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Imipramine blocks the transient outward potassium current in rat ventricular myocytes[1]

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KEY WORDS imipramine; myocardium; cultured cells; potassium channels; patch-clamp techniques

AIM: To examine the effects of imipramine on transient outward potassium current (I_{to}) in rat ventricular myocytes. **METHODS:** The patch-clamp whole-cell recording techniques were used. **RESULTS:** Imipramine resulted in a concentration-dependent inhibition of I_{to} with the IC_{50} of 6.0 $\mu mol \cdot L^{-1}$ and a concentration-dependent acceleration of I_{to} inactivation. The blocking showed no difference at different testing membrane potentials. Imipramine produced slight effects (about 3 and 4 mV, respectively) on steady-state activation and inactivation curves of I_{to}, and tended to prolong the recovery of I_{to} from inactivation ($\tau_{control} = 37 \pm 11$ ms; $\tau_{drug} = 58 \pm 17$ ms), but not significant ($n = 4$, $P > 0.05$). The inhibitory effect of imipramine on I_{to} was increased when the prepulses were prolonged progressively from 0 to 120 ms. ($\tau_{control} = 22 \pm 8$ ms; $\tau_{drug} = 14 \pm 5$ ms). **CONCLUSIONS:** Imipramine blocked I_{to} in concentration-dependent but voltage-independent manners, and with "open channel blocking" properties.

Imipramine is a tricyclic antidepressant drug which exhibits cardiotoxic effects. It inhibits the maximal upstroke velocity (V_{max}) of the action potential and has been classified as a class I_a anti-arrhythmic drug[1]. In guinea-pig ventricular myocytes, imipramine inhibited sodium current (I_{Na})[2], calcium current (I_{Ca})[3], delayed rectifier potassium current (I_k), but not the inward rectifier potassium current (I_{k1})[3]. In rabbit atrial myocytes[4], imipramine induced a concentration-dependent inhibition of transient outward current (I_{to}) and an increase in the inactivation rate of the peak currents.

The I_{to} is the major outward repolarizing current in the action potential of several mammalian hearts including human atrium[5,6], rabbit[7], and rat ventricle[8,9]. The I_{to} in human atrium and rat ventricle possess similar kinetics and pharmacological properties[5,6,10]. Rat ventricular myocytes are regarded as the best preparation for I_{to} study.

The present work is to investigate the effects and mechanism of imipramine on I_{to} in rat ventricular myocytes.

METHODS

Isolation of cardiac myocytes Ventricular myocytes were isolated from ♀ Wistar rats (230 - 260 g, from the Center of Experimental Animals, Chinese Academy of Medical Sciences) by enzymatic dissociation. Briefly, hearts were perfused with collagenase type I ($0.3 g \cdot L^{-1}$) from aorta for 15 min. Single cells were isolated by following incubation of heart tissue in the same enzyme solution[11]. Rod-shaped and without spontaneous contraction cells with clear striations were used for the whole-cell voltage-clamp studies.

Voltage-clamp experiments Transmembrane currents were recorded using the whole-cell recording techniques[12]. The currents were measured with an EPC-7 amplifier (List Medical). Via a digital-analog converter (TL-1-125, Axon Inc), computer program pCLAMP 5.51 (Axon Inc) were used to produce voltage clamping signals. Acquisition and analysis of membrane currents were also done with the program and with pCLAMP 6.0 (Axon Inc). The electrode resistances were 2 - 3 MΩ. They were filled with the solution containing KCl 140, $MgCl_2$ 0.5, egtazic acid 10, and HEPES 10 $mmol \cdot L^{-1}$, and the pH was adjusted to 7.2 with KOH. The chamber was perfused with modified Tyrode's solution containing NaCl 150, KCl 5.4, $CaCl_2$ 1.2, $MgCl_2$ 2, glucose 10, HEPES 5 $mmol \cdot L^{-1}$ at the rate of 1.5 $mL \cdot min^{-1}$. The pH of the solution was adjusted to 7.4 with NaOH. $CdCl_2$ 0.1 $mmol \cdot L^{-1}$ was added to inhibit Ca^{2+} current.

