Arrhythmogenic Action of Endothelin-$1_{1–31}$ Through Conversion to Endothelin-$1_{1–21}$

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Endothelin (ET)-$1_{1–21}$ is known to play an important role in the pathogenesis of acute ischemic arrhythmia. In the present study, we attempted to determine whether administration of ET-$1_{1–31}$ would result in arrhythmia in perfused isolated rat hearts. Forty-eight Sprague-Dawley rats weighing ~250–350 g were randomized into 6 groups. Heart was isolated and perfused in a Langendorff mode. The effects of ET-$1_{1–31}$ on arrhythmia, heart rate, coronary flow, and heart function were analyzed. Perfusion with 1 n*M* ET-$1_{1–31}$ resulted in frequent ventricular ectopic beats (VEBs) and ventricular tachycardia (VT). Overall VEB was 128.0 (~66.0–1015.0), and the arrhythmia score (AS) was 2.18 ± 0.87; both were significantly higher than those of the control group ($P < 0.01$). Pretreatment with perfusion of 10 n*M* of the ETA-receptor antagonist BQ_{123} markedly attenuated the occurrence of VEB and VT induced by ET-$1_{1–31}$. AS in 10 n*M* BQ_{123} group was significantly lower than that in 1 n*M* ET-$1_{1–31}$ group ($P < 0.01$). The arrhythmia induced by 1 n*M* ET-$1_{1–31}$ was partially but significantly reduced by phosphoramidon (1 μ*M*), a neutral endopeptidase/ET-converting enzyme inhibitor. ET-$1_{1–31}$ *per se* caused arrhythmia in perfused isolated rat hearts. This arrhythmogenic action is in part mediated by ET_A receptor and may be attributed mainly to the conversion of ET-$1_{1–31}$ to ET-$1_{1–21}$. Exp Biol Med 231:937–941, 2006

Key words: endothelin-$1_{1–31}$; arrhythmia; rats; hearts; phosphoramidon

Introduction

Endothelin (ET) is a 21-amino acid polypeptide that exhibits various physiological actions including vascular constriction, cardiac hypertrophy, mitogenesis, and apoptosis (1, 2). ETs comprise three isopetides, ET-1, ET-2, and ET-3, and are generated from big ETs through cleavage of the Trp^{22}-Val^{23} bond by ET-converting enzyme-1 and -2. However, $ETs_{1–31}$ are generated from big ETs through specific cleavage of the Tyr^{31}-Gly^{32} bond by human chymase (3). In addition, they may transiently be generated by other chymotrypsin-type proteases, such as human cathepsin G in granulocytes and rat mast cell chymases.

$ETs_{1–31}$ exhibit equivalent or lower contractile potencies in comparison with the 21-amino acid ETs, $ETs_{1–21}$, and the effects are dependent on species, vessel type, and vessel size. ET-$1_{1–31}$ increases intracellular free Ca^{2+} ($[Ca^{2+}]i$) in human mesangial cells, which are mediated by ET_A receptor or ET_A-like receptor (4, 5). ET-$1_{1–31}$ also increases intracellular free Ca^{2+} ($[Ca^{2+}]i$) in human cultured bronchial smooth muscle cells (BSMC), which is completely inhibited by phosphoramidon, a dual neutral endopeptidase (NEP)/ET-converting enzyme (ECE) inhibitor, and by thiorphan, a NEP inhibitor (6). The results suggest that the inrease in $[Ca^{2+}]i$ caused by ET-$1_{1–31}$ is mediated through cleavage to ET-$1_{1–21}$ by NEP in human BSMC. ET-$1_{1–31}$ causes contraction of isolated porcine coronary artery, rat aorta, and human umbilical artery (7, 8). ET-$1_{1–31}$ induces HCASMC, rat aortic smooth muscle cell, porcine coronary vascular smooth muscle cell, and rat zona glomerulosa cell proliferation. Furthermore, ET-$1_{1–31}$ activates the mitogen-activated protein kinase family (9, 10).

