Enhanced α -adrenoceptor responsiveness and receptor number during global ischaemia in the Langendorff perfused rat heart

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1 Idioventricular rate responses and adrenoceptor number have been examined in normal Langendorff hearts perfused at 70 cmH₂O and in 'ischaemic' hearts perfused at 10 cmH₂O.

2 In hearts perfused at normal pressure, ventricular rate responses to phenylephrine in the presence of propranolol were biphasic with low concentrations of phenylephrine reducing, and high concentrations increasing, ventricular rate. Both responses were abolished in the presence of prazosin (100 nM). In hearts perfused at low pressure, the negative chronotropic responses to phenylephrine were no longer present and positive chronotropic responses were enhanced compared with those of control tissues. The number of ventricular [³H]-prazosin binding sites was also significantly increased during ischaemia.

3 Idioventricular rate responses to isoprenaline were depressed in ischaemic tissues compared with controls, but [³H]-dihydroalprenolol binding was not altered in these hearts.

4 The incidence and duration of arrhythmias which occurred during 30 min of global ischaemia and reperfusion were not significantly altered in the presence of 100 nM prazosin.

5 These results demonstrate that reducing the perfusion pressure of rat isolated hearts enhances α -adrenoceptor-mediated responses and increases α -adrenoceptor density. Whether the increase in α -adrenoceptor responsiveness during ischaemia is involved in arrhythmogenesis requires further investigation.

Introduction

Ventricular arrhythmias form an important complication of acute myocardial ischaemia and reperfusion, but the factors involved in arrhythmogenesis have not yet been fully established. Enhanced sympathetic activity and locally induced noradrenaline release, have been identified as factors involved in the genesis of ventricular arrhythmias (Dart & Riemersma, 1985; Schomig, 1988) and activation of myocardial β -adrenoceptors with the resulting increase in cyclic AMP has been proposed as a critical event in the genesis of ventricular arrhythmias and fibrillation in the ischaemic heart (Podzuweit, 1982; Podzuweit *et al.*, 1989).

α -Adrenoceptors are also present in the myocardium and mediate positive inotropic responses (Bruckner *et al.*, 1985). Activation of these receptors in the cat heart increases ventricular automaticity and has been implicated in the genesis of the arrhythmias which occur during myocardial ischaemia and reperfusion (Sheridan *et al.*, 1980). In this species, ventricular α -adrenoceptor number (Corr *et al.*, 1981) and responsiveness (Sheridan *et al.*, 1980) are increased during periods of myocardial ischaemia and the α -adrenoceptor antagonists, prazosin and phentolamine, abolish ventricular fibrillation induced by coronary artery occlusion or reperfusion. The evidence supporting an increased role for α -adrenoceptors during myocardial ischaemia in other species is less clear. Ischaemia-induced increases in α -adrenoceptor density have been recorded in both the dog (Mukherjee *et al.*, 1980) and rat heart (Allely & Brown, 1988), but Dillon *et al.* (1988) were unable to confirm this finding in the rat. The mechanism for the anti-arrhythmic activity of α -adrenoceptor antagonists, demonstrated in many species (Benfey *et al.*, 1984; Sheridan & Culling, 1985; Williams *et al.*, 1987), has also been questioned and it has been suggested that these effects may be explained by membrane stabilizing properties (Dukes & Vaughan Williams, 1984; Daugherty *et al.*, 1986).

In the present study, we have examined α - and β -adrenoceptor responsiveness and receptor density in ischaemic and control perfused hearts of the rat. We have also examined

the effects of prazosin on the arrhythmias induced by global ischaemia and reperfusion in these isolated hearts. A preliminary account of this work has been presented to the British Pharmacological Society (Butterfield & Chess-Williams, 1989).

