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Electrophysiological and haemodynamic effects of endothelin ET_A and ET_B receptors in normal and ischaemic working rabbit hearts

*,[1]Christopher McCabe, [2]Martin N. Hicks, [1]Kathleen A. Kane & [3]Cherry L. Wainwright

[1]Department of Physiology & Pharmacology, University of Strathclyde, 27 Taylor Street, Glasgow G4 0NP; [2]Department of Medical Cardiology, University of Glasgow, Royal Infirmary, Glasgow G31 2ER and [3]School of Pharmacy, Robert Gordon University, Aberdeen AB10 1FR

1 The aims of this study were to determine if endothelin-1 (ET-1) under normal and ischaemic conditions exhibits a direct arrhythmogenic effect that is independent of its ability to cause coronary vasoconstriction, and to determine the contribution of the ET_A and ET_B receptor subtype.

2 $ET_{A/B}$ (with ET-1) and ET_A (ET-1 in the presence of BQ-788) receptor activation resulted in a significant reduction in both epi- and endocardial monophasic action potential duration ($MAPD_{90}$). ET_A receptor activation reduced both epi- and endocardial effective refractory period (ERP). This $MAPD_{90}$ and ERP shortening were associated with a reduction in coronary flow, myocardial contractility and induction of ventricular fibrillation (VF) during ERP measurement.

3 The ET_B agonist sarafotoxin (S6c) had no marked, or concentration-dependent, effect on $MAPD_{90}$, ERP, myocardial contractility or induction of arrhythmias.

4 Neither ET-1 nor S6c, given prior to coronary artery occlusion, significantly changed the ischaemia-induced dispersion of $MAPD_{90}$, ERP or the % incidence of VF.

5 In conclusion, neither ET_A nor ET_B receptor stimulation has a direct arrhythmogenic effect in isolated rabbit hearts under normal or ischaemic conditions. The ET-1-induced arrhythmogenic effect observed in nonischaemic hearts is likely to be the result of the associated coronary vasoconstriction caused by ET_A receptor stimulation resulting in myocardial ischaemia.
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Abbreviations: ERP, effective refractory period; ET-1, endothelin-1; $MAPD_{90}$, monophasic action potential duration; S6c, sarafotoxin; VF, ventricular fibrillation

Introduction

Endothelin-1 (ET-1) levels have been shown to be increased during myocardial ischaemia in humans (Miyauchi *et al*., 1989; Yasuda *et al*., 1990), pigs (Wang *et al*., 1995) and rabbits (Vitola *et al*., 1996). Exogenous administration of ET-1 results in myocardial ischaemia in dogs through its potent coronary vasoconstrictor ability (Salvati *et al*., 1991) and causes ventricular arrhythmias (Merkely *et al*., 1998). Antagonists at ET-1 receptors protect against ischaemia-induced arrhythmias (Horkay *et al*., 2000; Kiss *et al*., 2000), supporting the view that ET-1 may cause myocardial ischaemia and play a role in the resultant arrhythmogenesis.

ET-1 has also been shown to exhibit a direct arrhythmogenic effect, which is independent of its ability to cause myocardial ischaemia (Yorikane *et al*., 1991; Toth *et al*., 1995). However, the mechanism(s) that may underlie any such direct effect have not been clarified. Becker *et al*. (2000) showed that intracoronary administration of ET-1 induced ventricular arrhythmias that were not associated with changes in refractory periods or conduction delay, such changes usually being associated with myocardial ischaemia (Fozzard & Makielski, 1985). On the other hand, Szabo *et al*. (2000) reported that ET-1 induced arrhythmias and prolonged left ventricular (LV) monophasic action potential duration (MAPD) in dogs. It has been suggested that a mechanism, which may contribute to ET-1's direct arrhythmogenic effect, is regional heterogeneity in the electrophysiological effects of ET-1, increased dispersion of action potential duration and refractoriness and the generation of early after-depolarisations (EADs) (Geller *et al*., 1998; Duru *et al*., 2001). Thus, there exists controversy in the literature about the extent to which the arrhythmogenic effect of ET-1 is a consequence of the induction of myocardial ischaemia or a direct electrophysiological effect, and, if direct, what the mechanism is. To date, there have been no studies that have looked at the effect of ET-1 on dispersion of action potential duration and refractoriness; therefore, one of the aims of the current study is to determine if ET-1 modifies dispersion in such a way as to explain the arrhythmogenic effects of ET-1.

ET-1 acts as an agonist at both ET_A and ET_B receptors. The arrhythmogenic action of exogenous ET-1, the MAPD prolongation and EAD formation has been shown to be blocked by the ET_A selective antagonist, Lu 135.252, suggesting that these effects are mediated *via* ET_A receptors (Kiss *et al*., 2000). In contrast, stimulation of ET_B receptors with the selective agonist, sarafotoxin (S6c), exerted an antiarrhythmic

*Author for correspondence at: Division of Clinical Neuroscience, Wellcome Surgical Institute, Garscube Estate, University of Glasgow, Glasgow G61 1QH, U.K.; E-mail: cmc39v@clinmed.gla.ac.uk

action on ischaemia-induced arrhythmias *in vivo* (Crockett *et al*., 2000). Although ET_B receptors are found within the heart predominantly in the conducting system and endocardium (Molenaar *et al*., 1993), no studies appear to have examined what electrophysiological effects they produce in whole hearts, if any such effects are more pronounced in the endo- vs the epicardium and if these could explain an antiarrhythmic action of ET_B stimulation.

The aims of this study were to use an isolated working rabbit heart model in which $MAPD_{90}$, the effective refractory period (ERP) and conduction delay could be measured in both the endocardium and epicardium and correlated with haemodynamic variables to answer the following questions. Does ET-1, under normal and ischaemic conditions, have a direct arrhythmogenic effect that is independent of its ability to cause coronary vasoconstriction, and what is the contribution of ET_A and ET_B receptors to any observed effect? To address the latter question, studies were carried out using the selective ET_B receptor agonist, S6c, and the mixed agonist, ET-1. Since there is no potent selective ET_A agonist, the effects of ET-1 on ET_A receptors were determined in the presence of the ET_B antagonist, BQ-788 (Ishikawa *et al*., 1994). Regional myocardial ischaemia results in dispersion of action potential duration and refractoriness between the ischaemic and non-ischaemic areas and it is thought that this dispersion contributes to the increased arrhythmogenicity. Therefore, in the current study, we used a regional model of ischaemia in order to determine if ET-1 or S6c modified this ischaemia-induced dispersion.

