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Estradiol potentiates antiarrhythmic and antioxidative effects of intermittent hypoxic rat heart[1]

ZHANG Yi, ZHONG Ning, ZHOU Zhao-Nian[2]
(*Shanghai Institute of Physiology, Chinese Academy of Sciences, Shanghai 200031, China*)

KEY WORDS estradiol; intermittent hypoxia; superoxide dismutase; malondialdehyde; arrhythmia; myocardial reperfusion injury

ABSTRACT

AIM: To study the effects of estradiol (Est) on antiarrhythmic and antioxidative effects of intermittent hypoxia in rat heart. **METHODS**: Ligating and loosening the coronary artery of rat to induce ischemic and reperfusion arrhythmias, using arrhythmia score (AS) to evaluate the arrhythmias, measuring the activity of superoxide dismutase (SOD) and the content of malondialdehyde (MDA) in myocardium. **RESULTS**: AS of arrhythmia induced by ischemia and reperfusion in intermittent hypoxia 28-d group (IH28) and in intermittent hypoxia with Est group (IH14-Est) are lower than that in control group (CON), respectively. AS of ischemic arrhythmia but not reperfusion arrhythmia in Est treated group (ESTG) was lower than that in CON. No significant difference in AS of ischemia and reperfusion existed among CON, vehicle group (VEH), and intermittent hypoxia 14-d group (IH14). The activity of SOD was higher and the content of MDA was lower in IH28 and in IH14-Est compared with that in CON. No significant difference of the activity of SOD and the content of MDA existed among CON, VEH, IH14, and ESTG. **CONCLUSION**: Est potentiated the antiarrhythmic and antioxidative effects of intermittent hypoxia on rat heart.

INTRODUCTION

Estrogens play a key role in sex difference of cardiovascular diseases and have many effects on cardiovascular system[1-3]. It has been reported that estradiol (Est) has antiarrhythmia effects, reducing the occurrence of ischemic- and reperfusion-induced ventricular arrhythmias[4,5]. Our previous work showed that Est possessed obvious electrophysiological effects on myocardium, prolonging action potential duration of papillary muscle of guinea pig and inhibiting the inward rectifier and delayed rectifier K^+ currents in guinea pig ventricular myocytes[6,7].

It has been proved that prior adaptation to intermittent hypoxia (IH) in an altitude chamber appreciably protects the heart against ischemic injury and prevents the arrhythmia[8,9]. It seems that both Est and IH have beneficial effects on heart. But the relationship between their effects have not been reported. The present study was to observe the effects of Est on antiarrhythmic and antioxidative effects of IH in rat heart.

MATERIALS AND METHODS

Animal groups Male Sprague-Dawley rats (n = 60), originally weighing 160 ± 24 g and finally weighing 273 g ± 31 g, were divided into six groups: control group (CON), intermittent hypoxia 14-d groups (IH14), IH 28-d group (IH28), estradiol treated groups (ESTG), vehicle (alcohol) group (VEH), and IH 14-d with Est groups (IH14-Est). The rats in IH14 and IH28 were exposed to hypoxia in the hypobaric chamber at 5000-m altitude (oxygen 11.1 %) 6 h daily for 14 and 28 d, respectively. The rats in ESTG were treated for 14 d with a daily subcutaneous injection of 17β-estradiol (100 $\mu g \cdot kg^{-1} \cdot d^{-1}$, E Merck, Germany) and the rats in VEH were given the alcohol in the same concentration as of ESTG. The animals in IH14-Est were given both 17β-estradiol (100$\mu g \cdot kg^{-1} \cdot d^{-1}$) and IH for 14 d. Except for breathing room air, CON animals lived in the same environment as IH animals with free access to food and water.

Animal preparation Rats were anesthetized with 45 mg/kg ip injection of sodium pentobarbital. After

[1] Project supported by the National Natural Science Foundation of China, No 3950052.
[2] Correspondence to Prof ZHOU Zhao-Nian.
Phn 86-21-6431-3251, ext 8500. Fax 86-21-6474-6305.
E-mail znzhou@server.shcnc.ac.cn
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tracheotomy, they were ventilated with room air (stroke volume of 55 strokes/min). Routine arterial blood gases were measured (ABL3 pH/Blood Gas Analyzer, Denmark). p_{O_2}, Sa_{O_2}, p_{CO_2} and pH were (15.00 ± 0.03) kPa, 98.2 % ± 0.08 %, (4.76 ± 0.25) kPa, and 7.35 ± 0.01, respectively. Body temperature was maintained at 37.0 ± 0.5 ℃. ECG lead Ⅱ, together with the blood pressure at the carotid, was continuously monitored and recorded by using a data acquisition system (PowerLab/8 s, ADInstrument, Australia) and a computer (IBM/PC). Heart rate was calculated from the R-R interval in ECG.