Methods

Isolated perfused hearts

Male Wistar rats were killed by a blow to the head and exsanguinated. Hearts were rapidly removed, flushed with heparinized saline (20 units ml⁻¹), and perfused at 70 cmH₂O by the Langendorff method with Krebs-bicarbonate solution (composition in mM: NaCl 118.4, KCl 4.7, CaCl₂ 1.9, NaHCO₃ 25.0, MgSO₄ 1.2, KH₂PO₄ 1.2) at 37°C. The perfused hearts were mounted horizontally in an organ bath and superfused with Krebs solution to maintain the temperature at 37°C during periods of reduced coronary flow. The hearts were connected, via a thread and clip attached to the apex of the heart, to an isometric force transducer (Dynamometer UF1, 280g sensitivity). Developed tension was recorded on a Devices MX2 recorder. ECG and heart rate were displayed on a Hewlett Packard ECG monitor and recorded on a Devices MX2 recorder. Hearts were paced at 4 Hz (5 ms pulse-width, twice threshold voltage), with a Grass S48 stimulator and a bipolar silver electrode in contact with the right ventricle.

Preliminary experiments demonstrated that the tension developed by hearts was greatest when the resting tension on the tissue was 2 g and the perfusion pressure was 70 cmH₂O. These values were therefore used in all subsequent experiments.

Radioligand binding studies

Hearts were equilibrated for 20 min until developed tension was stable. The perfusion pressure was then reduced to 10 cmH₂O for 30 min ('ischaemic' perfusion) or maintained at 70 cmH₂O (control perfusion). At the end of this period, the ventricles were quickly immersed in ice-cold Tris buffer

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(50 mm) and a crude membrane fraction was prepared from the ventricular tissue by homogenizing the tissue in ice-cold Tris buffer (50 mM) for 15 s with an Ultra-Turrax homogenizer, followed by 4 times 4 strokes of a Teflon homogenizer. The homogenate was centrifuged at 1000g for 10 min in an M.S.E. Chillspin centrifuge. The supernatant was then recentrifuged at 30,000g for 20 min in an M.S.E.18 centrifuge. The pellet was finally resuspended in 3 ml of Tris buffer and used in a receptor binding assay. These procedures were carried out at 4°C.

The crude membrane fraction was assayed for α -adrenoceptor binding sites with [3 H]-prazosin and β -adrenoceptor binding sites with [3 H]-dihydroalprenolol (DHA). Samples, 100 μ l, of the membrane suspension were incubated at 37°C for 30 min with a range of radioligand concentrations (0.125–4.0 nM for [3 H]-prazosin and 0.5–16.0 nM for [3 H]-DHA) in a final volume of 250 μ l. Non-specific binding was determined by displacement with 10 μ M phentolamine in [3 H]-prazosin experiments or 200 μ M isoprenaline in [3 H]-DHA experiments. Incubations were terminated by the addition of 1 ml ice-cold buffer, followed by rapid filtration through Whatman GF/B glass fibres filters. The filters were rapidly washed with 3 times 5 ml ice-cold buffer. Radioactivity remaining on the filters was measured by standard scintillation counting techniques. The specific binding as a percentage of the total binding was approximately 60% for [3 H]-DHA and 80% for [3 H]-prazosin. Protein content of the membrane fraction was determined by the method of Lowry *et al.* (1951).

Idioventricular rate responses

To examine changes in ventricular automaticity induced by adrenoceptor stimulation, rat isolated hearts were set up as described previously. Before the hearts were paced, the atria were removed and the atrioventricular node was crushed. When the ventricular rate was stable, the tissues were paced at 4 Hz for an equilibration period of 20 min, followed by 30 min control (70 cmH₂O) or ischaemic (10 cmH₂O) perfusion. Without changing the perfusion pressure, the pacing stimulus was then removed, metanephrine (10 μ M) and cocaine (10 μ M) were added to the perfusate and the tissues were allowed to equilibrate until the ventricular rate was stable. Cumulative concentration-response curves were then constructed to isoprenaline or phenylephrine, by perfusing the tissue with a given concentration of drug until the ventricular rate had stabilized at the new level. The concentration of drug in the perfusate was increased in 3 fold increments until a maximum rate was obtained. When phenylephrine was the agonist, propranolol (0.1 μ M) was also added to the perfusate at the same time as the metanephrine and was then present throughout the rest of the experiment. At the end of the experiment, when the maximum response had been obtained to phenylephrine, a maximum rate response to isoprenaline was also obtained.