Methods

Whole heart preparation

Male New Zealand white rabbits (weight 2.5–3.4 kg) were used. The rabbits were euthanised with sodium pentobarbitone (100 mg kg^{-1}), containing heparin (2000 IU), administered through the ear vein. The hearts were rapidly excised and placed in a beaker of ice-cold Tyrode's solution. Within 4 min of removal, the aorta was cannulated and retrograde perfusion by the Langendorff technique was started. The perfusate was a modified Tyrode's solution (composition in mM: NaCl 112.9, $NaHCO_3$ 20, glucose 10.9, KCl 3.9, $MgCl_2$ 0.99, NaH_2PO_4 0.44, $CaCl_2$ 1.8) equilibrated with 95% O_2/5% CO_2 to provide a pH of 7.4. During Langendorff perfusion, the left atrium was cannulated to allow perfusion in the working heart mode. Once cannulation of the left atrium was complete, opening the left atrial inflow began perfusion in the working heart mode. The perfusion pressures were set at 10 cm H_2O for the preload and 75 cm H_2O for the afterload. The heart was enclosed in a temperature-controlled chamber and epicardial surface temperature was maintained at $35 \pm 0.1^{\circ}C$. The right atrium was paced at twice diastolic threshold by using a pair of platinum electrodes with a basic cycle length of 300 ms and a pulse width of 2 ms, generated by an isolated stimulator (model DS2; Digitimer Ltd, Welwyn Garden City, U.K.). For ischaemia/reperfusion studies, a loose loop of atraumatic silk (3/0 Mersilk) was placed through a snare around the marginal branch of the left coronary artery, to allow for occlusion of the coronary artery.

Electrophysiological measurements

Monophasic action potentials (MAPs) were recorded continuously from the apical epicardium and endocardium. In ischaemia/reperfusion studies, an additional epicardial MAP was recorded in the nonischaemic area (area above occlusion). Epicardial MAPs were recorded using custom-made suction electrodes (Ag–Ag/Cl), which were connected to an MAP amplifier. A pair of platinum electrodes was placed close to each epicardial MAP electrode for measurement of the local ERPs. Local ERP was determined during local ventricular pacing by the extrastimulus technique, by using square wave impulses of 2 ms duration at twice diastolic threshold. A train of eight regular stimuli (S1) with a cycle length of 300 ms, driven by a computer-based simulation programme, was followed by an early 'ineffective' extrastimulus (S2). This extrastimulus was introduced progressively later in 5 ms steps until it produced an action potential. The ERP was defined as the longest S1S2 interval at which S2 failed to produce an action potential.

Endocardial MAPs were recorded using a contact electrode catheter, which was located in the apical endocardium directly across from the epicardial electrode. ERPs were recorded from the endocardium by local ventricular pacing *via* bipolar stimulating electrodes present on the electrode catheter, *via* the extrastimulus technique as described previously.

Haemodynamic measurements

A 20-gauge venflon catheter was inserted into the left ventricle and connected to a pressure transducer to allow determination of LV systolic and LV end diastolic pressures. The differential of the pressure signal was also recorded as an index of contractility (dP/dt_{max}). Aortic and coronary flow was measured by using an inline flowmeter placed above the aorta and cardiac output was determined as the sum of the aortic and coronary flows. A minimum baseline forward flow of 80 ml min^{-1} was required for a preparation to be included in the experimental protocol.

Experimental protocol

The hearts were perfused in working heart mode for 50–60 min to allow for equilibration. At the end of this period, the solution and filter was renewed and a further equilibration period of 15 min was allowed. Then, baseline recordings were taken at three time points (0, 15 and 30 min). ERPs were recorded first in the epicardium followed by the endocardium at each of the above time points. MAPs and haemodynamic measurements were continuously recorded. If the extrastimulus used to measure ERP induced ventricular fibrillation (VF), then the hearts were electrically defibrillated and the protocol continued.

After baseline recordings were complete, the solution and filter was renewed for a second time and an equilibration period of 15 min was allowed. After the equilibration period, a baseline recording was obtained before vehicle (distilled H_2O), ET-1, S6c or BQ-788 + ET-1 administration commenced. ET-1, S6c or vehicle was added cumulatively directly into the Tyrode's solution with 15 min intervals between each dose (10^{-12} M, 3×10^{-12} M … 10^{-9} M). BQ-788 (30 nM) was added 15 min prior to ET-1 administration. All recordings were taken

15 min after the addition of vehicle or drug. This time point was chosen in that preliminary experiments showed that any observed electrophysiological or haemodynamic changes reached steady state by 5 min.

In a separate group of hearts, in which ischaemia/reperfusion was carried out once the baseline recordings were complete, the filter was renewed and the solution replaced with one containing either vehicle (distilled H_2O), 10^{-10} M ET-1 or 10^{-8} M S6c. After an equilibration period of 15 min, a baseline recording was taken prior to occlusion of the coronary artery. A 30 min period of ischaemia followed, during which MAP and haemodynamic variables were recorded continuously. ERPs were recorded after 15 min of ischaemia, first in the epicardial area at risk, and then in the endocardium followed lastly by the epicardial nonischaemic area. When any sustained arrhythmia appeared spontaneously, or was induced by the stimulation protocol, defibrillation with a DC shock was used after 30 s to restore normal rhythm. Time was subsequently allowed for stability to be re-established. Area at risk was assessed at the end of the reperfusion period by injection of Evans blue dye and expressed as a percentage of the left ventricle.

Data analysis

All signals were recorded onto videotape to allow for subsequent analysis off-line. The signals were analysed using the Whole-Cell Programme (WCP V3.12) supplied by John Dempster, University of Strathclyde. Before analysis, the signals were averaged in groups of 15 consecutive MAPs. APD was measured at 90% repolarisation ($MAPD_{90}$).

All data were expressed as mean ± s.e.m. All statistical comparisons were performed using GraphPad Prism analysis package. Significance was tested using a repeated-measures ANOVA followed by a Dunnet's multiple comparison post-test. All differences were taken as significant if $P<0.05$. For the % incidence of VF, a Fisher's exact test was performed.

Drugs

ET-1 (Sigma Chemical Co., Poole, Dorset, U.K.) and S6c (Alexis Biochemicals, Bingham, Nottingham, U.K.) were dissolved in distilled H_2O and added cumulatively into the circulating Tyrode solution. BQ-788 (Alexis Biochemicals, Bingham, Nottingham, U.K.) was dissolved in 10% DMSO and administered as a 3.5-ml aliquot into the circulating Tyrode's solution to obtain the desired final concentration of 30 nM.