Because ET-$1_{1–21}$ has been shown to play a significant role in the cardiovascular system, ET-$1_{1–31}$ may also participate in biological activities in the cardiovascular system. ET-$1_{1–21}$ has a proarrhythmogenic effect, which does not result from myocardial ischemia induced by coronary constriction. Our previous work showed that exogenous ET-$1_{1–21}$ increased the severity and incidence of acute ischemic arrhythmia in a dose-dependent manner in isolated rat hearts and in hearts of rat and cat *in vivo*. This

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effect can be antagonized by ET_A-receptor antagonist BQ_{123}, superoxide dismutase, verapamil, or Mg^{2+}. Similar to the effects of exogenous ET-1, increased endogenous ET-1 during ischemia tended to aggravate myocardial ischemia *via* coronary constriction, and to exert proischemic and proarrhythmogenic effects as well. The effects of ET-$1_{1–31}$ on heart and vessels *in vivo* have not been observed. In the present study, we examined the arrhythmogenic, coronary flow, and heart function effects of synthetic ET-$1_{1–31}$ on isolated rat hearts.

Materials and Methods

This study was performed in accordance with the guidelines for the care and use of laboratory animals published by U.S. National Academy Press in 1996 and the Guidelines for Animal Experiments of the Second Military Medical University of CPLA.

Forty-eight male Sprague-Dawley rats aged 80–100 days and weighing 250–350 g were randomized into the following groups: normal perfusion ($n = 8$), 0.01 n*M* ET-$1_{1–31}$ ($n = 8$), 0.1 n*M* ET-$1_{1–31}$ ($n = 8$), and 1 n*M* ET-$1_{1–31}$ ($n = 8$), 10 n*M* BQ_{123}+ 1 n*M* ET-$1_{1–31}$ ($n = 8$), and 1 μ*M* phosphoramidon + 1 n*M* ET-$1_{1–31}$ ($n = 8$).

Rats were anesthetized by intraperitoneal injection of 1 g/kg urethane with 750 U heparin. Hearts were rapidly excised and immersed in heparinized (500 U) ice-cold Krebs-Henseleit buffer (in m*M*: NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; $NaHCO_3$, 25; KH_2PO_4, 1.2; D-glucose, 11; and EDTA, 0.5). The aorta was attached to a metal cannula and the heart mounted on a Langendorff perfusion apparatus for retrograde perfusion with Krebs-Henseleit (KH) solution (37°C) bubbled with 95% O_2 and 5% CO_2 at a constant pressure of 75 cm H_2O, and a water balloon connected to a pressure transducer to measure intraventricular pressure was inserted into the left ventricle. A surface electrocardiogram (ECG) was recorded via electrodes placed on the right ventricle, right atrium, and left atrium. Heart rate in bpm was calculated from the ECG trace, and coronary flow (CF) was measured as the output from the pulmonary artery.

Experimental Protocol. Heart was preperfused for 15 mins to stabilize, and then was switched to KH solutions containing different concentrations of ET-$1_{1–31}$ for 15 mins. In some experiments, the perfusate was switched to KH solution containing ET_A receptor antagonist BQ_{123} or phosphoramidon, a NEP/ECE inhibitor, for 15 mins before being switched to KH solutions containing different concentrations of ET-$1_{1–31}$. The heart was then perfused with the KH solution for 60 mins.

Arrhythmia Analysis. According to the Lambeth convention (11), arrhythmia is classified as single ventricular premature beat, salvo, ventricular tachycardia (VT), and ventricular fibrillation (VF). Arrhythmia scoring (AS) was evaluated according to the Johnston criterion (12).

Statistical Analysis. The number of ventricular premature beats over 1-min intervals is expressed as mean ± SEM. The total number of ventricular premature beats is expressed as median (first quartile–third quartile), and was compared using Mann-Whitney nonparametric test. The incidences of ventricular tachycardia, total ventricular fibrillation, and irreversible ventricular fibrillation are expressed as a percentage incidence for the group, and statistical significance was assessed using Fisher's exact (χ^2 with Yates' correction) test. Coronary perfusion pressure and heart rate (mean ± SEM) were recorded and assessed within the group by one-way ANOVA and Dunnett's multiple comparison test. Variations in coronary perfusion pressure and heart rate between groups were compared by a two-tailed unpaired Student's *t* test. All differences were taken as significant if $P < 0.05$.

