依他酸；伊屋诺霉素；凝血酶；血小板

目的：研究凝血酶诱导的血小板活化中细胞内钙动员和 Na^+/H^+ 交换的关系。**方法**：Fura-2 负载测 $[Ca^{2+}]_i$ 和 BCECF 负载测 pH_i。**结果**：凝血酶 0.1 IU·L^{-1} 引起 $[Ca^{2+}]_i$ 和 pH_i 增加，$[Ca^{2+}]_i$ 增加先于 pH_i 增加。在无钠溶液中，Na^+/H^+ 交换被抑制而 $[Ca^{2+}]_i$ 增加不受影响；用尼日利亚菌素(1 mg·L^{-1})使胞内酸化可抑制 $[Ca^{2+}]_i$ 增加。用依他酸(EGTA)阻断外钙内流，胞浆碱化不受影响。伊屋诺霉素加 EGTA 耗竭胞内钙池时，胞浆碱化效应被取消，且静息 pH_i 更低，加入 1 mmol·L^{-1} 外钙重新充填钙池，胞浆碱化效应又被恢复。**结论**：细胞内钙动员调控 Na^+/H^+ 交换，后者需 $[Ca^{2+}]_i$ 增加达一定有效浓度。

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Blocking effects of phentolamine on L-type calcium current and ATP-sensitive potassium current in guinea pig ventricular myocytes[1]

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KEY WORDS phentolamine; prazosin; yohimbine; glyburide; patch-clamp techniques; calcium channels; potassium channels; myocardium

AIM: To study the effect of phentolamine on L-type calcium currents (I_{Ca}) and ATP-sensitive K^+ currents ($I_{K,ATP}$) in ventricular myocytes. **METHODS**: I_{Ca} and $I_{K,ATP}$ were observed using patch clamp techniques in whole-cell recording configuration. **RESULTS**: Phentolamine reduced I_{Ca} of ventricular myocytes in concentration-dependent and voltage-independent manners. Phentolamine 5, 25, and 100 μmol·L^{-1} decreased I_{Ca} from 370 ± 99 nA to 310 ± 95 nA (17 % block, $n = 6$, $P < 0.01$), from 230 ± 98 nA to 180 ± 73 nA (23 % block, $n = 5$, $P < 0.05$), and from 293 ± 66 nA to 206 ± 44 nA (30 % block, $n = 5$, $P < 0.01$), respectively, without affecting the current-voltage relationship. Prazosin 100 μmol · L^{-1} and yohimbine 100 μmol · L^{-1}, which were specific blockers of α_1 and α_2 adrenoceptors respectively, did not show the inhibitory effect on I_{Ca}. Phentolamine 100 μmol·L^{-1} also inhibited the $I_{K,ATP}$ induced by 2, 4-dinitrophenol (DNP) at 0 mV from 3.2 ± 0.6 nA to 0.8 ± 0.5 nA (75 % block, $n = 4$, $P < 0.01$). **CONCLUSION**: Phentolamine directly inhibits I_{Ca} and $I_{K,ATP}$ in guinea pig ventricular myocytes.

Phentolamine is a classical nonselective α adrenoceptor blocking agent. The potent anti-arrhythmic effects of phentolamine were shown in cat heart *in vivo*[1] and in rat[2] and guinea pig[3,4] hearts *in vitro*. Phentolamine can block single ATP-sensitive K^+ channels in ventricular myocytes of rabbit at the intracellular side of the channel[5]. But, there was no direct evidence that phentolamine can block K_{ATP} channels from the surface of cardiac myocytes and as one of targets of anti-arrhythmic drug, L-type Ca^{2+} channels should be a candidate[6]. Hence we studied the effect of phentolamine on ATP-sensitive K^+ currents ($I_{K,ATP}$) and L-type Ca^{2+} currents (I_{Ca}) in guinea pig cardiomyocytes.