Results

Effects of ET-1, ET-1 + BQ-788 and S6c on electrophysiological measurements in normal hearts

At the beginning of the experimental protocol, $MAPD_{90}$ was significantly longer in the endo- than the epicardium (131 ± 3 vs 119 ± 3 ms, $n=24$). In appropriate vehicle-treated time control hearts, there were no statistically significant haemodynamic or electrophysiological changes throughout the duration of the experimental protocol. As shown in the representative traces (Figure 1) and the mean data (Figure 2),

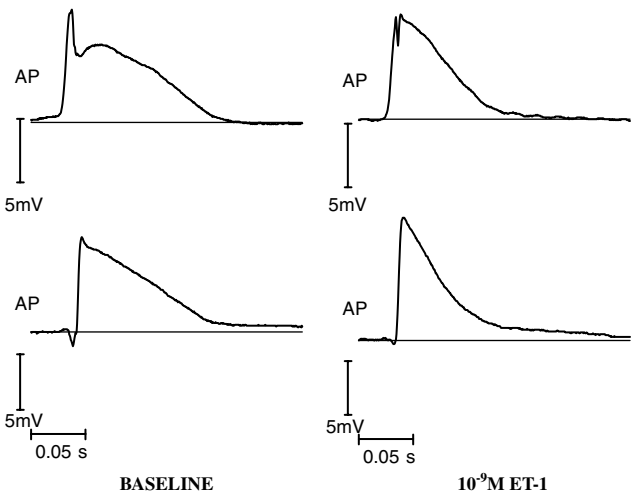

Figure 1 Representative MAP recordings from the endocardium (top) and epicardium (bottom) before (baseline) and after 10^{-9} M ET-1 in an isolated working rabbit heart.

ET-1 shortened $MAPD_{90}$, in a concentration-dependent manner, in both the endo- and epicardium with statistically significant decreases being observed at 10^{-10} M ET-1 and above. In the presence of the ET_B receptor antagonist, BQ-788, ET-1 also caused a reduction in $MAPD_{90}$ (at 10^{-11} M in the epicardium and 3×10^{-11} M in the endocardium) (Figure 2). In contrast, the ET_B receptor agonist S6c had little effect on $MAPD_{90}$ causing a slight prolongation in the endocardium but not the epicardium at the highest concentrations studied (Figure 2).

By and large, the changes in ERP paralleled the observed changes in $MAPD_{90}$. ET-1, in the presence of BQ-788, significantly reduced both endo- and epicardial ERP (at 10^{-10} M from 149 ± 2 to 134 ± 4 and from 153 ± 4 to 132 ± 4 ms in the endo- and epicardium, respectively). The ET_B agonist, S6c (at 3×10^{-9} M), in line with effects on $MAPD_{90}$, prolonged ERP in the endo- (139 ± 6–156 ± 7 ms) but not the epicardium (Figure 3). ET-1 alone tended to reduce ERP in the epicardium (from 149 ± 7 to 137 ± 7 and 109 ± 10 ms at 10^{-10} and 10^{-9} M, respectively), but these data failed to reach statistical significance (Figure 3). Both epicardial and endocardial conduction delay was increased by ET-1 with significant changes occurring at 10^{-10} M and above (from 107 ± 3 to 116 ± 6 and from 95 ± 3 to 104 ± 6 ms in the epi- and endocardium, respectively). Similarly, ET-1, in the presence of the ET_B receptor antagonist, BQ-788, also increased conduction delay in both the epi- and endocardium. At 3×10^{-11} M ET-1, in the presence of BQ-788, conduction delay was increased from 105 ± 3 to 112 ± 4 ms and from 89 ± 3 to 96 ± 3 ms in the epi- and endocardium, respectively. S6c did not significantly increase conduction delay in the endocardium, but at 10^{-9} M (and above), epicardial conduction delay was increased from 115 ± 2 to 123 ± 4 ms.

Table 1 summarises the effects of the drugs studied on the incidence of VF induced during the application of the extrastimulus to measure ERP. ET-1, at 10^{-10} M, induced VF in five out of six hearts studied, and, in the presence of BQ-788, VF occurred in four out of six hearts but at the lower concentration of 10^{-11} M. BQ-788 alone did not induce VF nor did the ET_B agonist, S6c.

