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Characterization of outward potassium current in embryonic chick heart cells[1]

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KEY WORDS chick embryo; myocardium; cultured cells; potassium channels; patch-clamp techniques; phosphorylation

ABSTRACT

AIM: To characterize a voltage-dependent outward K^+ current in cultured heart cells of 14 – 16-day-old embryos of yellow chick. **METHODS**: The patch-clamp technique in the whole-cell configuration was used. **RESULTS**: The kinetics and the pharmacology of the outward K^+ current in our cell mold were different from those described in white chick. Like the calcium-activated K^+ current, blocker of calcium channel, $CdCl_2$, eliminated the current of more than 95 %. Isoproterenol provoked an increase of peak amplitude (137 % ± 47 %, n = 16 cells) and acceleration of activation kinetics in the outward K^+ current (the time reaching a peak current reduced from 36 ms ± 10 ms to 16 ms ± 9 ms). This effect of isoproterenol was mimiced by cAMP. In addition, a frequency-dependent decrease in peak amplitude of the current occurred after cAMP-induced phosphorylation. **CONCLUSION**: There are species- and/or cell-type-specific difference in the K^+ channels properties. In embryonic yellow chick heart cells, the phospholation of channel could not only modulate the activation kinetic properties of the calcium-activated potassium channel, but also change their recovery kinetics.

INTRODUCTION

The embryonic chick heart cell is a good model for studying varieties of ionic channels associated the cardiac functions. Several types of ionic channels (such as a fast Na^+ inward current, L-type inward Ca^{2+} current, and a stress-sensitive Cl^- current) have been reported in detail on the embryonic heart cells of white Leghorn chick[1,2,3]. However, a few results about K^+ current in embryonic chick heart cell have been described, especially in yellow chick. In white Leghorn chick, single heart cells showed two types of K^+ current, an early outward current and a delayed outward rectifier K^+ current[4]. As we know, the gating kinetics and modulation of this K^+ current have not been described. In fact, K^+ conductance represents an important factor in control of Ca^{2+} influx, cell excitability and action potential firing in cardiovascular system and various types of cells[5,6]. In the present study, we examined an outward K^+ current in embryonic yellow chick heart cells.

MATERIALS AND METHODS

Culture of embryonic chick heart cells

Fertilized eggs (yellow Red Rob) were obtained from Institute of Animal and Veterinary Science, Shanghai Academy of Agricultured Science. Primary cultures of embryonic chick heart cell were performed as described previously[1]. 14- or 16-d-old chick embryos were extirpated from the egg. The ventricle was dispersed in sterile D-Hanks' solution containing 0.20 % – 0.25 % trypsin. The dispersed cells were pooled and centrifuged in the presence of 10 % fetal bovine serum at 100 × g for 5 min. The cells were resuspended in M-199 culture medium and centrifuged again to wash out the trypsin. The cells were then placed in an 100-mm plastic culture dish for 2 h to allow the fibroblasts to adhere on the plate. The heart cells that remained in suspesion were plated in 35-mm plastic culture dishes at a density of (2 – 5) × 10^5 cells · L^{-1}. The cultured

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cells were kept in a CO_2 incubator at 37 ℃, and used within 3 d after plating.

Current recordings and analysis Current recording was performed on cultured 2 – 3 d-old embryonic chick heart cells using the patch-clamp technique in the whole-cell configuration. For recording K^+ current, the culture medium was replaced with a bathing solution in (mol·L^{-1}): NaCl 145, KCl 2.5, $CaCl_2$ 1.0, HEPES 10, TTX 0.001, glucose 5.0 (pH was adjusted to 7.4 using NaOH). Soft glass patch pipettes were prepared by pulling capillary tubes in two steps with a vertical puller. The solution used to fill patch pipettes had the following composition (mol·L^{-1}): KCl 145, $MgCl_2$ 2.0, HEPES 10 (pH was adjusted to 7.4 using KOH). The resistance of the electrodes filled with this solution was 4 – 5 MΩ.

All current signals were recorded with an EPC-7 patch-clamp amplifier (List Electronic, Germany) operated in the voltage-clamp mode. Step voltage commands, data acquisition and analysis were performed with pClamp 6.01 software (Axon Instruments, USA). The currents were corrected on-line for leak and residual capacitance transients by a P/4 protocol.

Statistics Data were expressed as $\bar{x} \pm s$ and compared with t-test.