MATERIALS AND METHODS

Cell isolation Single ventricular myocytes were obtained by enzymatic dissociation[7] from Dunkin Hartley guinea pigs (235 ± 15 g) provided by Experimental Animal Center of Shanxi Medical University. Langendorff hearts were perfused through

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the coronary artery with solutions in the following sequence: 1) normal Tyrode's solution containing NaCl 139, $MgCl_2$ 1.0, KCl 5.4, $CaCl_2$ 1.8, NaH_2PO_4 1.0, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES) 5.0, and glucose 10 mmol·L^{-1} for 0.5 min; 2) Ca^{2+}-free Tyrode's solution containing NaCl 104, KCl 10, KH_2PO_4 1.0, $MgSO_4$ 5.0, glucose 20, taurine 50, and HEPES 5.0 mmol·L^{-1} for 5 min; 3) 30 mL of Ca^{2+}-free Tyrode's solution added, additionally, 1 mL of Tyrode's solution and 8 mg collagenase (type I Sigma, USA) for 8 min; 4) 80 mL Kraft-Brühe (KB) solution: KOH 85, *L*-glutamic acid 50, KCl 30, taurine 2.0, KH_2PO_4 30, $MgSO_4$ 3.0, HEPES 10, glucose 10, and egtazic acid 0.5 mmol·L^{-1}.

All solutions were saturated with O_2 at approximately 36 ℃, pH 7.4. The ventricular tissues were cut into pieces and *gently agitated with KB solution. These cell preparations* were stabilized for 1 h at 25 ℃, then stored at 10 ℃.

Electrophysiologic measurements Cell preparations were perfused with normal Tyrode's solution 2 mL·min^{-1} in a chamber (0.6 mL) on an inverted microscope (XDP-1, Shanghai). Only rod-shaped cells with a clear margin and striation were used. The tight-seal whole-cell recording techniques[8] were used. The pipette was filled with solution potassium aspartate 80, KCl 50, $MgCl_2$ 1.0, HEPES 5.0, egtazic acid 1.0, and Na_2 – ATP 3.0 mmol·L^{-1} (pH 7.4 adjusted with KOH 1 mol·L^{-1}, electrode resistance 3 – 5 MΩ). Transmembrane currents were recorded with a patch-clamp amplifier (Axopatch 200A, Axon Instruments, USA). The current signal was filtered at 2 Hz and stored via a Lab Master data acquisition system (Scientific Solution's Inc, USA) on a computer (AST 386) equipped with an AD converter. The sampling and data analysis were accomplished using a pCLAMP 5.5.1 software (Axon Instruments, USA).

Drugs Phentolamine (Regitine) (Ciba-Geigy, Basle, Switzerland); verapamil (Knoll AG, D-6700 Ludwigshafen, Germany/Alemania); prazosin and yohimbine (Sigma). All drugs were of 98 % purity. These drugs were prepared as stock solution of 1 mmol·L^{-1} in distilled water and diluted in the external solution.

Protocols After a 5-min period of Tyrode's solution perfusion for control measurements, the perfusate was changed to Tyrode's solution containing drugs, and data were collected again after 3 – 5 min. Cells were perfused again with drug-free solution to determine the reversibility of drug action. All data were expressed as $\bar{x} \pm s$ and compared with paired *t*-test.

RESULTS

L-type calcium current I_{Ca} was elicited by depolarization from the holding potential of – 40 mV to 0 mV. When cells were exposed to $CdCl_2$ 0.1 μmol·L^{-1} or verapamil 1.0 μmol·L^{-1} for 5 min, the elicited current was completely blocked, indicating the characteristic of I_{Ca}[9]. The amplitude of I_{Ca} was measured by subtracting the current at 100 ms of the depolarizing pulse from the inward peak value of I_{Ca} in order to eliminate the drug-induced alterations in outward K^+ current (if present)[6]. Phentolamine 5, 25, and 100 μmol·L^{-1} decreased I_{Ca} within 3 min by 17 % ($n = 6$), 23 % ($n = 5$), and 30 % ($n = 5$), respectively. After washout of phentolamine with normal Tyrode's solution for 5 min, I_{Ca} partially recovered (Tab 1, Fig 1).

Tab 1. Effect of phentolamine on I_{Ca} in guinea pig ventricular myocytes. n = **number of cells.** $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* **control.** $^eP < 0.05$, $^fP < 0.01$ *vs* **drug.**

Phentolamine/ μmol·L^{-1}	n	I_{Ca}/pA (relative value of block) Control	Drug	Washout
5	6	370 ± 40	310 ± 38^c (17 %)	330 ± 41^e
25	5	230 ± 44	180 ± 32^b (23 %)	210 ± 38^e
100	5	293 ± 29	206 ± 19^c (30 %)	244 ± 23^f

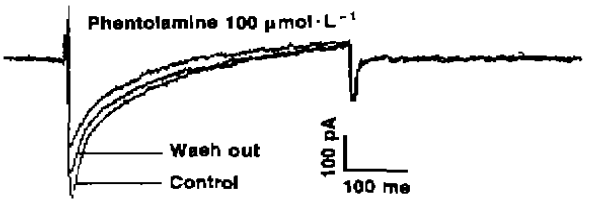

Fig 1. Effect of phentolamine 100 μmol·L^{-1} on I_{Ca}.