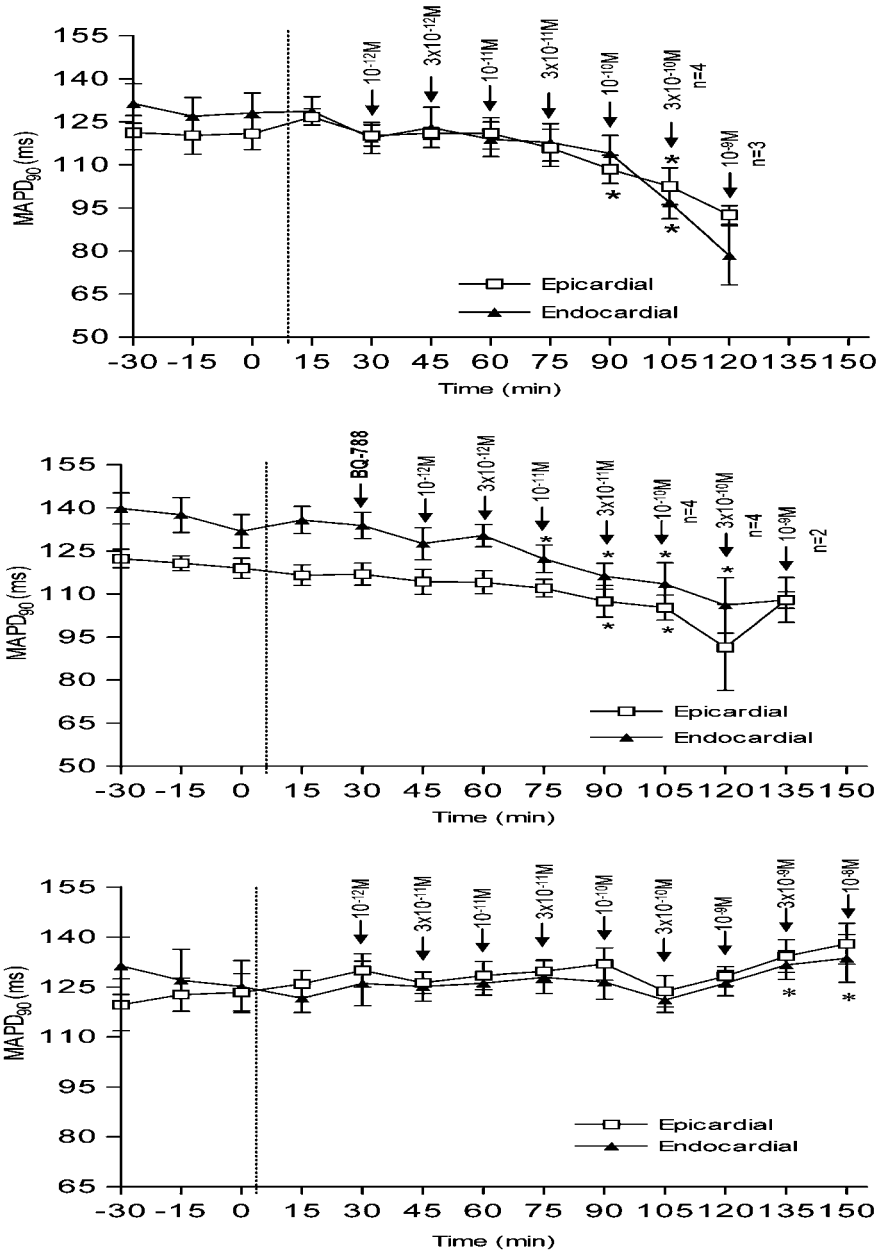

Figure 2 Effect of cumulative ET-1 (10^{-12}–10^{-9} M) (top), ET-1 in the presence of BQ-788 (3×10^{-8} M) (middle) and S6c administration (10^{-12}–10^{-8} M) (bottom) on LV epicardial and endocardial monophasic action potential duration ($MAPD_{90}$) measured at 90% repolarisation ($MAPD_{90}$, ms) in isolated working rabbit hearts. Dashed line indicates solution change. $^*P<0.05$ compared to baseline (15 min) values. $n=6$, unless otherwise stated.

Effects of ET-1, ET-1 + BQ-788 and S6c on haemodynamic variables in normal hearts

Figure 4 compares the effects of ET_A and ET_B receptor stimulation on coronary blood flow in normal rabbit hearts. ET-1, either alone or in combination with BQ-788, caused a concentration-dependent reduction in coronary blood flow (Figure 4). Coronary blood flow was significantly reduced by S6c at 10^{-10} M, but further reductions were not observed at higher concentrations. ET-1, alone or in the presence of BQ-788, also reduced aortic flow, cardiac output and myocardial contractility. At concentrations above 10^{-9} M ET-1, aortic flow was reduced to the extent that there was insufficient cardiac output to maintain coronary perfusion and the experiments had to be terminated. Unlike ET-1, S6c over the concentration range studied had no significant effect on myocardial contractility or LV systolic pressure (Table 2).

Table 2 summarises the effects of vehicle, 10^{-10} M ET-1, 10^{-8} M S6c and ET-1 in the presence of BQ-788 on aortic flow, cardiac output, LV diastolic and systolic pressures and dP/dt_{max}, an index of myocardial contractility. In vehicle-treated hearts, there was a trend towards a reduction in aortic flow and cardiac output at the later time points; however, this did not reach statistical significance. ET-1 alone and in the presence of BQ-788 exhibited a marked reduction in aortic flow, cardiac output and myocardial contractility. S6c administration

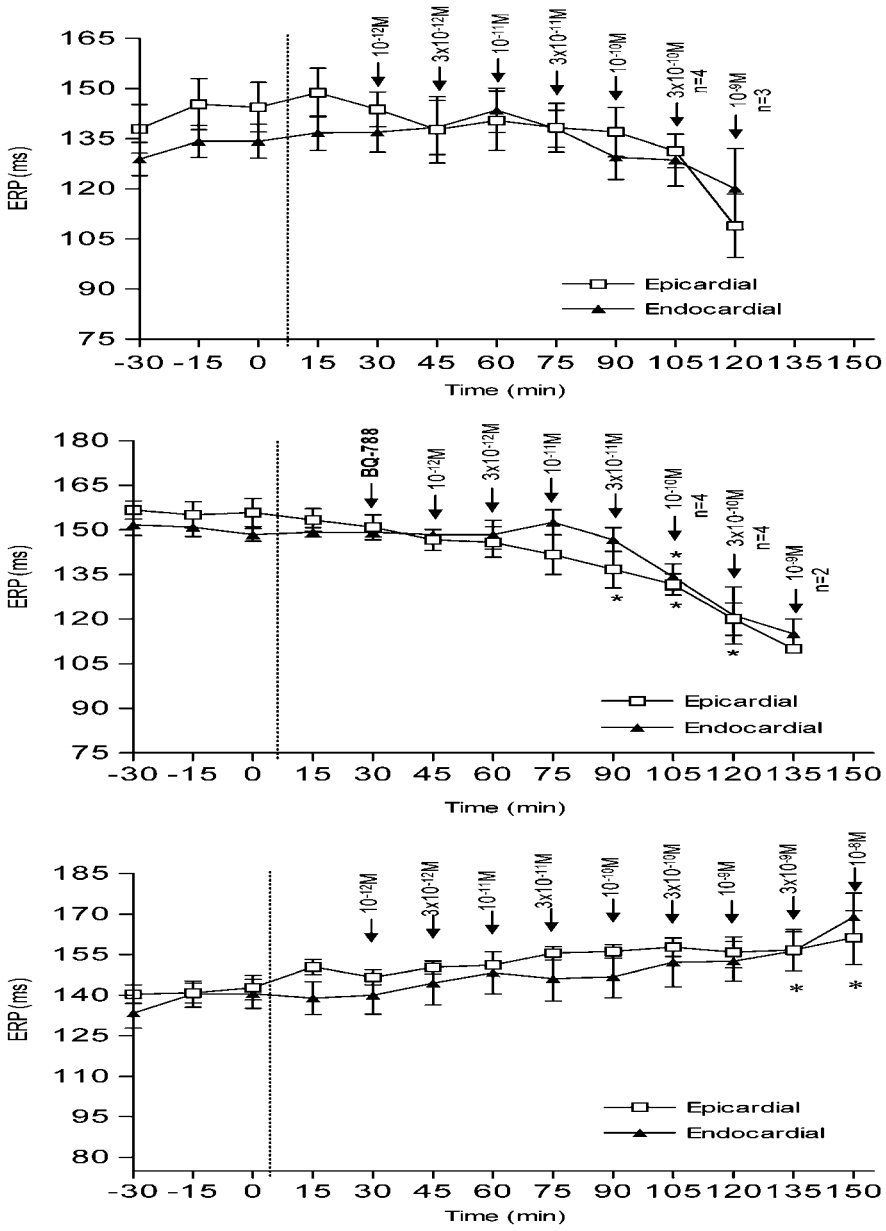

Figure 3 Effect of cumulative ET-1 (10^{-12}–10^{-9} M) (top), ET-1 in the presence of BQ-788 (3×10^{-8} M) (middle) and S6c administration (10^{-12}–10^{-8} M) (bottom) on LV epicardial and endocardial ERP (ERP, ms) in isolated working rabbit hearts. Dashed line indicates solution change. $^*P<0.05$ compared to baseline (15 min) values. $n=6$, unless otherwise stated.

Table 1 The % incidence of VF induced by an extrastimulus in normal and ischaemic hearts treated with vehicle, ET-1, ET-1 + BQ-788 or S6c

	% *Incidence VF*
Vehicle, normal	0
ET-1	83.3*
ET-1 + BQ788	66.6
BQ788 alone	0
S6c	0
Vehicle, ischaemia	42
ET-1 + ischaemia	50
S6c + ischaemia	33

$^*P<0.05$ indicates a statistically significant difference from vehicle treatment in normal hearts.

resulted in a trend towards a reduction in myocardial contractility that was associated with a significant reduction in aortic flow and cardiac output.