To record I_{to}, the cells were depolarized from the holding potential of −80 mV to −40 mV for 30 ms to inactivate

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I_{Na}, and then depolarized to test potential of 40 mV for 400 ms every 5 s. We used peak outward current as the I_{to} amplitude in our data analysis

Current-voltage relationship and activation curve of I_{to} were generated by applying 11 depolarizing pulses from a holding potential of −80 mV to −40 mV for 30 ms, and from −40 mV to 60 mV for 400 ms with a 10 mV increment at the rate of 0.1 Hz. The currents were transferred to conductance (G) and fitted to Boltzmann function to produce activation curve.

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 duration to potential between −80 mV and 0 mV were applied at the rate of 0.1 Hz. After a 15-ms step to −40 mV to inactivate I_{Na}, the cells were depolarized to +40 mV for 400 ms so as to determine the amplitude of I_{to} which could still be activated. I_{to} was normalized by comparing with maximal activation of the current.

To investigate directly the effect of imipramine on recovery of I_{to} from inactivation, we used paired pulses delivered at 0.1 Hz. Two 200-ms pulses (P_1 and P_2) to +40 mV from a holding potential of −80 mV were separated by a $P_1 - P_2$ coupling interval varying from 0 to 300 ms.

All experiments were carried out at room temperature (22 − 25 ℃). Series resistances were partially compensated (50 % − 70 %). Imipramine (Sigma) was dissolved in distilled water as a stock solution of 10 or 100 mmol·L^{-1}, and was added in perfusion solution before currents recording.

Data analysis To calculate dose-response curve, the total I_{to} was measured as the integral of the outward current measured from the end of capacitive current to 250 ms after the start of the pulse. The amplitude of I_{to} was measured as the difference between the peak of the I_{to} and the sustained current at the end of the pulse. The results were expressed as $\bar{x} \pm s$. Paired and unpaired t tests were applied.

RESULTS

Concentration-dependent inhibition of I_{to} by imipramine The outward currents activated by depolarization voltage steps consisted of a rapidly inactivating component I_{to} and a sustained component I_{sus}. The amplitude of I_{to} was 1514 ±342 pA in 8 cells before exposed to imipramine. Imipramine 1 − 100 μmol·L^{-1} caused a concentration-dependent inhibition of I_{to}, with IC_{50} 6.0 μmol·L^{-1} (Fig 1).

Imipramine 10 μmol·L^{-1} or more obviously accelerated I_{to} inactivation. In control conditions, the inactivation processes of I_{to} were well fitted by a single exponential equation. However, in the presence of imipramine, the inactivation processes were better fitted by a double exponential equation, including a fast and a slow component. The time constant of fast inactivation component (τ_f) decreased correspondingly and significantly with the increment of imipramine concentration. ($P < 0.01$, unpaired t-test) (Tab 1).

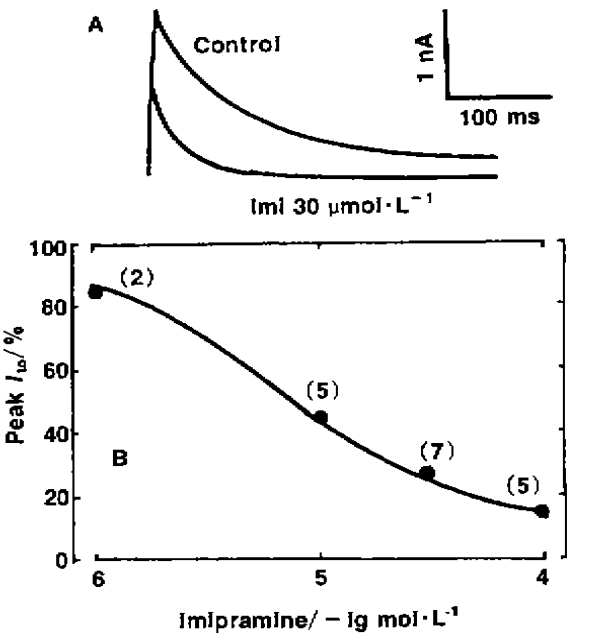

Fig 1. Block of I_{to} by imipramine. The numbers in the parentheses express amount of cells for each point.

