Antiarrhythmic effect of endothelin-A receptor antagonist on acute ischemic arrhythmia in isolated rat heart[1]

XU Hong, LIN Li, YUAN Wen-Jun[2]

Department of Physiology, Second Military Medical University, Shanghai 200433, China

KEY WORDS endothelin receptors; arrhythmia; myocardial ischemia; rats

ABSTRACT

AIM: To observe the effects of endothelin receptor subtype A (ET_A) and B (ET_B) antagonists on acute ischemic arrhythmia in isolated rat heart, and to determine whether endogenous endothelin (ET) was implicated in the pathophysiological process of arrhythmia induced by acute myocardial ischemia. **METHODS:** Fifty-three SD male rats were randomized into 8 groups. Heart was isolated and perfused in Langendorff mode and acute ischemia model was established by ligation of the left anterior descending (LAD) coronary artery. The effects of ET_A receptor antagonist PD156707 and ET_B receptor antagonist IRL1038 on arrhythmia, heart function, the myocardial activity of superoxide dismutase (SOD), and the content of melondialdehyde (MDA) during the acute 60-min ischemic phase were analyzed. **RESULTS:** Pretreatment with PD156707 (20-500 nmol/L) dose-dependently improved the ischemic isolated heart function, enhanced SOD activity and decreased MDA content in the ischemic myocardium, and suppressed the acute ischemic arrhythmia. Conversely pretreatment with IRL1038 did not change the heart function, SOD activity, MDA content, and the acute ischemic arrhythmia significantly as compared with the occlusion control. **CONCLUSION:** ET_A receptor antagonist effectively improved heart function, enhanced anti-oxidative function of the myocardium and reduced arrhythmia during the acute ischemic phase in isolated rat hearts, while ET_B receptor antagonist did not exert protective effects, suggesting that endogenous ET-1, acting through ET_A receptor, may be one of the factors implicated in arrhythmia and impairment to heart function during the acute ischemic phase.

INTRODUCTION

Endothelin (ET) is believed to be involved in a variety of pathophysiological cardiovascular activities including myocardial infarction, heart failure, hypertension, and myocardial ischemic/reperfusion injury. Endothelin receptors, including ET_A and ET_B subtypes, are distributed abundantly in cardiac cells and coronary arteries. Intracoranary administration of ET-1 resulted in severe arrhymia in pigs, severity and mortality of which were dose-dependent[1]. Similarly ET-1 could induce arrhythmia in rat hearts both *in vivo* and *in vitro*[2,3]. The arrhythmogenic effect of ET-1 was proposed to be attributed to its proarrhythmic effects[4]. We previously described the arrhythmogenic effect of exogenous ET-1 in cats, which was mainly mediated by ET_A receptor[5]. We observed that BQ123, a specific ET_A

[1] Project supported by the Major State Basic Research Development Program of People's Republic of China (No. G2000056905) and the National Natural Science Foundation of China (No. 30070306).

[2] Correspondence to Prof YUAN Wen-Jun.
Phn/Fax 86-21-2507-0262. E-mail yuanwj@smmu.edu.cn

Received 2001-12-31 Accepted 2002-10-14

receptor antagonist, significantly suppressed the acute ischemic arrhythmia induced by occlusion of the left anterior descending coronary artery in cats, while ET_B receptor antagonist IRL 1038 failed to be antiarrhythmic[6]. Besides, the study has shown that ET-1 mRNA anti-sense oligonucleotide prevented the acute ischemic arrhythmia in rats[7].

Whether ET is implicated in arrhythmia under the pathophysiological condition of increased endogenous ET such as in myocardial ischemia is a problem of great interest. Most previous studies on ischemic/reperfusion arrhythmia have been focused much attention on arrhythmia during the reperfusion following ischemia, with little attention on arrhythmia during ischemia. As severe ischemic arrhythmia is the main cause of death in clinical acute myocardial infarction, study on the role and mechanism of endogenous ET in acute ischemic arrhythmia is of great significance.

In an attempt to shed more light on the pathophysiological significance of ET-1 in ischemic arrhythmia, the present study observed the effects of ET receptor antagonists on acute ischemic arrhythmia caused by occlusion of the left anterior descending (LAD) coronary artery in isolated rat heart.

