British Journal of Pharmacology (2001) 134, 38–45

www.nature.com/bjp

Angiotensin II reduces infarct size and has no effect on post-ischaemic contractile dysfunction in isolated rat hearts

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1 In order to test the hypothesis that angiotensin II exacerbates myocardial ischaemia-reperfusion (IR) injury, we examined the effects of graded angiotension II concentrations of angiotensin II on IR injury in both working and non-working (Langendorff) isolated rat hearts.

2 Non-working hearts were subjected to 30 min aerobic perfusion (baseline) then 25 min of global, no-flow ischaemia followed by 30 min of reperfusion either in the absence (control, $n=7$) or presence of 1 ($n=6$) or 10 nM ($n=5$) angiotensin II). Recoveries of LV developed pressure and coronary flow after 30 min reperfusion in control hearts (58 ± 9 and $40\pm8\%$ of baseline levels, respectively) were no different from hearts treated with 1 or 10 nM angiotensin II. Infarct size (determined at the end of reperfusion by triphenyltetrazolium chloride staining) was reduced by angiotensin II in a concentration-dependent manner (from a control value of 27 ± 3 to $18\pm4\%$ and $9\pm3\%$ of the LV, respectively).

3 Working hearts were subjected to 50 min pre-ischaemic (pre-I) aerobic perfusion then 30 min of global, no-flow ischaemia followed by 30 min of reperfusion either in the absence (control, $n=14$) or presence of 1 ($n=8$), 10 ($n=7$) or 100 nM ($n=7$) angiotensin II). In controls, post-ischaemic (post-I) left ventricular (LV) work and efficiency of oxygen consumption were depressed (43 ± 9 and $42\pm10\%$ of pre-I levels, respectively). The presence of angiotensin II throughout IR had no effect on LV work compared with control.

4 Thus, angiotensin II reduces infarct size in a concentration-dependent manner but has no effect on contractile stunning associated with IR in isolated rat hearts.

British Journal of Pharmacology (2001) **134,** 38–45

Keywords: Angiotensin II; ischaemia-reperfusion injury; Langendorff perfusion; working heart perfusion; myocardial infarction

Abbreviations: ACE, Angiotensin converting enzyme; BSA, Bovine serum albumin; CF, Coronary flow; CK, Creatine kinase; CO, Cardiac output; CVC, Coronary vascular conductance; ECG, Electrocardiogram; HR, Heart rate; IR, Ischaemia-reperfusion; LVDP, Left ventricular developed pressure; LVEDP, Left ventricular end diastolic pressure; LV Work, Left ventricular minute work; MVO_2, Myocardial oxygen consumption; PKC, Protein kinase C; Pre-I, Pre-ischaemia; Post-I, Post-ischaemic; PSP, Peak systolic pressure; RAS, Renin-angiotensin system

Introduction

Therapeutic strategies that inhibit the renin-angiotensin system (RAS) are actively promoted for use in the treatment of ischaemia-reperfusion (IR) injury. However, the influence of angiotensin II *per se* on the severity of IR injury has not been extensively tested. Angiotensin II mediates a number of responses which might be expected to worsen IR injury including coronary vasoconstriction, enhanced release of noradrenaline from presynaptic sympathetic nerve terminals (Brasch *et al*., 1993) and increased intracellular Ca^{2+} concentration (Shao *et al*., 1998). However, angiotensin II has also been linked to activation of protein kinase C (PKC) (Liu *et al*., 1995; Taubman *et al*., 1989) which itself has been linked to protection of the heart from IR injury (Cohen & Downey, 1996).

Angiotensin II is generated locally in the heart (Dzau, 1993). Therefore, in theory, angiotensin converting enzyme (ACE) inhibitors could be used to reduce endogenous levels of angiotensin II and so allow the study of the role of angiotensin II in the pathogenesis of IR injury. Unfortunately, ACE inhibition also leads to an increase in bradykinin concentration that also affects IR injury (Brew *et al*., 1995; Bugge & Ytrehus, 1996; Zhu *et al*., 1995). In addition to the effects of bradykinin, it has become apparent that other enzymes, in addition to ACE, are capable of converting angiotensin I into angiotensin II (Akasu *et al*., 1998; Urata *et al*., 1993). Because of these two factors, results obtained with ACE inhibitors do not provide reliable data about the role of angiotensin II on the severity of IR injury.