RESULTS

A voltage-dependent K^+ current in embryonic chick heart cells Voltage-dependent outward K^+ current evoked by a train of depolarizing pulse. The cell was held at – 80 mV and depolarized by 15 mV steps from – 60 to 45 mV at 10-s intervals. The K^+ current began activating about – 45 mV of depolarized pulse and their amplitude was increased with the depolarized voltage between – 60 and 45 mV. In most of the cells tested, this current slowly reached a peak amplitude (36 ms ± 10 ms, n = 20 cells) and showed a very slightly inactivation with time at depolarizing pulse above 15 – 30 mV, thus having a separation between the peak current and the late current (Fig 1). However, in other cells, the current did not reach its peak amplitude and had no inactivation during the voltage step period of 200 ms using the same voltage protocol (n = 3 cells). In general, this current resembled the late, slowly decaying outward current I_k which had previously been characterized in mammalian heart cells and other type cells [7].

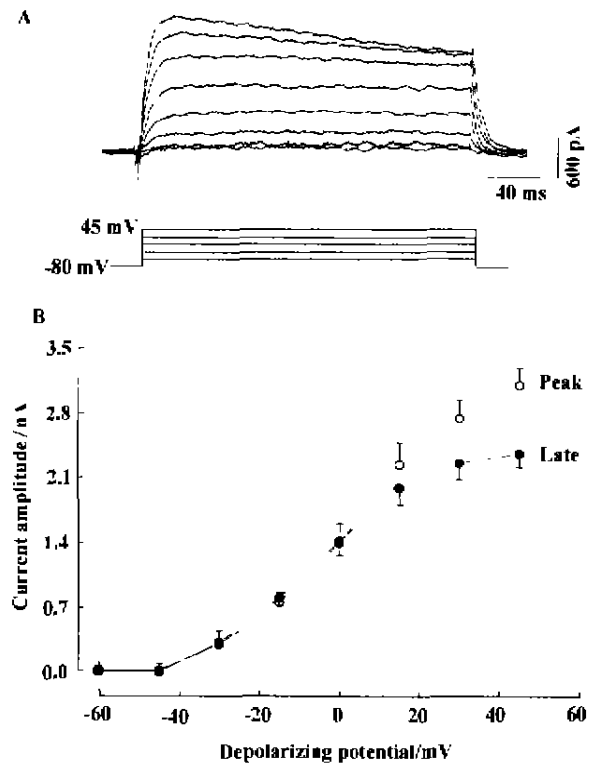

Fig 1. Voltage-dependent outward K^+ current in an embryonic chick heart cell. A) Current traces evoked by a series of 200 ms voltage steps from – 60 mV to 45 mV in 15 mV increment. B) *I-V* relationship.

Pharmacological profile of outward K^+ in embryonic chick heart cells To characterize the components of the outward K^+ current, different blockers of K^+ channels were tested. The outward K^+ current was evoked from a holding potential of – 80 mV by a 200 ms depolarizing step to 30 mV. The cells were first incubated with tetrathylammonium (TEA) which is as a relatively specific blocker of the delayed outward K^+ current[8]. Addition of TEA 20 mol·L^{-1} in the solution, the outward K^+ current amplitude did not significantly differ from the control currents (Fig 2, upper tracings). The mean peak amplitude was (543 ± 118) pA and (518 ± 164) pA in the presence and absence of TEA, respectively (P > 0.05, n = 8 cells). Conversely, 4-aminopyridine (4-AP) which is a relatively specific blocker of the transient outward potassium current[9], resulted in a

marked inhibitory effect in the outward K^+ current. Addition of 4-AP 4 mol·L^{-1} to the solution completely abolished the K^+ current within 2 – 3 min (Fig 2, middle tracings). The inhibitory effect induced by 4-AP in the outward K^+ current was 86 % – 96 % (91.5 % ± 6.2 %, n = 5 cells). Besides, a blocker of calcium channels $CdCl_2$ also attenuated the outward K^+ current (Fig 2, Lower tracings). In six cells tested, the mean inhibition on the K^+ current by $CdCl_2$ was > 95 %.

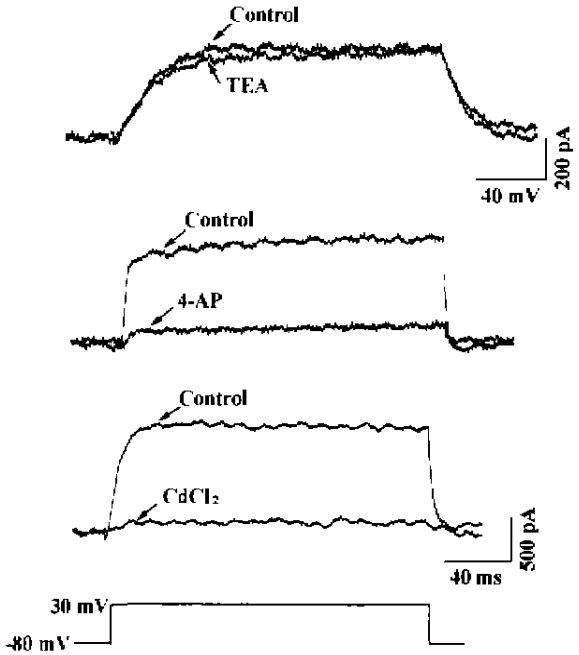

Fig 2. Effect of 4-AP, TEA, and $CdCl_2$ on outward K^+ current in embryonic chick heart cells.