Effect of ET-1 and S6c in the presence of myocardial ischaemia/reperfusion

Concentrations of ET-1 and S6c that resulted in significant electrophysiological effects were chosen based on the previous concentration response experiments; therefore, 10^{-10} M ET-1 and 10^{-8} M S6c was used. Appropriate time control experiments were carried out where single concentrations of either ET-1 (10^{-10} M) or S6c (10^{-8} M) were given without ischaemia. Any haemodynamic and electrophysiological changes observed

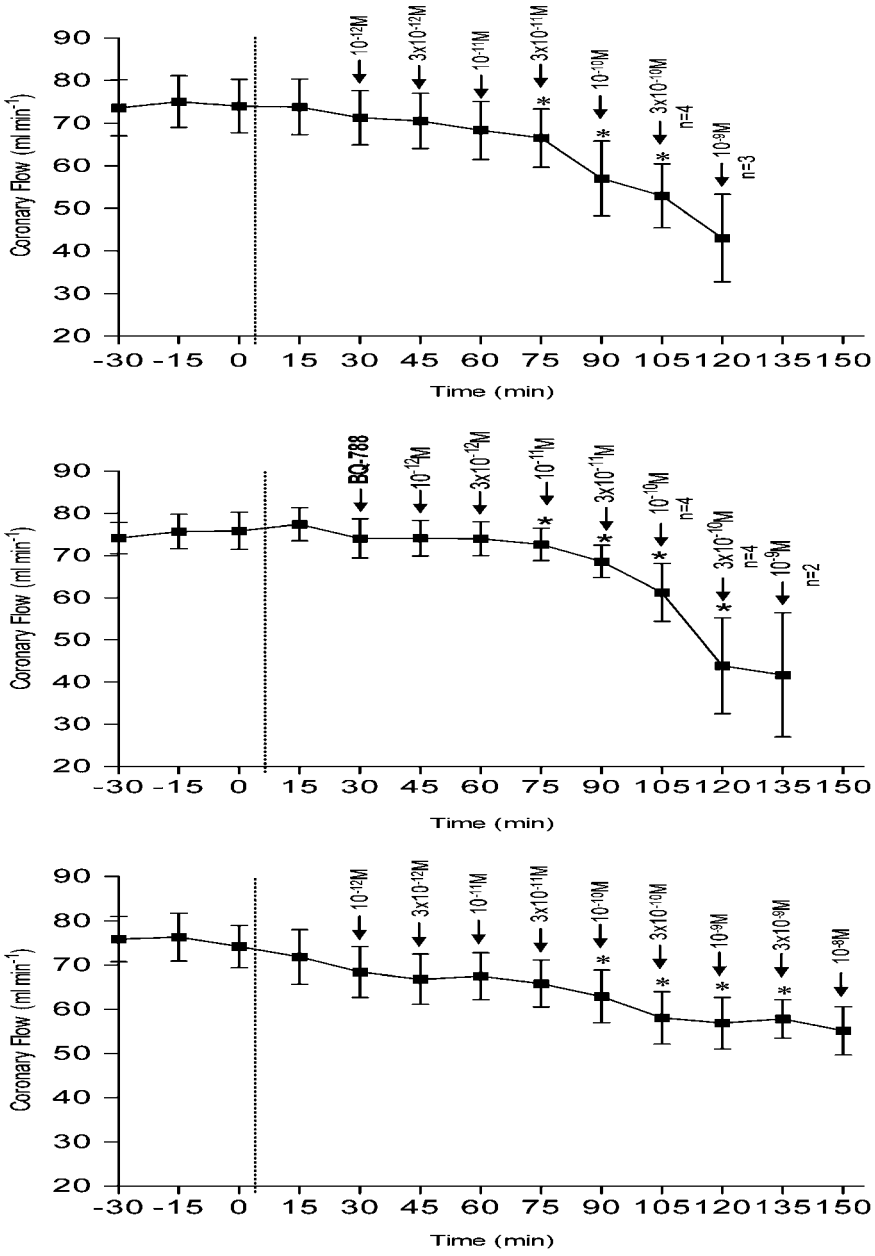

Figure 4 Effect of cumulative ET-1 (10^{-12}–10^{-9} M), ET-1 (10^{-12}–10^{-9} M) in the presence of BQ-788 (3×10^{-8} M) and S6c (10^{-12}–10^{-8} M) administration on coronary flow in isolated working rabbit hearts. Dashed line indicates solution change. $^*P<0.05$ compared to baseline (15 min) values. $n=6$, unless otherwise stated.

Table 2 Effects of ET-1, ET-1 + BQ788 and S6c on haemodynamic variables in normal working hearts

	Aortic flow (ml min^{-1})	*Cardiac output* (ml min^{-1})	*LV diastolic pressure* (mmHg)	*LV systolic pressure* (mmHg)	dP/dt_{max} (mmHg s^{-1})
Baseline vehicle	159±5	223±10	7±3	92±4	714±32
90 min postvehicle	148±6	208±11	9±3	89±4	647±36
Baseline ET-1	195±9	269±15	6±1	104±4	804±42
ET-1 10^{-10} M	134±25*	191±34*	12±2*	88±6*	647±71*
Baseline ET-1 + BQ788	225±5	302±5	6±1	114±3	952±28
ET-1 10^{10} M + BQ788	144±22*	205±27*	13±2*	96±9	752±94*
S6c baseline	173±10	245±16	8±1	94±3	680±43
S6c 10^{-8} M	132±15*	188±18*	11±2*	89±5	630±60

$^*P<0.05$ indicates a statistically significant difference from appropriate baseline groups.

