ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Jul; 20 (7): 631-634
http://www.simm.ac.cn; www.chinainfo.gov.cn/periodical

Inhibitory effects of estradiol on inward rectifier and delayed rectifier K^+ currents in guinea pig ventricular myocytes[1]

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KEY WORDS estradiol; patch-clamp techniques; myocardium; potassium channels; action potentials

ABSTRACT

AIM: To study the effects of estradiol (Est) on inward rectifier K^+ (I_{K1}) and delayed rectifier K^+ (I_K) channels in isolated guinea pig ventricular myocytes. **METHODS**: Using whole cell patch-clamp recording techniques. **RESULTS**: Est 10 $\mu mol \cdot L^{-1}$ and 100 $\mu mol \cdot L^{-1}$ decreased the action potential duration, APD_{50}, from (474 ± 71) ms to (330 ± 75) ms and (229 ± 67) ms ($n = 7$ cells of 7 guinea pigs, $P < 0.05$), respectively. Est 100 $\mu mol \cdot L^{-1}$ also decreased APD_{90} from (587 ± 60) ms to (418 ± 79) ms ($n = 7$, $P < 0.05$). Est inhibited I_K tail current (I_K · tail) concentration-dependently. I_K · tail was depressed 53 % ($n = 5$, $P < 0.05$) at 10 $\mu mol \cdot L^{-1}$ and 80 % ($n = 5$, $P < 0.01$) at 100 $\mu mol \cdot L^{-1}$ compared with control. Est $\geqslant 10$ $\mu mol \cdot L^{-1}$ blocked I_{K1}. The maximal inhibition of inward current of I_{K1} occurred at −100 mV test potential was 49 % ($n = 5$, $P < 0.01$) and outward current of I_{K1} at −40 mV was 72 % ($n = 5$, $P < 0.01$). The reverse potential shifted negatively, from −70 to −76 mV. **CONCLUSION**: Est possessed blocking effects on both I_{K1} and I_K channels in guinea pig ventricular myocytes.

INTRODUCTION

Estrogens played a key role in sex difference of cardiovascular diseases[1]. It protected the heart against coronary disease and ischemia in some conditions and acted as a coronary risk factor in another condition[2-4]. Est inhibited contraction of isolated rabbit heart, guinea pig papillary muscle and guinea pig ventricular myocytes, antagonized experimental arrhythmia and influenced action potentials (AP) of isolated guinea pig papillary muscle[5-7]. The present study was to observe the effects of Est on AP, inward rectifier K^+ current (I_{K1}) and delayed rectifier K^+ current (I_K) in cardiomyocytes.

MATERIALS AND METHODS

Preparation of ventricular myocytes Single ventricular myocytes from guinea pigs (♂, $n = 11$, weighing 261 g ± s 47 g) were prepared by enzymatic dissociation[8]. Briefly, the heart was rinsed in an oxygenated Ca^{2+}-free Tyrode's solution. The aorta was cannulated and the heart was retrogradely perfused on a Langendorff apparatus at 37 ℃. A perfusion with Ca^{2+}-free Tyrode's solution for 5 min was followed by low Ca^{2+} (50 $\mu mol \cdot L^{-1}$) Tyrode's solution containing 0.03 % collagenase and 1 % bovine serum albumin (BSA) for 5 min. The ventricles were cut, minced, and gently triturated with a pipette in the low-Ca^{2+} Tyrode's solution containing BSA at 37 ℃ for 10 min. The cells were filtered through 200-μm nylon mesh, resuspended in the Tyrode's solution in which the Ca^{2+} concentration gradually increased to 1.0 $\mu mol \cdot L^{-1}$. Only the cells with rod shaped and clear cross striation were used for experiments.

