacy, the molecular mechanism underlying its action has not been elucidated[1,2]. Previous studies have shown that lithium is a potent inhibitor of GSK and specifically inhibits GSK-3β[3−5]. Moreover, it has been shown that lithium may exert its effects on the central nervous system. Hong *et al.*[6] demonstrated that lithium reduces the phosphorylation of tau, enhances the binding of tau to microtubules and promotes microtubules assembly through. Recently it was reported that lithium could inhibit amyloid deposition. Decreased β-amyloid (Aβ) secretion in lithium-treated cells was observed in a dose-dependent manner[7]. The hyperphosphorylation of tau and Aβ deposition are believed to be critical events in the pathogenesis of Alzheimer's disease (AD)[8,9]. Since lithium can inhibit both tau and amyloid pathologies, it is possible that lithium can protect neurons against Aβ toxicity and slow the progression of AD[7].

Abnormalities of potassium channel function have been shown in cultured cells from patients with AD[10,11] and potassium channel dysfunction was a very clear and specific marker for AD. However, the effects of lithium chloride on potassium channel in central neurons are uncertain. The hippocampus is critically involved in learning and memory. Hippocampal lesions cause some deficits in the ability to transfer information from short-term to long-term stores, thus preventing the formation of new memories[12]. In the current study, we examined the effects of lithium on voltage-gated outward potassium currents in rat-dissociated hippocampal CA1 pyramid neurons. This provides new insight into how lithium mediates its effects on the central nervous system, and these findings might be an interpretation on its neuroprotective actions.

1 Materials and methods

1.1 Isolation of neurons

Single rat hippocampal pyramids neurons were acutely isolated by enzymatic digestion and mechanical dispersion from 7-d-old Wistar rats as previously described[13]. Hippocampal CA1 region was cut into 300−500 μm thick slices and incubated for 30 min at 32℃ in artificial cerebrospinal solution (ACS) and successively transferred into ACS containing 0.5 mg·mL^{-1} protease and digested at 32℃ for 35 min. Throughout the entire procedure the media were continuously saturated with a 95%O_2−5%CO_2 gas mixture to maintain a pH of 7.4. After digestion, the tissue pieces were washed 3−4 times with ACS and neurons were triturated through a series of fire-polished glass

pipettes with opening diameter from 0.1 to 0.5 mm. The cell suspension was transferred into a 35-mm culture dish, filled with 1.5 mL extracellular solution. 20 min later, the neurons were attached to the bottom of the culture dish and were ready for experiments.

1.2 Whole-cell patch-clamp recording

The pyramidal neurons with a diameter of 15—30 μm were identified by their characteristic bright pyramid-shaped soma under a phase contrast microscope and two or three short-branched dendrites and a long axon. The patch electrodes (BJ-40, diameter 1.5±0.1 mm, Beijing) were pulled in two steps by a microeletrode puller (PP-830, Narrishage, Japan) and had tip resistance of 5—8 MΩ when filled with pipette solution. Whole-cell currents were recorded with Axopatch 200B patch clamp amplifier (Axon Instrument, USA). After forming a conventional "gigaseal", the membrane was ruptured with a gentle suction to obtain the whole-cell voltage clamp configuration. Liquid junction potential between the pipette solution and extracellular solution was compensated after the pipette tipped into the external solution.

1.3 Preparation of experimental solutions (mmol·L^{-1})

Artificial cerebrospinal solution (ACS): NaCl 124, KCl 5, KH_2PO_4 1.2, $MgSO_4$ 1.3, $CaCl_2$ 2.4, glucose 10, $NaHCO_3$ 26, pH adjusted to 7.4 with tris. Extracellular solution: NaCl 150, KCl 5, $MgCl_2$ 1.1, $CaCl_2$ 2.6, glucose 10, HEPES 10, pH adjusted to 7.4 with tris (final concentration 4). To record potassium current, 1 μmol·L^{-1} TTX and 0.2 mmol·L^{-1} $CdCl_2$ were added to extracellular solution before the electrophysiological recording. Pipette solution: KCl 65, KOH 5, KF 80, $MgCl_2$ 2, HEPES 10, EGTA 10, Na_2ATP 2, pH adjusted to 7.3 with KOH. Protease, TEA-Cl, HEPES, TTX were purchased from Sigma Company.

