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# Effects of isoprenaline on delayed rectifier potassium current in isolated guinea pig ventricular myocytes

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**AIM**: To study the effects of isoprenaline (Iso) on the delayed rectifier potassium current ($I_k$) in isolated guinea pig ventricular myocytes. **METHODS**: Single cells were isolated from guinea pig ventricle. $I_k$ was studied under voltage clamp conditions. **RESULTS**: When $I_k$ was activated by depolarizing pulses to +40 mV of increasing duration (40—300 ms), Iso 1 μmol·L$^{-1}$ caused an enhancement in $I_k$ which was larger for longer pulses (150—300 ms). This was also seen when the intracellular calcium was buffered by 1,2-bis(2-aminophenoxy) ethane-*N*, *N*, *N'*, *N'*-tetraacetic acid (BAPTA). Intracellular application of BAPTA caused a decrease in $I_k$ activated by longer pulses. **CONCLUSION**: There were two components of $I_k$, one of which was modulated by Iso and intracellular $Ca^{2+}$.

**KEY WORDS** isoproterenol; calcium, myocardium; potassium channels

The delayed rectifier potassium current ($I_k$) was increased by β-adrenergic stimulation[1-4]. In guinea pig heart cells $I_k$ may have 2 separate components, $I_{kr}$ and $I_{ks}$[5,6]. $I_{kr}$ activates rapidly and is blocked by E-4031; $I_{ks}$ is a much larger current which activates very slowly and is not blocked by E-4031. In this study, the aim was to study the effects of isoprenaline (Iso) and intracellular calcium on $I_k$ in isolated guinea pig ventricular myocytes.

## MATERIALS AND METHODS

Experiments were done on single guinea pig ventricular myocytes which were isolated by using collagenase (type II, Worthington Biochemical Corp)[7] and superfused at 36 ℃ with a 95 % $O_2$+5 % $CO_2$ gassed solution containing: NaCl 118.5, $NaHCO_3$ 14.5, KCl 4.2, $KH_2PO_4$ 1.2, $MgSO_4$ 1.2, glucose 11.1 and $CaCl_2$ 2.5 mmol·L$^{-1}$.

Electric recordings in most experiments were made via microelectrodes containing $K_2SO_4$ 0.5 mol·L$^{-1}$ + KCl 10 mmol·L$^{-1}$. In experiments where intracellular calcium was buffered at a low level, 1,2-bis(2-aminophenoxy)ethane-*N*, *N*, *N'*, *N'*-tetraacetic acid (BAPTA, Sigma) 75 mmol·L$^{-1}$ was added to the electrode solution. In some experiments BAPTA was injected into cells after recordings in their absence. In these experiments cells were impaled with "theta" microelectrodes. Recordings of electric activity were made from one barrel of the electrode filled KCl 3 mol·L$^{-1}$ and injection of BAPTA made from another barrel of the electrode[8].

A switch voltage-clamp method with a single-electrode system (Dagan 8500) was used. $I_k$ was activated by depolarizing pulses from a holding potential of −40 mV to a constant test potential of +40 mV. Upon repolarization from an activating voltage step an outward tail current was observed which is thought to represent the slow deactivation of $I_k$ and serve as an index of $I_k$ current. The calcium current was blocked by addition of nisoldipine (Sigma) 2 μmol·L$^{-1}$ to the bath solution and the sodium current was voltage-inactivated by maintaining the holding potential at −40 mV. The currents were recorded on a modified digital audio tape recorder (Sony DTC-55ES) and displayed on a digital storage oscilloscope.

## RESULTS

Cells were depolarized to +40 mV for various durations, and the outward $I_k$ tails on repolarization to the holding potential of −40 mV were recorded. The envelope of de-

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activating outward tails measured in this way showed that $I_k$ was increased as the pulse duration was lengthened from 40 to 300 ms, reflecting the time dependence of activation of $I_k$. In the presence of Iso 1 $\mu mol \cdot L^{-1}$ there was no significant changes in $I_k$ tail current with shorter depolarizing steps, however, the tail current of $I_k$ with longer pulses was increased (Fig 1 & 2).

