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The effects of endothelin-1 on ischaemia-induced ventricular arrhythmias in rat isolated hearts

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Abstract

We have shown previously that a small bolus dose of endothelin-1, given intravenously before coronary occlusion, exerts a marked antiarrhythmic effect in anaesthetised rats. The aim of the current study was to determine whether or not this is due to a direct effect of endothelin-1 on the heart by assessing the antiarrhythmic effect of endothelin-1 against occlusion-induced arrhythmias in rat isolated hearts. Rat isolated hearts were perfused in Langendorff mode (constant flow) and subjected to coronary artery occlusion for 30 min. Coronary perfusion pressure and a surface electrocardiogram (ECG) were monitored throughout the experiment. In the first series of studies, the effects of three 5-min infusions of endothelin-1 (0.1–10 nM), given prior to coronary occlusion, were assessed. A second series of hearts was given a single bolus dose of endothelin-1 (10 pmol) 5 min prior to ischaemia. A third series of experiments was performed using a modified (low K^+) Krebs Henseleit solution to increase the incidence of ischaemia-induced ventricular fibrillation (VF). In these hearts, endothelin-1 (0.1 or 2 pmol) was administered as a bolus injection 5 min before ischaemia. Infusion of endothelin-1 prior to ischaemia did not modify the incidence or severity of arrhythmias at any of the concentrations used. Bolus administration of endothelin-1 (10 pmol) in hearts perfused with Kreb's Henseleit solution containing normal K^+ (4.4 mM) was found to cause a small rise in coronary perfusion pressure, with no preceding depressor response. Under these conditions, endothelin-1 exerted only a very moderate reduction in arrhythmias, by reducing the arrhythmia count in the 21–30-min post-occlusion period. Furthermore, in hearts perfused with low K^+ solution, bolus injection of endothelin-1, in a dose that either had no effect on coronary perfusion pressure (0.1 pmol) or produced a significant vasodilator effect with no significant pressor effect (2 pmol), had no effect on ventricular fibrillation. Thus, in concentrations that are sufficient to exert effects on the coronary vasculature, endothelin-1 fails to modify arrhythmias in an isolated heart preparation. These results suggest that the antiarrhythmic effects of endothelin-1 previously observed in vivo are not due to a direct effect on either the myocardium or the coronary blood vessels.

Keywords: Endothelin-1; Arrhythmias, ischaemic; Heart; (Rat); Isolated

1. Introduction

As a potent vasoconstrictor substance, endothelin-1 has been implicated in a range of cardiovascular disorders including ischaemic heart disease (Winkles et al., 1993; Rubanyi and Polokoff, 1994; Omland et al., 1994). Early studies demonstrated that extension of myocardial injury could be reduced by a monoclonal antibody to endothelin-1 (Watanabe et al., 1991). Subsequent reports using either endothelin ET_A selective or mixed ET_A/ET_B receptor antagonists also demonstrated a favourable effect against infarct size (Grover et al., 1993; Wang et al., 1995). In addition to a role in the development of myocardial injury following ischaemia/reperfusion, several studies have shown that endothelin-1 exerts a pro-arrhythmic effect, both in the absence (Yorikane and Koike, 1990; Becker et al., 2000) and presence (Garjani et al., 1995) of acute myocardial ischaemia in experimental animal models. Indeed, endothelin-1 has been shown to exert direct cardiac electrophysiological actions which would explain these effects (Becker et al., 2000; Yorikane et al., 1991). Furthermore, both selective endothelin ET_A and non-selective ET_A/ET_B receptor antagonists are antiarrhythmic under conditions of acute myocardial ischaemia in rats (Garjani et al., 1995; Sharif et al., 1998) and rabbits (Vitola et al., 1996). Taken together, these findings present a strong argument for a detrimental effect of endogenous endothelin-1 in acute ischaemic states.

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However, recent evidence has emerged that suggests that endothelin-1 may, under certain conditions, exert a protective effect against myocardial infarct size (Hide et al., 1995; Wang et al., 1996; Bugge and Ytrehus, 1996) as a form of 'pharmacological preconditioning'. More recently, we demonstrated for the first time that endothelin-1 is profoundly antiarrhythmic when given as a very low bolus dose, insufficient to cause a significant pressor effect, prior to coronary occlusion in anaesthetised rats (Sharif et al., 1998). Interestingly, this response was not reversed by either a selective endothelin ET_A or ET_A/ET_B receptor antagonist. This finding, however, was due to the fact that these agents protect against the pro-arrhythmic effects of endogenous endothelin-1. Thus, the use of these drugs as tools to provide any insight into the mechanism of the antiarrhythmic effect of exogenous endothelin-1 is limited.

