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ORIGINAL PAPER

Enhancement of Lanthanum (III) on Sodium Currents in Acutely Isolated Hippocampal CA1 Neurons of Rat

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Abstract The effects of lanthanum (III) (La^{3+}) on voltage-gated sodium channel currents (I_{Na}) in freshly dissociated rat hippocampal CA1 neurons were studied using the whole-cell patch clamp techniques. La^{3+} reversibly enhanced I_{Na} in a concentration- and voltage-dependent manner. The 50% enhancement concentration (EC_{50}) of La^{3+} on I_{Na} was 9.93 μM. In addition, 10 μM La^{3+} shifted the steady state activation curve of I_{Na} towards positive potential and the steady state inactivation curve towards negative potential without changing the slope factor. These results indicated that La^{3+} could increase the amplitudes of I_{Na} and change the activation and inactivation courses of I_{Na} even in very low concentration.

Keywords Lanthanum (III) · Sodium current · Hippocampal CA1 neurons · The whole-cell patch clamp techniques

Introduction

The lanthanide elements (Ln) are f-block inner transition elements belonging to the group III B of the periodic table. They occur abundantly in the lithosphere, widely used in organic synthesis, bioorganic chemistry, homogeneous catalysis industry, stockbreeding and medicine, especially as trace fertilizers in agriculture, and be concentrated by food chain. The impact of Ln on human health is still largely unknown. Therefore, understanding the effects of Ln on health has become more and more important. Whether Ln is toxic is mostly dose-related. Ln generally is considered to be toxic in micro-molar concentrations [1, 2]. Ln exerts diverse biological effects mainly by their resemblance to calcium. While the inhibitory effects of Ln on calcium-dependent physiological processes are well-known, their relevance for the pharmacological properties and mechanism remains to be elucidated. Moreover, there is increasing interest in the use of Ln in medicine. Experimental results are summarized to show that the appropriate uses of Ln would be useful in therapeutical application [3]. The therapeutic use of lanthanides as an anticancer agent has been proposed. Daily i.p. injection of 2.5 mg $LaCl_3$ retarded the growth of sarcoma in rats [4]. The influences of lanthanide ions (Ln^{3+}) on cancer cell proliferation have been investigated for several cell lines. For example, in the presence of Ln^{3+}, growth rates of B16 melanoma cells were significantly lower than that of the control cells [5]. Xiao et al. [6] and Ji et al. [7] found that Ln^{3+} had certain suppression effects on the proliferation of the human gastric cancer cell PAMC82 and leukemic cell K562. Lanthanum (III) salts have been reported to exert moderate anti-proliferative effects in vitro [8, 9] and in vivo [4].

Previous studies have shown that the transient K^+ current is blocked by lanthanum in Drosophila neurons [10]. Trivalent lanthanides such as lanthanum (La^{3+}) and gadolinium (Gd^{3+}) have been demonstrated that they may affect voltage-gated K^+ currents directly [11]. Micro-molar concentrations of La^{3+} ions have been reported to block a component of the delayed rectifier current of guinea-pig ventricular myocytes [12]. In addition to their reported effects on K^+ currents, similar concentrations of La^{3+} have been shown to modify Na^+ channel gating in clonal pituitary GH3 cells [13]. Furthermore, Ln has also been

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demonstrated to affect the threshold for spike initiation. La^{3+} can not enter ventricular myocytes through L-type calcium channel, but it can enter the cells via Na^{+}–Ca^{2+} exchange [14, 15], which is in consistence with the subsequent reports [16]. La^{3+} (0.1–1,000 μM) in the external solution inhibited transient outward potassium current ($I_{K(A)}$) in a concentration- and voltage-dependent manner in acutely isolated rat hippocampal pyramidal neurons [17]. La^{3+} (10 μM) in the external solution inhibited Ca^{2+} independent voltage-activated outward K^{+} current in ventricular myocytes of rats [18].

