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Characteristics of transient outward K^+ current in human atrial cardiomyocytes[1]

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KEY WORDS potassium channels; patch-clamp techniques; heart atrium; myocardium; 4-aminopyridine

AIM: To study the properties of transient outward potassium current (I_{to}) in human atrial myocytes. **METHODS:** The patch-clamp whole-cell recording techniques were used. When the calcium inward current was blocked by $CdCl_2$ (0.1 mmol·L^{-1}), the transient outward potassium current (I_{to}) was recorded by depolarizing cells. **RESULTS:** The current was voltage dependent, it was activated quickly and inactivated rapidly too. 4-Aminopyridine (4-AP) 10 mmol·L^{-1} (a selective blocker of I_{to}) blocked the current completely. The IC_{50} (95 % confidence limits) of 4-AP on I_{to} were 0.67 (0.58 - 0.80) mmol·L^{-1}, 4-AP 1 mmol·L^{-1} shifted the activation curve of I_{to} to positive potential, therefore a higher membrane potential was required to activate I_{to}. **CONCLUSION:** I_{to}, a major K^+ channel current in human atrial cells, is blocked by 4-AP.

The transient outward potassium current (I_{to}) was the major outward repolarizing current in the action potential of several mammalian hearts including human atrium[1,2], rat[3], dog[4], and rabbit[5], ventricles. Some anti-arrhythmic drugs have been known to influence I_{to} in animals[6]. 4-Aminopyridine (4-AP), is a classic K^+ channel blocker and shows a relative selectivity to I_{to}. It was used in our study. The present work was to investigate the characterizations of I_{to} in human atrial myocytes.

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MATERIALS AND METHODS

Human cardiac specimens Specimens of human right atrial appendage were obtained from hearts on 11 patients [6 M, 5 F, age (35 ± 9) a] undergoing cardiopulmonary bypass. All patients did not take drugs which blocked ionic channels in 2 wk before operation. Nine patients had valvular heart disease and two had congestive heart disease. Specimens were placed in blood cardioplegic solution (4 ℃), blood crystalline solution = 41. The crystalline solution contained: KCl 20, NaCl 130, $CaCl_2$, sodium lactate, 28 (mmol·L^{-1}), pH 6.08. The processing was completed within 1 h.

Cell isolation Single human atrial cells were enzymatically isolated[7]. Specimens were minced and washed in Ca^{2+}-free solution: NaCl 100, KCl 10, $MgSO_4$ 5, taurine 50, 3-(*N*-Morpholino)propanesulfonic acid (Mops) 5, and glucose 20 mmol·L^{-1}, pH 7.0. The tissue was incubated in oxygenated Ca^{2+}-free solution containing collagenase 1 g·L^{-1} (Sigma, type I), protease 0.5 g·L^{-1} (Sigma, type XXIV), and bovine albumin 0.5 g·L^{-1} at 37 ℃ with a magnetic stirring bar for 45 min. The chunks were incubated again with collagenase 1 g·L^{-1} (Sigma, type Ⅰ) and $CaCl_2$ 0.15 mmol·L^{-1}.

When the yield of isolated cells appeared to be maximal, the chunks were spun at (12 - 15) × *g* for 2 min and then stored at 20 - 25 ℃ in Ca^{2+}-free solution containing 0.1 % bovine albumin. The isolation procedure produced an initial yield of 40 % - 50 % rod-shaped, calcium-tolerant cells. Myocytes could be used for 6 - 8 h.

Solutions Tyrode's solution: NaCl 150, KCl 5.4, $CaCl_2$ 1.2, $MgCl_2$ 2, HEPES 5, and glucose 15 mmol·L^{-1}, adjusted to pH 7.3 with NaOH. Pipette solution: KCl 140, $MgCl_2$ 0.5, egtazic acid 10, and HEPES 10 mmol·L^{-1}, adjusted to pH 7.2 with KOH. For I_{to}

measurements, Ca^{2+} currents was suppressed by $CdCl_2$ 0.1 mmol·L^{-1} in Tyrode's solution. All experiments were carried out at 20 – 25 ℃.