MATERIALS AND METHODS

Preparation of isolated rat heart Fifty-three Sprague-Dawley male rats aged 80-100 d and weighing 250-320 g were randomized into the following groups: normal perfusion group (n=7), occlusion control group (n=7), ET_A receptor antagonist PD156707 (a gift from Prof SHEN You-Tang, USA) 20 nmol/L, 100 nmol/L, and 500 nmol/L groups (n=7), and ET_B receptor antagonist IRL 1038 (a gift from Prof ZHU Yuan-Xiang, USA) 20 nmol/L, 100 nmol/L, and 500 nmol/L groups (n=7).

The rats were anesthetized with ip injection of 60 mg/kg pentobarbital with heparin 750 U. The heart was removed right after anesthesia and perfused in the Langendorff mode with the Tyrode's solution (37 °C) bubbled with 95 % O_2 and 5 % CO_2 at a constant pressure of 100 cm H_2O, the left ventricle was inserted with a water-ballon connected to a pressure transducer to measure intraventricular pressure. Three 0.1-mm in diameter silver wire electrodes were placed in the right ventricle, right atrium, and left atrium to record electrocardiogram. A 6/0 nylon thread was placed around the left anterior decending coronary artery. The suture point was 1.0 mm away from both sides of LAD and 3 mm away from the starting point of LAD. The acute myocardial ischemia was induced by LAD occlusion resulting from thread ligation.

Experimental protocol Heart was pre-perfused for 15 min to stablize, and then PD156707 or IRL1038 were added to the perfusion fluid. Five minnutes after drug administration, LAD was occluded. Thirty minutes later, normal Tyrode's solution was used instead of drug, and the electrocardiogram and intraventricular pressure were monitored for another 30 min. At the end of the experiment, the left ventricular anterior wall, partial side wall, and apex were sampled off to measure the activity of myocardial superoxide dismutase (SOD)[8] and the content of malonyldialdehyde (MDA) by the microtrihydroxy benzene and trimethylolpropane (TMP) methods, respectively[9].

Arrhythmia analysis According to the Lambeth convention[10], arrhythmia is classified as single ventricular premature beat (VPB), salvo, ventricular tachycardia (VT), and ventricular fibrillation (VF). Arrhythmia scoring (AS) was done according to Johnston criterion[11].

Statistical analysis All data were expressed as mean±standard deviation and statistically analyzed with ANOVA followed by the Newman-Keuls test.

RESULTS

Changes of heart rate after LAD occlusion in isolated rat heart LAD occlusion point of time is regarded as the zero point of the time axis. In the normal perfusion group, heart rate began to fall about 40 min after perfusion; in the occlusion control group, heart rate began to slow down markedly 20 min into occlusion; in the PD156707 group, decrease in heart rate began much later, and in the IRL1038 group, there was no significant difference in heart rate as compared with the occlusion control group (Tab 1).

Effects of PD156707 and IRL1038 on ischemic isolated heart function In the occlusion control and PD156707 groups (Fig 1), LAD occlusion resulted in significant decrease in left ventricular systolic pressure (LVSP) and coronary flow (CF) as compared with the normal control group (P<0.01). However, 20 min after occlusion, LVSP began to rise in the PD156707 500 nmol/L and 100 nmol/L groups, to a level significantly higher than that of the occlusion control (P< 0.01); 30 min after occlusion, CF began to rise, show-

Tab 1. Heart rate (HR) before and during LAD occlusion in isolated rat hearts pretreated with PD156707 and IRL1038. *n*=7 isolated rat hearts. Mean±SD. $^{c}P<0.01$ *vs* normal control group. $^{f}P<0.01$ *vs* occlusion control group. $^{i}P<0.01$ *vs* pre-occlusion (-2 min).