An alternative strategy to investigate the role of angiotensin II in the pathogenesis of IR injury is to increase its concentration by the addition of exogenous angiotensin II. Despite its simplicity, this approach has not been extensively used. To date, only one study has examined the effect of exogenous angiotensin II on IR injury in isolated hearts. Yoshiyama *et al*. (1994) used hearts perfused by the

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Langendorff method at a constant flow. However, infarct size in that study was not measured directly and the results are open to alternative interpretations.

In this study we have used two models of IR in isolated hearts to examine the effects of graded concentrations of angiotensin II, in a range that will occupy from 50–99% of both AT_1 and AT_2 receptors, on the severity of myocardial injury. In a non-working (Langendorff) model, hearts were perfused at a constant pressure *via* the aorta, thereby circumventing confounding experimental artefacts arising from angiotensin II-mediated increases in coronary perfusion pressure and potential peroxynitrite formation consequent to elevated shear stress. This experimental model has been used extensively to study IR-induced infarction (de Leiris *et al*., 1984; Galinanes & Hearse, 1990). The isolated working heart model, adapted from Neely *et al*. (1967), was also used. In this model, perfusate is delivered to the left atrium and ejected from the left ventricle (LV) against a set afterload *via* the aorta. Hearts perfused by this method have near-physiological levels of workload and oxygen consumption which are higher than those of non-working (Langendorff) hearts (de Leiris *et al*., 1984; Galinanes & Hearse, 1990). In addition, we have previously found that the coronary circulation of working hearts is less susceptible to angiotensin II-mediated coronary vasoconstriction than non-working (Langendorff) hearts (Ford *et al*., 1998; Yoshiyama *et al*., 1994).

The aim of this study was to investigate the direct effects of angiotensin II on two indices of IR injury, namely infarction and IR-induced post-ischaemic contractile dysfunction.

Methods

All animals were housed and treated in accordance with guidelines of the Canadian Council and the American Physiological Society on Animal Care. Male Sprague-Dawley rats (250–350 g), that had been fed *ad libitum*, were killed with an overdose of sodium pentobarbital. Hearts were rapidly excised and placed in ice-cooled Krebs-Henseleit solution.

Langendorff perfusions

After cannulating the aorta, constant pressure (80 mmHg) Langendorff perfusion was commenced. The perfusate, consisting of a modified Krebs-Henseleit solution (mM: NaCl 118, HCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, Ca^{2+} 2.5, glucose 11, 100 mu l^{-1} insulin), was maintained at a temperature of 37°C and continuously bubbled with a gas mixture of 95% O_2/5% CO_2. Hearts were immersed in a chamber containing perfusate at a constant temperature of 37°C. During periods of perfusion, hearts were electrically paced *via* bipolar platinum electrodes at a frequency of 5 Hz (Palmer Bioscience stimulator 100).

Left ventricular developed pressure (LVDP) was measured by means of a pressurized balloon (Harvard Apparatus) inserted into the left ventricle and connected to a pressure transducer and inflated to a level such that end diastolic pressure was set to a value between 5–10 mmHg. Coronary flow was measured by ultrasonic flow probes (Transonic H4X). Electrocardiogram (ECG, lead 3) was continuously recorded by means of two stainless steel needles inserted into the ventricular apex and base, connected to an ECG amplifier (Grass EKG/Tachograph model 7P4). Hearts that could not be electrically paced at 300 beats min^{-1} after the first 10 min of reperfusion were excluded from further analysis. All parameters were continuously recorded using a PowerLab 800 (ADI instruments) and stored using a Macintosh PowerPC.