Tab 1. The time constant of fast decaying component of I_{to} before and after imipramine. $\bar{x} \pm s$.
$^cP < 0.01$ *vs* control.

Imipramine/ μmol·L^{-1}	Number of Cells	Inactivation constant/ms
0	23	77 ± 24
1	2	57 ± 4^c
10	5	23 ± 7^c
30	7	20 ± 6^c
100	5	10 ± 3^c

Effects of imipramine on current-voltage relationship and steady-state activation and inactivation of I_{to} I_{to} started to be activated at −20 mV and continued to increase with depolarization. The amplitude of I_{to} after exposure

to imipramine 30 μmol · L^{-1} showed lower than predrug level at membrane potential − 10 mV to +60 mV ($P < 0.05$, paired t-test). But the values for percent block of I_{to} by imipramine 30 μmol · L^{-1} at different membrane potentials showed no significant difference, $P > 0.05$ (Fig 2).

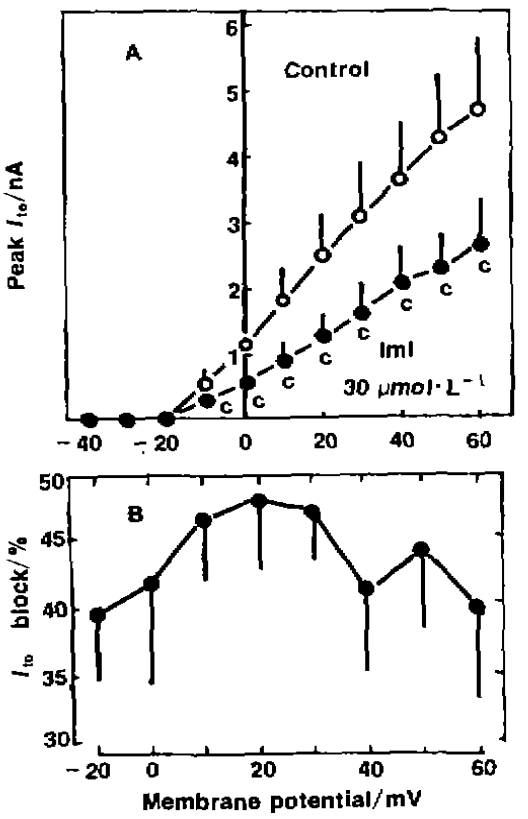

Fig 2. A) Current-voltage relationships (I - V curve). (n = 5 cells, *$P < 0.05$). B) Effect of imipramine 30 μmol·L^{-1} on I_{to} at different membrane potentials.

The activation curve was fitted by the Boltzmann function as follows:

$$G/G_{max} = 1/[1 - \exp(V - V_{\frac{1}{2}})/k]$$

G = membrane conductance transferred from membrane current. $V_{\frac{1}{2}}$ = the membrane potential of half maximal activation. k = slope factor. Imipramine 30 μmol · L^{-1} produced a slight effects on the steady-state activation of I_{to} and had no effects on slope factors. The $V_{\frac{1}{2}}$ before and after imipramine were 19.1 ± 2.0 and 22.2 ± 2.5 mV (n =5, $P > 0.05$, paired t-test), respectively. The k were 14.1 ± 2.7 under control conditions and 14.8 ± 1.1 in the presence of imipramine (Fig 3).

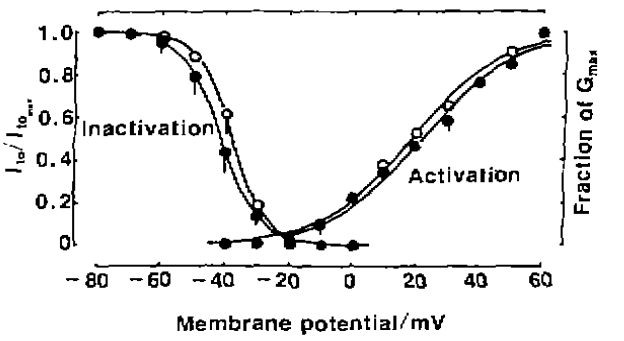

Fig 3. Steady-state activation and inactivation of I_{to} before (○, n = 5) and after imipramine 30 μmol·L^{-1}(●, n = 5).