Group	HR/beat·min^{-1}								
	-15 min	-2 min	5 min	10 min	20 min	30 min	40 min	50 min	60 min
Normal control	256±7	259±6	255±6	260±8	260±7	248±5	237±8 ^{i}	226±9 ^{i}	220±10^{i}
Occlusion control	255±7	255±7	254±7	255±8	221±14^{ci}	212±12^{ci}	193±11^{ci}	184±6^{ci}	184±7^{ci}
PD156707 20 nmol/L	262±9	258±8	261±14	260±15	234±9^{ci}	214±4^{ci}	194±13^{ci}	179±9^{ci}	181±5^{ci}
100 nmol/L	262±7	258±5	257±8	254±8	259±7^{f}	238±7^{cfi}	218±6^{cfi}	204±7^{cfi}	207±6^{cfi}
500 nmol/L	263±9	261±2	259±10	258±11	253±5^{f}	232±10^{cfi}	220±11^{cfi}	206±6^{cfi}	204±5^{cfi}
IRL1038 20 nmol/L	260±8	260±6	260±4	258±5	223±7^{cf}	214±8^{cf}	194±10^{cf}	191±10^{cf}	182±10^{cf}
100 nmol/L	261±8	261±6	260±5	257±5	224±8^{cf}	215±8^{cf}	194±10^{cf}	192±9^{cf}	182±10^{cf}
500 nmol/L	262±10	261±7	258±10	260±10	219±9^{cf}	215±7^{cf}	199±14^{cf}	188±13^{cf}	181±6^{cf}

ing significant difference from the occlusion control ($P<0.01$). Twenty minutes after occlusion, the left ventricular diastolic pressure (LVDP) of the occlusion control and drug groups rose significantly as compared with the normal perfusion group ($P<0.01$), and 30 min after occlusion, the rise was more evident in the occlusion control and PD156707 20 nmol/L group as compared with the PD156707 500 nmol/L and 100 nmol/L group ($P<0.01$).

Before LAD occlusion, LVSP and CF showed no significant difference between the normal control, occlusion control, and IRL1038 groups. After occlusion, LVSP and CF decreased to 60 percent of the values before occlusion. LVSP and CF of the occlusion control and IRL1038 groups were significantly lower than those of the normal control (Fig 2). Forty minutes after occlusion, LVSP of the occlusion control and IRL1038 groups began to rise significantly as compared with the normal control ($P<0.01$). There was no significant difference between the occlusion control and IRL1038 groups.

Assessment of ischemic arrhythmia No salvo, ventricular tachycardia, ventricular fibrillation, or prolonged arrest were observed before LAD occlusion. In the normal perfusion control, only ventricular premature beats were observed; there was no VT or VF, AS=0.17±0.37 (Tab 2). VT or VF occurred after LAD occlusion in all animals of the occlusion control group ($n=7$), and the AS averaged 3.3±0.5, higher than the normal perfusion control. Ischemic arrhythmia occurred on a multi-phase base between 3 and 25 min, the peak being between 19 and 22 min; there were only a few VPB after 25 min (Tab 2). Short-duration VT was observed only in one heart of the PD156707 500 nmol/L group, and there was no significant difference in the AS (0.5±0.8) and the counts of arrhythmia as com-

Tab 2. Effect of PD156707 and IRL1038 on the acute ischemic arrhythmia in isolated rat hearts. *n*=7 isolated rat hearts. Mean±SD. $^{b}P<0.05$, $^{c}P<0.01$ *vs* normal control group. $^{e}P<0.05$, $^{f}P<0.01$ *vs* LAD occlusion control group.

Arrhythmia	Arrhythmia counts				VT duration/s	Incidence/%		Arrhythmia score
	Single VPB	Salvos	VT	Total		VPB	VT/VF	
Normal control	33±8	3±1	0±0	40±10	0±0	100.0	0	0.17±0.37
Occlusion control	62±8^{c}	9±3^{c}	214±36^{c}	297±33^{c}	21±3^{c}	100.0	100.0^{c}	3.3±0.5^{c}
PD156707 20 nmol/L	63±7^{c}	8±3^{b}	234±69^{c}	311±72^{c}	23±7^{c}	100.0	100.0^{c}	3.1±0.4^{c}
100 nmol/L	36±9^{e}	6±2	0±0^{f}	48±13^{f}	0±0^{f}	100.0	0	0.5±0.5
500 nmol/L	39±10^{e}	7±3	4±8^{f}	56±22^{f}	1±1^{f}	100.0	16.7	0.5±0.8
IRL1038 20 nmol/L	66±11^{c}	12±4^{c}	298±152^{c}	534±149	42±14^{c}	100.0	100.0^{c}	3.0±1.4^{c}
100 nmol/L	69±15^{c}	12±4^{c}	361±110^{c}	456±106^{c}	35±12^{c}	100.0	100.0^{c}	3.2±0.4^{c}
500 nmol/L	65±15^{c}	14±5^{c}	427±96^{c}	522±91^{c}	41±10^{c}	100.0	85.7^{c}	3.0±0.6^{c}