Working heart perfusions

After cannulating the aorta, a temporary Langendorff perfusion was commenced using Krebs-Henseleit solution (pH 7.4, gassed with 95% O_2/5% CO_2) at a hydrostatic pressure of 60 mmHg for 10 min. During this time, extraneous tissue was removed and the pulmonary artery and left atrium were cannulated. The Langendorff perfusion was then terminated and working heart perfusion commenced with 100 ml of modified Krebs-Henseleit solution (mM: NaCl 118, HCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, Ca^{2+} 2.5, glucose 11, 100 mu l^{-1} insulin and 1.2 mM palmitate pre-bound to 3% bovine serum albumin). In the working mode, perfusate was delivered to the left atrium at a constant preload of 11.5 mmHg and ejected *via* the aorta against a hydrostatic afterload of 80 mmHg. During aerobic perfusion, atrial pacing was applied to the hearts at 5 Hz (Grass S88 stimulator). Hearts that could not be electrically paced at 300 beats min^{-1} after the first 10 min of reperfusion were excluded from further analysis. Working hearts were perfused in a closed recirculating system at 37°C using an oxygenator with a large surface area in constant contact with a 95% O_2-5% CO_2 gas mixture. The O_2 content of the coronary effluent was measured continuously using a probe (YSI, model number 5331) placed in the pulmonary artery outflow line, which was connected to an O_2 meter (YSI, model number 5300). Coronary effluent drained back into the perfusate reservoir.

Heart rate and systolic and diastolic pressures were measured with a P23Db pressure transducer (Gould) connected to the aortic outflow line (see Ford *et al*., 1998) and recorded on a Grass model 7D polygraph. Cardiac output and aortic flow were measured using ultrasonic flow probes (Transonic T206) placed in the left atrial and aortic lines, respectively. Coronary flow was calculated as the difference between cardiac output and aortic flow. Mechanical function was expressed as LV minute work [(aortic systolic pressure − left atrial pressure) × cardiac output × 0.133 Joules]. Myocardial efficiency was calculated as LV work expressed as a percentage of total potential work [based on oxygen consumption (MVO_2, μmol min^{-1} g dry wt^{-1}) as mols of oxygen/coronary flow]. Coronary vascular conductance (CVC, ml min^{-1} $mmHg^{-1}$) was calculated as the ratio of coronary flow and mean aortic pressure.

Langendorff perfused hearts

Non-working (Langendorff) perfused hearts were randomly assigned to one of three experimental groups. Hearts were initially perfused aerobically for 20 min (baseline) before being randomly assigned to receive either Krebs-Henseleit solution (control) or angiotensin II (final concentration of either 1 or 10 nM) infused for 10 min at a flow rate controlled

to be 1% of coronary flow (Figure 1). Global no-flow ischaemia was initiated and maintained for 25 min followed by 30 or 120 min of reperfusion. At the end of reperfusion, the intraventricular balloon was deflated and constant pressure perfusion was terminated. Hearts were then perfused with a 1% solution of triphenyltetrazolium chloride in Krebs-Henseleit solution at a flow rate of 5 ml min^{-1} for 10 min. Stained hearts were stored overnight in 10% formalin solution (BDH, U.K.). Determinations of infarct size were carried out by an investigator blinded to the treatment the heart had received. The right ventricle and connective tissues were removed and the left ventricle cut into sections of 2 mm thickness. Areas with an absence of red stain (infarcted area) were carefully separated from areas of red staining and weighed. Infarct size is reported as the wet weight of the area without red staining expressed as a percentage of the weight of the entire left ventricle.