Prazosin 100 μmol·L^{-1} and yohimbine 100 μmol·L^{-1}, which were specific blockers of α_1 and α_2 adrenoceptor respectively, did not show the inhibitory effect on I_{Ca} (Tab 2).

Tab 2. Effects of phentolamine, prazosin, and yohimbine on I_{Ca} in ventricular myocytes. n = **number of cells.** $\bar{x} \pm s$. $^aP > 0.05$, $^cP < 0.01$ *vs* **control.** $^dP > 0.05$, $^fP < 0.01$ *vs* **medication.**

Drug (100 μmol·L^{-1})	n	I_{Ca}/pA Control	Medication	Washout
Phentolamine	5	293 ± 29	206 ± 19^c	244 ± 23^f
Prazosin	6	270 ± 43	240 ± 34^a	230 ± 34^d
Yohimbine	6	330 ± 41	320 ± 43^a	310 ± 44^d

Cells were depolarized from a holding potential of −40 mV to +60 mV with 10 mV increment, resulting in a progressive activation of I_{Ca}. Under control condition, the maximal peak current was attained at potential of 10 mV. Phentolamine decreased the peak I_{Ca} (measured as initial peak inward currents) without shifting the potential (10 mV) at which maximal peak I_{Ca} was elicited (Fig 2).

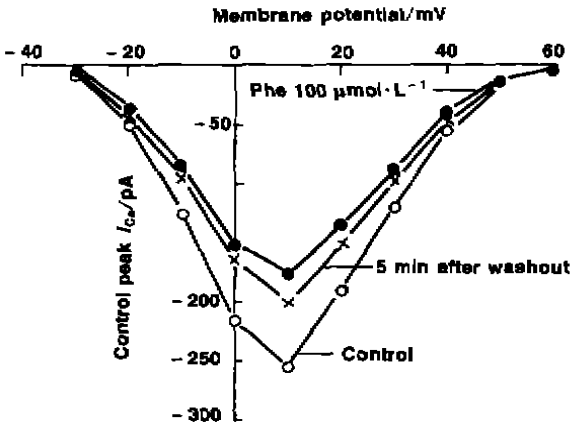

Fig 2. Effect of phentolamine 100 μmol·L^{-1} on $I-V$ relationship of Ca^{2+} current in ventricular myocytes.

ATP-sensitive potassium current 2,4-dinitrophenol (DNP), an uncoupler of oxidative phosphorylation, activated the $I_{K,ATP}$, which was characterized by an increase in the time independent outward current at positive potentials[10]. We used a ramp depolarized pulse from −100 mV with velocity of 8 mV·s^{-1} to +60 mV, and obtained a quasi-steady state current-voltage ($I-V$) relationship. During equilibration in normal Tyrode's solution, the $I-V$ relationship had a characteristic N shaped contour. After a 6 – 10 min exposure to Tyrode's solution containing DNP 50 μmol·L^{-1}, the outward current was increased dramatically and the $I-V$ relationship became almost linear. This DNP-induced outward current (I_{DNP}) which can be measured by subtracting the control current from the outward current after 6 – 10 min exposure to DNP 50 μmol·L^{-1} was completely abolished within 3 min after the subsequent application of glibenclamide 1 μmol·L^{-1} (Fig 3), a specific inhibitor of K_{ATP} channels[11], indicating that I_{DNP} is the $I_{K,ATP}$[11,12]. Phentolamine 100 μmol·L^{-1} obviously decreased the I_{DNP} within 5 min from 3.2 ± 0.6 nA to 0.8 ± 0.5 nA

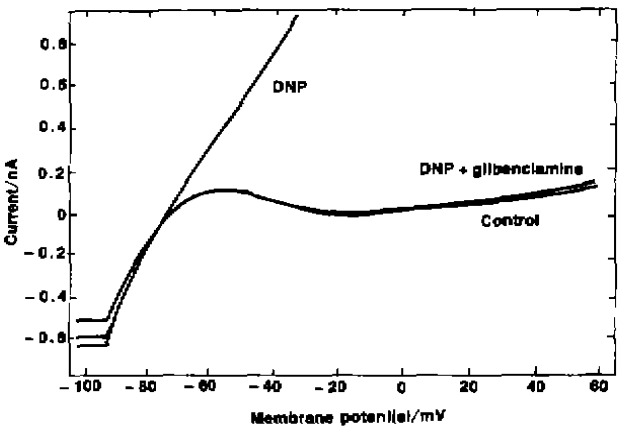

Fig 3. Effect of glibenclamine 1 μmol·L^{-1} on $I_{K,ATP}$ induced by DNP 50 μmol·L^{-1}.