Effect of prazosin on ventricular arrhythmias

To investigate the effect of prazosin on ventricular arrhythmias in this model, Langendorff perfused hearts were set up as previously described and paced at 4 Hz. Following 20 min equilibration, the perfusion pressure was reduced to 10 cmH₂O for 30 min, before the tissue was reperfused by returning the perfusion pressure to 70 cmH₂O. The arrhythmias occurring during the 30 min perfusion at reduced pressure and during the first 10 min after reperfusion were recorded and classified according to the Lambeth Conventions (Walker *et al.*, 1988). The incidence, time of onset and duration of ventricular tachycardia (VT) and ventricular fibrillation (VF) were recorded and also the incidence and time of onset of premature ventricular complexes (PVCs). Experiments were performed in the absence or in the presence of prazosin (100 nM) in the perfusate.

Statistical analysis

The dissociation constants (K_d) and the maximum number of sites (B_{max}) for [3 H]-prazosin and [3 H]-DHA binding were determined by Scatchard analysis of saturation curves. The number of binding sites was expressed as the number of sites per mg protein and the mean values with s.e.mean were calculated. Students unpaired *t* test was employed to test for statistically significant differences between control and ischaemic tissue.

In experiments in which idioventricular rate responses were examined, increases in rate were plotted as a percentage of individual maximum responses and EC₅₀ values (concentration for a half-maximal response) were determined. Geometric mean EC₅₀ values with 95% confidence limits were then calculated and differences between values for control and ischaemic tissues examined by performing Student's *t* test on individual logarithmic EC₅₀ values. For phenylephrine, responses were also plotted as a percentage of the maximum isoprenaline response.

In arrhythmia experiments, the incidences of VT, VF and PVCs were compared between prazosin and control groups by use of Chi square test. The duration and time of onset of VT and VF in control and test animals were compared with a Mann Whitney U-test.

Drugs used

Prazosin was kindly supplied as a gift by Pfizer Central Research, Sandwich, Kent. ($\pm$)-Isoprenaline, (–)-phenylephrine, ($\pm$)-metanephrine and ($\pm$)-propranolol were obtained commercially as the hydrochloride salts from Sigma. [3 H]-prazosin (23 Ci mmol⁻¹) and [3 H]-DHA (110 Ci mmol⁻¹) were obtained from the Radiochemical Centre, Amersham.

Results

Idioventricular rate responses

Removing the atria from perfused hearts resulted in a significant fall in the spontaneous rate of beating (Figure 1) which was reduced even further when the atrioventricular node was crushed, the final resting rate stabilising at 75.9 ± 6.7 beats min⁻¹. Perfusing the ventricles for 30 min at 70 cmH₂O,

Figure 1 Mean spontaneous rates (beats min⁻¹) of tissues perfused at 70 cmH₂O (A) intact hearts, (B) hearts with the atria removed and (C) hearts with the atria removed and the atrioventricular node crushed. *Significantly different from intact hearts ($P < 0.001$), **Significantly different from hearts with atria removed ($P < 0.001$). Bars show s.e.mean.

Figure 2 Mean concentration-response curves to isoprenaline of rat isolated perfused ventricles during normal (70 cmH₂O) perfusion (●) and during 'ischaemic' (10 cmH₂O) perfusion (■). Responses are plotted as the mean increase in spontaneous rate. Vertical lines show s.e.mean.

while pacing at 4 Hz did not affect the spontaneous rate, the resting spontaneous rate being 72.8 ± 6.0 beats min⁻¹ when the pacing stimulus was removed. However, when the perfusion pressure was reduced to 10 cmH₂O, the spontaneous rate on removing the pacing stimulus after 30 min pacing was significantly reduced to 39.1 ± 2.0 beats min⁻¹ ($P < 0.05$). When isoprenaline (1–300 nM) was perfused through the hearts the idioventricular rate was increased in both the control and the ischaemic tissues (Figure 2). The EC₅₀ values for isoprenaline were similar in the two groups of tissues (3.5 (1.5–8.3) nM and 1.8 (0.5–6.3) nM in control and ischaemic tissues respectively), but the maximum increase in rate was greater ($P < 0.05$) in the tissues perfused at 70 cmH₂O (86.3 ± 7.5 beats min⁻¹) than in the tissues perfused at 10 cmH₂O (53.5 ± 8.2 beats min⁻¹).