Coronary occlusion and reperfusion The chest was opened and pericardium was incised. A 6/0 nylon suture attached to a fine needle was placed under the left descending coronary artery. Regional myocardial ischemia could be produced by pulling the two ends of the suture through a plastic tube and pressing the tube against the surface of the myocardium, and then clamping the tube together with the suture. Reperfusion was initiated by declamping and removing the tube[10]. After a 15-min stabilization, rats were subjected to 15-min coronary artery occlusion followed by a 15-min reperfusion. Successful coronary arterial occlusion was indicated by an ischemic sign, ST-segment elevation in ECG, and reperfusion was confirmed by the reversal of the ST segment elevation immediately upon release of the ligation[11]. Ventricular arrhythmias during ischemia and reperfusion were defined in accordance with the guideline of the Lambeth Conventions for analysis of experimental arrhythmias[12] and quantified with Arrhythmia Score (AS) according to Johnston standard[13].

Measurement of serum Est Blood (1 mL) was collected from animals before coronary occlusion and reperfusion. Serum Est concentration was measured using enzyme immunoassay method[14].

Measurement of SOD and MDA in heart The hearts were quickly removed from the animal after experiments and homogenized. The supernatant was assayed by spectrophotometry. SOD activity was determined by inhibition of pyrogallol antioxidation[15] and MDA was measured according to Ohkawa method[16].

Statistics All data were presented as $\bar{x} \pm s$. The t-test was used to determine statistical significance. A P value < 0.05 was considered significant.

RESULTS

Serum Est levels Serum levels of Est in CON and IH14 were (121 ± 23) and (99 ± 18) ng/L, respectively, whereas animals that received Est had slightly higher levels of Est [(182 ± 32) ng/L in ESTG and (153 ± 25) ng/L in IH14-Est].

Effects of Est and IH on the arrhythmias No significant difference existed between IH14 and CON in AS during ischemia and reperfusion (2.6 ± 0.5 and 3.6 ± 0.3 in IH14 *vs* 4.3 ± 0.9 and 3.5 ± 0.7 in CON, $P > 0.05$). AS during ischemia and reperfusion in IH28 (1.2 ± 0.5 and 1.0 ± 0.5) were lower than CON ($P < 0.05$, Fig 1).

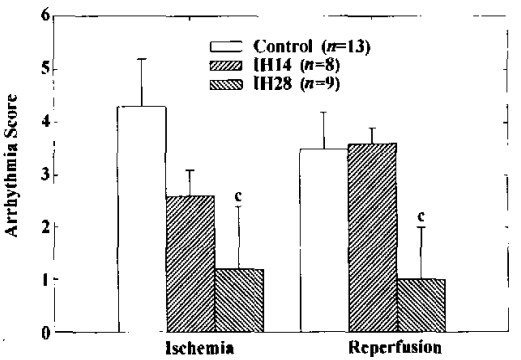

Fig 1. Effects of intermittent hypoxia on arrhythmia induced by ischemia and reperfusion in rat. $^cP < 0.01$ *vs* control.

AS of ischemic arrhythmia in VEH and reperfusion arrhythmia in VEH and ESTG were 3.2 ± 0.6, 3.7 ± 0.3, and 2.3 ± 0.3, respectively, showing no significant difference compared with CON ($P > 0.05$). AS of ischemia in ESTG was greatly lower (1.0 ± 0.4) compared with CON ($P < 0.05$, Fig 2).

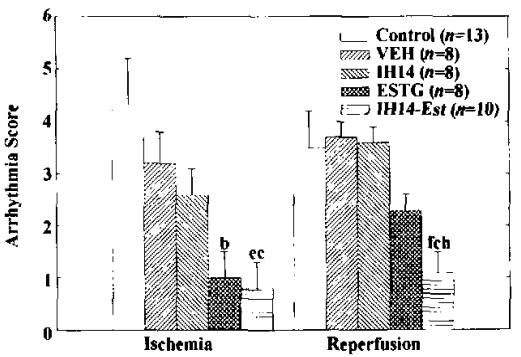

Fig 2. Effects of IH and Est on the arrhythmia during ischemia and reperfusion in rat. $^bP < 0.05$, $^cP < 0.01$ *vs* IH14. $^eP < 0.05$, $^fP < 0.01$ *vs* control. $^bP < 0.05$ *vs* ESTG.

AS of ischemia and reperfusion arrhythmias in IH14-Est were 0.8 ± 0.5 ($P < 0.05$) and 1.1 ± 0.5 ($P < 0.01$), respectively, significantly lower than CON.