Sodium channels play a crucial role in regulating the electrical excitability of neuron cells and maintaining cell integrity and function, being primarily responsible for the depolarization phase of the action potential [19]. At the ion channel level, the effects of La^{3+} on Na^{+} channels in hippocampal neurons are uncertain. In the present study, we examined the effects of La^{3+} on I_{Na} in freshly dissociated rat hippocampal CA1 neurons using the whole-cell patch clamp techniques.

Materials and Methods

Preparation of Single Neuron Cell

Single rat hippocampal pyramidal neurons were acutely isolated by enzymatic digestion and mechanical dispersion [20]. Wistar rats of 7–10 days were purchased from the Experimental Animal Center of Shanxi Medical University (Grade II, Certificate No. 070101). All experiments conformed to local and international guidelines on ethical use of animals and all efforts were made to minimize the number of animal used and suffering. Briefly, 400–600 μm thick brain slices were cut from hippocampal region in ice-cold artificial cerebrospinal solution (ACS). These tissue pieces were incubated for at least 30 min at 32°C in ACS, and then transferred into ACS containing 0.5 mg/ml protease at 32°C for 35 min. Throughout the entire procedure the media were continuously saturated with a 95% O_2–5% CO_2 gas to maintain a pH value as 7.40. After digestion, the tissue pieces were washed three times with ACS. Through a series of Pasteur pipettes with decreasing tip diameter, neurons were isolated by triturating the brain fragments. Then, the cell suspension was maintained at room temperature in extracellular solution, and was ready for the electrophysiological experiment. All experiments were performed within 4 h after isolation.

Patch-Clamp Techniques

The cell suspension was transferred into an experimental chamber mounted on the stage of an inverted microscope (Chongqing, China) with 1 ml extracellular solution. After 20 min, pyramidal cells settled on the bottom of the chamber. Sodium currents (I_{Na}) were recorded with an Axopatch 200B patch clamp amplifier (Axon Instruments, Foster City, CA, USA). Glass microelectrodes were made using micropipette puller (PP 830, Narishige, Japan) and had a resistance of 7–12 MΩ when filled with electrode internal solution. Neurons with bright, smooth appearance and apical dendrites were selected for recording. Liquid junction potential between the pipette solution and external solution was corrected after the pipette tipped into the external solution. After forming a conventional 'gigaseal', the membrane was ruptured with a gentle suction to obtain the whole cell voltage clamp. To minimize the duration of capacitive current, membrane capacitance and series resistance were compensated after membrane rupture. Evoked currents were low-pass filtered at 2 kHz, digitized at 10 kHz, command pulses were generated by a Digidata 1200B (Axon) controlled by pCLAMP version 6.0.4 software (Axon Instruments, CA, USA), and on-line acquired data stored in a PC486 computer for subsequent analysis. All experiments were carried out at room temperature (20–26°C).

Solutions

Artificial cerebrospinal solution (ACS) contained (in mM): NaCl 124, KCl 5, KH_2PO_4 1.20, $MgSO_4$ 1.30, $CaCl_2$ 2.40, Glucose 10, $NaHCO_3$ 26, pH 7.4. External solution contained (in mM): NaCl 150, KCl 5, $MgCl_2$ 1.1, $CaCl_2$ 2.6, Glucose 10, HEPES 10, pH 7.40. Before the recording, 0.2 mM $CdCl_2$ was added to the external solution. Electrode internal solution contained (in mM): CsCl 75, NaF 15, $MgCl_2$ 2, HEPES 10, EGTA 2.5, Na_2ATP 3, pH 7.3.

Statistical Analysis

All data were analyzed by the use of pCLAMP 6.0 and Origin 5.0 software (Microcal software, USA). All values were presented as mean ± SD, and statistical comparisons were made using the paired Student's t test and one-way ANOVA procedure, and the probabilities <0.05 were considered significant.