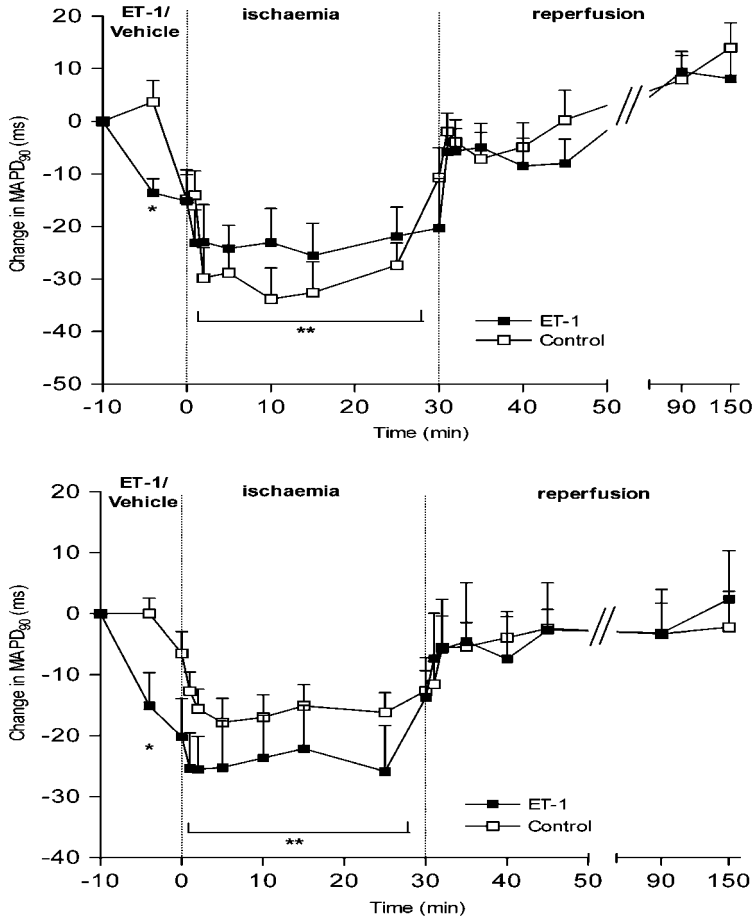

Figure 5 Change in $MAPD_{90}$ in the epicardium (AAR) (top) and endocardium (bottom) of working rabbit hearts treated with 10^{-10} M ET-1 or vehicle before and during acute regional ischaemia and reperfusion. *Significantly different from predrug value. **Statistically significant differences from respective preischaemic values ($P<0.05$ in each case) ($n=6$–12).

following drug administration occurred within 5 min and were maintained throughout the duration of these experiments. Coronary artery occlusion caused a significant reduction in $MAPD_{90}$ in both the epicardium and in the endocardium (shown in Figure 5). ET-1, at 10^{-10} M, administered prior to coronary artery occlusion also abbreviated $MAPD_{90}$ and the consequent coronary artery occlusion induced fall in $MAPD_{90}$ was less than in vehicle-treated hearts. This is illustrated for the epicardium within the area at risk in Figure 5, where the absolute occlusion-induced reductions in $MAPD_{90}$ at 15 min postischaemia were significantly different (38 ± 6 vs 12 ± 5 ms in vehicle and ET-1-treated hearts, respectively). In the endocardium, ET-1 also abbreviated $MAPD_{90}$ prior to coronary artery occlusion and occlusion itself caused a further reduction in $MAPD_{90}$ that was less than the absolute reduction observed in vehicle-treated hearts. However, in the endocardium, this difference was not statistically significant (16 ± 3 vs 7 ± 6 ms in vehicle and ET-1-treated hearts, respectively). In contrast to ET-1, S6c (10^{-8} M) had no statistically significant effect on $MAPD_{90}$ either before or during coronary artery occlusion/reperfusion in the endo- or epicardium (Figure 6). Neither ET-1 nor S6c had any statistically significant effect on ERP prior to occlusion of the coronary artery. Upon occlusion of the coronary artery, a significant decrease in epi- and endocardial ERP was observed and the magnitude of this ischaemia-induced decrease was similar in the epi- and endocardium with respective decreases in vehicle (-22 ± 5 and -14 ± 4 ms), ET-1 (-21 ± 7 and -14 ± 10 ms) and S6c (-13 ± 5 and -15 ± 9 ms) pretreated hearts.

It can be seen in Figures 5 and 6 that the decreases in ischaemia-induced abbreviation of $MAPD_{90}$ was greater in the epi- than the endocardium. This results in an increased dispersion of $MAPD_{90}$ between the two areas at 15 min post-occlusion. The endo-epicardial dispersion was 4 ± 4 ms postvehicle and 26 ± 5 ms ($P<0.5$) at 15 min postischaemia. Similarly, there was increased dispersion of $MAPD_{90}$ between the epicardial area above occlusion and area at risk at 15 min postischaemia (26 ± 9 ms) vs postvehicle (-15 ± 10 ms). However, administration of ET-1 and S6c did not significantly modify either endo-epicardial or transepicardial dispersion of $MAPD_{90}$. No spontaneous arrhythmias were observed in any of the groups during the experimental protocol; however, introduction of the extrastimulus to measure ERP at 15 min postischaemia induced VF in 42% of the control hearts. Neither ET-1 nor S6c modified the % incidence of VF that occurred during ischaemia when compared to vehicle-treated hearts subject to ischaemia (Table 1). In the ET-1 and S6c time control experiments in which ET-1 or S6c was administered but coronary artery occlusion was not carried out, 50 and 0% of hearts, respectively, fibrillated during the ERP measurement ($n=6$).