Change of SOD and MDA in ESTG and IH

The activity of SOD and the content of MDA between IH28 and CON had significant difference [(1783 ± 170) U/g and (131 ± 11) nmol/g *vs* (1120 ± 178) U/g and (182 ± 7) nmol/g, $P < 0.05$]. In IH14-Est, the activity of SOD (1678 U/g ± 158 U/g) was higher and content of MDA (102 nmol/g ± 10 nmol/g) was significantly lower than CON ($P < 0.05$, $P < 0.01$). Both of the above in IH14, VEH, and ESTG had no significant differences compared with CON ($P > 0.05$, Tab 1).

Tab 1. Effects of Est and IH on the activity of SOD and the content of MDA in the myocardium of rat. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ ***vs* control.** $^dP < 0.05$ ***vs* IH14.** $^eP < 0.05$ ***vs* ESTG.**

Group	*n*	SOD (U/g)	MDA (nmol/g)
Control	8	1120 ± 178	182 ± 7
VEH	6	1210 ± 102[a]	170 ± 23[a]
IH28	8	1783 ± 170[b]	131 ± 11[b]
IH14	6	1470 ± 180[a]	147 ± 19[a]
ESTG	6	1492 ± 154[a]	150 ± 19[a]
IH14-Est	6	1678 ± 158[b]	102 ± 10[cdh]

DISCUSSION

In this report, we demonstrated firstly that Est potentiated antiarrhythmic and antioxidative effects of intermittent hypoxia in rat. The results clearly showed that intermittent hypoxia had antiarrhythmic and antioxidative effects during ischemia and reperfusion in rat heart, which is in accordance with some earlier reports[8,9]. Both effects of intermittent hypoxia developed earlier after treating with a low level of Est that only had antiarrhythmic effect during ischemia. Antiarrhythmic effects of Est have been reported in recent years[4,5], but there is no agreement on the dose of Est that produce antiarrhythmia. Some have reported that only pharmacological doses of estradiol exerted protective and antiarrhythmic effects[17], others reported that physiological level of Est had effects against the arrhythmia[5]. The level of serum Est of rats in our experiment was in the physiological range[18] according to the assay of serum Est, which suggested that physiological level of Est had an effect against ischemic arrhythmia. But Est showed more powerful effect to potentiate the antiarrhythmic effect of intermittent hypoxia. It also suggested that Est in the lower level had no antioxidative effect.

The mechanism by which Est potentiated antiarrhythmic and antioxidative effects of intermittent hypoxia is not known. One mechanism involved may be the direct effect of Est on heart. It had been reported that Est prolonged the action potential duration in papillary muscle of guinea pig and inhibited the inward rectifier and delayed rectifier K^+ currents, as well as Ca^{2+} current, in guinea pig ventricular myocytes[6,7,19]. All above mentioned effects may constitute the electrophysiological basis of Est effects. Est has an antioxidant potential that also contributes to its antiarrhythmic effect[4]. It has been also reported that Est regulated the activity of sympathetic nervous system and inhibited the catecholamine release[20]. Thus this regulatory action of Est may constitute another mechanism. Estrogen receptor has been found in heart, which implies heart to be an estrogen target organ[21]. Further studies are needed to elucidate whether estrogen receptor in heart is involved in mediating the potentiation of Est on antiarrhythmic and antioxidative effects of intermittent hypoxia.

It is well known that ischemic heart diseases are common in clinic and ischemic arrhythmia is one of leading cause of death. Up to now, all kinds of antiarrhythmic drugs have not been satisfactory and have many side effects. The combination of intermittent hypoxia and Est, may a newmethod, to prevent and arrest the arrhythmia induced by ischemia.

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雌二醇加强大鼠心脏间歇性低氧抗心律失常及抗氧化作用[1]

张 翼, 钟 宁, 周兆年[2]
(中国科学院上海生理研究所, 上海 200031, 中国)

关键词 雌二醇; 间歇性低氧; 超氧化物歧化酶; 丙二醛; 心律失常; 心肌再灌注损伤

目的: 观察雌二醇对大鼠心脏间歇性低氧抗心律失常及抗氧化作用的影响. **方法**: 结扎和放松冠脉造成心脏缺血及再灌注, 以诱发心律失常; 并测定心室肌超氧化物岐化酶(SOD)活性和丙二醛(MDA)含量. **结果**: 与对照组相比较, 间歇性低氧 28 天组和间歇性低氧 14 天加雌二醇组在缺血期和再灌注期, 心律失常评分(AS)都显著降低. 单纯雌二醇处理组的缺血期 AS 明显降低, 而再灌注期 AS 无变化. 间歇性低氧 14 天组、溶剂组的 AS 无显著变化. 间歇性低氧 28 天组和间歇性低氧 14 天加雌二醇组的 SOD 活性明显升高, MDA 含量明显下降. 在间歇性低氧 14 天组、溶剂组和单纯雌二醇处理组, SOD 活性和 MDA 含量与对照组相比较无明显差异. **结论**: 雌二醇可加强间歇性低氧大鼠抗缺血、再灌注心律失常及心肌的抗氧化作用.

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