Current recording Ionic currents were recorded using the whole cell recording technique[8] with an amplifier (EPC-7, List Germany). The myocytes were perfused with Tyrode's solution. The resistance of the microelectrodes was 2 – 4 MΩ. Recordings were filtered at 3 kHz and the series resistance was partially compensated about 60 % – 80 %. Test pulses were generated by pClamp 5.5.1 software. Data acquisition and analysis were made on the same software supported by TL-1 (A/D & D/A converter, Axon Co, USA).

Statistical analysis Data were presented as $\bar{x} \pm s$, and compared with t-test.

RESULTS

I_{to} To record I_{to}, the cells were depolarized from the holding potential of – 80 mV to – 40 mV for 30 ms to inactivate I_{Na}, and then depolarized to test potential of 60 mV for 400 ms every 5 s. We used peak outward current as the I_{to} amplitude in our data analysis. Current-voltage relationship of I_{to} was generated by applying 11 test depolarizing pulses from – 20 mV to 80 mV for 400 ms with a 10 mV increment. The outward currents reached the maximum quickly and then inactivated rapidly too, showing property of the current. Activation of the current was voltage-dependent (Fig 1). The current was inactivated faster at higher test potential. The time constant (τ value) of the inactivation of I_{to} was (161 ± 49) ms. While at 80 mV the τ value was (111 ± 19) ms, much shorter ($P < 0.05$). This meant that the current's decay was voltage dependent too. All above showed that the currents were transient outward potassium currents (I_{to}).

Kinetic properties of I_{to} The activation curve of I_{to} was generated by applying 11 depolarizing pulses from a holding potential of – 80 mV to – 40 mV for 30 ms, and from – 20 mV to 80 mV for 400 ms with a 10 mV increment. The currents were transferred to conductance (G) and fitted to Boltzmann function to produce activation curve. The Boltzmann equation was described below:

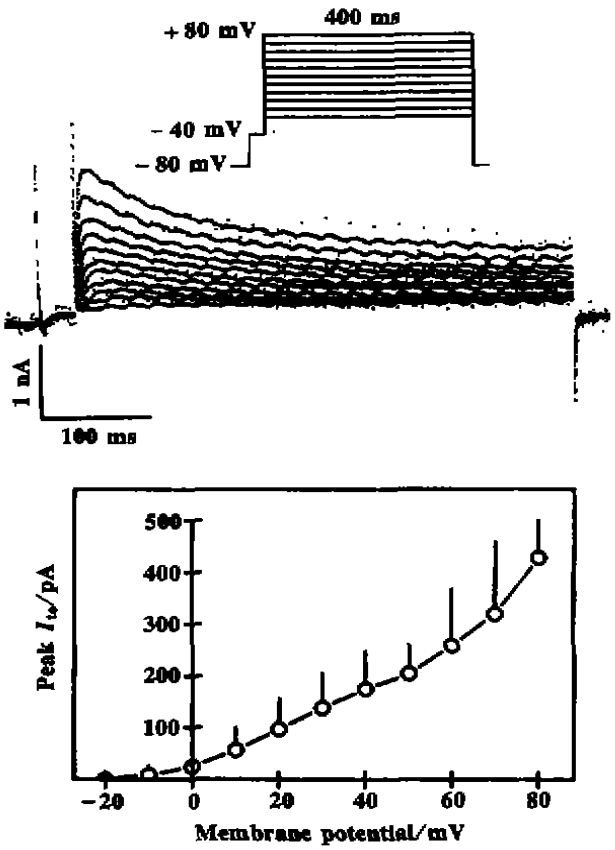

Fig 1. The oringinal recording of the I_{to} and the current-voltage relationship of I_{to} ($n = 11$ cells).

$$G = 1/\{1 + EXP[-(V - V_{1/2})/k]\}$$

The membrane potential for half maximal activating ($V_{1/2}$) was (25 ± 5) mV, the value of k (slope factor of the curve) was 13 ± 2 (Fig 2A).