In our previous in vivo study, it was not possible to distinguish between a direct effect of endothelin-1 on the heart, or an indirect mechanism of protection, mediating the protective effects of endothelin-1 against arrhythmias. This was because the administration of endothelin-1 prior to ischaemia resulted in marked haemodynamic effects that could possibly account for the antiarrhythmic effect. The primary aim of the current study, therefore, was to assess the ability of endothelin-1 to reduce ventricular arrhythmias in a rat isolated heart model of coronary artery occlusion. Our first series of experiments employed a protocol which had previously been successful in demonstrating the cardioprotective effect of repeated short infusions of endothelin-1, prior to the onset of ischaemia, against infarct size in rat isolated hearts (Bugge and Ytrehus, 1996). In addition, to make a direct comparison with our previous in vivo studies we also determined the effect of a single bolus injection, rather than repeat short infusions, of endothelin-1 on ventricular arrhythmias in hearts perfused with normal (4.4 mM) and low (3.2 mM) K^+ solution. This allowed us to distinguish between effects on the less severe arrhythmias (ventricular premature beats) seen under normal perfusion conditions and on the life-threatening arrhythmia, ventricular fibrillation, which is more evident in the presence of low extracellular K^+ (Demiryürek et al., 1998).

2. Methods and materials

2.1. Surgical procedure

Male Sprague–Dawley rats (300–450 g) were anaesthetised with sodium pentobarbitone (60 mg kg^{-1} i.p.). A transverse laparotomy and a left and right anterolateral thoracotomy were performed. On removal of the pericardium, a 6/0 braided silk suture (attached to a 10-mm micropoint reverse cutting needle) was placed around the left main coronary artery. The heart was rapidly excised and immersed in heparinised (500 i.u.) ice-cold Krebs-Henseleit buffer (in mM: NaCl 118, KCl 3.2, $CaCl_2$ 2.5, $MgSO_4$ 1.7, $NaHCO_3$ 27, KH_2PO_4 1.2, D-glucose 5.6, Na-pyruvate 2). The aorta was attached to a metal cannula and the heart mounted on a Langendorff perfusion apparatus for retrograde perfusion. Temperature was maintained at 37 °C using a heated jacket and hearts were perfused with a Krebs Henseleit solution (pH 7.4) at a constant flow rate (12 ml min^{-1}) using a roller pump (Watson-Marlow 505S). The coronary perfusion pressure was measured using a pressure transducer (Gould etc) via a side arm just proximal to the aortic cannula. A surface electrocardiogram (ECG) was recorded via electrodes placed on the right atrium and left ventricle and the coronary perfusion pressure and ECG traces were continuously recorded on a two-channel chart recorder (Gould TA 240). Heart rate (in beats per min; bpm) was calculated from the ECG trace.

2.2. Induction of myocardial ischaemia and arrhythmia analysis

Following a stabilisation period determined by the experimental protocol, the coronary artery was occluded by threading the ends of the ligature around the coronary artery through a short length of polythene tubing to form a snare. The snare was then tightened and held in place with a small artery clip. Successful coronary occlusion was determined by a rapid rise in coronary perfusion pressure within 1 min of occlusion. The consequent ventricular arrhythmias were monitored for 30 min. Ventricular arrhythmias were analyzed (from the ECG trace) according to the guidelines of the Lambeth Conventions for the determination of experimental arrhythmias (Walker et al., 1988). Arrhythmias were classified as single ventricular premature beats, salvos (couplets or triplets), and ventricular tachycardia (defined as a run of four or more ventricular premature beats). The total number of ventricular premature beats (calculated as the sum of individual arrhythmias) was only counted in hearts that survived the 30-min period of ischaemia. Ventricular fibrillation was defined as when individual QRS complexes could no longer be distinguished and successive waves were inconsistent both in amplitude and rhythm. The percent incidence of ventricular tachycardia, total (reversible + irreversible) ventricular fibrillation and irreversible ventricular fibrillation (any period of ≥ 5 min ventricular fibrillation) was noted for each group. The onset time and the time spent in reversible ventricular fibrillation was also calculated.

2.3. Experimental protocols

2.3.1. Assessment of the effects of repeated short infusions of endothelin-1 prior to ischaemia on ventricular arrhythmias

Previous studies using isolated hearts to determine the preconditioning effects of endothelin-1 on myocardial in-

farct size (Bugge and Ytrehus, 1996) employed a protocol of three 5-min periods of infusion of endothelin-1 into the coronary perfusate prior to induction of regional ischaemia. In order to determine whether this protocol also protected against ischaemic arrhythmias, a similar protocol was employed in the present study. Following a 15-min stabilisation period endothelin-1 was infused, via a siliconised side-arm in the aortic cannula and at a rate of 5% of the coronary perfusion rate ($\sim$ 0.35–0.45 ml min^{-1}), three times for 5-min periods, separated by 5-min washout. The coronary artery was occluded after the third and last 5-min washout period. Rat hearts were allocated to one of five groups; buffer infusion (control; $n = 16$) or endothelin-1 infusion at 0.1 nM ($n = 7$), 1 nM ($n = 15$), 5 nM ($n = 9$) or 10 nM ($n = 10$).