In contrast, idioventricular rate responses to phenylephrine in control perfused hearts were biphasic: low concentrations of phenylephrine ($< 10 \mu\text{M}$) reducing spontaneous rate; higher concentrations ($> 10 \mu\text{M}$) increasing spontaneous ventricular rate (Figure 3). Both the positive and the negative chronotropic responses to phenylephrine were abolished in the pre-

Figure 3 Mean concentration-response curves to phenylephrine of rat isolated perfused ventricles during normal (70 cmH₂O) perfusion (●) and during 'ischaemic' (10 cmH₂O) perfusion (■). Responses are plotted as the change in spontaneous rate in beats min⁻¹ (a) and also as a percentage of the isoprenaline maximum response (b). Vertical lines show s.e.mean.

Table 1 The mean number of binding sites (B_{max}) and dissociation constants (K_d) for [³H]-dihydroalprenolol ([³H]-DHA) and [³H]-prazosin binding to crude membrane fractions prepared from control and ischaemic perfused ventricles of the rat

Tissue	B_{max} (fmol mg ⁻¹ protein)	K_d (nM)	n
[³H]-DHA			
Control	52.1 ± 4.6	1.4 ± 0.1	5
Ischaemic	48.0 ± 4.5	1.9 ± 0.8	5
[³H]-prazosin			
Control	123.6 ± 9.9	0.09 ± 0.04	9
Ischaemic	$177.9 \pm 16.2^*$	0.10 ± 0.03	6

Data shown are means $\pm$ s.e.mean. n is the number of saturation curves studied. Individual hearts were used in each assay procedure. * $P < 0.01$.

sence of prazosin (100 nM). In hearts perfused at 10 cmH₂O, phenylephrine elicited positive chronotropic responses only. The EC₅₀ values for these responses in ischaemic tissues (3.0 (0.8–18.0) μM) were significantly lower ($P < 0.05$) than those obtained in control tissues (28.8 (12.4–67.0) μM), indicating an increase in tissue sensitivity in tissues perfused at only 10 cmH₂O. The maximum increase in spontaneous rate to phenylephrine was also greater in ischaemic tissues (54.8 ± 8.2 beats min⁻¹) than in controls (44.8 ± 11.9 beats min⁻¹), although this was not statistically significant. However, when expressed as a percentage of the isoprenaline maximum response in each tissue, the change was significant ($P < 0.05$), the maximum increasing from $49.6 \pm 9.6\%$ to $73.0 \pm 5.6\%$.

Radioligand binding experiments

In membranes prepared from the ventricular tissue of control hearts perfused at 70 cmH₂O for 30 min, the number of [³H]-prazosin binding sites was significantly greater ($P < 0.001$) than the number of [³H]-DHA binding sites (Table 1). Perfusing hearts at a reduced perfusion pressure of 10 cmH₂O for 30 min resulted in a 30% increase in the number of [³H]-prazosin binding sites, without a change in the dissociation constant for [³H]-prazosin binding. Reducing coronary flow had no effect on either the number or the affinity of [³H]-DHA binding sites (Table 1).

Effect of prazosin on arrhythmias

An analysis of the arrhythmias occurring during 30 min of low pressure perfusion and reperfusion, failed to demonstrate an antiarrhythmic effect of prazosin in this model. In control experiments ($n = 10$), PVCs occurred in 70% of the hearts, but the incidence was identical in hearts perfused with 100 nM prazosin ($n = 10$). The time of onset of PVCs and the actual number of PVCs occurring during the 30 min of perfusion at 10 cmH₂O were also similar in control (714 ± 56 s; 32 ± 8 PVCs) and prazosin-perfused (587 ± 147 s; 35 ± 11 PVCs) hearts. No PVCs were recorded during reperfusion in either group of hearts.

Prazosin also failed to alter the incidence or duration of VT and VF induced during low pressure perfusion and reperfusion of hearts (Table 2). The times of onset of VT and VF during ischaemia and reperfusion in the two groups were also not significantly different.