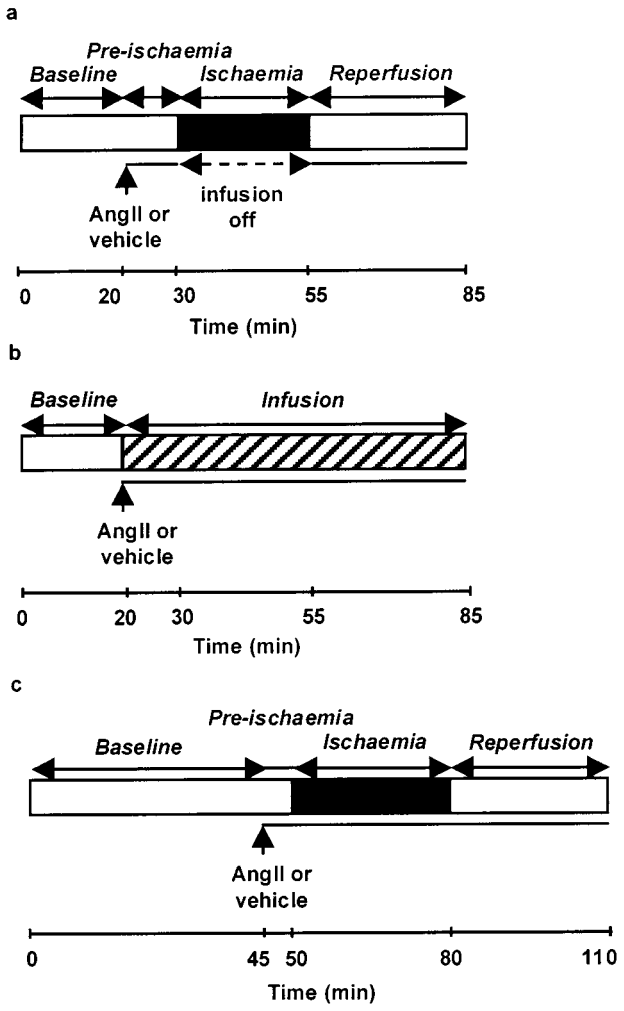

Figure 1 Experimental protocols used for non-working (Langendorff) heart perfusions (a and b) and working heart perfusions (c). Langendorff hearts were either perfused according to the ischaemic-reperfusion (IR) protocol (a) or the aerobic infusion protocol (b). The Langendorff IR protocol (a) consisted of a 20 min aerobic baseline perfusion followed an infusion of either vehicle (control) or angiotensin II (final concentration 1 or 10 nM). After 10 min equilibration (pre-ischaemia), global no-flow ischaemia was induced. Infusions were terminated at the start of the ischaemic period. Following ischaemia, hearts were reperfused and infused with angiotensin II or vehicle for a further 30 min. The Langendorff aerobic infusion protocol (b) consisted of 20 min aerobic baseline perfusion followed by a 65 min infusion of either vehicle or angiotensin II (final concentration 10 nM). Perfused working hearts were perfused for 45 min of aerobic baseline period (c). Hearts assigned to receive angiotensin II (1, 10 or 100 nM) received a bolus at 45 min. After 5 min equilibration (pre-ischaemia), hearts were subjected to 30 min global no-flow ischaemia followed by 30 min reperfusion. Where administered, angiotensin II was present throughout ischaemia and reperfusion.

In a separate series of experiments, hearts were perfused aerobically for 20 min (baseline) and then randomly assigned to receive a 60 min infusion of Krebs-Henseleit solution (control) or angiotensin II (final concentration 10 nM) at a rate controlled to be 1% of coronary flow.

Perfused working hearts

Isolated working hearts were randomly assigned to one of four experimental groups. Hearts were either untreated (control) or received 1, 10 or 100 nM angiotensin II (Figure 1). All hearts were perfused aerobically for 45 min (baseline) followed by 5 min in the absence or presence of angiotensin II. Global, no-flow ischaemia (30 min) was induced after 50 min of perfusion and was followed by 30 min of aerobic reperfusion.

Drugs and reagents

Angiotensin II and palmitate were obtained from Sigma. Insulin (porcine) and bovine serum albumin (BSA fraction V, hsp) were obtained from Boehringer Mannheim. Reagents and their sources were as follows: Formalin (10%, technical grade, BDH), triphenyltetrazolium chloride (Sigma). All salts were obtained from BDH and were of AnalR grade.

Statistical analysis

All data are expressed as mean $\pm$ s.e.mean. Paired data was compared using a Student's *t*-test. Multiple comparisons against a single control were made using analysis of variance (ANOVA) supported by Dunnett's *post hoc* test. The comparison of time course data was analysed using ANOVA with repeated measures. Statistical significance was set at $P<0.05$.

Results

Langendorff perfused hearts

Parameters of mechanical function were stable during baseline aerobic perfusion in control hearts. Left ventricular contracture was observed during the 25 min global, no-flow ischaemia reaching a maximum of 45 ± 5 mmHg after 22 ± 1 min (Table 1). After 30 min reperfusion, LVDP and coronary flow partially recovered to 58 ± 9 and 40 ± 8% of pre-I, respectively (Table 1 and Figure 2a). During reperfusion, LV diastolic function was impaired such that after 30 min of reperfusion LV end diastolic pressure (LVEDP) remained elevated at 41 ± 5 mmHg (Table 1 and Figure 3). In control hearts after 30 min reperfusion, 27 ± 3% of the left ventricle was infarcted (Table 1 and Figure 4).