Modulation of outward K^+ channels by cAMP-dependent phosphorylation After applying the β-adrenergic receptor agent, isoproterenol (Iso), 1 μmol · L^{-1} in bath solution, the peak amplitude of outward K^+ current markedly augmented within 1 – 2 min. The augmentation in peak amplitude caused by Iso was 75 % – 150 % (137 % ± 47 %, n = 16 cells). In addition, activation kinetics of the K^+ current was also modified. The time values reaching a maximal amplitude reduced from (36 ± 10) ms of control to (16 ± 9) ms ($P < 0.05$, n = 16 cells). Typically, the current, after applying Iso, showed an inactivation during a depolarized voltage step period of 200 ms (Fig 3, upper tracings). The decay phase of the current was best fitted by a single exponential, of which time constant was (26 ± 3) ms. This current resembled a transient outward current I_{to} which had been characterized in mammalian cardiac cells. Similar results were obtained when a saturating concentration of the membrane-permeable cAMP analogue (dibutyryl cAMP 1 mol·L^{-1}, n = 6 cells) was included in the solution (Fig 3, lower tracings). This result suggested that the phosphorylation of channels could be involved.

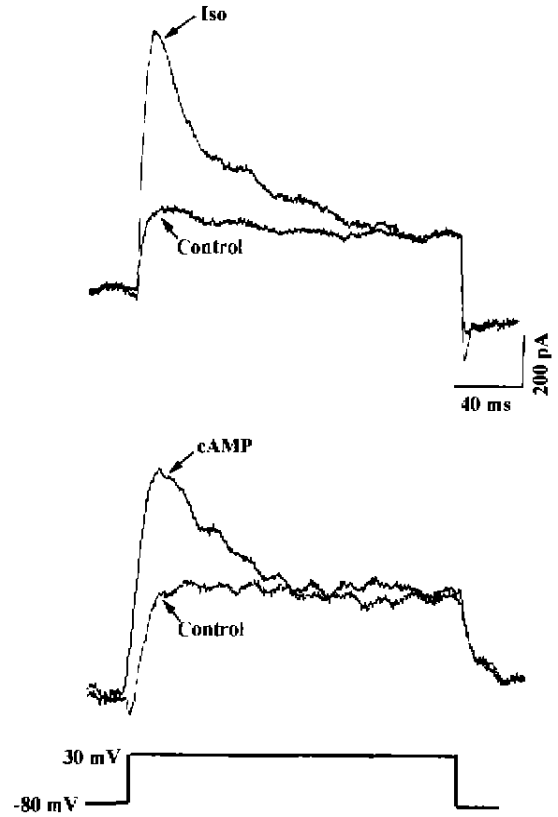

Fig 3. Modulation of K^+ current in embryonic chick heart cells by isoproterenol and cAMP.

A frequency-dependent decrease of peak K^+ current followed the K^+ channel phosphrylated by Iso or cAMP. When heart cells were repetitively depolarized using a train of depolarizing pulse at the same interval of 10 s, after a rest period of 30 s at a holding potential of – 80 mV, a slight decrease in the K^+ peak amplitude was observed. A steady state attained at the second depolarized pulse. The effect of frequency-dependent decrease in peak amplitude of K^+ current occurred in 21 out of 22 cells tested. If the

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time of interval was prolonged to 20 s, the decay of outward K^+ current disappeared. In some cells, 30 s of interval was needed for eliminating the frequency-dependent decrease in peak amplitude of the K^+ current (Fig 4).

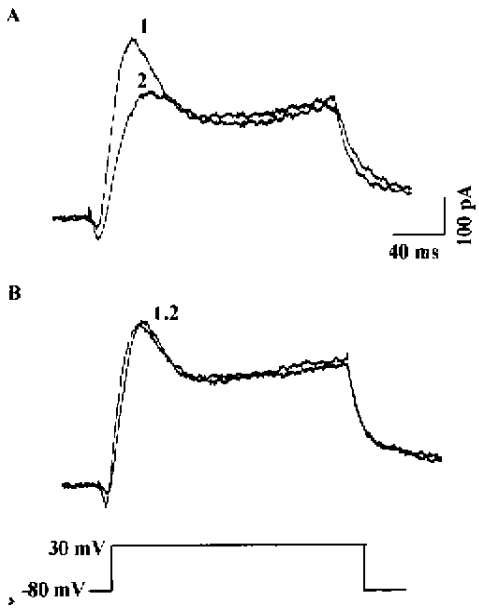

Fig 4. Frequency-dependent decrease of peak amplitude after modifying the K^+ channels by cAMP and Iso. Superimposed tracings were outward K^+ currents evoked at the 1st and 2nd depolarizing pulse The time intervals between 1st and 2nd depolarizing pulse was 10 s (A) or 20 s (B).