The inactivation curve was also fitted to the Boltzmann equation as follows:

$$\text{Normalized } I_{to} = 1/[1 + \exp(V - V_{\frac{1}{2}})/k]$$

$V_{\frac{1}{2}}$ = the membrane potential of half maximal inactivation and k = slope factor. During control conditions, the $V_{\frac{1}{2}}$ was − 38 ± 3 mV and k was 5.4 ± 1.1. Imipramine 30 μmol · L^{-1} produced a minor effects on the inactivation curve; $V_{\frac{1}{2}}$ was −42 ± 4 mV (n = 5, $P > 0.05$, paired t-test) and k was 6.7 ± 0.9 (Fig 3).

Kinetics of pulse-dependent blocking and unblocking Imipramine 30 μmol · L^{-1} tended to prolong the recovery of I_{to} from inactivation with time constant (τ) of 37 ± 11 ms under control conditions and 58 ± 17 ms in the presence of imipramine, but not significant (n = 4, $P > 0.05$, unpaired t-test) (Fig 4).

The onset of blocking during a depolarizing pulse was also studied with a paired-pulse protocol, in which a 200 ms test pulse (P_2) from a holding potential of −80 mV to +40 mV was preceded by a conditioning pulse (P_1) over the same voltage range with a duration varying from 0 to 120 ms. The P_1 − P_2 interval was fixed at 50 ms. Pairs of pulses were delivered every 10 s. I_{to} elicited by P_2 were normalized by P_1 and showed a function of P_1 duration. The time constant were 22 ± 8 ms and 14 ± 5 ms (n = 4, $P < 0.05$, unpaired t-test) before and after exposure to imipramine 30 μmol · L^{-1}, respectively (Fig 5).

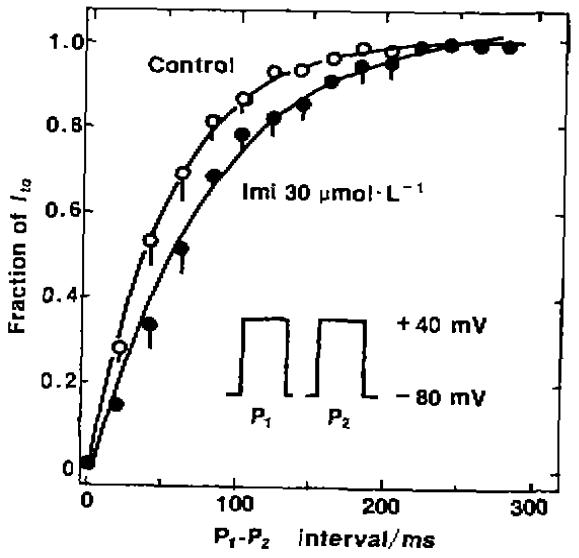

Fig 4. Effects of imipramine on recovery of I_{to}. (○: $\tau = 37 \pm 11$ ms; ●: $\tau = 58 \pm 17$ ms. $P > 0.05$, $n = 4$.