Table 1 Effect of angiotensin II on IR injury in Langendorff perfused hearts

		Control (n = 7)	*Angiotensin II* (1 nM) (n = 6)	*Angiotensin II* (10 nM) (n = 5)
LVDP (mmHg)	Baseline	101 ± 8	107 ± 7	106 ± 3
	Pre-I	100 ± 8	102 ± 6	98 ± 5
	Post-I	56 ± 8*	51 ± 4*	53 ± 14*
Coronary flow (ml.min^{-1})	Baseline	15 ± 1	16 ± 2	15 ± 2
	Pre-I	14 ± 1	13 ± 2	11 ± 1
	Post-I	6 ± 1*	7 ± 4*	4 ± 1*
HR*LVDP (mmHg min^{-1})	Baseline	302 ± 25	320 ± 21	318 ± 8
	Pre-I	299 ± 25	305 ± 7	295 ± 14
	Post-I	168 ± 23*	154 ± 11*	158 ± 41*
Max contracture (mmHg)		45 ± 5	39 ± 3	36 ± 6
Time to max (s)		1341 ± 60	1379 ± 96	1144 ± 82
Infarct size (% of LV)		27 ± 3	18 ± 4	9 ± 3†
[Fibrillation data removed].				

Contractile function, coronary flow, ischaemic contracture and infarct size in Langendorff perfused hearts are reported for different stages of the IR protocol: before infusion (Baseline), before (Pre-I) and after 30 min reperfusion (Post-I) following 25 min of global no-flow ischaemia. *$P<0.05$ vs respective Pre-I (paired Student's *t*-test) †$P<0.05$ vs time matched control value (ANOVA supported by Dunnett's *post hoc* test for multiple comparisons against a single control).

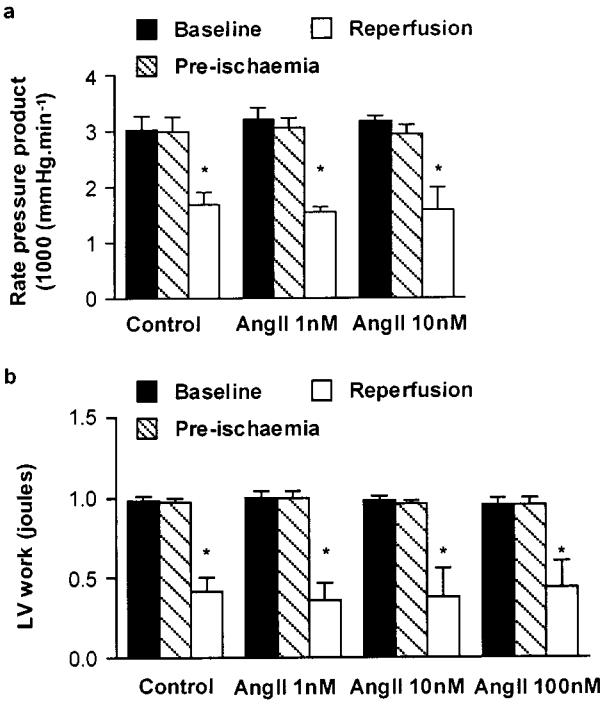

Figure 2 Left ventricular (LV) contractile function at the end of baseline (filled bars), pre-ischaemia (open bars), and reperfusion (hatched bars) time periods in non-working (Langendorff) perfused hearts (a) and perfused working hearts (b). In Langendorff perfused hearts LV contractile function is expressed as the rate pressure product (mmHg min^{-1}) for control ($n=7$), 1 nM angiotensin II ($n=6$) or 10 nM angiotensin II ($n=5$). In perfused working hearts LV contractile function is expressed as LV work (joules) for control hearts ($n=14$), 1 nM angiotensin II ($n=8$), 10 nM angiotensin II ($n=7$) and 100 nM angiotensin II ($n=7$). * $P<0.05$ vs pre-ischaemia control (ANOVA followed by Dunnett's *post hoc* test for comparisons against a single control).