Chemicals and solutions Est was purchased from Sigma Co and dissolved in ethanol to make a 10 $mmol \cdot L^{-1}$ stock solution. The maximal concentration of ethanol in perfusate was 0.2 %. All of BSA, collagenase type II, taurine, HEPES, egtazic acid,

[1] Project supported by the Hebei Science Foundation, No 395356.
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Received 1998-06-16 Accepted 1998-12-10

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Jul; 20 (7)
E-mail aps@server.shcnc.ac.cn Phn/Fax 86-21-6474-2629

Na_2ATP and K_2ATP were products of Sigma. 3-(*N*-morpholino)-propanesulfonic acid (MOPS) was purchased from Shanghai Boao Biotech Co. The composition of the Ca^{2+}-free Tyrode's solution was: NaCl 100, KCl 10, NaH_2PO_4 1.2, $MgSO_4$ 5.0, glucose 20, taurine 10, MOPS 10 mmol·L^{-1}; pH was adjusted with KOH to 7.2. Test solution for AP recording was composed of: NaCl 137, KCl 5.4, $MgCl_2$ 1.0, $CaCl_2$ 1.8, HEPES 10, glucose 20 mmol·L^{-1}; pH was adjusted with KOH to 7.4. Test solution for K^+ currents recording: AChCl 137, KCl 5.4, $MgCl_2$ 1.0, HEPES 10, glucose 10 mmol·L^{-1}; pH was adjusted with KOH to 7.4. The electrode internal solution for AP recording: KCl 140, $MgCl_2$ 2.0, egtazic acid 2.0, HEPES 5.0, Na_2ATP 4.0 mmol·L^{-1}; pH was adjusted with KOH to 7.4. The electrode internel solution for K^+ currents recording: KCl 140, $MgCl_2$ 0.5, egtazic acid 10, HEPES 10, K_2ATP 5.0 mmol·L^{-1}; pH was adjusted with KOH to 7.4.

Potential and currents recording Myocytes were placed in a 500 μL chamber on stage of inverted microscope (Olympus CK2). The chamber was continuously superfused with test solutions 2 mL·min^{-1} at 25 ℃. Membrane currents and AP were recorded using the whole-cell patch-clamp techniques with a patch-clamp amplifier (CEZ 2300, Nihon Kohden, Japan)[9]. Patch electrodes were pulled with a vertical puller (PB-7, Narishige, Tokyo, Japan) and had a resistance of 2 – 3 MΩ when filled with electrode internal solution. After gigaseal produced and patch ruptured, AP was recorded in current clamp mode and currents were recorded in voltage clamp mode. Experimental protocols, data acquisition and storage were accomplished with Pclamp 5.6 (Axon Instrument, USA) running on a personal computer.

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

RESULTS

AP AP in guinea pig ventricular myocytes was evoked by a step current pulse of 90 pA, 10 ms duration at the frequency of 1 Hz. Est 10 μmol·L^{-1} decreased APD_{50}, from (474 ± 71) ms to (330 ± 75) ms ($n = 7$ cells of seven guinea pigs, $P < 0.05$). Est 100 μmol·L^{-1} decreased APD_{50} to (229 ± 67) ms ($n = 7$, $P < 0.01$) and APD_{90} from (587 ± 60) ms to (418 ± 79) ms ($P < 0.05$). The effects were not canceled completely after Est was washed out. RP and other parameters of AP had no significant change (Tab 1). Ethanol 0.2 % had no significant effect on AP, RP as well as currents.

I_K · tail I_K · tail in guinea pig ventricular myocytes was obtained by a depolarizing step pulse from the holding potential (E_h) of −40 mV to 30 mV at the frequency of 0.2 Hz. The step pulse duration was 5 s. Est inhibited I_K · tail concentration-dependently, 53 % (from 247 ± 51 to 117 ± 27 pA, $n = 5$ cells of 4 guinea pigs, $P < 0.05$) at 10 μmol·L^{-1} and 80 % (from 248 ± 51 to 57 ± 13 pA, $P < 0.01$) at 100 μmol·L^{-1}. After washout of Est, the inhibition of I_K · tail was partly recovered (from 57 ± 13 to 84 ± 24 pA) (Fig 1).