The steady-state inactivation of I_{to} was evaluated by using a two-pulse protocol. From a holding potential of – 80 mV, conditioning clamp steps of 2 s were applied between – 80 mV and – 10 mV. After a 15 ms step to – 40 mV to inactivate I_{Na}, the cells were depolarized to + 80 mV for 400 ms to activate the I_{to}. Then the I_{to} was normalized by comparing with maximal activation of the current. Inactivation curve was obtained by fitting the curve with the equation described below:

$$I = 1/\{1 + EXP[(V - V_{1/2})/k]\}$$

The inactivation curve was shown in Fig 2B. Potential for half inactivating of I_{to} ($V_{1/2}$) of the inactive curve was (– 41 ± 9) mV, the value of k (slope factor of the curve) was 6.6 ± 2.5

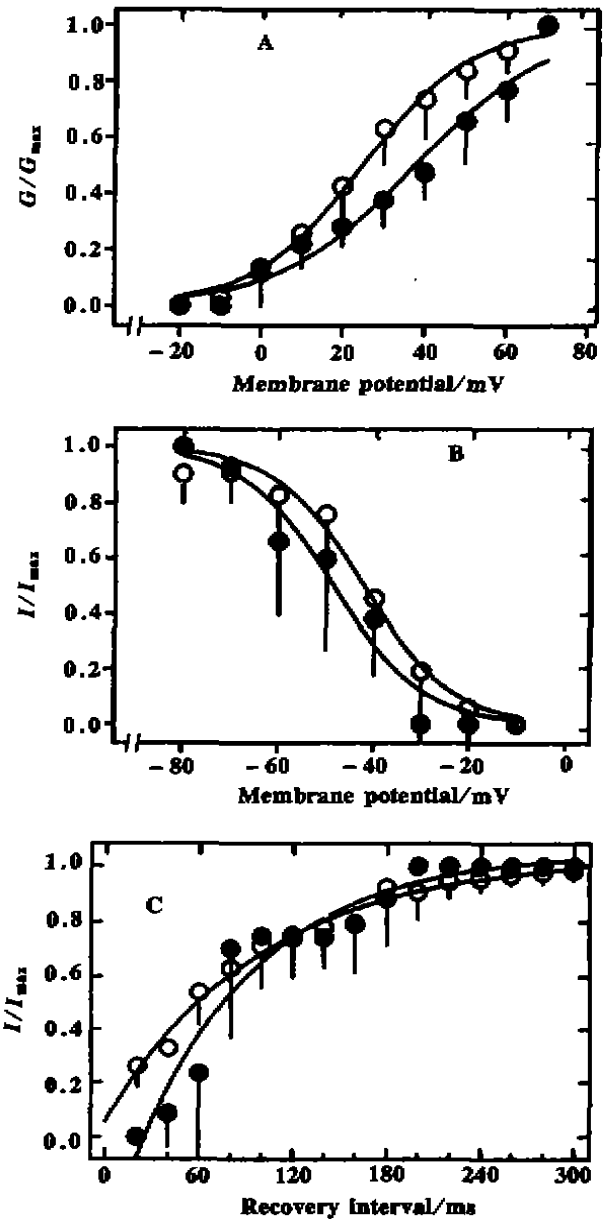

Fig 2. I_{to} A) Activation of I_{to} before (○) and after adding 4-AP 1 mmol·L^{-1} (●) (n = 3 cells). B) Inactivation of I_{to} before (○) and after adding 4-AP 1 mmol·L^{-1} (●) (n = 3 cells). C) Recovery of I_{to} before (○) and after (●) adding 4-AP 1 mmol·L^{-1} (n = 3 cells).

(n = 7).

To investigate the recovery process of I_{to} from inactivation, we used paired pulses delivered. Two 200 ms pulses (P_1 and P_2) to + 80 mV from a holding potential of − 80 mV were separated by a $P_1 - P_2$ coupling interval varying from 0 to 300 ms. The data were well fitted by the equation described below:

$$I = A + B \times EXP(-t/\tau)$$

It showed that the recovery time constant (τ) of human atrial I_{to} was 98 ± 10 (n = 4) (Fig 2C).

Effect of 4-AP on I_{to} The I_{to} was reduced by 4-AP in a concentration-dependent manner. The IC_{50} 95 % confidence limits of 4-AP were 0.67 (0.58 − 0.80) mmol·L^{-1} (Fig 3A). 4-AP 10 mmol·L^{-1} blocked I_{to} completely, while 4-AP 1 mmol·L^{-1} inhibited I_{to} to 60 % [before adding 4AP, I_{to} was (549 ± 119) pA] (Fig 3B).