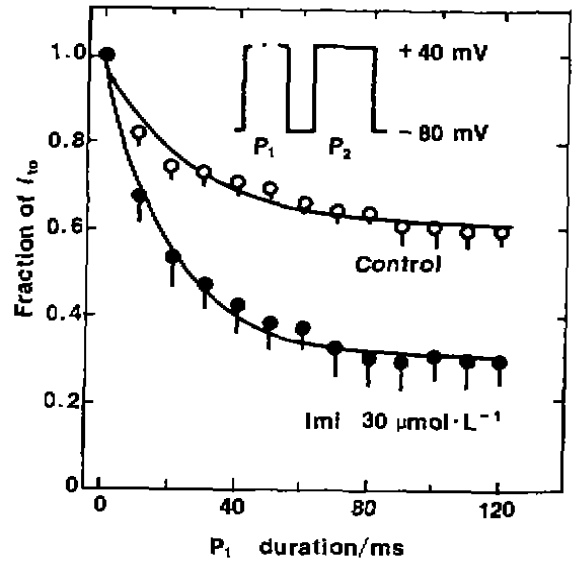

Fig 5. Influence of duration of a depolarizing prepulse on imipramine-induced inhibition of I_{to}. ○: $\tau = 22 \pm 8$ ms; ●: $\tau = 14 \pm 5$ ms. $P < 0.05$, $n = 4$.

DISCUSSION

In general, I_{to} has two types[6]. In our experiment, egtazic acid 10 mmol·L^{-1} in electrode solution and Cd^{2+} 0.1 mmol·L^{-1} ascertained us that the current components we recorded were Ca^{2+}-independent I_{to}.

Our results demonstrated that imipramine resulted in a concentration-dependent inhibition of I_{to} with the IC_{50} of 6.0 μmol·L^{-1}. The therapeutic plasma concentrations of imipramine are 0.53 − 1.07 μmol·L^{-1}[13]. But imipramine had a selective affinity for cardiac muscle, where it can reach concentrations 20 − 200 times greater than that in plasma[14]. Because I_{to} is a major potassium outward current in human heart, the inhibition of I_{to} may be partly responsible for the therapeutic effect of imipramine on arrhythmia.

In our study, imipramine produced slight shifts in the steady-state activation and inactivation curves of I_{to}, which were found to be statistically insignificant. More, the values for percent blocking of I_{to} by imipramine showed no difference at different testing membrane potential from − 10 mV to + 60 mV. These results demonstrated that the blocking effect of imipramine on I_{to} was voltage-independent.

Like other I_{to} blockers such as quinidine and flecainide[5], imipramine not only inhibited I_{to} amplitude but also accelerated the inactivation of I_{to}. Acceleration of inactivation can be a result of interaction with the open state of the channel[15]. Exposure to imipramine exhibited no significant change in the time course of I_{to} recovery from inactivation , indicating that the inhibition has no relation with $P_1 - P_2$ period (close- or rest-state). Whereas when P_1 progressively prolonged from 0 to 120 ms, the amplitude of I_{to} elicited by P_2 was significantly reduced by imipramine in P_1 duration dependent manner, indicating that channel open is required for the channel blocking. All these may indicate that imipramine affected on "open channel".

In summary, imipramine resulted in a concentration-dependent but voltage-independent inhibition of I_{to} and favored to interact with the open channel in rat ventricular myocytes.

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丙咪嗪对大鼠心室细胞瞬间外向钾电流的抑制作用

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关键词 丙咪嗪；心肌；培养的细胞；钾通道；膜片箝技术

目的：研究丙咪嗪对大鼠心室细胞瞬间外向钾电流(I_{to})的抑制作用. 方法：膜片箝全细胞记录法. 结果：丙咪嗪对 I_{to} 有浓度依赖性抑制作用，IC_{50} 为 6.0 $\mu mol \cdot L^{-1}$，并明显加速该电流的灭活时程. 在不同的测试电位下，丙咪嗪对该电流的抑制百分率没有差别. 丙咪嗪对 I_{to} 的稳态激活和灭活曲线的半数膜电位都无明显影响，对 I_{to} 灭活后的再复活时程有延长趋势，但不显著($\tau_{control} = 37 \pm 11$ ms，$\tau_{drug} = 58 \pm 17$ ms，$P > 0.05$)，但可明显加速条件刺激时程对 I_{to} 的灭活($\tau_{control} = 22 \pm 8$ ms，$\tau_{drug} = 14 \pm 5$ ms，$P < 0.05$). 结论：丙咪嗪对大鼠心室细胞瞬间外向钾电流有浓度依赖性和电压非依赖性抑制作用，作用特点属于开放通道阻断.