Results

Effects of ET-$1_{1–31}$ on Heart Rate (HR) and Heart Function of Isolated Rat Hearts. In all experimental groups, HR decreased slightly, though there were no significant differences between the ET-$1_{1–31}$–treated groups and the control. The lower concentrations of ET-$1_{1–31}$ (0.01 and 0.1 n*M*) did not significantly affect CF, left ventricular systolic pressure (LVSP), or maximum left ventricular pressure increase and decrease rate (±dp/dt). However, 1 n*M* ET-$1_{1–31}$ significantly reduced CF, LVSP, and ±dp/dt and increased left ventricular end diastolic pressure (LVEDP) (Table 1), suggesting that 1n*M* ET-$1_{1–31}$ inhibited the heart function. In 1n*M* ET-$1_{1–31}$ group, CF, LVSP, +dp/dt and –dp/dt decreased to 71% ± 16%, 67% ± 22%, 63% ± 22%, and 69% ± 23% of the values before administration of ET-$1_{1–31,}$ respectively. However, CF, LVSP, LVEDP, and ±dp/dt remained almost unchanged in the control, 0.01 n*M* ET-$1_{1–31}$, and 0.1 n*M* ET-$1_{1–31}$ groups.

Effects of ET-$1_{1–31}$ on Cardiac Rhythm. In the normal perfusion control, only ventricular premature beats were observed; there was no salvo, ventricular tachycardia, ventricular fibrillation, or prolonged arrest; AS = 0.11 ± 0.33 (Table 2). In contrast, the incidence of VT in the 0.01 n*M*, 0.1 n*M*, and 1 n*M* ET-$1_{1–31}$–perfused groups was 13%, 17%, and 64%, respectively. In the 1 n*M* ET-$1_{1–31}$ group, AS averaged 2.18 ± 0.87, higher than that of the normal perfusion control. Arrhythmia occurred on a multiphase basis between 10 and 25 mins, with the peak being between 30 and 45 mins (Fig. 1).

Effects of BQ_{123} and Phosphoramidon on ET-$1_{1–31}$–Induced Arrhythmias. The effects of BQ_{123} (10 n*M*) and phosphoramidon (1 μ*M*) on the percentage incidences of single VEBs, salvos, and ventricular tachycardia were compared with the 1 n*M* ET-$1_{1–31}$ group in Table 2. Pretreatment with perfusion of 10 n*M* BQ_{123} markedly attenuated the occurrence of VEB and VT induced by 1 n*M* ET-$1_{1–31}$. AS in the 10 n*M* BQ_{123} group was significantly lower than that in the 1 n*M* ET-$1_{1–31}$ group ($P < 0.01$) (Table 2). The arrhythmia induced by 1n*M* ET-$1_{1–31}$ was

Table 1. Effect of ET-1_{1-31} on HR and Heart Function of Isolated Rat Hearts[a]

	Time (min)	Normal control	0.01 n*M* ET-1_{1-31}	0.1 n*M* ET-1_{1-31}	1 n*M* ET-1_{1-31}
HR (bpm)	0	282 ± 43	272 ± 36	278 ± 38	289 ± 43
	15	270 ± 35	266 ± 31	272 ± 32	274 ± 39
CF (ml)	0	11.35 ± 1.31	11.18 ± 1.20	11.64 ± 1.56	11.03 ± 1.69
	15	11.01 ± 1.28	10.73 ± 1.32	11.17 ± 1.54	7.88 ± 1.76*
LVSP (mmHg)	0	76.80 ± 6.94	78.92 ± 7.71	75.63 ± 9.12	80.12 ± 8.83
	15	73.73 ± 7.82	73.40 ± 6.34	69.58 ± 8.85	54.49 ± 7.98*
LVEDP (mmHg)	0	7.05 ± 1.60	7.32 ± 1.31	6.98 ± 1.40	7.60 ± 1.25
	15	7.45 ± 1.65	7.47 ± 1.28	7.27 ± 1.52	9.50 ± 1.21*
+dp/dt	0	2325 ± 437	2176 ± 383	2238 ± 239	2275 ± 352
	15	2120 ± 332	2067 ± 365	2103 ± 267	1431 ± 386*
−dp/dt	0	1528 ± 258	1504 ± 321	1457 ± 213	1483 ± 230
	15	1466 ± 279	1413 ± 346	1388 ± 225	1021 ± 203*

[a] ET$_{1-31}$, endothelin-1_{1-31}; HR, heart rate; CF, coronary flow; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end diastolic pressure; dp/dt, maximum left ventricular pressure increase and decrease rate.
* $P < 0.01$ compared with normal control.

partially but significantly reduced by 1 μ*M* phosphoramidon. In the 1 μ*M* phosphoramidon group, AS (0.78 ± 0.83) was also significantly lower than that in the 1 n*M* ET-1_{1-31} group ($P < 0.01$) (Table 2).

Discussion

Endogenous chemicals such as K^+, Ca^{2+}, cAMP, and ET are responsible for ischemic arrhythmia. ET has unambiguous proarrhythmogenic effects. Nonspecific ET receptor antagonist, specific ET_A receptor antagonist, ET monoclone antibody, or ET converse enzyme inhibitor can reduce infracted area and improve heart function and the function of vascular endothelium.