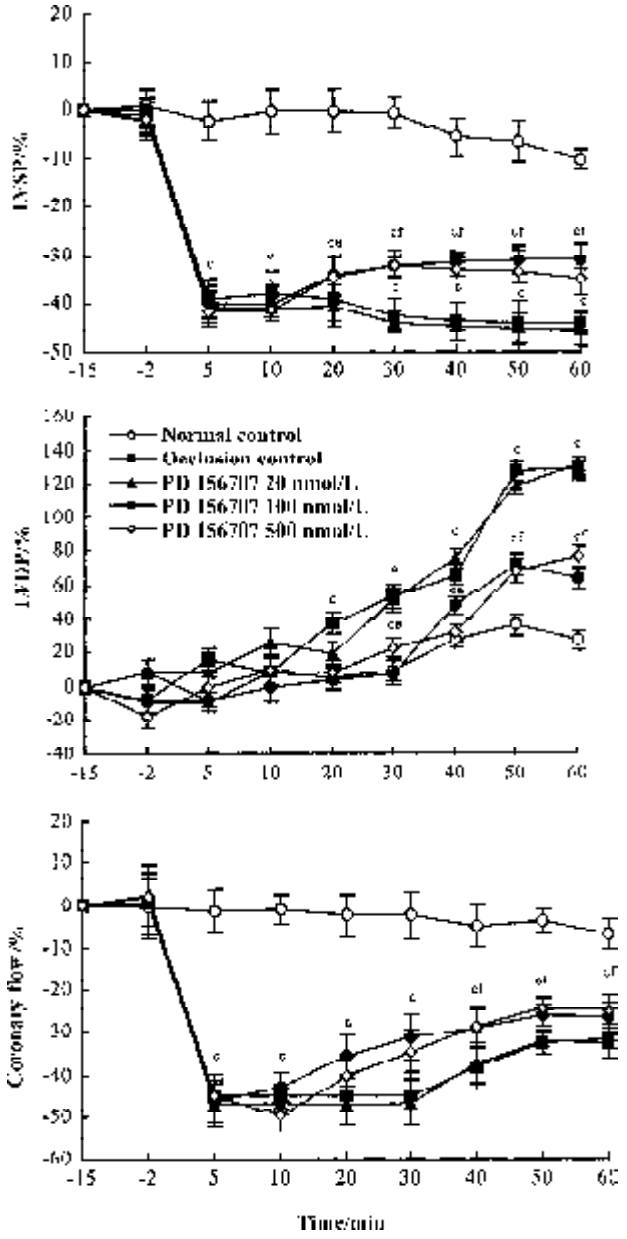

Fig 1. The percentage of heart function changes before and during LAD occlusion in isolated rat hearts pretreated with PD156707. n=7 isolated rat hearts. $^cP<0.01$ *vs* normal control group. $^eP>0.05$, $^fP<0.01$ *vs* occlusion control group.