Results

La^{3+} Enhanced I_{Na} in a Concentration-Dependent Manner

The cell was held at −100 mV and 12 ms depolarizing potentials at 10 mV steps applied at 0.5 Hz activated inward currents which were completely and reversibly

blocked by 1 mM TTX. Therefore, these inward currents were attributed to Na^+ currents (I_{Na}).

I_{Na} was measured at their peaks. In control conditions, the peak amplitudes of I_{Na} decreased by 6.33 ± 1.5% ($n = 8$). The peak amplitudes of I_{Na} increased 28.4 ± 3.75% and 44.4 ± 2.95% by La^{3+} at 1 and 10 μM, respectively. After cessation of the drug and washout, I_{Na} recovered by 87 and 89% (Fig. 1a). Upon the application of La^{3+} in the external solutions, the peak amplitudes of I_{Na} increased, and this action progressed with increment in concentrations of La^{3+} from 0.1 to 1,000 μM (including: 0.1, 1, 10, 100 and 1,000 μM, respectively). Concentration-response curve was obtained by plotting the incremental percent against the concentration of La^{3+} (Fig. 1b), and the curve was fitted with the Hill function: $y = 144.86 + 18.96x$ ($R = 0.9913$). The results indicated that La^{3+} reversibly enhanced I_{Na} in a concentration-dependent manner. The 50% enhancement concentration (EC_{50}) of La^{3+} on I_{Na} was 9.93 ± 0.85 μM.

The Effects of La^{3+} on Current–Voltage Relationships of I_{Na}

I_{Na} were evoked by a series of 10 mV voltage steps to potentials between −100 and 0 mV from a holding potential of −100 mV. In the experiments where 12 ms test pulses were applied, currents were measured at their peaks. As Fig. 2a, Current–voltage (I–V) curves of I_{Na} generated by plotting the current peak amplitudes as a function of the test potentials. In the presence of 10 μM La^{3+}, the amplitude of I_{Na} was significantly increased at the test potentials between −40 and 30 mV, relative to the control conditions, and the amplitudes of I_{Na} increased differently at different membrane potentials ($n = 5$).

As shown in Fig. 2a, the threshold for activation of I_{Na} was approximately −70 mV and the peak amplitude of I_{Na} was maximal at approximately −30 mV in the absence or presence of 10 μM La^{3+}. The relationship between the increment ratio on I_{Na} and the depolarizing potential was linear; and enhancement effects did not vary with changes of membrane potential from −40 to +30 mV (Fig. 2b), which indicated that the amplitudes of I_{Na} increased in a voltage-independent manner. The results imply that La^{3+} does not sense the electric field in the pore.

The Effects of La^{3+} on Activation of I_{Na}

The effects of La^{3+} on activation of I_{Na} were detected by conductance–voltage relationship. I_{Na} were evoked by a series of 10 mV voltage steps to potentials between −100 and 60 mV from a holding potential of −100 mV, by 12 ms test pulses. The activation curves for I_{Na} in the absence and presence of 10 μM La^{3+} were shown in Fig. 3. Conductance–voltage relationship curves were constructed by plotting G/G_{max} versus membrane potentials, and the curves were fitted by a Boltzmann equation:$G/G_{max} = 1/\{1 + \exp[(V - V_{1/2})/k]\}$, where G is conductance,