The ET_A receptor antagonist BQ_{123} (10 mg/kg) attenuated arrhythmia by reducing total ventricular ectopic count. The higher dose of BQ_{123} (100 mg/kg) increased arrhythmia by significantly increasing the incidence of irreversible VF (13). ET-1 released endogenously during ischemia is arrhythmogenic, whereas exogenous application of ET-1 may be antiarrhythmic under certain conditions (14). BQ_{123}, but not ET_B-receptor antagonist IRL 1038, can attenuate acute ischemic arrhythmia in anesthetized cats. Preproendothelin-1 mRNA antisense oligodeoxynucleotides significantly attenuated acute ischemic arrhythmia elicited by occlusion of the left anterior descending coronary artery (15, 16). ET-1_{1-21} is generated from big ET-1 through cleavage at the Trp^{21}-Val/Ile^{22} bond by a specific ET-converting enzyme, ECE-1. Big ET-1 may also be selectively cleaved in humans by a chymase at the Tyr^{31}-Gly^{32} bond to produce ET-1_{1-31} without any further degradation.

In this project, we examined whether ET-1_{1-31} is arrhythmogenic, and if so, whether this effect is dependent on conversion to ET-1_{1-21}. The present study demonstrated that exogenous ET-1_{1-31} caused arrhythmia and increased the incidence of arrhythmia dose-dependently. The question

Table 2. Arrhythmia Induced by ET-1_{1-31} in Perfused Isolated Rat Hearts Pretreated with or Without BQ_{123} or Phosphoramidon[a]

		Arrhythmia counts				
Group	AS	Single VEBs	Salvos	VT	Total VEBs	VT duration (sec)
Normal control	0.11 ± 0.33	18.00 (16.00–29.00)	0.00 (0.00–2.00)	0.00 (0.00–0.00)	17.00 (14.00–37.00)	0.00 (0.00–0.00)
0.01 n*M* ET-1_{1-31}	0.50 ± 0.76	34.00 (14.75–53.25)	0.00 (0.00–3.00)	0.00 (0.00–0.00)	35.00 (17.75–54.75)	0.00 (0.00–0.00)
0.1 n*M* ET-1_{1-31}	0.67 ± 1.21	35.50 (16.25–55.50)	1.00 (0.00–3.50)	0.00 (0.00–0.00)	36.50 (16.25–59.00)	0.00 (0.00–0.00)
1 n*M* ET-1_{1-31}	2.18 ± 0.87**	211.00 (83.50–734.00)**	12.00 (6.50–114.00)**	8.00 (2.00–135.50)**	128.00 (66.00–1015.00)**	1.00 (0.00–9.00)*
10 n*M* BQ_{123} + 1 n*M* ET-1_{1-31}	0.33 ± 0.52****	30.00 (20.75–46.75)****	1.00 (0.00–2.00)****	0.00 (0.00–0.00)***	35.00 (23.25–48.25)****	0.00 (0.00–0.00)***
Phosphoramidon 1 μ*M* +1 n*M* ET-1_{1-31}	0.78 ± 0.83****	47.00 (36.00–70.00)***	2.00 (0.00–6.00)***	0.00 (0.00–0.00)***	47.00 (38.00–70.00)***	0.00 (0.00–0.00)

[a] ET$_{1-31}$, endothelin-1_{1-31}; AS, arrhythmia score; VEBs, ventricular ectopic beats; VT, ventricular tachycardia.
* $P < 0.05$ compared with normal control; ** $P < 0.01$ compared with normal control; *** $P < 0.05$ compared with 1 n*M* ET-1_{1-31}; **** $P < 0.01$ compared with 1 n*M* ET-1_{1-31}.