2.3.2. Determination of the effects of a single bolus injection of endothelin-1 prior to ischaemia on ventricular arrhythmias

Our previous in vivo studies (Sharif et al., 1998) demonstrated that a single bolus injection of endothelin-1 insufficient to cause a sustained pressor effect, given 5 min before coronary occlusion, markedly reduced ventricular arrhythmias. Thus, to test the hypothesis that this effect is due to a direct action on the heart we employed a similar protocol in the isolated heart model. However, the choice of dose was important, since high doses of endothelin-1 that cause significant coronary constriction have been shown to induce arrhythmias (Yorikane and Koike, 1990). Thus, an appropriate concentration of endothelin-1 was determined from a cumulative dose–response curve to endothelin-1 (1–240 pmol, where 1 pmol = 2.5 ng endothelin-1) performed in a group of hearts ($n = 7$). Bolus injections were given via the side-port and were separated by 4-min intervals. From this dose–response curve, a bolus dose of 10 pmol endothelin-1, which produced an approximate 25% increase in coronary perfusion pressure, was chosen to study its effects on ischaemic arrhythmias. Thus, following a 20-min stabilisation period hearts were administered either buffer ($n = 9$) or 10 pmol endothelin-1 ($n = 6$) 5 min prior to coronary occlusion. The injection volume was 0.1 ml.

2.3.3. The effects of a single bolus injection of endothelin-1 in a high ventricular fibrillation incidence model of coronary occlusion

The model of coronary occlusion perfused with standard Krebs Henseleit solution (described above) resulted in the development of a large number of ventricular arrhythmias (i.e. single ventricular premature beats, salvos and ventricular tachycardia) but a relatively low incidence of ventricular fibrillation. Thus, to increase the incidence of ventricular fibrillation a modified perfusion protocol was employed to reduce the concentration of K^+ in the Krebs solution (Demiryürek et al., 1998). After a 30-min stabilisation period in normal Krebs the perfusion medium was changed to a modified Krebs Henseleit solution (in mM: NaCl 118, KCl 2, $CaCl_2$ 1.23, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, D-glucose 11, Na-pyruvate 0) for the remainder of the experiment. The hearts were then stabilised for a further 45-min period, to allow equilibration of the coronary perfusion pressure and the extracellular K^+ concentration. This modified perfusion resulted in an increase in baseline coronary perfusion pressure and, thus, a further cumulative dose–response curve to endothelin-1 ($n = 5$) was performed to determine the most appropriate concentration for administering prior to occlusion under these perfusion conditions. Ultimately two concentrations (0.1 and 2 pmol) were selected on the basis that the lower concentration had no effect on coronary perfusion pressure, while the higher dose caused an initial (approximately 12%) decrease in coronary perfusion pressure and a subsequent approximate 25% (not significant) increase in coronary perfusion pressure. Three groups of hearts were then subjected to coronary occlusion with injections of buffer ($n = 15$) or endothelin-1 (0.1 pmol; $n = 10$ or 2 pmol; $n = 10$) given 5 min prior to coronary occlusion.

2.4. Exclusion criteria

Any hearts that developed persistent spontaneous ventricular arrhythmias before the administration of endothelin-1 or vehicle was excluded from the study. Hearts were also excluded if the tightening of the ligature described in the methodology did not produce an immediate and sustained increase in coronary perfusion pressure. Under these criteria, one heart from the control group in the modified perfusion experiments was excluded.

2.5. Statistical analysis

The number of ventricular premature beats over 1-min intervals is expressed as mean $\pm$ standard error of the mean (S.E.M.). The total numbers of ventricular premature beats are expressed as median (Q1–Q3), and were compared using Mann–Whitney non-parametric 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 Yate's correction) test. coronary perfusion pressure and heart rate (mean $\pm$ S.E.M.) were recorded and assessed within the group by one-way analysis of variance (ANOVA) and Dunnett's multiple comparison test determined significant differences. 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 $P \leq 0.05$.