1.4 Data analysis

Currents were amplified by using an amplifier (Axopath 200B), and digidata 1200B interface (Axon Instrument, USA) and Pclamp version 6.0.4 software (Axon Instrument, USA) were used to produce protocols, acquire and process data. All data were analyzed by the use of Clampfit procedures (Axon Instrument, USA) and MICROCAL-ORIGIN (5.0). All values were presented as mean ±S.D., and statistical comparisons were made using the paired student's *t*-test with $P<0.05$ being statistically significant.

2 Results

2.1 Separation of I_A and I_K

Step depolarization to potential from −50 to +60 mV (10 mV steps) following a hyperpolarizing prepulse of 400 ms to −110 mV clearly activated two components of outward current (Fig. 1(a)). First, a rapidly activating and inactivating current, sensitive to 4-AP, was referred to as I_A, and then a delayed rectifier current, sensitive to TEA, was named I_K. To study the effects of lithium chloride on I_A and I_K respectively, a signal subtraction procedure was used to separate I_K from the total potassium current. By holding at −50 mV and stepping to more positive potentials, I_A undergoes steady-state inactivation, therefore, I_K could be activated nearly uncontaminated with I_A[14]. With this protocol, I_K was activated slowly to a plateau with minimal time-dependent inactivation (Fig. 1(b)). Subtraction of I_K from total potassium current revealed I_A (Fig. 1(c)). Currents at the end of the depolarizing pulse were referred to as I_K. The peak of the subtracted currents was referred to as I_A.

2.2 Effects of lithium chloride on I_A and I_K

To determine the potential effects of lithium chloride on two components of outward potassium current, lithium chloride of various concentrations was added to the bath solution at final concentrations of 1×10^{-6}, 5×10^{-6}, 2.5×10^{-5}, 1.25×10^{-4}, 6.25×10^{-4} mol·L^{-1}. Application of 25 μmol·L^{-1} lithium chloride increased the amplitudes of I_A drastically (Fig. 2), but the amplitudes of I_K were not affected (Fig. 3). The increase in I_A developed slowly and 15 min for bath application was necessary before reaching a steady state, which may be attributed to the slow penetration of lithium chloride in the slice. In addition, lithium chloride produced a concentration-dependent enhance of I_A (Fig. 4). The concentration-dependent curve was fitted by the Logical equation: $Y=(A_1-A_2)/$ yangpin@ sxu.edu.cn $(x/x_0)^p]+A_2$, where A_1 is initial value of the increase of I_A by lithium chloride, A_2 is final value of the increase of I_A by lithium chloride, x is the concentration of lithium chloride, x_0 is the center, and p is the power. The half enhancement concentration (EC_{50}) of lithium chloride was 22.80±5.45 μmol·L^{-1}. Fig. 5 shows the effect of lithium chloride on the current-voltage (I-V) curve of I_A. In the presence of 25 μmol·L^{-1} lithium chloride, the amplitude of I_A was significantly increased at potential −10 mV through +60 mV.