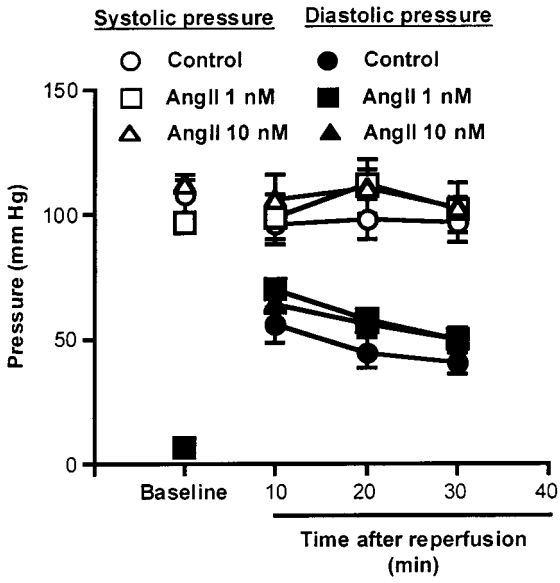

Figure 3 Sytolic and diastolic pressures (mmHg) obtained at baseline and during reperfusion in hearts treated with vehicle (control, $n=7$), 1 nM angiotensin II ($n=6$) or 10 nM angiotensin II ($n=5$).

Infusion of 1 or 10 nM angiotensin II for 10 min before ischaemia reduced pre-I coronary flow by 14 ± 4 and 22 ± 3%, respectively, without affecting LVDP (Table 1 and Figure 2a). The time for development of maximum contracture was reduced by 10 nM but not 1 nM angiotensin II (Table 1) although the maximal extent of contracture was unaffected by either concentration compared to control. After 30 min reperfusion, LVDP and coronary flow were impaired compared with baseline levels but did not differ from recovery observed in control hearts (Table 1 and Figure 2a). LVEDP was elevated during reperfusion to a similar extent (50 ± 5 mmHg after 30 min reperfusion) as in controls (Table 1 and Figure 3).

Angiotensin II concentration-dependently reduced infarct size. In the presence of 1 nM angiotensin II infarct size tended to be less (18 ± 4% of the left ventricle) than that observed in control hearts (Table 1 and Figure 4), although the difference did not attain statistical significance, whereas 10 nM angiotensin II significantly reduced infarct size by 66% of the control level.

Coronary flow did not change significantly during 60 min of vehicle infusion (Figure 5). Angiotensin II infusion (10 nM) reduced coronary flow to 71±3% of baseline levels after 2 min that recovered to 82±2% of baseline levels after 10 min. Thereafter coronary vasoconstriction was maintained for the remaining 50 min of infusion.

Perfused working hearts

In control hearts, parameters of mechanical function and oxygen consumption were stable during 40 min of baseline perfusion. After 30 min reperfusion of control hearts, peak aortic systolic pressure (66±9%), cardiac output (49±10%), coronary flow (77±13%), LV work (43±9%), myocardial oxygen consumption (68±14%) and efficiency (42±10%) were depressed compared with values obtained after 50 min of aerobic baseline perfusion (Table 2 and Figure 2b). In control hearts, CVC at the end of reperfusion was similar to values obtained at the end of aerobic baseline perfusion (84±11% of baseline).

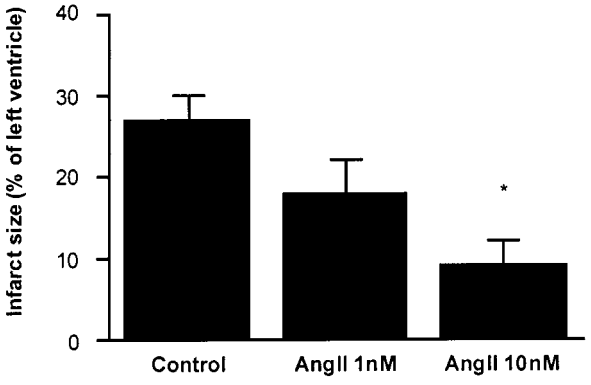

Figure 4 Infarct size as determined by 1% triphenyltetrazolium chloride staining after 30 min reperfusion in Langendorff perfused hearts. Infarct size is expressed as a percentage of left ventricular wet weight for hearts infused with either vehicle (control, $n=7$), 1 nM angiotensin II ($n=6$) or 10 nM angiotensin II ($n=5$). *$P<0.05$ vs control (ANOVA followed by Dunnett's *post hoc* test for comparisons against a single control).