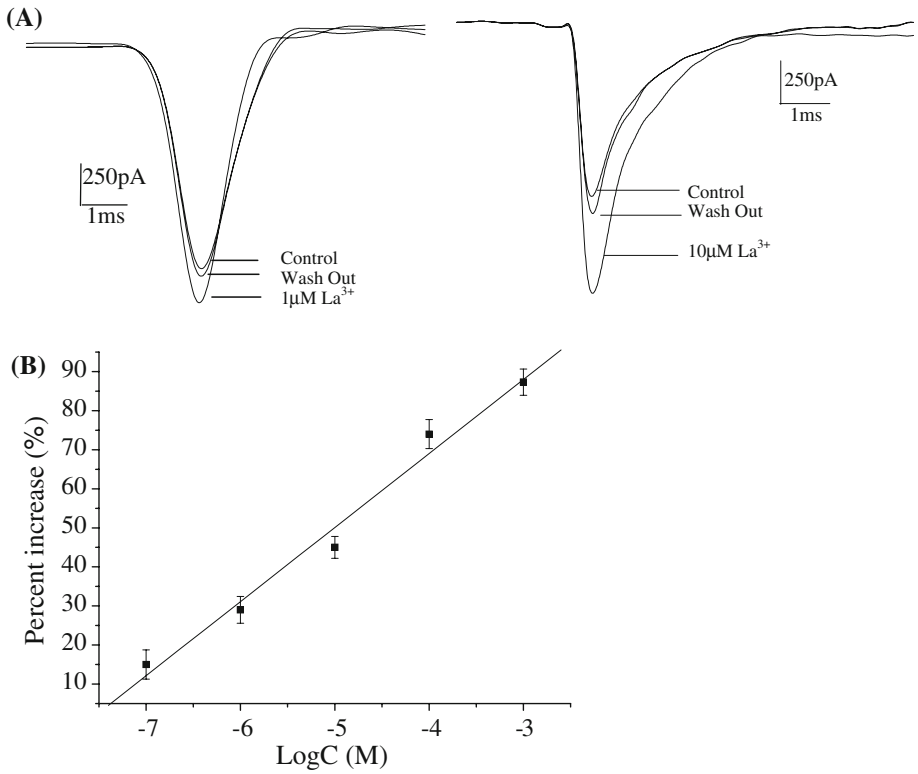

Fig. 1 The enhancement effects of La^{3+} on I_{Na}. **a** *Left*: Current trances of I_{Na} in control, 1 μM La^{3+} treated, and after wash. *Right*: Current trances of I_{Na} in control, 10 μM La^{3+} treated, and after wash. **b** Concentration-response curve of the enhancement effects of La^{3+} on I_{Na}. EC_{50} value is 9.93 μM, with R of 0.9913. Each point represents mean of five cells

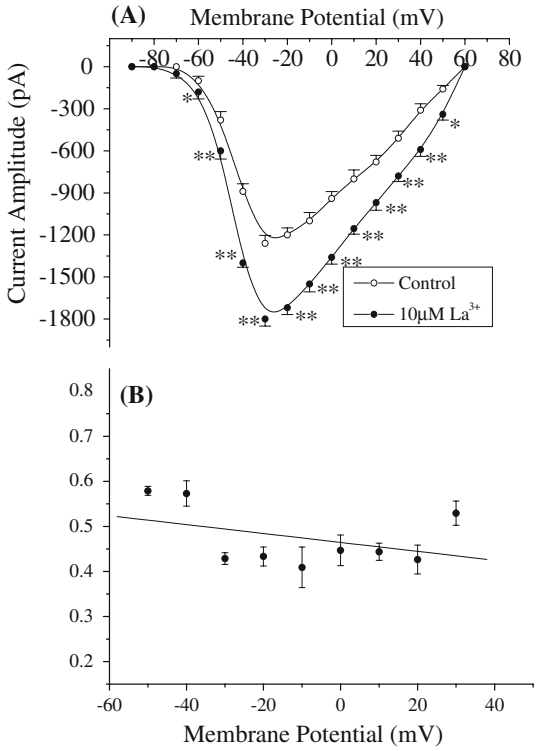

Fig. 2 Effect of 10 μM La^{3+} on Current–voltage curves of I_{Na}. $*P < 0.05$, $**P < 0.01$ vs. control. Each point represents mean of five cells. **a** Current–voltage (I–V) curves of I_{Na} in the absence (○) and presence (●) of 10 μM La^{3+}. **b** The increment ratio in I_{Na} caused by 10 μM La^{3+} application as a function of depolarizing potentials. The relationship between the increment ratio on I_{Na} and the depolarizing potential was linear; and incremental effects did not vary with changes of membrane potential from −40 to +30 mV, which indicated that the amplitudes of I_{Na} increased in a voltage-independent manner