4-AP also influenced the kinetic properties of I_{to}. At the concentration of 4-AP 1 mmol·L^{-1} shifted the voltage-dependent activation of I_{to} to the positive membrane potential. The parameter $V_{1/2}$ of the activation curve changed from (25 ± 5) mV to (37 ± 8) mV ($P < 0.05$, n = 3) and k from 13 ± 2 changed to 17 ± 2 ($P < 0.05$, n = 3) (Fig 2A). The inactivation curve did not move obviously (Fig 2B). The parameters of the recovery curves had no significant difference before and after adding 4-AP 1 mmol·L^{-1} (Fig 2C).

DISCUSSION

In almost all cells which we had studied, there existed the current of I_{to}. The current might conclude two components, Ca^{2+}-independent I_{to1} and Ca^{2+}-dependent I_{to2}. Due to the peptide solution containing egtazic acid 10 mmol·L^{-1} and Ca^{2+} current being blocked by extracellular Cd^{2+}, we ensured that the I_{to} we recorded was the Ca^{2+} dependent I_{to1} (I_{to} was used below) (Fig 1).

The kinetic properties of I_{to} in human atrial myocytes were different from the I_{to} in rat ventricular myocytes[9-11]. The $V_{1/2}$ value of the activation curve of human I_{to} was (24.8 ± 4.8) mV, while the value in rats was (10.8 ± 1.1) mV. It suggested that to activate human atrial I_{to} required more depolarization of membrane potential than that to activate rat heart I_{to}. In this study the $V_{1/2}$ of inactivation curve was (−41.1 ± 9.0) mV in human atrial cells. And in previous study, $V_{1/2}$ was found to be (−49.8 ± 1.8) mV in rat heart cells. It showed

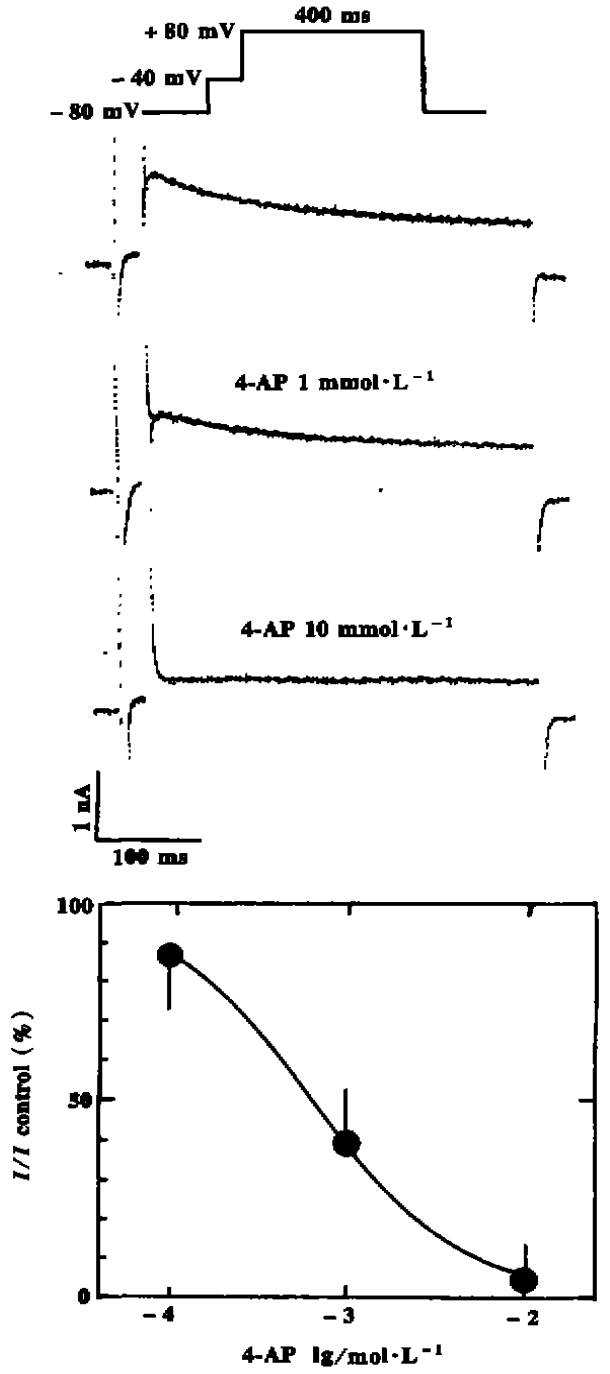

Fig 3. Inhibitory effect of 4-AP on I_{to}.
$n = 5$ cells from 5 patients, $\bar{x} \pm s$.

the channel in human heart would be inactivated at more positive membrane potential level. The time constants (τ value) of the current inactivation at 40 mV in human cells and in rat cells were (161 ± 49) ms and (41.0 ± 2.0) ms[9] respectively, indicated that the I_{to} inactivating in rat cells was much more quickly than in human cells.