2.6. Materials

Sodium chloride and sodium pyruvate were purchased from Sigma. Potassium dihydrogen orthophosphate, sodium

Table 1
The effects of 3 × 5-min infusions of endothelin-1 on coronary perfusion pressures before and after coronary occlusion in rat isolated hearts

Group	Time after occlusion (min)							
	−30	0	1	3	5	10	15	30
Control	36 ± 1	43 ± 1[a]	71 ± 3[b]	72 ± 3	73 ± 3	70 ± 3	68 ± 4	72 ± 4
Endothelin-1								
0.1	34 ± 3	42 ± 4	62 ± 5[b]	62 ± 5	61 ± 5	60 ± 4	59 ± 4	64 ± 4
1	34 ± 1	41 ± 1[a]	63 ± 3[b,c]	64 ± 3[c]	59 ± 5[c]	62 ± 3	59 ± 2	60 ± 3[c]
5	37 ± 2	51 ± 3[a,c]	74 ± 5 [b]	75 ± 5	78 ± 6	80 ± 8	78 ± 8	81 ± 8
10	35 ± 1	53 ± 3[a,c]	80 ± 6 [b]	81 ± 6	80 ± 6	78 ± 6	78 ± 6	80 ± 6

[a] $P < 0.05$ compared to −30-min time point (i.e. before infusion protocol).
[b] $P < 0.05$ compared to pre-occlusion value (both by one-way ANOVA). All subsequent time points were also statistically significant from pre-occlusion value.
[c] $P < 0.05$ compared to the same time point in the control group (unpaired Students' *t*-test).

hydrogen carbonate, potassium chloride, calcium chloride, magnesium sulphate, and glucose were all purchased from BDH Laboratory Supplies (Poole, BH15 1TD, England). Endothelin-1 was purchased from Novabiochem (England).

3. Results

3.1. The effects of 3 × 5-min infusions of endothelin-1 prior to and during coronary occlusion in rat isolated hearts

Table 1 summarizes the coronary perfusion pressure in all groups of hearts before and after the infusion protocol and during coronary occlusion. Coronary perfusion pressure rose during the infusion protocol in all groups of hearts. The coronary perfusion pressure in hearts perfused with 5 or 10 nM endothelin-1 was significantly greater than controls at the end of the infusions and immediately before coronary occlusion ($P < 0.05$). The lower concentrations of endothelin-1 (0.1 and 1 nM) did not significantly increase coronary perfusion pressure relative to the controls. Following coronary occlusion, there was an immediate and sustained increase in coronary perfusion pressure in all groups. In the 1 nM endothelin-1 group, the coronary perfusion pressure was significantly lower than that in control hearts at most of the time points throughout the period of coronary occlusion. The two groups of hearts receiving the higher concentrations of endothelin-1 (5 and 10 nM) both tended to have a higher coronary perfusion pressure during occlusion, compared to controls, although this was not statistically significant. Heart rate did not alter significantly in any group throughout the experimental procedure (data not shown). Coronary artery occlusion in controls resulted in a large number of ventricular arrhythmias, occurring as single ventricular premature beats, salvos and ventricular tachycardia (Fig. 1). The incidence of ventricular fibrillation (both reversible and irreversible) was low in this model (21% and 14%, respectively). None of the concentrations of endothelin-1 infusions modified either total VPB count (Fig. 1) or the incidence of ventricular tachycardia or ventricular fibrillation (Table 2).

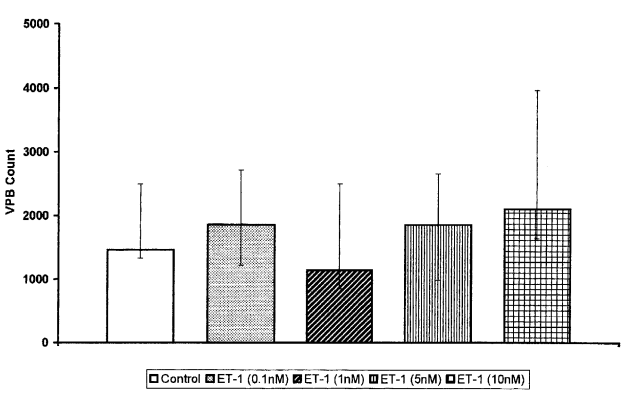

Fig. 1. The effects of 3×5-min infusions of endothelin-1 (0.1–10 nM), given before the onset of ischaemia, on total ventricular premature beat (VPB) counts during a 30-min period of coronary artery ligation in rat isolated hearts. Values are median (Q1, Q3).

3.2. Effects of a single bolus injection of endothelin-1, under normal perfusion conditions, before and during coronary occlusion

The cumulative dose–response curve to endothelin in rat hearts perfused with normal Krebs demonstrated that, under these perfusion conditions, only a pressor response

Table 2
The effects of 3×5-min infusions of endothelin-1, prior to coronary occlusion, on percent incidence of ischaemia-induced ventricular tachycardia (ventricular tachycardia) and ventricular fibrillation (ventricular fibrillation) in rat isolated hearts perfused with normal Krebs

	% Ventricular tachycardia	% Total ventricular fibrillation	% Irreversible ventricular fibrillation
Control	100	38	13
Endothelin-1 (nM)			
0.1	100	14	0
1	100	33	0
5	100	44	0
10	100	30	0