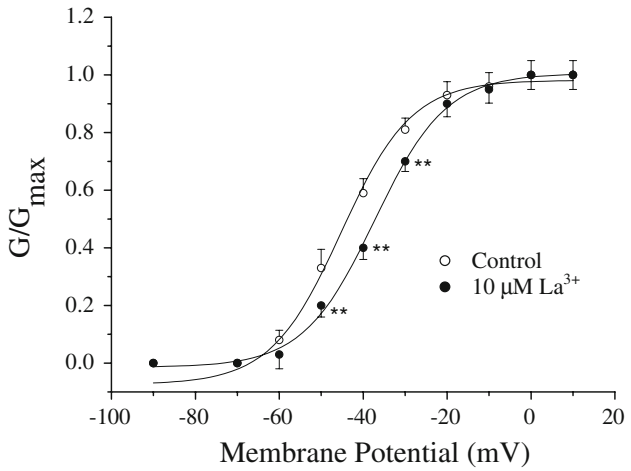

Fig. 3 Normalized steady-state activation *curves* of I_{Na} in the absence (○) and presence (●) of 10 μM La^{3+} were plotted as a function of membrane potential. The *curves* were fitted with Boltzmann function (see text). The value of each point is mean of five cells. $*P < 0.05$, $**P < 0.01$ vs. control

G_{max} is maximum conductance, V is membrane potential, $V_{1/2}$ is the potential for half-maximal activation, and k is the slope factor; G was calculated by using the equation:$G = I/(V - V_{Na})$, where I is current amplitude and V_{Na} is the reversal potential. In the absence and presence of 10 μM La^{3+}, the value of $V_{1/2}$ was (-45.25 ± 0.91) and (-37.26 ± 0.59) mV ($n = 5$, $P < 0.01$), with k of (-8.40 ± 0.89) and (-8.51 ± 0.54) mV ($n = 5$, $P > 0.05$). 10 μM La^{3+} shifted the steady state activation curve of I_{Na} towards positive potential without changing the slope factor.

The Effects of La^{3+} on Steady-State Inactivation of I_{Na}

Figure 4 shows the effects of La^{3+} on the voltage-dependence of I_{Na} inactivation using a double-pulse protocol. 500 ms hyperpolarizing pre-pulse between −120 and −10 mV from a holding potential of −100 mV were followed by a 12 ms test pulse to −30 mV with a 1 ms gap. The inactivation curves were obtained by plotting the normalized I_{Na} against the prepulse voltages. The plots were well fitted with a single Boltzmann function:$I/I_{max} = 1/\{1 + \exp[(V - V_{1/2})/k]\}$, where I/I_{max} is the normalized data, V is the prepulse potential, $V_{1/2}$ is the potential where normalized I was reduced to one-half and k is the slope factor. In the absence and presence of 10 μM La^{3+}, the value of $V_{1/2}$ was (-75.42 ± 1.11) mV and (-83.14 ± 0.87) mV ($n = 5$, $0.01 < P < 0.05$), with k of (-10.54 ± 0.42) mV and (-9.99 ± 0.76) mV ($n = 5$, $P > 0.05$). La^{3+} (10 μM) caused a negative shift of the inactivation curve of I_{Na} along the potential axis. At the same time, the slope factor k remained unchanged.

Discussion

Voltage-gated sodium channel is responsible for both initiation and propagation of action potentials of the neurons in the hippocampus and throughout the CNS. Therefore, modulation of the functional properties of voltage-gated sodium channels would be expected to alter the activity and function of CNS neurons. The voltage-gated sodium channel is a key molecular component responsible for both action potential generation and propagation and therefore plays an important role in regulating neuronal excitability in the CNS. The voltage dependence of the activation of sodium channels implies that the transition from a resting, closed conformation to an open conformation is accompanied by the outward translocation of several positive charges across the membrane [21].