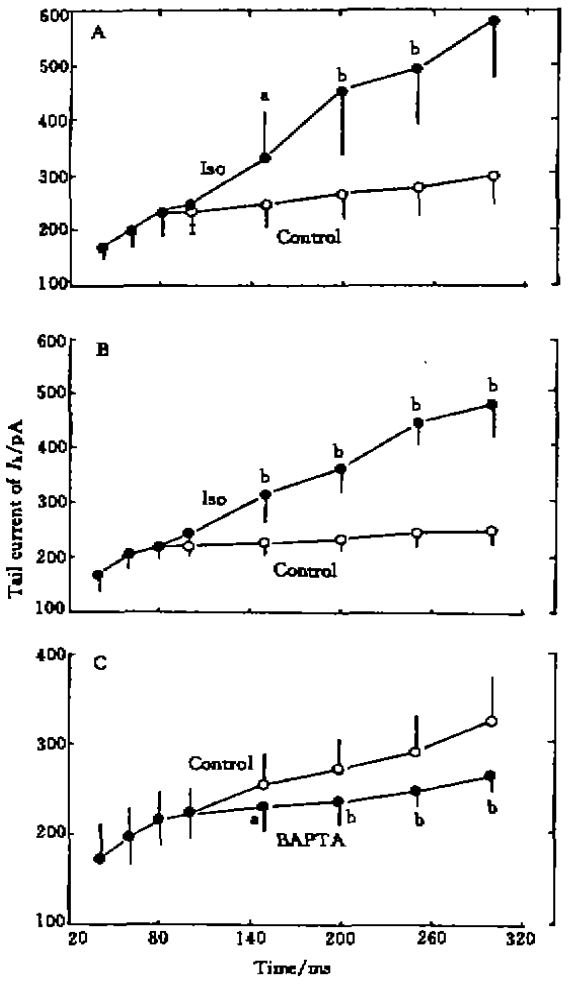


**Fig 1. Effects of isoprenaline 1 $\mu mol \cdot L^{-1}$ in the absence (A)/presence (B) of intracellular BAPTA and intracellular application of BAPTA (C) on tail current of $I_k$ activated with a depolarization to +40 mV (duration 40—300 ms). $n=6-8$, $\bar{x} \pm s$. $^aP<0.05$. $^bP<0.01$.**

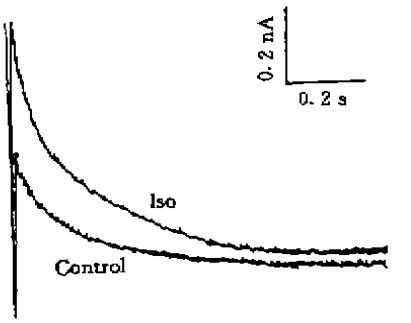


**Fig 2. Effects of isoprenaline 1 $\mu mol \cdot L^{-1}$ on tail current of $I_k$ activated with a depolarization of 250 ms to +40 mV.**

To minimize calcium transient in the cell which might have the effect on action of Iso, BAPTA was used to buffer intracellular calcium at a low level[18,19], and a depressed tail current was recorded. Under these conditions Iso showed a similar pattern of action on tail current of $I_k$ (Fig 1).

Effects of intracellular calcium on $I_k$ were examined because the $I_k$ was decreased in the presence of BAPTA as described above. The same protocol was applied to activate $I_k$. After intracellular application of BAPTA the tail current of $I_k$ activated with the longer but not the shorter pulses was apparently decreased (Fig 1).

## DISCUSSION

It has been shown that in the presence of Iso E-4031 had a small effect on action potential duration which might result from the smaller contribution of $I_{kr}$ relative to the augmented repolarizing current. The results implied that Iso could increase $I_{ks}$ without significant effect on $I_{kr}$[13]. In our experiment we found that Iso only increase the tail current of $I_k$ activated with longer pulses. This indicates that Iso could only modulate one of components of $I_k$, which was in agreement with the assumption discussed above. This Iso-

sensitive component corresponds to $I_{ss}$.

One of the components of $I_k$, $I_{ss}$, has been reported not to decrease in the absence of extracellular calcium[11]. Our results that $I_k$ activated by the shorter but not longer pulses was not changed after BAPTA was injected into the cells further indicate that this component of $I_k$ was not modulated by intracellular Ca.

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## 异丙肾上腺素对豚鼠离体心室肌细胞延迟整流钾电流的影响

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**目的**：观察异丙肾上腺素对豚鼠离体心室肌细胞延迟整流钾电流($I_k$)的作用。**方法**：分离单个离体豚鼠心室肌细胞，采用电压钳技术观察$I_k$。**结果**：在用不同去极化时程脉冲(40—300 ms)激活$I_k$的条件下，异丙肾上腺素(1 μmol·$L^{-1}$)仅增加长时程脉冲(150—300 ms)激活的$I_k$，这一作用在预先用BAPTA缓冲细胞内钙的条件下仍然存在，而细胞内注射BAPTA则降低长时程脉冲激活的$I_k$。**结论**：豚鼠离体心室肌细胞$I_k$有二个成分，其中一个成分受异丙肾上腺素和细胞内钙的调控。

**关键词** 异丙肾上腺素；钙；心肌；钾通道