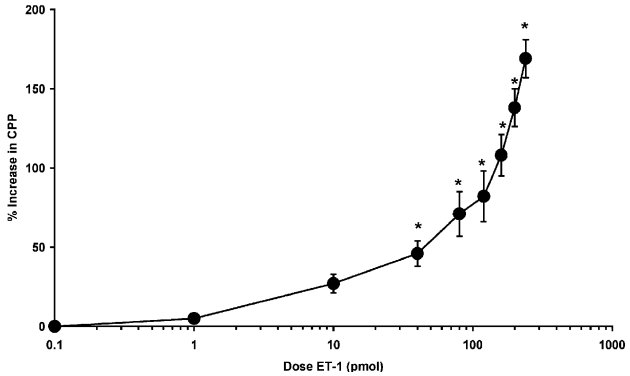

Fig. 2. Cumulative dose–response curve to endothelin-1 in rat isolated hearts perfused with Krebs containing normal (4.4 mM) K^+. The increase in coronary perfusion pressure is expressed as a percent change from a baseline coronary perfusion pressure of 36 ± 2 mm Hg in a group of seven hearts. $^* P < 0.05$ compared to baseline (one-way ANOVA).

to endothelin-1 could be produced (Fig. 2). The threshold dose for inducing a pressor response was determined from the dose–response curve as 10 pmol (~25% increase in coronary perfusion pressure) and was therefore used as the dose with which to prime the heart prior to coronary occlusion. When endothelin-1 (10 pmol) was given as a single bolus dose 5 min prior to coronary occlusion, there was no effect on coronary perfusion pressure compared to controls (pre-occlusion coronary perfusion pressures were 36 ± 3 and 36 ± 1 mm Hg in endothelin-1 and control hearts, respectively). Furthermore, both groups of hearts showed similar changes in coronary perfusion pressure following occlusion (data not shown). This dose of endothelin-1 had no significant effects on either the total VPB count (Fig. 3) or the incidences of ventricular tachycardia (100% in both groups), irreversible ventricular fibrillation (13% and 0% in control and endothelin-1 groups, respectively) or total ventricular fibrillation (50% and 17%; P = ns). However, although the total VPB count was not significantly reduced by endothelin-1, the distribution of ventricular premature beats over the 30-min period was modified in that the phase 1b arrhythmias (i.e. those appearing between 20 and 30 min) were markedly and significantly attenuated (Fig. 4). This was probably as a result of decreasing the number of arrhythmias occurring as ventricular tachycardia (not significant; Fig. 3).

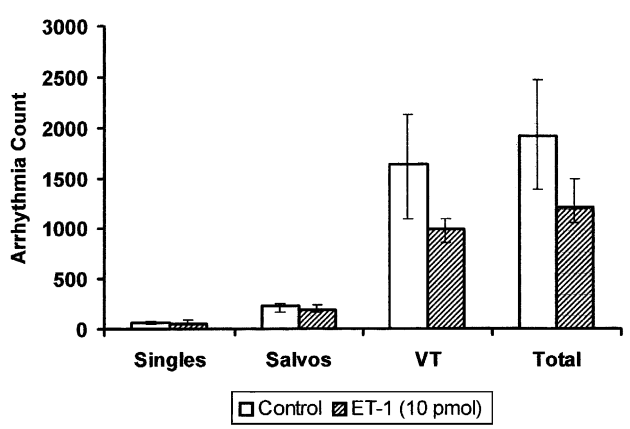

Fig. 3. The effects of a single bolus injection of endothelin-1 prior to coronary artery ligation on the number of the different types of arrhythmias seen during 30 min of ischaemia in rat isolated hearts perfused with Krebs containing normal (4.4 mM) K^+. Values are median (Q1, Q3).

3.3. Effects of a single bolus injection of endothelin-1, under perfusion conditions of low extracellular K^+, before and during coronary occlusion

Perfusion with a low K^+ Krebs solution raised the perfusion pressure in all hearts, for example, from 34 ± 5 mm Hg prior to switching to modified perfusate to 64 ± 7 mm Hg ($P < 0.05$) immediately before occlusion (i.e. 45 min after switching perfusate) in the control coronary occlusion group. The cumulative dose–response curve to endothelin-1 in hearts perfused with low K^+ demonstrated that both vasodilator and vasoconstrictor responses could be observed (Fig. 5). Thus, two doses of endothelin-1 (0.1 and 2 pmol) for bolus injection were selected based on the finding that the lower dose had no significant effects on