Discussion

In the present study, we have established a model for the examination of ventricular spontaneous rate in the rat. Ventricular automaticity during ischaemia has previously been examined in the anaesthetized cat, where idioventricular rate was recorded during stimulation of the vagus nerve (Sheridan

Table 2 Incidence (%), time of onset (s) and duration (s) of ventricular tachycardia (VT) and ventricular fibrillation (VF) during 30 min ischaemia and 10 min reperfusion of Langendorff rat hearts perfused with Krebs solution alone ($n = 10$) or Krebs solution containing 100 nm prazosin ($n = 10$)

	Control		Prazosin	
	VT	VF	VT	VF
Ischaemia				
Incidence (%)	100	30	90	40
Onset (s)	725 $\pm$ 100	1473 $\pm$ 99	699 $\pm$ 109	1008 $\pm$ 140
Duration (s)	859 $\pm$ 121	88 $\pm$ 32	743 $\pm$ 147	81 $\pm$ 36
Reperfusion				
Incidence (%)	80	60	100	60
Onset (s)	40 $\pm$ 35	162 $\pm$ 73	18 $\pm$ 8	124 $\pm$ 47
Duration (s)	337 $\pm$ 78	397 $\pm$ 89	322 $\pm$ 65	327 $\pm$ 63

Data shown are means $\pm$ s.e.mean.

et al., 1980). However, this method is not ideal for studying adrenergic responses, since muscarinic stimulation itself may alter adrenergic responsiveness (Simpson *et al.*, 1981). We have recorded ventricular rate following removal of the sinoatrial and atrioventricular pacemakers. In preliminary experiments ($n = 4$), the tension developed by hearts before (2.7 ± 0.1 g) and after (2.6 ± 0.1 g) this procedure was similar, indicating that the ventricular tissue was not damaged. Furthermore, the idioventricular rate and developed tension remained stable over a period of several hours of control perfusion.

Idioventricular rate responses to phenylephrine were found to be biphasic in rat isolated perfused ventricles, low concentrations reducing spontaneous ventricular rate, while concentrations greater than $10 \mu\text{M}$ caused an increase in rate. Negative chronotropic effects of phenylephrine have previously been demonstrated on isolated Purkinje fibres from the dog, the response being mediated via α -adrenoceptors (Rosen *et al.*, 1984). The rate response of canine Purkinje fibres to adrenaline is also biphasic, low concentrations reducing spontaneous rate via α -adrenoceptor activation, while higher concentrations increase ventricular rate via β -adrenoceptor stimulation (Rosen *et al.*, 1977). In the present study, phenylephrine was employed as a selective α -adrenoceptor agonist. However, this drug also has weak β -adrenoceptor agonist activity (Chess-Williams *et al.*, 1989) and responses to phenylephrine were therefore performed in the presence of propranolol. The antagonism of responses by low concentrations of prazosin, which abolished both the negative and positive chronotropic ventricular responses, confirmed that both components of the response to phenylephrine were mediated via α -adrenoceptor activation.

In experiments where perfusion pressure was reduced, positive chronotropic responses to phenylephrine were enhanced. This supports previous *in vivo* studies in the cat (Sheridan *et al.*, 1980; Corr & Crafford, 1981) and dog (Juhász-Nagy & Aviado, 1976), where ventricular chronotropic responses to α -adrenoceptor stimulation were potentiated during periods of ischaemia. The mechanism for the enhanced α -adrenoceptor responsiveness, may be explained at least partially, by an increase in the density of [^3H]-prazosin binding sites in the ischaemic ventricle, indicating an increase in the number of ventricular α -adrenoceptors. Such an increase in the density of α -adrenoceptor binding sites induced by ischaemia has been demonstrated in the cat (Corr *et al.*, 1981; Corr & Crafford, 1981; Dillon *et al.*, 1988) and the dog heart (Mukherjee *et al.*, 1980). No such change can be demonstrated in the guinea-pig heart (Broadley *et al.*, 1985), although the number of ventricular α -adrenoceptors in this species is low, making the demonstration of small changes in receptor number difficult, Dillon *et al.* (1988) were similarly unable to find a change in [^3H]-prazosin binding during myocardial ischaemia in the rat. However, the present results disagree, and support the findings of Allely *et al.* (1988) who obtained an increase in α -adrenoceptor number after 30 min of ischaemia in the perfused

heart of the rat. This ischaemia-induced increase in α -adrenoceptor number may combine with the lack of a negative chronotropic response to enhance ventricular automaticity during periods of ischaemia.