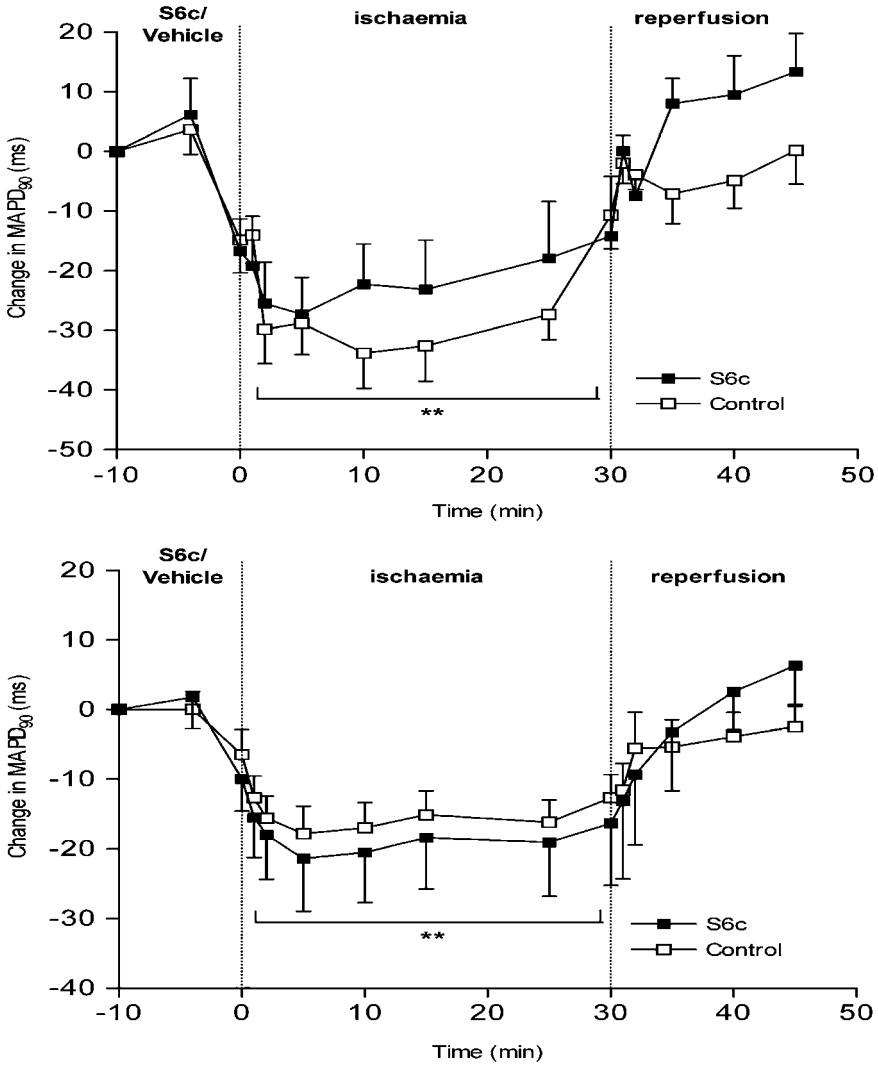

Figure 6 Change in $MAPD_{90}$ in the epicardium (AAR) (top) and endocardium (bottom) of working rabbit hearts treated with 10^{-8} M S6c or vehicle before and during acute regional ischaemia and reperfusion. **Statistically significant differences from respective preischaemic values ($P<0.05$ in each case) ($n=6$–12).

Figure 7 shows the changes in coronary flow that occurred in control, ET-1- and S6c-treated hearts subjected to myocardial ischaemia. ET-1, but not S6c, reduced coronary flow prior to ischaemia. Coronary flow was reduced following coronary artery occlusion, but at 15 min postischaemia, the fall in coronary flow was similar in ischaemia alone and ischaemia + ET-1-treated hearts with respective decreases of -11 ± 2 and -16 ± 4 ml min^{-1}. In line with effects on coronary flow, coronary artery occlusion and ET-1 both reduced myocardial contractility, LV systolic pressure, aortic flow and cardiac output (Table 3). When combined, there were further significant reductions in aortic flow and cardiac output but not LV systolic pressure and myocardial contractility. S6c did not affect the haemodynamic variables before coronary artery occlusion and during occlusion the changes were similar to those in control occluded hearts.

Reperfusion of the coronary artery in control and drug-treated hearts caused the $MAPD_{90}$ (Figure 5) and ERP (data not shown) to return to preocclusion values. There were no differences in these variables during reperfusion between control and drug-treated hearts. Measurement of the ERP at 15 min postreperfusion did not induce VF in any of the hearts studied. Postreperfusion there was partial recovery of coronary flow in control and S6c-treated hearts. Area at risk expressed as a percentage of the left ventricle was not significantly different between the three treatment groups (vehicle + ischaemia, $27\pm3\%$; ET-1 + ischaemia, $26\pm4\%$; S6c + ischaemia, $30\pm1\%$).

Discussion

The results of the present study support the view that stimulation of ET_A, but not ET_B, receptors is arrhythmogenic in isolated rabbit hearts as a consequence of a coronary vasoconstrictor rather than a direct cardiac electrophysiological action. ET-1 an agonist at both ET_A and ET_B receptors reduced, in a concentration-dependent manner, both epi- and

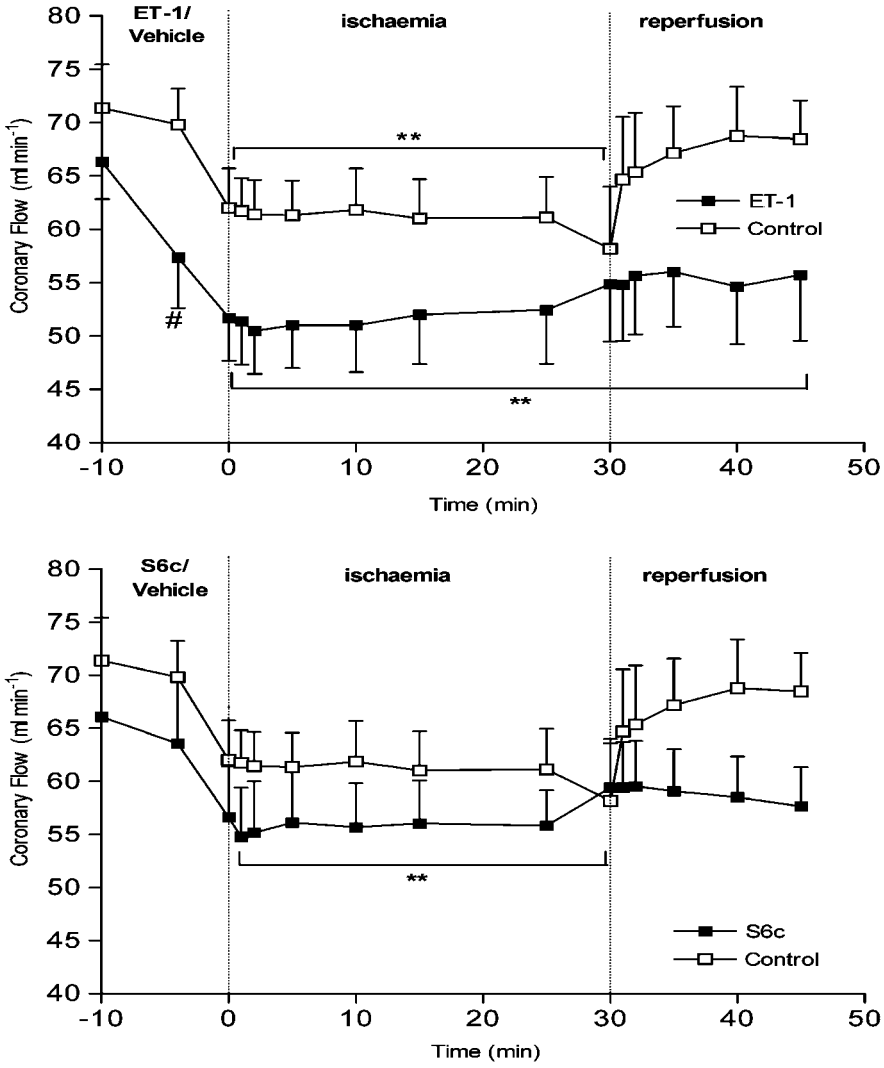

Figure 7 Coronary flow in working rabbit hearts treated with vehicle, 10^{-10} M ET-1 (top) or S6c (10^{-8} M) (bottom) before and during acute regional ischaemia and reperfusion. #Statistically significant difference from control ligation values. **Statistically significant difference from respective preischaemic values ($P<0.05$ in each case) ($n=6$–12).