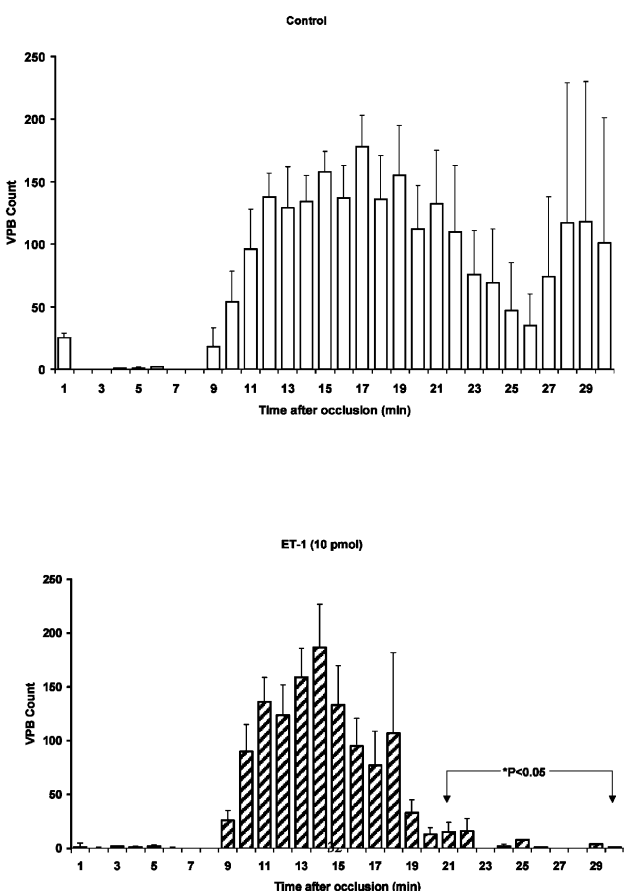

Fig. 4. Distributions of ventricular premature beats over 1-min intervals during 30 min myocardial ischaemia in control rat isolated hearts and hearts administered a single bolus injection of endothelin-1 5 min prior to ischaemia. Values shown are mean ± S.E.M. $^* P < 0.05$ compared to the entire 21–30 min time period in controls (Mann–Whitney test).

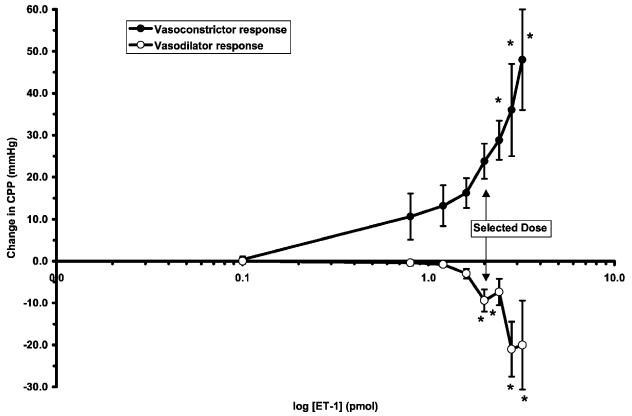

Fig. 5. Dose–response curve to endothelin on coronary perfusion pressure in rat isolated hearts perfused with modified, low K^+ (3.2 mM) perfusate. The increase in coronary perfusion pressure is the pressor response to endothelin-1 and is calculated from a baseline of 60 ± 3 mm Hg in a group of five hearts. The decreases in coronary perfusion pressure represent the transient vasodilator response to endothelin-1 injection and were calculated as a change from the coronary perfusion pressure immediately prior to injection of the dose of endothelin-1. $^{*}P < 0.05$ compared to corresponding baseline coronary perfusion pressure (one-way ANOVA).

coronary perfusion pressure, while the higher dose produced a significant fall in coronary perfusion pressure with no significant pressor effect. When given 5 min prior to coronary occlusion, 2 pmol endothelin-1 induced a significant, transient fall in coronary perfusion pressure (Fig. 6), while the 0.1 pmol dose had no effect on coronary perfusion pressure. During coronary occlusion in the low dose endothelin-1 group, the changes in coronary perfusion pressure were of a similar magnitude to those in the control hearts (data not shown). However, in the 2 pmol endothelin-1 group the coronary perfusion pressure prior to occlusion was significantly higher than in controls

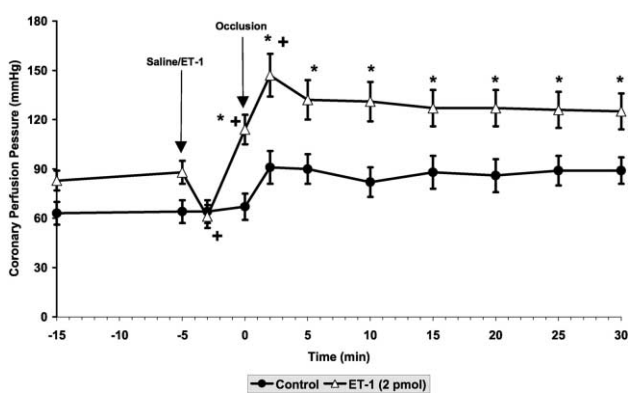

Fig. 6. The effects of bolus administration of endothelin-1 on coronary perfusion pressure (coronary perfusion pressure) in rat isolated hearts, perfused with modified (3.2 mM K^+) perfusate, before and during coronary artery ligation. Saline or endothelin-1 was injected via the aortic cannula 5 min before coronary occlusion (performed at time 0). $^{*}P < 0.05$ compared to same time point in controls (unpaired *t*-test). $\dagger P < 0.05$ compared to -5-min value (one-way ANOVA).