Ventricular rate responses to isoprenaline were also altered during ischaemic perfusion. Although EC_{50} values were similar in control and ischaemic tissues, maximum responses to isoprenaline were reduced during low pressure perfusion. This may be due to the general depression of spontaneous rate during ischaemia, or to a depressed adenosine 3':5'-cyclic monophosphate (cyclic AMP) response to β -adrenoceptor stimulation. Short periods of ischaemia have been shown to result in a depressed cyclic AMP response to β -adrenoceptor stimulation (Krausse & England, 1982).

Thus, during ischaemia, β -adrenoceptor responses were depressed while α -adrenoceptor responses were enhanced and the maximum phenylephrine response relative to isoprenaline was increased from 50% to 73%. A similar situation also occurs in hypothyroidism and results in the inotropic response to noradrenaline being mediated via α -adrenoceptors, whereas in the normal hearts of euthyroid animals responses to noradrenaline are mediated solely via β -adrenoceptors (Chess-Williams & Critchley, 1987). The observations made in the present study may therefore explain the change in the idioventricular rate response to sympathetic nerve stimulation during ischaemia in the cat heart, where responses were β -mediated in the normal heart but α -mediated during ischaemia (Sheridan *et al.*, 1980).

Whether the enhanced ventricular α -adrenoceptor responsiveness contributes to the genesis of arrhythmias is unclear at the present time. Manning *et al.* (1984) have found that the increase in cardiac α -adrenoceptor density during ischaemia does not coincide with the onset of arrhythmias, suggesting a dissociation of the two events. On the other hand, α -adrenoceptor activation has been shown to play an important role during reperfusion, where α -adrenoceptor antagonists significantly attenuate calcium uptake (Sharma *et al.*, 1983). α -Adrenoceptor antagonists have also been shown to prevent reperfusion-induced arrhythmias in the cat (Sheridan *et al.*, 1980), dog (Benfey *et al.*, 1984), guinea-pig (Sheridan & Culling, 1985) and rat (Williams *et al.*, 1987), although it has been suggested that these effects may be due to membrane stabilizing properties (Dukes & Vaughan-Williams, 1984; Daugherty *et al.*, 1986). In the present study, 100 nm prazosin failed to reduce the incidence of premature ventricular complexes, ventricular tachycardia or ventricular fibrillation induced during periods of low pressure perfusion and reperfusion. This concentration of prazosin completely abolished chronotropic responses to phenylephrine in perfused ventricles, thereby confirming that this concentration of prazosin produced a substantial blockade of α -adrenoceptors. The increase in α -adrenoceptor responsiveness therefore, does not appear to contribute to arrhythmogenesis in this model of global ischaemia.

It has been suggested that where myocardial ischaemia is localised to small regions, local α -adrenoceptor mediated prolongation of action potential duration may combine with β -adrenoceptor-mediated shortening of action potential duration in areas of normal coronary flow, to produce the electrical inhomogeneity suitable for the emergence of ventricular arrhythmias (Uprichard *et al.*, 1988). If this is correct, the ischaemia-induced increase in α -adrenoceptor responsiveness may not be an important factor in the genesis of arrhythmias in a model of global ischaemia, where α -mediated prolongation of action potential duration may be more uniform. Whether an α -adrenoceptor antagonist would have been more effective against ischaemia- and reperfusion-induced arrhythmias following coronary artery occlusion is unknown. Manning *et al.* (1984) could not demonstrate a protective effect for prazosin in coronary artery occluded isolated hearts of the rat, but Bernauer & Ernenputsch (1988) have found the α_1 -selective antagonist corynanthine to be effective in this model.

α_1 -selective antagonist corynanthine to be effective in this model.

The present study has therefore demonstrated both positive and negative idioventricular rate responses to α -adrenoceptor stimulation, the positive chronotropic response being potentiated during ischaemia. The increase in α -adrenoceptor sensitivity during ischaemia is associated with an increase in the

number of [^3H]-prazosin binding sites, but further work is required to establish the relationship between these receptor changes and the genesis of arrhythmias during myocardial ischaemia and reperfusion.

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