In this study, the sodium currents we recorded in hippocampal CA1 neurons could be completely abolished by 0.5 μM TTX (data not shown), indicating the sodium

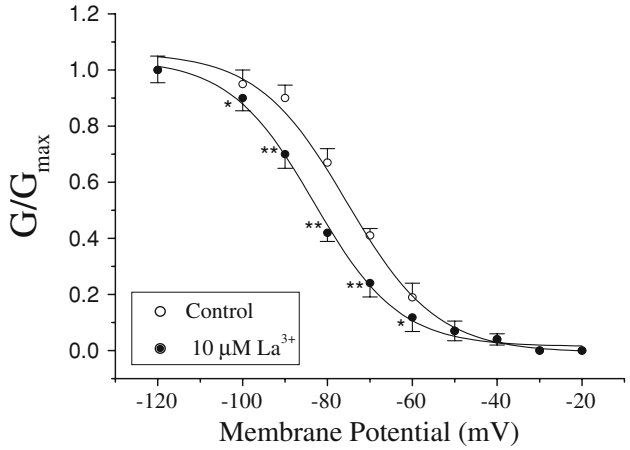

Fig. 4 Normalized steady-state inactivation *curves* of I_{Na} in the absence (○) and presence (●) of 10 μM La^{3+} were plotted as a function of membrane potential. The *curves* were fitted with Boltzmann function (see text). The value of each point is mean of five cells. $*P < 0.05$, $**P < 0.01$ vs. control

channels expressed in hippocampal CA1 neurons are TTX-sensitive sodium channels.

Previous studies have shown that La^{3+} attacks multiple targets extracellularly or intracellularly, including some kinds of voltage-gated calcium channels [14] and potassium channels [18]. In our observation, La^{3+} may modulate voltage-gated sodium channel in rat hippocampal CA1 neurons. La^{3+} enhanced I_{Na} in a concentration- and voltage-dependent manner (EC_{50} was 9.93 μM). After cessation of the drug and washout, I_{Na} completely recovered, indicating possible binding site on the extracellular side of the channel.

La^{3+} shifted the activation curve to positive potentials ($V_{1/2}$ from −45.24 to −37.26 mV), which would increase the threshold voltage for the action potential generation and reduce the excitability of neurons. La^{3+} also caused a negative shift of the inactivation curve ($V_{1/2}$ from −75.42 to −83.14 mV), which means a lower membrane potential threshold for closing these channels and would reduce the excitability of neurons.

In addition, increasing I_{Na} may cause intracellular Na overload, which reversed the action of the Na^+, K^+-ATPase and stimulated ATP turnover, facilitating energy expenditure. Na loading also reversed Na^+/Ca^{2+} exchange, resulting in an increase of intracellular Ca^{2+}. Activity of Ca^{2+}-ATPase was decreased because of energy deprivation and Ca^{2+} efflux was reduced, which caused Ca^{2+} overload, triggering a cascade of harmful events via activation of protease, phospholipases and endonucleases, even cell death [22].

La^{3+} is a very potent modulator of gating activity, and has been called a "supercalcium" [23]. The mechanism by which La^{3+} affected sodium channel currents is not fully understood at this time. The effects by trivalent cations on the gating properties of voltage-dependent ion channels are usually explained in terms of this mechanism [24]. La^{3+} affecting Na^+ channels might involve that La^{3+} could rapidly enter Na^+ channels with high selectivity in the course of traversing the membrane by replacing other bivalent cations like Ca^{2+}, combine with the amino acid residues in the transmembrane protein, and close the voltage sensor. These probable results all might contribute to the change of Na^+ channel properties by La^{3+} [25, 26].

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