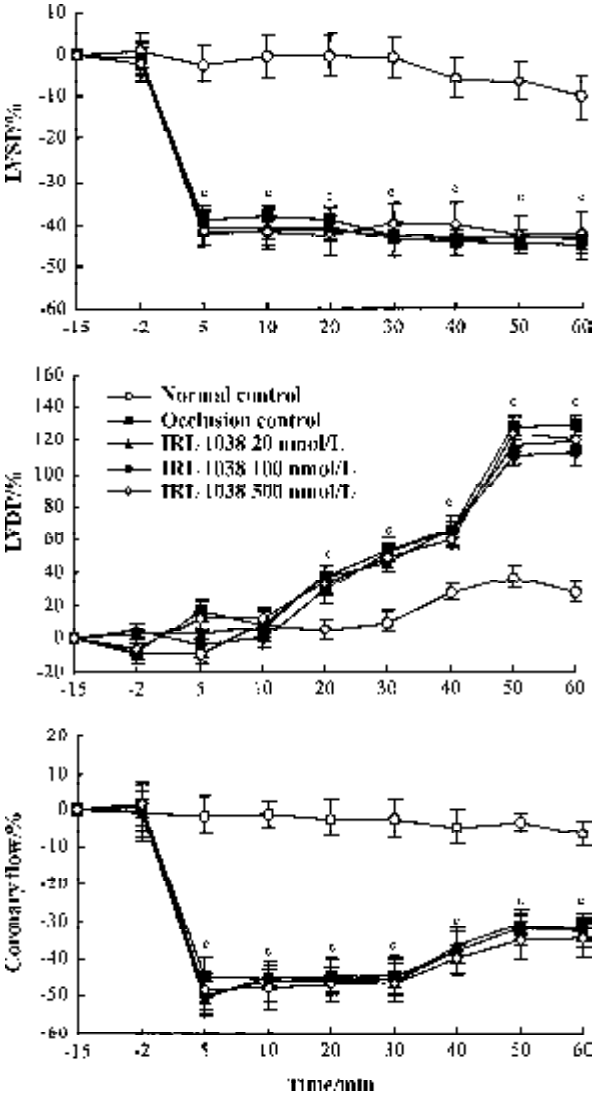

Fig 2. The percentage of heart function changes before and during LAD occlusion in isolated rat hearts pretreated with IRL 1038. n=7 isolated rat hearts. $^cP<0.01$ *vs* normal control group.

pared with the normal control group, but the number of VPBs and VT decreased significantly as compared with the occlusion control group (Fig 3). There was no VT/VF in the PD156707 100 nmol/L group, and AS (0.5±0.5) and arrhythmia counts showed no significant difference from the normal perfusion control, but lower than the occlusion control group (P<0.01). In the PD156707 20 nmol/L group, VT/VF was observed in all 7 hearts, and the AS (3.1±0.4) and arrhythmia counts were greater than that of the normal perfusion control (P<0.01) but was not different from that of the occlusion control. In the IRL1038 500 nmol/L group, the AS (3.0±0.6) was higher than the normal perfusion control (P<0.01), but was insignificantly different from the occlusion control, and VT happened in 6 of the 7 hearts. In the IRL1038 100 nmol/L group, the AS (3.2±0.4) was different from the normal perfusion control (P<0.01) but was not different from the occlusion control; distribution of arrhythmia was much the same as that of the occlusion control; VT happened in all 7 hearts, including reversible VF in 2 hearts. In the IRL1038 20 nmol/L group, AS=3.0±1.4, and VT happened in 4 hearts, including reversible VF in one heart. There was no difference in the numbers of arrhythmia between