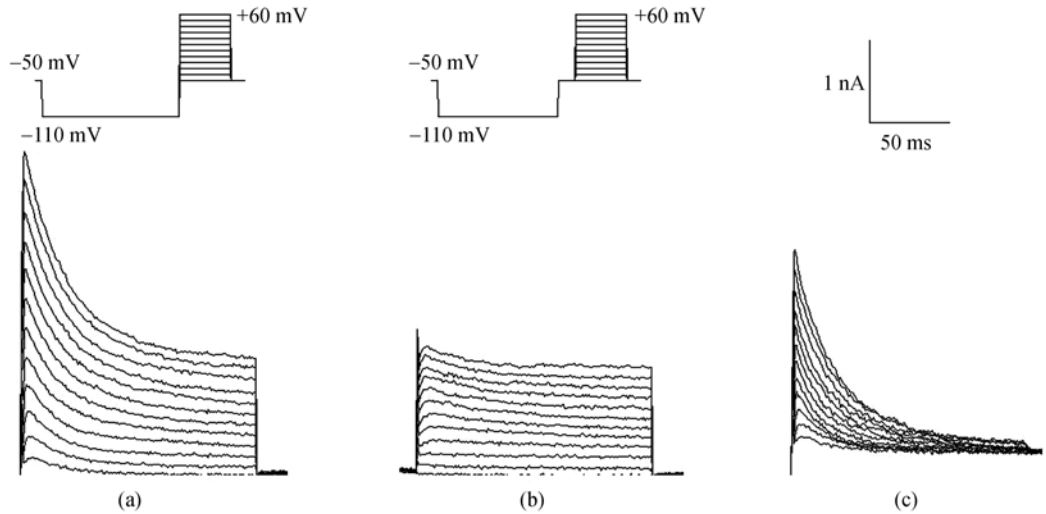

Fig. 1. Outward potassium current families in a hippocampal pyramidal neuron. (a) Total outward potassium current; (b) I_K stimulated with similar protocol as in (a), except for a 50 ms interval at −50 mV, was inserted after the prepulse (inset); (c) isolated I_A by subtracting current traces of (b) from those of (a).

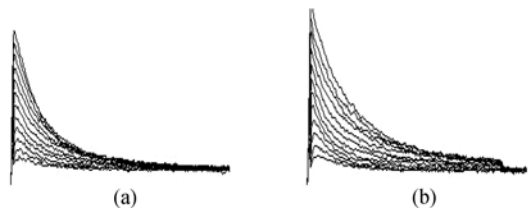

Fig. 2. Effects of 25 μmol·L^{-1} lithium chloride on I_A. (a) Control; (b) 25 μmol·L^{-1} lithium chloride.

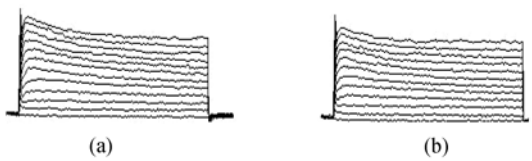

Fig. 3. Effects of 25 μmol·L^{-1} lithium chloride on I_K. (a) Control; (b) 25 μmol·L^{-1} lithium chloride.

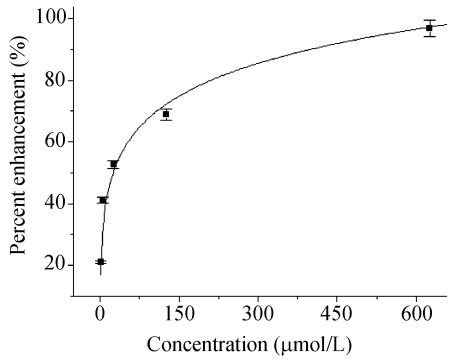

Fig. 4. Concentration-response curve of the lithium enhancement on I_A.

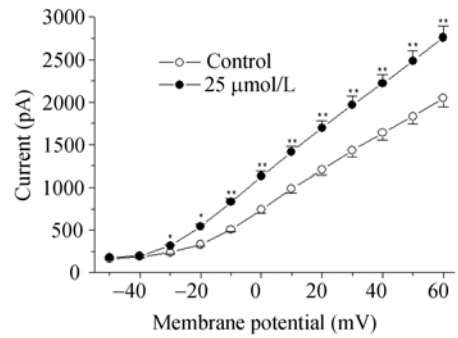

Fig. 5. Current-voltage relationships in the absence and presence of 25 μmol·L^{-1} lithium chloride.

