**Effect of La\(^{3+}\) on myocardial potassium channels revealed by patch-clamp technique**

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**Abstract** The effect of La\(^{3+}\) on potassium channels in rat ventricular myocytes was investigated using the whole-cell patch-clamp recording mode. The Ca\(^{2+}\)-independent voltage-activated outward K\(^+\) current was activated by the depolarizing pulse in enzymatically isolated rat ventricular myocytes. After addition of different concentrations La\(^{3+}\) to the bath solution, the outward K\(^+\) current was depressed gradually. The inhibition effect was in a concentration-dependent manner. The phenomena of the outward K\(^+\) current, being the main repolarizing current suppressed by La\(^{3+}\), suggest that the effect of lanthanides on myocardial function should be exploited further.

Keywords: whole-cell patch-clamp recording, ventricular myocytes, potassium channel, La\(^{3+}\).

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Lanthanides have been widely used and have effects in the field of agriculture, livestock husbandry and medicine. Meanwhile, concerns about the negative effects of lanthanides are also rising. For the biological effects and the action mechanisms of lanthanides on animals and plants still remain puzzled, it is urgent to evaluate the safety of lanthanides in agriculture and therapeutical application. In recent years, the researches go deep into at the cell and molecular levels. Cell membranes are one of the main targets after the lanthanides enter the living body, so it is useful to illustrate the biological effects of lanthanides on the cell membranes. Many researches have been reported in recent years, and the results are controversial. Yang Pin and his group studied the effects of lanthanum ion on L-type calcium channels and sodium-calcium exchangers in rat ventricular myocytes and the results indicated that lanthanum ion could across the cell membrane through sodium-calcium exchangers but not through L-type calcium channels.

The lanthanides content in human body has close relations to diseases, and patients with myocardial infarction have more lanthanides content than healthy people. Cardiovascular diseases are seriously hazard to people’s health, and many heart diseases have close relations to abnormal activities of myocytes ion channels that changed the balance of cardiac electrophysiology and induced cardiac arrhythmia. Functional changes of ion channels are the basis of the changes of cardiac myocyte electrophysiology. It was confirmed that potassium current and calcium current decreased in myocytes of patients with cardiac diseases such as ischemia, congestive failure, and dilated and hypertrophic cardiomyopathy. Therefore, the mechanism of lanthanides on myocytes was meaningful to study. In this report, we studied the effect of La\(^{3+}\) on outward potassium current in rat ventricular myocytes using whole-cell patch-clamp technique to illustrate effect of La\(^{3+}\) on cardiac function at channel level.

1 Materials and methods

1.1 Preparation of single ventricular myocyte

Single ventricular myocyte was isolated from Wistar rats (250—300 g, male or female, from the Animal House of Institute of Radiation Prevention, Chinese Academy of Sciences) as previously described. In brief, hearts were quickly moved and mounted on a Langendorff apparatus for retrograde perfusion at 37\(^{\circ}\)C, first with free-Ca\(^{2+}\)-Tyrode’s solution for 5 min, then with low-Ca\(^{2+}\) solution containing 0.03% collagenase (1), 0.25% Taurine and 50 μmol/L Ca\(^{2+}\). Afterwards, the hearts were incubated in KB solution (100% O\(_{2}\) saturated) and minced and dispersed with a pipette. The suspension was filtered through 190 μm nylon mesh, and kept at RT for 4 h before use.

1.2 Patch-clamp recording

The whole-cell patch-clamp recording mode was used by Hamill’s patch-clamp methods. Patch-clamp pipettes (1—2 μm) were prepared from glass capillaries (BJ-40, diameter 1.5±0.1 mm, Beijing), and pulled on a multi-stage programmable puller (NATISHZGE, Japan). Before the experiment, they were filled with pipettes solution (pipette resistance 2-5 MΩ), then mounted on the holder which connected Ag-AgCl reference electrode, and was then screwed. The cell suspensions were put in a chamber mounted on the stage of an inverted microscope. After being settled to the bottom of the chamber for 15 min, cells were superfused with bath solution at a rate of 2 mL/min for 30 min to wash the cell surfaces. After 30 min, only the cells with rod-shape and clear striations were used in experiments. Giga-Ω seals between electrode and the cell membrane (above 2 GΩ) were obtained by gentle suction. After membrane was broken, the whole-cell recording mode was formed. Currents were amplified by using an amplifier (Digidata 1200), and pClamp 6.0.4 software was used to produce protocols, acquire and process data. Data were analyzed and figures were plotted with Clampfit and software MICROCAL-ORIGIN (5.0).