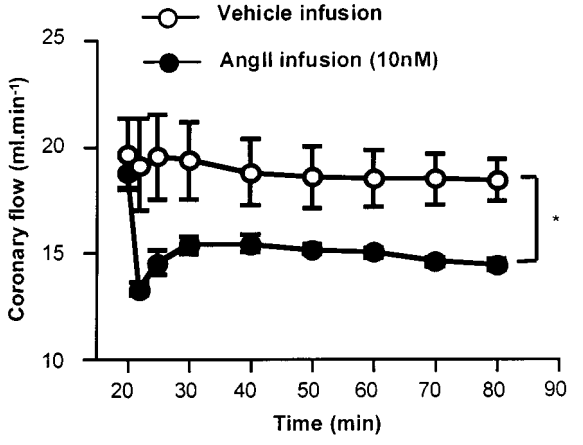

Figure 5 Time course of the response to a 65 min infusion of either vehicle (control, $n=3$) or 10 nM angiotensin II ($n=4$). *$P<0.05$ vs control (ANOVA with repeated measures).

Pre-ischaemic parameters of mechanical function, coronary flow, CVC and oxygen consumption were unaffected by 1, 10 or 100 nM angiotensin II (Table 2 and Figure 2b). Compared with time-matched control hearts, angiotensin II had no effect on the recovery of mechanical function, coronary flow, CVC or oxygen consumption after 30 min reperfusion at any concentration used (Table 2 and Figure 2b).

Discussion

The main finding in this study is that the presence of angiotensin II during ischaemia and reperfusion in isolated non-working (Langendorff) perfused rat hearts reduced infarct size in a concentration-dependent manner (ranging from 1 to 100 nM). In addition, angiotensin II had no deleterious effect on post-ischaemic contractile dysfunction (stunning) in either isolated working or non-working rat hearts measured after 30 min reperfusion.

There are very few reports where the effects of elevated angiotensin II concentrations during IR on the severity of injury *in vitro* have been studied. As far as we are aware, the only other study is that of Yoshiyama *et al.* (1994) who studied the effect of exogenous angiotensin II on IR injury in Langendorff-perfused hearts. Their main finding was that angiotensin II increased IR-mediated creatine kinase (CK) release. However the source of CK was not identified and may have been released from the coronary endothelium, rather than cardiac myocytes, due to damage by angiotensin II-mediated elevation of coronary perfusion pressure. In addition, angiotensin II-mediated increase in coronary perfusion pressure is likely to have stimulated nitric oxide release from the coronary endothelium due to increased shear stress (Stewart *et al.*, 1994). Nitric oxide production has been shown to cause peroxynitrite formation during reperfusion (Yasmin *et al.*, 1997) which damages the heart (Ma *et al.*, 1997; Yasmin *et al.*, 1997). It has been demonstrated that alterations in coronary vascular tone do not cause significant amounts of nitric oxide release in the constant pressure Langendorff model (Curtis & Ellwood, 1998). We estimated myocardial damage by means of direct measurements of infarct size, using triphenyltetrazolium chloride (TTC) staining, rather than indirectly *via* CK release. With TTC staining, angiotensin II reduced infarct size associated with IR in a concentration-dependent manner. Infarct sizes were estimated after 30 min reperfusion in our study. Although this is a relatively short period of reperfusion we found that infarcts in control hearts reperfused for up to 2 h following 25 min global, no-flow ischaemia were not significantly larger than those reperfused for 30 min (unpublished observations). Furthermore, the reduction in infarct size associated with 10 nM angiotensin II was maintained when the duration of reperfusion was extended to 2 h. In agreement with Yoshiyama *et al.* (1994), angiotensin II treatment did not alter LVDP after 30 min of reperfusion in our study. The increase in fibrillation reported by Yoshiyama *et al.* (1994) is surprising given that a later study demonstrated a reduction in IR-associated electrical dysrhythmias with angiotensin II treatment (Thomas *et al.*, 1996).

Our study is not the only one where angiotensin II treatment has been associated with infarct size reduction. Infarct size was reduced by angiotensin II given as a