DISCUSSION

Our results obtained by the whole-cell recording techniques demonstrate that there is an outward K^+ current evoked by depolarized pulse in embryonic yellow chick heart cells, but their characterizations are different from those outward K^+ current described in embryonic white chick heart cell[4]. In white Leghorn chick, the early outward K^+ current evoked by a step voltage from -80 mV to -45 mV could be blocked completely by bretylium tosylate. The delayed outward rectifer K^+ current evoked by a step voltage from -80 mV to 4 mV was sensitive to TEA. In contrast, in our cell model, the activation kinetics of outward K^+ current evoked by depolarizing pulse below $+20$ mV was similar to those described for the delayed outward rectifer K^+ current, I_{to}-like outward K^+ current only appeared at the more depolarized potential. However, this current exhibited the same pharmacological profile as the I_{to} current previously characterized in mammalian cardiac cells as well as some neuron[10]. Therefore, the outward K^+ current discovered in embryonic yellow chick heart cells might be neither I_k current nor I_{to} current. Another important property which must be mentioned was that Ca^{2+} was necessary for activating the outward K^+ current since it was abolished when $CdCl_2$ was added to the external medium. The results indicated that gating kinetics of the outward K^+ channels required the binding of Ca^{2+} ions to open the channels. Moreover, the evidence has been put forward recently that the sensitivity to TEA or 4-AP varies in the Ca^{2+}-dependent K^+ current from different types of cells[11]. It thus appears that this outward K^+ current found in embryonic yellow chick heart cells would be a Ca^{2+}-activated K^+ current although this K^+ current has not yet been reported in chick heart cells.

Some investigations have recently shown that, in vascular smooth muscle cells, cAMP-dependent protein kinase (PKA) could induce the phospholation of the Ca^{2+}-activated K^+ channels and increase the activity of this channels[12]. There was also an evidence that the stimulation of β-adrenergic receptor resulted in the opening of Ca^{2+}-activated K^+ channels in airway smooth muscle by the phosphorylation of the channels[13]. Our experiments demonstrated that in embryonic yellow chick heart cells, isoproterenol provoked an increase of peak amplitude and acceleration of activation kinetics in outward K^+ current. Moreover, this effect can be mimicked by cAMP, suggesting that the outward K^+ channels could be modulated by the phosphorylation. This finding was in good agreement with the report in smooth muscle cells, and it thus supported that the outward K^+ current presently characterized in yellow chick heart cells corresponded to a Ca^{2+}-activated K^+ current.

The present results have also shown for the first time that a frequency-dependent decrease in peak amplitude of Ca^{2+}-activated K^+ current is mediated by a phosphorylation of the channel. The frequency-dependent facilitation of calcium current in rat heart cells had been reported in which Ca^{2+} influx and phosphorylation of the Ca^{2+} channel were involved[14]. However, in our experiment, no modulation in calcium

channels could be found after using Iso and cAMP. This frequency-dependent decrease of outward K^+ current accounted for that the recovery time of K^+ channel from inactivation was prolonged after it was phosphorylated. It is possible that, in yellow embryonic chick heart cells, the phospholation of channel can not only modulate the activation kinetic properties of the Ca^{2+}-activated K^+, but also change their recovery kinetic properties.

In conclusion, the present study showed that embryonic yellow chick heart cells exhibited a calcium-activated potassium current which is sensitive to 4-AP. This K^+ channel could be activated and showed a frequency-dependent decrease in peak amplitude of the current mediated by cAMP-induced phosphorylation. The specific properties described in our experiment also revealed that there were species- and/or cell-type-specific differences in the K^+ channels properties.

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鸡胚心肌细胞膜上外向钾电流的特征

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心肌细胞膜

关键词 鸡胚；心肌；培养的细胞；钾通道；膜片箝技术；磷酸化

钙拮抗剂

目的：研究原代培养黄羽鸡胚心肌细胞上存在的外向钾电流特性. **方法**：膜片箝技术的"全细胞"记录. **结果**：此细胞模型上的钾电流动力学和药理学特征均不同于白羽鸡胚心肌细胞上的报道. 钙通道阻断剂镉离子可消除此电流，异丙肾上腺素和 cAMP 通过磷酸化增加电流的峰值和缩短达到最大峰电流的时间. 通道被磷酸化修饰后出现频率依赖性负性效应. **结论**：黄羽鸡胚心肌细胞上存在着钙激活的钾通道，磷酸化修饰不仅改变其开放速率还延长通道失活后再复活的时间. 此结果也说明，同一种动物其细胞膜上离子通道的特性可因不同种系而各异.

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