I_{K1} I_{K1} was elicited by a number of step pulses (300 ms) from the E_h of −40 mV to test potential between −100 and +30 mV with step 10 mV. Addition of Est 10 μmol·L^{-1} induced a remarkable

Tab 1. Effects of Est on AP. $n = 7$ **ventricular myocytes of 7 guinea pigs.** $\bar{x} \pm s$. [a]$P > 0.05$, [b]$P < 0.05$, [c]$P < 0.01$ *vs* **control.**

	RP/mV	APA/mV	OS/mV	V_{max}/V·s^{-1}	APD_{50}/ms	APD_{90}/ms
Solvent	−82 ± 5[a]	150 ± 18[a]	68 ± 21[a]	38 ± 7[a]	439 ± 56[a]	558 ± 62[a]
Control	−81 ± 6[a]	145 ± 10[a]	64 ± 14[a]	33 ± 5[a]	474 ± 71[a]	587 ± 60[a]
Est/μmol·L^{-1}						
1	−82 ± 6[a]	148 ± 17[a]	65 ± 18[a]	32 ± 8[a]	407 ± 32[a]	522 ± 45[a]
10	−83 ± 5[a]	145 ± 18[a]	62 ± 19[a]	30 ± 8[a]	330 ± 75[a]	460 ± 95[a]
100	−82 ± 7[a]	140 ± 28[a]	58 ± 23[a]	20 ± 12[a]	229 ± 67[c]	418 ± 79[b]
Washout	−85 ± 2[a]	149 ± 15[a]	64 ± 15[a]	21 ± 6[a]	294 ± 65[b]	495 ± 95[a]

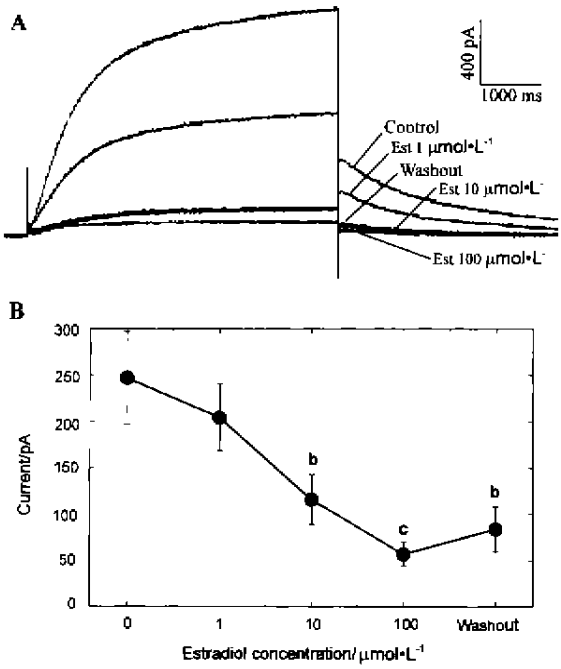

Fig 1. Block of I_K · tail by Est in guinea pig ventricular myocytes. A) Current tracings from a representative cell. B) concentration-dependent block of I_K·tail by Est. $n = 5$. $^bP < 0.05$, $^cP < 0.01$ *vs* control.

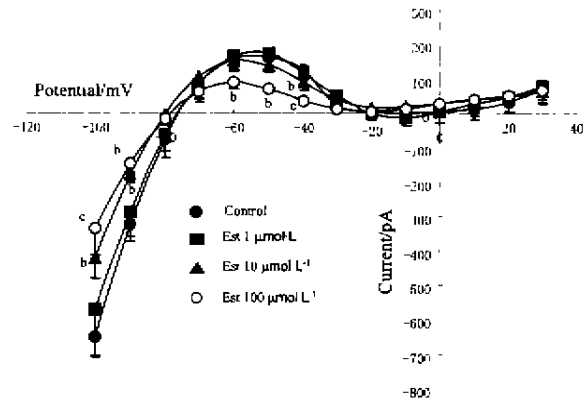

Fig 2. Effects of Est on I_{K1}. $n = 5$ ventricular myocytes of 5 guinea pigs. $^bP < 0.05$, $^cP < 0.01$ *vs* control.