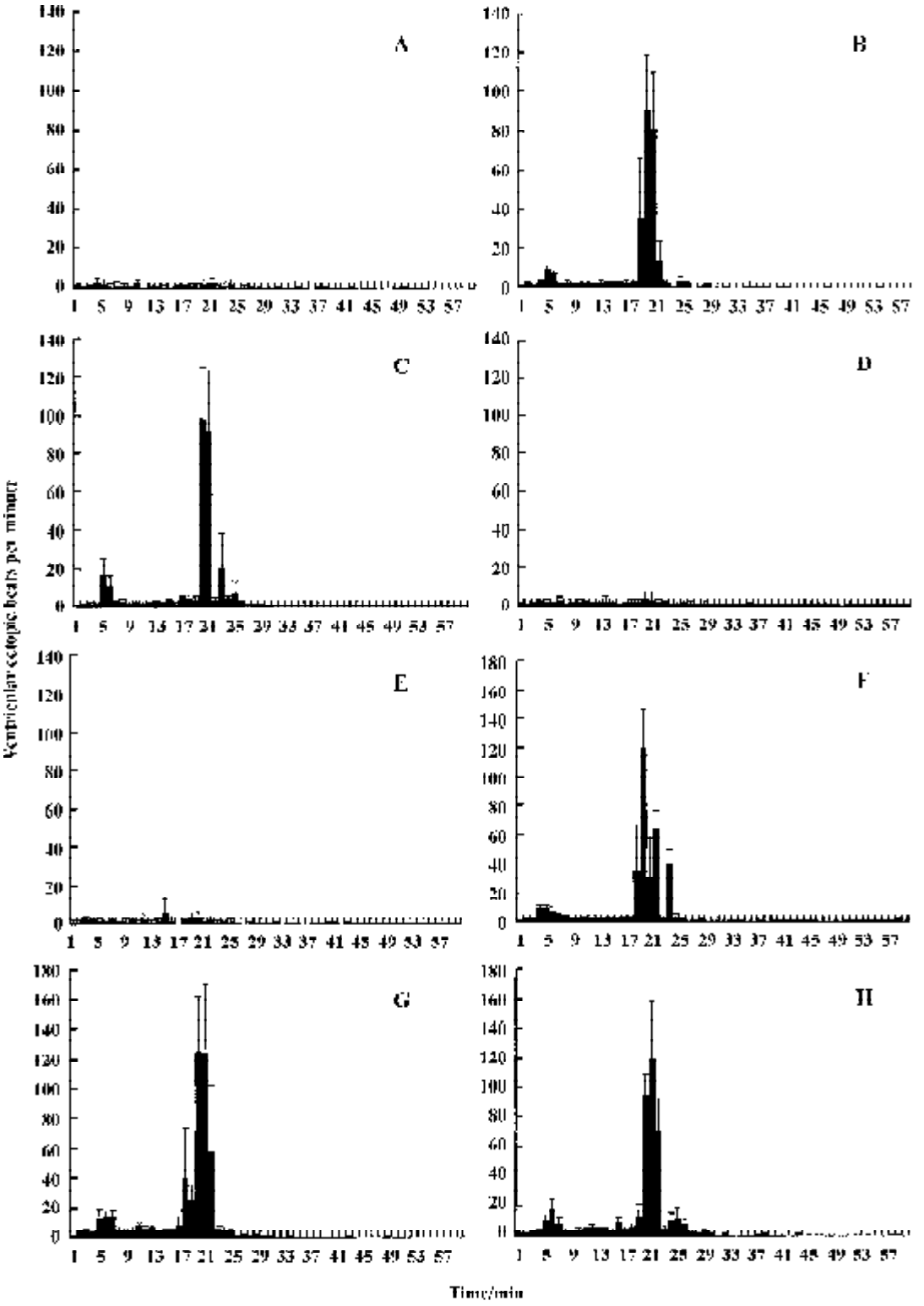

Fig 3. Distribution of the ventricular ectopic beats in 1-min intervals during 60-min occlusion of the LAD in perfused rat hearts. A) Normal control; B) LAD occlusion control; C) PD156707 20 nmol/L; D) PD156707 100 nmol/L; E) PD156707 500 nmol/L; F) IRL1038 20 nmol/L; G) IRL1038 100 nmol/L; H) IRL1038 500 nmol/L. *n*=7. Mean±SD.

the occlusion control and IRL1038 groups.

Effects of PD156707 and IRL1038 on SOD activity and MDA level of ischemic myocardium in isolated rat heart Ischemic myocardial SOD activity

was (1144±134) U/g in the occlusion control group (Tab 3), significantly lower than that of the normal perfusion group ($P<0.01$), and MDA was (180±10) nmol/g, significantly higher than that of the normal perfusion group ($P<0.01$). SOD activity of the PD156707 100 nmol/L and 500 nmol/L groups was (1469±138) U/g and (1470±163) U/g, respectively, significantly lower than that of the normal perfusion group ($P<0.01$) but higher than that of the occlusion control ($P<0.05$), and MDA was (120±20) nmol/g and (160±7) nmol/g, significantly higher than that of the normal perfusion group ($P<0.01$) but lower than that of the occlusion control ($P<0.05$). In the PD156707 20 nmol/L group, SOD was (1178±103) U/g and MDA was (162±12) nmol/g, both showing significant difference from the normal perfusion group ($P<0.01$) and insignificant difference from the occlusion control. SOD of the IRL1038 20 nmol/L, 100 nmol/L, and 500 nmol/L groups was (1159±168) U/g, (1244±146) U/g, and (1181±116) U/g, respectively, and MDA was (176±34) nmol/g, (187±14) nmol/g, and (186±15) nmol/g, respectively, showing significant difference from the normal perfusion group, but the difference was not significant as compared with the occlusion control.