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

Free-Ca\(^{2+}\) Tyrode’s solution: NaCl 135, MgCl\(_{2}\) 1, KCl 5.4, NaH\(_{2}\)PO\(_{4}\) 0.33, HEPES 10, Glucose 10, pH adjusted
to 7.3 with NaOH. Tyrode’s solution: added 1 mmol·L⁻¹ CaCl₂ into Free-Ca²⁺ Tyrode’s solution. KB solution: KCl 70, KH₂PO₄ 20, MgCl₂ 5, Glucose 10, HEPES 10, EGTA 0.5, Taurine 20, L-Glutamic acid 50, pH adjusted to 7.3 with KOH. Bath solution: NaCl 135, KCl 5.4, MgCl₂ 1, Na₂HPO₄ 0.33, HEPES 10, Glucose 10, CdCl₂ 0.1, pH = 7.4. Pipettes solution: KCl 130, MgCl₂ 1, EGTA 10, HEPES 10, Glucose 5, MgATP 3, pH = 7.2. Collagenase I, HEPES, EGTA, Taurine, L-Glutamic acid were purchased from Sigma Company.

2 Results and discussion
2.1 Recording of outward K⁺ current

Fig. 1 shows a series of outward current in rat ventricular myocytes obtained by whole-cell patch-clamp mode. Holding potential was clamped to −40 mV (sodium current inactivated at the potential), and test potential delivered in 10 mV increments up to 60 mV. The outward current was not affected when calcium current was blocked by Cd²⁺. The current vanished after replacing K⁺ with equimolar Cs⁺ in the pipette solution. The membrane potential is also called reverse potential when ion channels tail current is at zero. Under the imposed ionic conditions, reverse potential was close to the theoretical equilibrium potential for the permeable ions. Fig. 2(a) shows tail current of the outward current, and Fig. 2(b) shows current-voltage relationship of tail current. According to Fig. 2(b), the reverse potential of −69 mV was near to K⁺ equilibrium potential (there exists some 10 mV error for the liquid potential not calibrated[2]). The above indicates that the current was Ca²⁺-independent voltage-activated outward K⁺ current[9].

2.2 Effect of La³⁺ on K⁺ current

After establishing the pattern of K⁺ current in rat ventricular myocytes, we introduced the La³⁺ in the system and examined the influence of La³⁺ on the pattern of K⁺ current subsequently. Upon addition of La³⁺ at different concentrations to bath solution, the effect of La³⁺ on K⁺ current was recorded. As shown in Fig. 3, when the holding potential was −40 mV and the membrane potential was 60 mV, the K⁺ current was recorded at varying La³⁺ concentrations of 0 (control), 10 μmol·L⁻¹, 100 μmol·L⁻¹, 1 mmol·L⁻¹. The maximum amplitude of the control current activated at 60 mV is 2498 pA, while it diminished to 1419, 1117, 817 pA with the bath solution containing La³⁺ of 10 μmol·L⁻¹, 100 μmol·L⁻¹, 1 mmol·L⁻¹ respectively. At each concentration, the data were taken based on the average values in 5 separate cells at least. According to Fig. 3, the application of La³⁺ to the bath solution blocked the K⁺ channel in a concentration-dependent manner. At the same voltage protocol in Fig. 1, whole-cell K⁺
channels. So many studies favored the binding sites mainly for the adverse effects of metal ion act on ion cells. But there are defects in the surface charge theory, to screen membrane surface negative charge in cardiac sites theory. Agus et al. [11] strongly favored that there are divalent-specific binding sites from the finding that the wide divergence in the potency of divalents effects outward K⁺ current in rat ventricular myocytes. By contrast, Sanguinetti et al. [12] showed that La³⁺ blocked a specific component of delay rectifier outward K⁺ current in guinea pig ventricular myocytes and favored that La³⁺ mainly acts to screen membrane surface negative charge in cardiac cells. But there are defects in the surface charge theory, mainly for the adverse effects of metal ion act on ion channels. So many studies favored the binding sites theory [13,14]. Whereas our data do not allow us to distinguish between the two theories, and we found in this experiment that K⁺ current in rat ventricular myocytes was depressed gradually with the increased concentrations of La³⁺ in the bath solution, indicating that La³⁺ modulated transport of K⁺ through outward K⁺ channels. Further investigation is required to elucidate the action mechanism between metal ion and channels.

The outward K⁺ current is the main repolarizing current in cardiac myocytes, which plays an important role in maintaining the action potential and restoring the membrane potential to its resting level of electronegativity [15]. So the cardiac action potential could be affected by the inhibition of outward K⁺ current with La³⁺, which suggested the relations between lanthanides and cardiomyopathy should be given attention.

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References


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