2.3 Effect of lithium chloride on the activation kinetics of I_A and I_K

I_A and I_K were converted into conductance by use of the equation $G=I/(V-V_K)$, where G is conductance, V is membrane potential, and V_K is reversal potential (calculated to be −87 mV with Nernst equation $E_{rev}=(-2.303\ RT/zF)\times\log([K^+]_i/[K^+]_o)$). The activation curves shown in Fig. 6 were obtained by plotting the normalized conductance against the membrane potentials. The curves were fitted with a Boltzmann equation: $G/G_{max}=1/\{1+\exp[-(V-V_h)/k]\}$, where V_h is membrane potential at half-activation, and k is slope factor. Before and after application of 25 μmol·L^{-1} lithium chloride, the values of V_h for I_K (Fig. 6(a)) were −2.36±2.76 mV and −6.85±2.45 mV (n=5, P>0.05), with k being 23.55 ±3.50 mV and 21.52±2.66 mV (n=5, P>0.05), which

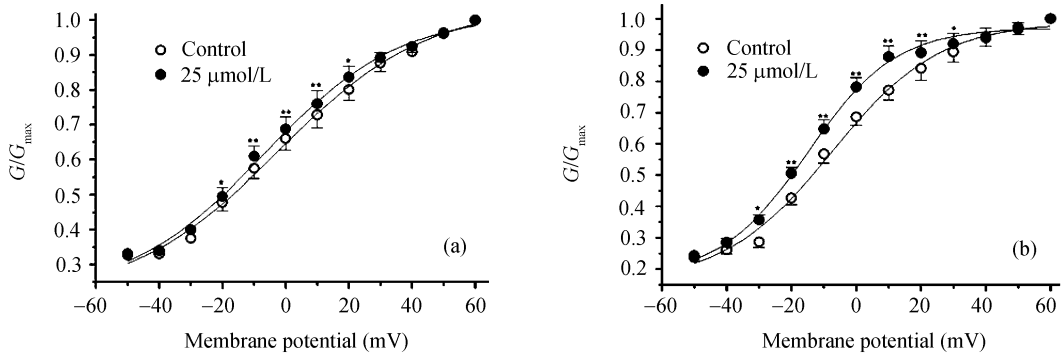

Fig. 6. Effects of lithium chloride on the steady-state activation curves of I_K (a) and I_A (b).

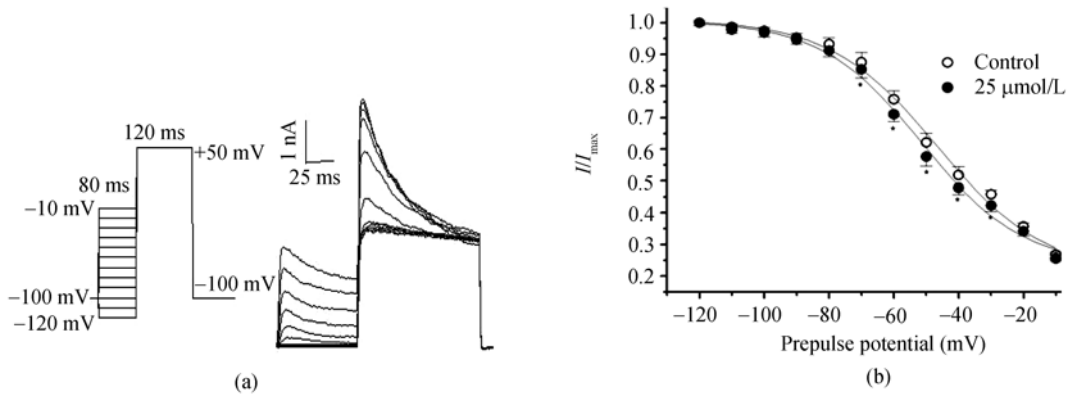

Fig. 7. Effects of lithium chloride on the steady-state inactivation curves of I_A. (a) A selection of the I_A traces examined with a double-pulse protocol (inset); (b) peak amplitudes for I_A currents in the absence and presence of lithium chloride were normalized and plotted versus prepulse potentials.

indicated that the activation process of I_K was not affected by lithium chloride. Before and after application of 25 μmol·L^{-1} lithium chloride, the values of V_h for I_A (Fig. 6(b)) were −7.58±2.16 mV and −15.05±1.59 mV (n=5, P<0.01), with k being 16.68±2.14 mV and 13.53±1.35 mV (n=5, P<0.05), which indicated that the activation curve of I_A was shifted toward negative potential.