Tab 3. Effect of PD156707 and IRL1038 on the activity of SOD and content of MDA in ischemic myocardium from isolated rat hearts. n=7 isolated rat hearts. Mean±SD. $^{b}P<0.05$, $^{c}P<0.01$ *vs* normal control group. $^{e}P<0.05$, $^{f}P<0.01$ *vs* LAD occlusion group.

Treatment	SOD/U·g^{-1}	MDA/nmol·g^{-1}
Normal control	1955±103	97±10
Occlusion control	1144±134^{c}	180±10^{c}
PD156707 20 nmol/L	1178±103^{c}	162±12^{c}
100 nmol/L	1469±138^{ce}	120±20^{bf}
500 nmol/L	1470±163^{ce}	160±7^{ce}
IRL1038 20 nmol/L	1159±168^{c}	176±34^{c}
100 nmol/L	1244±146^{c}	187±14^{c}
500 nmol/L	1181±116^{c}	186±15^{c}

DISCUSSION

ET_A and ET_B receptors have been determined to be distributed in rat heart. Activation of ET_A and ET_B receptors results in different cardiovascular effects. Non-specific ET receptor antagonist, specific ET_A receptor antagonist, ET monoclonal antibody, and ET converting enzyme inhibitor may favorably affect the infarction size of acute ischemia/reperfusion myocardium, myocardial function, and endothelial function. ET_A receptor antagonist BQ123 and BQ610 prevented the ischemic arrhythmia induced by LAD occlusion in cats, while the same effect was not observed in ET_B receptor antagonist IRL1038[11,12]. BQ123 has also been described to protect the ultrastructure of ischemic myocardium in cats[13]. Alexiou *et al*[14] also reported that endogenous ET might be the main cause of arrhythmia. ET-1 may induce myocardial ischemia, resulting in arrhythmia directly[15]. Becker *et al*[16] studied ventricular arrhythmia under the condition of LAD occlusion and believed that ET played a key role in this process. It is reported that ET_A receptor antagonist could lessen ventricular arrhythmia induced by intracardial injection of ET-1[17].

To exclude the constitutional influence of innervation and humoral factors, the present study used isolated rat heart to establish the acute ischemia model by ligating LAD, whereby occurrence of arrhythmia and heart function change were observed before and during drug intervention. The results showed that ET_A receptor antagonist PD156707 reduced the incidence of heart arrest, VT, VF, and other forms of ventricular arrhythmias. Conversely, pretreatment of the acute ischemic isolated heart with ET_B receptor antagonist IRL1038 failed to antagonize the acute ischemic arrhythmia and improve the ischemic heart function.

Oxygen free radical produced in myocardial ischemia/reperfusion is an important factor of myocardial impairment. ET_A receptor antagonist PD156707 can maintain the activity of SOD in ischemic myocardium at a certain extent. The result clewed that mechanism of PD156707 to reduce acute ischemic arrhythmia may be related to scavenge of oxygen free radical. The present result coincided with the previous report that radical scavengers alleviated reperfusion-induced arrhythmia[18], and that ET receptor antagonist maintained myocardial SOD activity and improve heart function in guinea pigs with ischemic dysfunction[19]. We previously observed that the arrhythmogenic effect of exogenous ET-1 was related to the increase of free radicals. The present experiment observed whether pretreatment with ET receptor antagonist had any effect on the anti-oxidative process in the ischemic myocardium of the isolated rat heart, and for the purpose of finding out the relations between oxidative mechanism and endogenous ET-1.

These results showed that ET_A receptor antagonist dose-dependently prevented the acute ischemic arrhythmia induced by LAD occlusion, maintained SOD activity of the ischemic myocardium, facilitated scavenge of radicals, and reduced accumulation of lipid perioxide. Differently, ET_B receptor did not exert the same effects, suggesting that endogenous ET plays an important role in the genesis of ischemic arrhythmia and injuries in isolated rat heart, among which ET_A receptor may work as a functional receptor.

ACKNOWLEDGMENT We are very grateful to Prof ZHANG Shun-Xing for his revision of the manuscript.