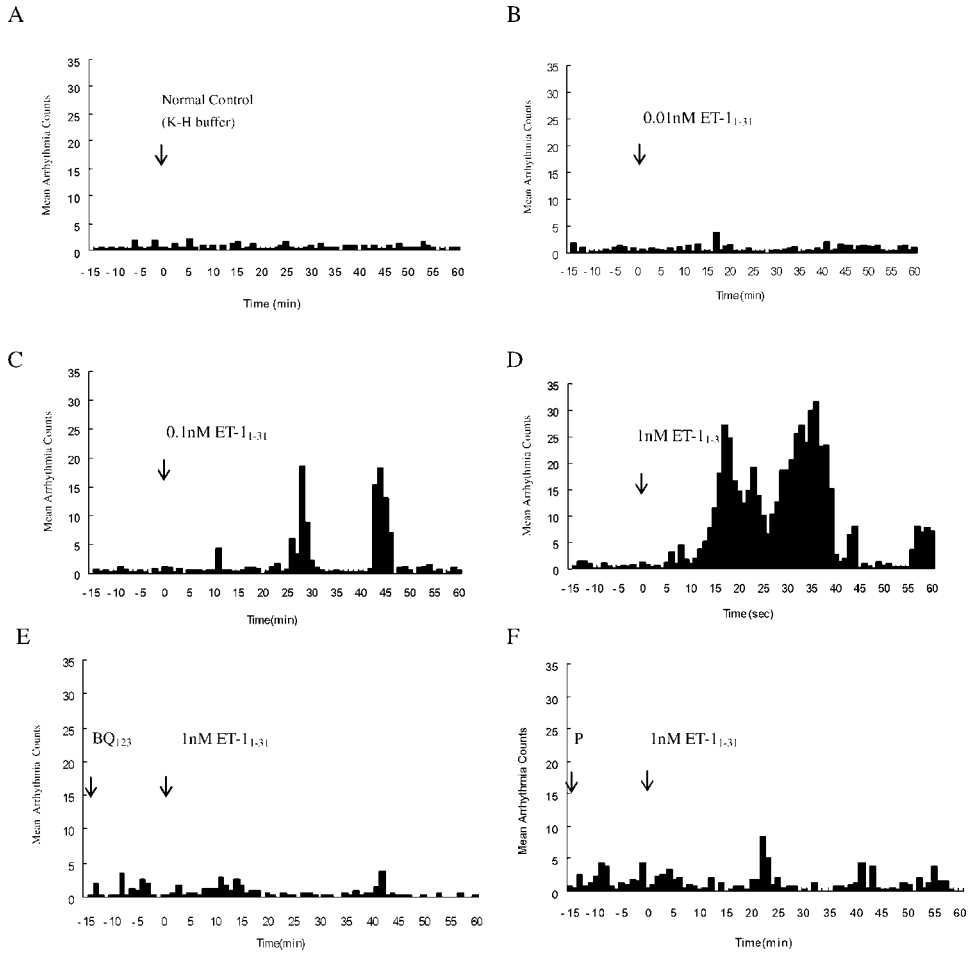

Figure 1. Distribution of mean ventricular ectopic beats (VEBs) at 1-min intervals during 75-min perfusion in isolated rat hearts receiving (A) KH buffer, (B) 0.01n*M* ET-$1_{1–31}$, (C) 0.1 n*M* ET-$1_{1–31}$, (D) 1 n*M* ET-$1_{1–31}$, (E) 10 n*M* BQ_{123} + 1n*M* ET-$1_{1–31}$, and (F) 1 μ*M* Phosphoramidon (P) +1n*M* ET-$1_{1–31}$, respectively.

arises as to which receptor mediated these arrhythmogenic effects. In rats and dogs, ET-induced ventricular arrhythmia proved sensitive to ET_A blockade, whereas a contribution of ET_B receptors could be excluded. In the present study, BQ_{123}, a ET_A antagonist, almost completely abolished this proarrhythmogenic effect of exogenous ET-$1_{1–31}$. Although there is no evidence that the receptor of ET-$1_{1–31}$ is identical to that of ET-$1_{1–21}$, the findings suggest that the proarrhythmogenic effect of ET-$1_{1–31}$ is mediated through ET_A or ET_A-like receptors. Further studies are needed to clarify what types of receptors are involved in the ET-$1_{1–31}$ induced phenomenon.

It is important to elucidate whether the effect of ET-$1_{1–31}$ is a result of its conversion to ET-$1_{1–21}$, or whether ET-$1_{1–31}$ itself acts directly on the heart. As shown in Figure 1, phosphoramidon NEP/ECE inhibitor largely but not completely abolished the proarrhythmogenic effect of ET-$1_{1–31}$, suggesting that this arrhythmogenic action is attributed mainly to the conversion of ET-$1_{1–31}$ to ET-$1_{1–21}$. With respect to the potential clinical implications of our findings, attention should be directed to severe ventricular arrhythmia. We hypothesize that $ET_{1–21}$ in general and ET-$1_{1–31}$ in particular may act as an important mediator of arrhythmogenesis.

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