4-AP 10 mmol·L^{-1}, a relatively selective I_{to} blocker, could inhibit the current completely. The IC_{50} of 4-AP to block human atrial I_{to} in this study was similar to the results reported by Nabauer *et al*[12]. 4-AP 1 mmol·L^{-1} made the activation curve of I_{to} shift to positive potential. This meant that 4-AP could affect voltage dependent opening of the channel in human myocyte. 4-AP did not affect the recovery of I_{to} from current inactivation, suggested that 4-AP had little effect on closed state channels. In our research 4-AP 1 mmol·L^{-1} did not influence the inactivation curve of I_{to}. Wang *et al* also reported that 4-AP showed a low affinity for the inactivated state[4]. All these indicated that blockade of I_{to} by 4-AP was only open-channel blockade.

Many drugs, such as quinidine, some class I_c antiarrhythmic agents, nifedipine and so on could block cardiac I_{to} in animals[6]. The physiological function and the pharmacological significance of I_{to} in human heart cells were little known but of much valuable to be further studied. The researches directly in the human myocytes may be sure to provide important information to clinical experiences.

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人心房肌细胞瞬间外向钾电流的特性[1]

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关键词 钾通道；膜片箝技术；心房；心肌；4-氨基吡啶

目的：研究人的心房肌细胞瞬间外向钾电流（I_{to}）的特性. **方法**：采用膜片箝全细胞记录法观察人心房肌通道电流的变化，在用 $CdCl_2$ 0.1 mmol·L^{-1} 阻断钙电流的情况下，细胞膜去极化引出瞬间外向钾电流（I_{to}）. **结果**：I_{to}为电压依赖的电流，迅速激活，迅速失活. 4-AP（4-氨基吡啶）10 mmol·L^{-1}（选择性的 I_{to}的阻断剂）能完全阻断此电流. 其 IC_{50}值为 0.67 mmol·L^{-1}. 4-AP 1 mmol·L^{-1}能使 I_{to}的激活曲线明显向正电位移动，使 I_{to}的激活电压提高，且较难激活 I_{to}. **结论**：在人心房肌细胞，I_{to}是一种主要的钾电流，它可被 4-AP 阻断.

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Inhibitory effects of *S*-nitrosocaptopril on vasomotor tone[1]

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KEY WORDS nitric oxide; *S*-nitrosocaptopril; captopril; renal circulation; thoracic aorta; angiotensin-converting enzyme inhibitor; vascular endothelium; phenylephrine; arginine; vasodilation

AIM: To study effects of captopril (Cap) and *S*-nitrosocaptopril (CapNO) on vascular tension. **METHODS**: Tension of rabbit aortic rings and perfusion pressure of rat renal artery (PP_r) were examined to evaluate the effects of CapNO. **RESULTS**: CapNO (3 nmol·L^{-1} – 10 μmol·L^{-1}) produced concentration-dependent vasorelaxation response in the rings of rabbit thoracic aortas. Endothelium denudation did not alter the relaxations to CapNO. In contrast, Cap had no vasorelaxing effect on the rings precontracted with phenylephrine. CapNO (10 nmol·L^{-1}) decreased rat PP_r *in vivo* ($P < 0.01$), and the effect was concentration-dependent and reversible. Cap showed a reduction in rat PP_r only at the concentration 1000 nmol·L^{-1} ($P < 0.05$). The relaxing potency of CapNO was 100 times higher than that of Cap in this respect. Pre-perfusion of rat renal arteries with N^G-monomethyl-*L*-arginine monoacetate (*L*-NMMA, 0.03 nmol·L^{-1}) or *L*-arginine (3 nmol·L^{-1}) did not significantly blocked the relaxing effect induced by CapNO. **CONCLUSION**: CapNO had a direct vasodilatory effect.

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