2.4 Effect of lithium chloride on the inactivation kinetics of I_A

The steady-state inactivation was examined by changing the prepulse potentials at levels between −120 and −10 mV (80 ms duration) before depolarization to a test pulse of +50 mV (duration of 120 ms). A selection of the I_A traces from a typical experimental series is shown in Fig. 7(a). Peak amplitudes for I_A currents were normalized and plotted versus prepulse potentials (Fig. 7(b)). The curves were well fitted with a Boltzmann equation: $I/I_{max}=1/\{1+\exp[(V-V_h)/k]\}$, where V_h is membrane potential at half-inactivation, and k is slop factor. During control conditions, average V_h was −46.77±2.38 mV and k was 16.58±2.08 mV. After the addition of 25 μmol·L^{-1} lithium chloride, the inactivation curve was shifted to more negative potential: V_h was −51.46±2.02 mV (n=5, P<0.05) and k was 15.61±1.93 mV (n=5, P>0.05). But the shift of k was not statistically significant.

3 Discussion

Voltage-gated potassium currents play crucial roles in modifying neuronal cellular and network excitability and activity[15]. Potassium currents control action potential duration. I_A and I_K contribute to action potential repolarization. While the I_A, in addition, plays an important role in repetitive firing and backpropagation of action potentials into dendrites[6]. Blockage of potassium channels causes membrane depolarization, enhances neuronal excitability, and lead to increase Ca^{2+} influx, intracellular Ca^{2+} accumulation, thus begin a

cascade of harmful events that eventually result in neuronal dysfunction and death[16]. There are reports that Aβ selectively inhibits I_A in acutely dissociated or cultured hippocampal or cortical neurons[17,18], sustains increased Ca^{2+} influx[18,19] and causes a loss of function or structural deficits in synapses of the hippocampus. The hyperexcitability is expected to occur before cell death induced by calcium influx, but it can lead to cognitive deficits of early or mid-stage AD. It is very important to find a way to compensate or/and eliminate the lasting Aβ-induced membrane depolarization[20], since this can lead to hyperexcitability of affected neurons in the brain.

An increase in potassium conductance inhibits excitable cells by shifting the membrane potential toward the potassium equilibrium potential and away from the threshold of the action potential. Activation of potassium channels would be expected to attenuate membrane depolarization and Ca^{2+} influx. Stimuli that activate potassium conductance hyperpolarize cells and lower the excitatory inputs[21]. Activation of potassium channels by a cyclic GMP-mediated mechanism appears to be involved in the mechanism whereby amyloid precursor proteins protect neurons against excitotoxicity[22,23]. In addition, K^+ channels openers prevent hippocampal CA1 neurons from cell death in rats and protect hippocampal neurons against oxidative injury and Aβ toxicity presumably because of their ability to hyperpolarize the plasma membrane and reduce Ca^{2+} influx[24,25]. We assume that increase of potassium current by lithium chloride could reduce cell excitability and Ca^{2+} influx, which contributed to its neuroprotective actions. But we do not yet known how much enhancement would be needed to restore normal cell excitability. One important reason is that the molecular mechanisms of lithium have not yet been fully understood and lithium could affect multiple cellular targets. Further investigation is required to elucidate the action mechanism between metal ions and channel proteins.

In summary, activation of I_A and acceleration of its activation process, membrane hyperpolarization and suppression of Ca^{2+} influx might be interpretations on lithium chloride's neuroprotection against damages induced by Ca^{2+} overload. Our findings, as well as results obtained from biochemical and neurochemistry studies about the link between lithium and central neurons suggest that the function of lithium chloride should be re-evaluated. The more we understand the action mechanisms of lithium chloride, the more it will be used.

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