depression of I_{K1}. The inward currents of I_{K1}, at E_t −100 mV and −90 mV, were depressed 36 % (from −652 pA ± 54 pA to −420 pA ± 60 pA, $P < 0.01$) and 44 % (from −321 pA ± 53 pA to −178 pA ± 24 pA, $P < 0.05$) of the control, respectively and outward currents, at E_t of −40 mV, 29 % (from 126 pA ± 25 pA to 90 pA ± 23 pA, $P < 0.05$, $n = 5$ cells of 5 guinea pigs). Est 100 μmol · L^{-1} had a more remarkable blocking effect on I_{K1}. The inward currents of I_{K1} at E_t of −100 mV and −90 mV were depressed respectively 49 % (from −652 pA ± 54 pA to −334 pA ± 78 pA, $P < 0.01$) and 55 % (from −321 pA ± 53 pA to −144 pA ± 27 pA, $P < 0.05$) and outward currents, at E_t of −60 mV, −50 mV and −40 mV, 43 % (from 150 pA ± 39 pA to 91 pA ± 20 pA, $P < 0.05$), 55 % (from 163 pA ± 23 pA to 72 pA ± 20 pA, $P < 0.05$) and 72 % (from 126 pA ± 25 pA to 38 pA ± 11 pA, $P < 0.05$) of control ($n = 5$, Fig 2).

DISCUSSION

Effects of estrogen on cardiovascular system are diverse, irregular and even contradictory with each other in some condition[10], which means the mechanism of estrogen is very complex. Although estrogen receptors were found in heart[11] and the direct effects of Est on heart were observed, the mechanism of the hormone is not clear yet. In the present study, we firstly demonstrated the inhibitory effects of Est on I_{K1} and I_K in single ventricular myocytes of guinea pig in a concentration-dependent manner. It suggested that the inhibitory effects of Est on K^+ channels might play an important role in the effects of Est on heart.

I_{K1} and I_K channels of myocardium are very important in maintaining normal RP and APD in electrophysiology of heart. Some reported that the blockade of K^+ channels could protect against arrhythmia[12]. The inhibitory effects of Est on I_{K1} and I_K may be another ionic basis of antagonizing experimental arrhythmia, besides I_{Ca} channel blocking[13]. In the experiment, APD decreasing and K^+ channels blocking seems contradictory because blockade of K^+ channels usually cause the APD shortening. Some reported that Est has a Ca^{2+} blocking effect[13] and the results of study in our laboratory definitely supported that. It suggested that Est blocked Ca^{2+} channel stronger than K^+ channels at the same time, which might be the main cause of APD shortening in myocytes. Interestingly, Est shortened APD in single cardiomyocytes[13,14], but in isolated papillary muscle we previously used[7], Est prolonged the APD significantly. Different effect of Est on APD

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ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Jul; 20 (7)
E-mail aps@server.shcnc.ac.cn Phn/Fax 86-21-6474-2629

between papillary muscles and myocytes has not been understood yet and should be paid attention to.

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雌二醇抑制豚鼠心室肌细胞内向整流和延迟整流钾通道电流[1]

R965.2

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关键词 雌二醇; 膜片箝技术; 心肌; 钾通道; 动作电位

目的: 研究雌二醇(Estradiol, Est)对心室肌细胞动作电位(AP)、内向整流钾通道电流(I_{K1})及延迟整流钾通道电流(I_K)的影响. **方法**: 全细胞膜片箝技术. **结果**: EST 10 μmol·L^{-1}使豚鼠心室肌细胞AP时程明显缩短, APD_{50}由给药前(474 ± 71) ms 缩短至(330 ± 75) ms ($P < 0.05$), Est 100 μmol·L^{-1}使APD_{50}缩短至(229 ± 67) ms ($P < 0.01$), 使APD_{90}由(587 ± 60) ms 缩短至(418 ± 79) ms ($P < 0.05$). Est 浓度依赖性地抑制 I_K 尾电流(I_K·tail), 10 μmol·L^{-1}浓度下, I_K·tail 减少 53 % ($P < 0.05$), 100 μmol·L^{-1}浓度下, I_K·tail 减少 80 % ($P < 0.05$). 10 μmol·L^{-1}以上浓度 Est 明显抑制 I_{K1}, 在 - 100 mV 刺激电压下, 内向电流最大抑制为 49 % ($P < 0.01$); 在 - 40 mV 刺激电压下, 外向电流最大抑制为 72 % ($P < 0.01$). 同时, Est 使 I_{K1}翻转电位向负电位方向移位(由 - 70 mV 变为 - 76 mV). **结论**: Est 对豚鼠心室肌细胞 I_{K1}和 I_K 通道具有明显的抑制作用.

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