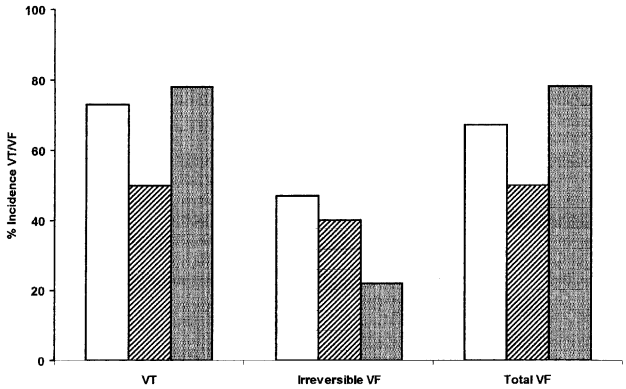

Fig. 7. The effects of bolus administration of endothelin-1, prior to acute coronary ligation in isolated rat hearts perfused with modified (3.2 mM K^+) perfusate, on the incidence of ischaemia-induced ventricular tachycardia and ventricular fibrillation. Total ventricular fibrillation includes those hearts that experienced periods of reversible and/or irreversible ventricular fibrillation.

and remained so throughout the 30-min occlusion period (Fig. 6).

Coronary occlusion in control hearts perfused with low K^+ Krebs resulted in a higher incidence of ventricular fibrillation (60%) and a lower VPB count (41 (11,171)) than in controls perfused with normal Krebs (see Section 3.1). Bolus injection of 0.1 or 2 pmol endothelin-1 had no effect on the percent incidence of ventricular tachycardia, or the incidence of reversible or total ventricular fibrillation (Fig. 7). The low arrhythmia counts precluded further analysis.

4. Discussion

We have previously shown that endothelin-1, given as a small intravenous bolus dose prior to the onset of ischaemia, has a marked antiarrhythmic effect against ventricular ischaemic arrhythmias in an in vivo rat model of coronary occlusion. While the mechanism(s) of this protection remain far from clear, one possibility is that the marked haemodynamic effects that accompany the administration of endothelin-1 in vivo may play a role. This study aimed to determine whether the antiarrhythmic effects of endothelin-1 could be reproduced in vitro and, if so, whether effects on coronary perfusion pressure were important in any observed effects.

The first series of experiments aimed to mimic the protocol by which endothelin-1 had previously been demonstrated to reduce myocardial infarct size in an in vitro rat model of ischaemia/reperfusion (Bugge and Ytrehus, 1996). Endothelin-1, infused over a range of concentrations for three 5-min periods prior to occlusion, had no effects on ventricular arrhythmias, irrespective of any effects on coronary perfusion pressure prior to the onset of ischaemia. This is in contrast to the results of the infarct study by Bugge and Ytrehus, where three infusions of 1nM

endothelin-1 reduced infarct size. These contrasting findings probably reflect the different mechanisms by which preconditioning protects against myocardial injury and arrhythmias. In the Bugge and Ytrehus study, they went on to demonstrate that the infarct-reducing effect of endothelin-1 could be inhibited by a K_{ATP} channel blocker. However, the evidence to date suggests that K_{ATP} channels are not involved in the antiarrhythmic effect of preconditioning (Lu et al., 1993; Vegh et al., 1993). Thus, the results of the present study support the wide body of evidence to suggest that the mechanism(s) underlying the infarct-reducing effect of preconditioning (pharmacological or ischaemic) is different from the mechanism responsible for the antiarrhythmic effect of preconditioning (Wainwright and Sun, 1996).