REFERENCES

1 Ezra D, Goldstein RE, Czaja JF, Feuerstein GZ. Lethal ischemia due to intracoronary endothelin in pigs. Am J Physiol 1989; 257: H339-43.
2 Tang J, Tang ChSh, Li ZhSh. Effect of endothelin on the cardiovascular function in rats. Acta Physiol Sci 1989; 9: 22-6.
3 Yorikane R, Koike H. The arrhythmogenic action of endothelin in rats. Jpn J Pharmacol 1990; 53: 259-63.
4 Salvati P, Chierchia S, Dho L, Ferrario RG, Parenti P, Vicedomini G, *et al*. Proarrhythmic activity of intracoronary endothelin in dogs: relation to the site of administration and to changes in regional flow. J Cardiovasc Pharmacol 1991; 17: 1007-14.
5 Yuan WJ, Fu WJ, Rong WF, Xia JH, Zhu BW, Han H. Antagonistic effect of verapamil on the arrhythmia produced by administration of endothelin-1 in rats. CAPS News Commun 1991; 10 (1 Suppl 6): 39-40.
6 Yuan WJ, Rong WF, Yan Y, Zhu BW, Han H. Arrhythmia produced by administration of endothelin in left anterior descending coronary artery in cats. Pathophysiology 1994; 1 Suppl: 481-3.
7 Lin L, Yuan WJ, Chu XL, Xu H, Li L, Ren AJ. Preventive effect of endothelin-1 mRNA antisense oligonucleotide on acute myocardial ischemic arrhythmia in rats. Acta Physiol Sin 1999; 51: 533-40.
8 Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 1974; 47: 469-74.
9 Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95: 351-8.
10 Walker MJ, Curtis MJ, Hearse DJ, Campbell RW, Janse MJ, Yellon DM, *et al*. The Lambeth Conventions: guidelines for the study of arrhythmias in ischaemia infarction, and reperfusion. Cardiovasc Res 1988; 22: 447-55.
11 Lin L, Yuan WJ, Zhu BW, Rong WF, Han H. Antagonism of BQ610 on the acute myocardial ischemic arrhythmia in cats. Acad J Sec Milit Med Univ 1999; 20: 146-51.
12 Lv Ping, Yuan WJ. The effect of BQ123 on the heart function of acute ischemic myocardia. Chin J Cardiac Funct 1994; 6: 172-5.
13 Lin L, Yuan WJ. Protective effect of endothelin receptor subtype A antagonist BQ610 on the ultrastructure of acute ischemic myocardium in cats. Chin J Pathol 1999; 28: 281-4.
14 Alexiou K, Dschietzig T, Simsch O, Laule M, Hundertmark J, Baumann G, *et al.* Arrhythmogenic effects induced by coronary conversion of pulmonary big endothelin to endothelin: aggravation of this phenomenon in heritable hyperlipidemia. J Am Coll Cardiol 1998; 32: 1773-8.
15 Szabo T, Geller L, Merkely B, Selmeci L, Juhasz-Nagy A, Solti F. Investigating the dual nature of endothelin-1: ischemia or direct arrhythmogenic effect? Life Sci 2000; 66: 2527-41.
16 Becker R, Merkely B, Bauer A, Geller L, Fazekas L, Freigang KD, *et al*. Ventricular arrhythmias induced by endothelin-1 or by acute ischemia: a comparative analysis using three-dimensional mapping. Cardiovasc Res 2000; 45: 310-20.
17 Merkely B, Szabo T, Geller L, Kiss O, Horkay F, Raschack M, *et al*. The selective endothelin-A-receptor antagonist LU 135.252 inhibits the direct arrhythmogenic action of endothelin-1. J Cardiovasc Pharmacol 2000; 36 (5 Suppl 1): S314-6.
18 Woodward B, Zakaria MN. Effect of some free radical scavengers on reperfusion induced arrhythmias in the isolated rat heart. J Mol Cell Cardiol 1985; 17: 485-93.
19 Maczewski M, Beresewicz A. The role of endothelin, protein kinase C and free radicals in the mechanism of the post-ischemic endothelial dysfunction in guinea-pig hearts. J Mol Cell Cardiol 2000; 32: 297-310.

Xu H *et al* / Acta Pharmacol Sin 2003 Jan; 24 (1): 37-44

~~~~~~
~~~~~~