Table 3 Effects of ischaemia alone and in the presence of ET-1 and S6c on haemodynamic variables in isolated rabbit hearts

	Aortic flow (ml min^{-1})	*Cardiac output* (ml min^{-1})	*LV systolic pressure* (mmHg)	dP/dt_{max} (mmHg s^{-1})
Ischaemia				
Baseline	172±12	240±14	95±4	688±52
15 min ischaemia	124±15*	183±17*	86±4*	590±44*
15 min reperfusion	135±17*	202±18*	90±4	657±38
ET-1 + ischaemia				
Baseline	202±13	268±17	98±2	719±30
ET-1	150±27*	207±31*	87±5*	620±54*
ET-1 + ischaemia	117±22*	169±27*	79±5*	613±68*
ET-1 + reperfusion	137±27*	193±33*	83±6*	618±59*
S6c + ischaemia				
Baseline	179±13	245±17	93±3	577±27
S6c	191±17	255±22	92±4	576±35
S6c + ischaemia	139±13*	195±16*	90±2*	522±15*
S6c + reperfusion	139±11*	197±13*	84±5*	503±57

*$P<0.05$ indicates a statistically significant difference from appropriate baseline groups.

endocardial $MAPD_{90}$, increased conduction delay and these electrophysiological changes were accompanied by reductions in coronary flow and myocardial contractility. The observed electrophysiological effects of endothelin receptor stimulation are independent of the negative chronotropic action of ET-1 that has been reported in rabbit sinoatrial node (Ono *et al*., 2001), since they were observed in hearts that were paced at a constant frequency. The profile of electrophysiological changes seen with ET-1 is similar to that observed following coronary artery occlusion in this isolated working heart model. Abbreviation of the action potential duration and ERP in the ischaemic zone led to increased dispersion of these variables between the endo- and epicardium and across the epicardium (area at risk vs normal) as described previously (Wolk *et al*., 1999). Furthermore, both ET-1 and coronary artery occlusion led to the occurrence of VF following the introduction of the extrastimulus to measure ERP. We, therefore, found no evidence to suggest that ET-1 may have a direct arrhythmogenic effect independent of a reduction in coronary flow. These data do not agree with previously published work which shows that intracoronary administration of low doses of ET-1 in anaesthetised dogs causes prolongation of ventricular MAPs, dispersion of repolarisation and cardiac arrhythmias without a marked effect on coronary flow (Merkely *et al*., 1998; Kiss *et al*., 2000; 2004). There are several possible explanations for the observed differences between the present and previous studies. For example, species differences may be relevant but of more likely importance is the fact that our work was carried out in isolated hearts devoid of neurohumoral influences of blood or nervous system.

The negative inotropic effect observed with ET-1 in the current study contrasts with the positive inotropic effect observed in isolated myocytes (Kelso *et al*., 2000) or muscle (Talukder Hassan *et al*., 2001) and may be explained by the concomitant reduction in coronary flow and resulting myocardial ischaemia. Ischaemia, induced by coronary artery occlusion, was shown to cause a similar profile of haemodynamic changes to that of ET-1. In a recent study in anaesthetised rats, ET_A receptor stimulation was shown to result in a negative inotropic effect, which was associated with a reduction in cardiac output and ejection fraction (Beyer *et al*., 2004). In that study, even when the vasoconstrictor effect of ET-1 was attenuated with adenosine, no positive inotropic effect was observed and the negative inotropic effect was attributed to ET_A receptor-induced vasoconstriction and myocardial ischaemia.

The data obtained in this study also supports the view that the electrophysiological, arrhythmogenic and coronary vasoconstrictor effects of ET-1 are ET_A receptor mediated. S6c, the ET_B receptor agonist, had no concentration-dependent effect on either endo- or epicardial action potentials, coronary flow or myocardial contractility and did not induce VF. This lack of effect of S6c on haemodynamics has previously been reported (Sargent *et al*., 1994), but there is no published data on the electrophysiological effect of ET_B receptor stimulation in whole hearts. In the presence of the ET_B receptor antagonist, ET-1 produced its electrophysiological and vasoconstrictor effects at a lower concentration than when given alone, suggesting that ET_B receptor stimulation with ET-1 may cause some counteracting coronary vasodilation. Overall, however, our data suggest that in isolated working rabbit hearts stimulation of ET_B receptors does not exert a direct electrophysiological effect and that ET-1-induced arrhythmias are ET_A receptor mediated. This conclusion is in line with *in vivo* studies, which have demonstrated that ET-1-induced arrhythmias are prevented in the presence of an ET_A receptor antagonist (Merkely *et al*., 2000). It would be of interest to look at the effects of ET-1 in the presence of an ET_A selective antagonist devoid of direct electrophysiological effects and/or in the presence of a vasodilator to further confirm that the proarrhythmic effects of ET-1 are indeed mediated through the ET_A receptor.

It has previously been shown by our laboratory that ligation of the coronary artery in the isolated working rabbit heart results in regional myocardial ischaemia and causes an abbreviation of the $MAPD_{90}$ in the ischaemic zone while the nonischaemic area is unaffected (Wolk *et al*., 1998; 2000). When given prior to coronary artery occlusion as a single dose, ET-1 but not S6c reduced coronary flow and abbreviated $MAPD_{90}$. The subsequent abbreviation in $MAPD_{90}$ caused by coronary artery occlusion was less in ET-1-pretreated than in vehicle-control hearts, supporting the view that ET-1 itself had caused myocardial ischaemia. It has previously been established that there is a direct correlation between action potential shortening and myocardial ischaemia with the greater the degree of ischaemia causing a more pronounced abbreviation of the action potential duration (Downar *et al*., 1977; Franz *et al*., 1984). The ischaemia-induced dispersion in $MAPD_{90}$ and ERP between the endo- and epicardium and across the epicardium was not modified by the presence by ET-1 nor was the incidence of ischaemia-reduced VF. These data further support the conclusion that ET_A but not ET_B receptor stimulation causes cardiac electrophysiological changes that are a consequence of myocardial ischaemia. In agreement with the present study, it has been shown in anaesthetised rabbits that infusion of ET-1 during coronary artery occlusion and reperfusion did not affect the incidence of VF compared to control animals, albeit that there was an increased incidence in premature ventricular contractions in the ET-1 group (Vitola *et al*., 1996). In the isolated rabbit heart, however, ectopic arrhythmias did not occur in the presence of the endothelin agonists and/or coronary artery occlusion. It has previously been shown in the *in vivo* rat that both ET-1 (Sharif *et al*., 1998) and S6c (Crockett *et al*., 2000) can reduce ischaemia-induced arrhythmogenesis. The present study did not yield any evidence for a direct electrophysiological effect of either ET_A or ET_B receptor stimulation that might explain such an antiarrhythmic action.

In conclusion, it has been demonstrated that, in isolated working rabbit hearts, stimulation of ET_A, but not ET_B, receptors is proarrhythmic as a consequence of coronary vasoconstriction and the resultant ischaemia-induced changes in cardiac electrophysiology.

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