The second series of experiments aimed to emulate the conditions in vivo where endothelin-1 is antiarrhythmic by administering a bolus dose of endothelin-1 that was too low to cause a significant pressor response, but sufficient to produce a vasodilator response. In the rat isolated heart perfused with normal (4.4 mM) K^+, we found that endothelin-1 (1–240 pmol) was only able to elicit a pressor response, without the initial transient vasodilator response that is characteristic of its haemodynamic profile in vivo (Yanagisawa et al., 1988). However, under these perfusion conditions the coronary perfusion pressure was fairly low (typically 35–40 mm Hg) in non-ischaemic hearts, possibly reflecting maximal coronary vasodilatation. Indeed, we have similarly been unable to demonstrate a coronary vasodilator response to the endothelium-independent nitric oxide donor SIN-1 under these same experimental conditions (unpublished observations). Other studies have previously demonstrated that cumulative bolus doses of endothelin-1 in isolated hearts produced a transient coronary vasodilatation followed by a sustained constriction of the coronary vessels (Baydoun et al., 1990; Sakuma et al., 1993; Kusumoto et al., 1996). However, these studies were performed in constant pressure models where the coronary perfusion pressure was maintained above 80 mm Hg. In the absence of any vasodilator response in the present study, a dose of 10 pmol was identified as a threshold dose that did not induce a marked coronary constriction. Administration of this dose prior to coronary occlusion did not modify the total VPB count, but did reduce the arrhythmias seen in the 21–30-min period post-occlusion (phase 1b arrhythmias). This suggests a very moderate antiarrhythmic effect of bolus injection of endothelin-1 in vitro, but it in no way mimics the very marked reduction in both ventricular arrhythmia count and incidence of ventricular fibrillation seen in vivo (Sharif et al., 1998). While it could be argued that the lack of antiarrhythmic effect on arrhythmias could be due to the dose of endothelin-1 being insufficient to have a pharmacological effect, the low K^+ studies demonstrated a vasodilator response with 2 pmol endothelin-1, confirming that we were within an effective concentration range.

Since perfusion of hearts with normal K^+ resulted in a low coronary perfusion pressure, preventing the vasodilator effect of endothelin-1 from occurring, and a low incidence of ventricular fibrillation, which precluded assessment of any antifibrillatory effect of endothelin-1, we studied the effects of bolus injection of endothelin-1 further in hearts perfused with low K^+ solution. Decreasing K^+ concentration had two effects: to increase perfusion pressure in non-ischaemic hearts and to increase the incidence of ventricular fibrillation in controls during occlusion. From the dose–response curve to endothelin-1 in low K^+-perfused hearts, two doses of endothelin-1 were selected for testing against the development of ventricular fibrillation during ischaemia. Neither dose of endothelin-1 had any effect on the incidence of either ventricular tachycardia or ventricular fibrillation, despite the fact that 2 pmol endothelin-1 caused a significant, transient fall in coronary perfusion pressure prior to ischaemia and raised coronary perfusion pressure during coronary occlusion. This effect on coronary perfusion pressure is similar to the effect seen on arterial blood pressure during acute myocardial ischaemia in the in vivo rat model (Sharif et al., 1998). These findings suggest that the marked antiarrhythmic effects seen with endothelin-1 in vivo are more complex than a direct action on the heart and may involve systemic haemodynamic effects and/or release of another mediator from the periphery, which is ultimately responsible for the antiarrhythmic effect. This clearly warrants further investigation, and studies to investigate the in vivo antiarrhythmic effect of endothelin-1 are currently underway.

One further explanation for the failure of endothelin-1 to markedly reduce arrhythmias in vitro may be that, as a non-selective agonist at both endothelin ET_A and ET_B receptors, endothelin-1 may have opposing actions at these two receptors. This hypothesis is supported by our recent demonstration that the endothelin ET_B receptor agonist sarafotoxin 6c reduces arrhythmias (both ventricular tachycardia and ventricular fibrillation) both in vivo and in isolated hearts, under experimental conditions identical to those used in the present study (Crockett et al., 2000), suggesting that activation of endothelin ET_B receptors is antiarrhythmic. Thus, in a scenario where sarafotoxin 6c exerts an antiarrhythmic effect, endothelin-1 does not. One explanation for this may be that simultaneous activation of endothelin ET_A receptors by endothelin-1 may exert a pro-arrhythmic action, which overrides any ET_B-receptor mediated antiarrhythmic effect, although this hypothesis clearly remains to be tested. In addition, the site of endothelin receptor subtypes on the heart, the mechanism by which they influence electrical activity of the heart and the relative access of exogenous endothelin-1 (given prior to the onset of ischaemia) and endogenous endothelin-1 (released during ischaemia) to the different receptor subtypes, all require further investigation.

The major limitation of these studies is that it was not possible to replicate exactly in vitro the concentration of

endothelin-1 that reached the coronary circulation in our previous studies in vivo. From our previous study, we estimate, assuming no breakdown occurred, that approximately 50 pmol of the intravenous bolus dose of endothelin-1 (0.16 nmol 100 g^{-1}) reached the coronary circulation. Here the highest concentrations we could deliver in vitro were 10 pmol in the high K^+ study and 2 pmol in the low K^+ study, due to marked coronary vasoconstriction and development of spontaneous arrhythmias with higher concentrations. Thus, although the concentrations were not identical, they were within the same order of magnitude as that given in vivo.

In conclusion, we have shown that, in contrast to its marked antiarrhythmic effect in vivo, pre-treatment of rat isolated hearts with endothelin-1 prior to the onset of acute myocardial ischaemia does not result in a similar antiarrhythmic effect. This suggests that the antiarrhythmic effect of endothelin-1 observed previously in vivo is not due simply to a direct action of endothelin-1 on the heart.

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