(block 75 %, $n=4$) at 0 mV (Tab 3).

Tab 3. Effect of phentolamine on $I_{K,ATP}$ in myocytes. $n=4$ cells from 4 guinea pigs. $\bar{x} \pm s$. $^cP<0.01$ *vs* control. $^fP<0.01$ *vs* DNP 50 μmol·L^{-1}.

DNP/ μmol·L^{-1}	Phe/μmol·L^{-1}	Outward current/nA
0	0 (Control)	0.6 ± 0.3
50	0	3.2 ± 0.6^c
50	100	0.8 ± 0.5^f

DISCUSSION

In our experiment condition, phentolamine obviously suppressed the cardiac L-type calcium current in a concentration dependent manner. $I-V$ relationship of I_{Ca} showed that the peak amplitude of I_{Ca} was decreased by phentolamine at all potentials tested, without shifting the potential (10 mV) at which the maximum peak I_{Ca} was elicited. This indicated the blocking effect of phentolamine on Ca^{2+} channels was voltage-independent. The mechanism underlying this effect did not, at least, relate with the blocking of α_1 and α_2 adrenoceptor since prazosin, a specific α_1 antagonist, and yohimbine, a specific α_2 antagonist, failed to block the I_{Ca}. That is, blocking effect of phentolamine on I_{Ca} was independent of α adrenoceptors. It is possible that the so-called "run-down" phenomenon comes into existence. Thus, we observed the change in amplitude of I_{Ca} with time using the same experimental condition and specimen

($n = 5$). The results showed that the amplitude of I_{Ca} did not decline significantly within initial 3 min.

In this study, the blocking effect of phentolamine on $I_{K,ATP}$ has been shown in whole cell patch clamp configuration. Comparing with the study of Wilde *et al*[5], the drug concentration (100 μmol·L^{-1}) needed for blockade in whole cell model was higher than that in inside-out patch configuration (5 μmol·L^{-1}). Probably this is due to the fact that the drug can not block the channels from outside of membrane and needs to pass the sarcolemma and then the blockade occurs at intracellular side of the channel. The higher concentration of phentolamine needed for blocking $I_{K,ATP}$ channel shows that this blocking action might have no clinical benefit for anti-arrhythmic therapy.

In conclusion, phentolamine inhibited I_{Ca} of guinea pig ventricular myocardium, but prazosin and yohimbine did not show any effects on I_{Ca}. Therefore, the effect of phentolamine on L-type calcium channels is a direct action. In this whole cell patch-clamp configuration, much higher concentration of phentolamine can block $I_{K,ATP}$ from the surface of myocardium.

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酚妥拉明对豚鼠心室肌细胞 L-型钙电流及 ATP 敏感钾电流的抑制作用[1]

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关键词 酚妥拉明; 哌唑嗪; 育亨宾; 格列本脲; 膜片箝技术; 钙通道; 钾通道; 心肌

目的: 研究酚妥拉明对豚鼠心室肌细胞 L-型钙电流及 ATP 敏感钾电流的作用. **方法**: 用膜片钳的全细胞记录方式观察钙电流和 ATP 敏感钾电流. **结果**: 酚妥拉明 5, 25 和 100 μmol·L^{-1}对钙电流呈浓度依赖性和非电压依赖性的抑制作用, 抑制率分别为 17 %, 23 %和 30%, 而对电流-电压关系没有影响. 这一抑制作用与酚妥拉明对 α_1 和 α_2 受体的作用无关. 酚妥拉明 100 μmol·L^{-1}可显著抑制 DNP 诱导产生的 ATP 敏感钾电流, 抑制率为 75 %. **结论**: 酚妥拉明显著抑制豚鼠心室肌细胞 L-型钙电流和 ATP 敏感钾电流.