Table 2 Effect of Angiotensin II on IR injury in perfused working hearts

		Control (n = 14)	*Angiotensin II* (1 nM) (n = 8)	*Angiotensin II* (10 nM) (n = 7)	*Angiotensin II* (100 nM) (n = 7)
PSP (mmHg)	Baseline	120 ± 1	124 ± 1	125 ± 2	120 ± 2
	Pre-I	120 ± 1	124 ± 1	124 ± 3	120 ± 2
	Post-I	79 ± 11*	74 ± 18*	61 ± 22*	70 ± 19*
CO (ml min^{-1})	Baseline	67 ± 1	66 ± 2	65 ± 2	66 ± 2
	Pre-I	67 ± 1	66 ± 2	64 ± 2	66 ± 2
	Post-I	33 ± 6*	27 ± 8*	26 ± 11*	35 ± 11*
CF (ml min^{-1})	Baseline	23 ± 1	26 ± 2	24 ± 1	25 ± 3
	Pre-I	23 ± 1	26 ± 2	22 ± 3	23 ± 2
	Post-I	17 ± 3	16 ± 4	12 ± 5	19 ± 6
LV work (Joules)	Baseline	0.98 ± 0.03	1.00 ± 0.04	0.98 ± 0.03	0.95 ± 0.05
	Pre-I	0.97 ± 0.03	1.00 ± 0.04	0.96 ± 0.02	0.95 ± 0.05
	Post-I	0.41 ± 0.11*	0.36 ± 0.12*	0.38 ± 0.18*	0.44 ± 0.16*
CVC (ml min^{-1} $mmHg^{-1}$)	Baseline	0.29 ± 0.01	0.30 ± 0.02	0.30 ± 0.02	0.32 ± 0.04
	Pre-I	0.28 ± 0.01	0.30 ± 0.02	0.27 ± 0.02	0.29 ± 0.03
	Post-I	0.23 ± 0.03	0.23 ± 0.04	0.20 ± 0.5	0.34 ± 0.09*
MVO_2 (μmol g dry wt^{-1})	Baseline	53 ± 3	61 ± 3	55 ± 4	62 ± 8
	Pre-I	53 ± 3	61 ± 3	52 ± 5	59 ± 8
	Post-I	33 ± 7*	33 ± 9*	24 ± 10*	44 ± 10
Efficiency (%)	Baseline	16 ± 1	15 ± 1	15 ± 1	15 ± 2
	Pre-I	16 ± 1	15 ± 1	16 ± 1	15 ± 2
	Post-I	7 ± 2*	6 ± 2*	6 ± 3*	7 ± 3*

Contractile function, coronary flow, ischaemic contracture and infarct size in perfused working hearts are reported for different stages of the IR protocol: before infusion (Baseline), immediately before ischaemia (Pre-I) and after 30 min reperfusion (Post-I) following 30 min of global no-flow ischaemia. *$P < 0.05$ vs respective Pre-I (paired Student's *t*-test).

preconditioning stimulus (5 min transient exposure prior to ischaemia) in isolated perfused rabbit hearts (Liu *et al*., 1995). However, the authors reported a sustained depression of coronary flow immediately prior to ischaemia with the implication being that AT_1 receptors were activated at the onset, and for an unknown period during, ischaemia. Furthermore, it has been reported that stimulation of AT_1 receptors by angiotensin II can inhibit apoptosis (Haendeler *et al*., 2000; Pollman *et al*., 1996). This evidence, taken together with reports of apoptotic cell death following ischaemia that can be blocked by inhibition of caspase (Holly *et al*., 1999; Ma *et al*., 1999; Musat-Marcu *et al*., 1999) supports our finding that angiotensin II reduces infarct size. However, angiotensin II is proapoptotic in endothelial cells (Dimmeler *et al*., 1997) and can cause myocyte necrosis (Tan *et al*., 1991) although necrosis was only observed with long-term *in vivo* administration of angiotensin II in the absence of myocardial IR injury.

Numerous studies have investigated the effect of reducing angiotensin II tone on IR injury using either angiotensin converting enzyme (ACE) inhibitors or angiotensin II receptor antagonists. However, there are a number of reasons why the results of these studies might not accurately reflect the effect of angiotensin II itself on IR injury. ACE inhibitors increase bradykinin concentration and bradykinin itself has been shown to reduce IR injury (Brew *et al*., 1995; Bugge & Ytrehus, 1996; Zhu *et al*., 1995). Furthermore, bradykinin antagonists reverse the beneficial effects of ACE inhibition (Ehring *et al*., 1994; Hartman, 1995; Linz *et al*., 1993). Therefore the effects of ACE inhibitors on IR injury are not specific to angiotensin II. Similarly, caution needs to be exercised in interpreting the results of selective antagonism of either AT_1 or AT_2 receptors. Firstly, non-peptide AT_1 receptor antagonists are structurally derived from losartan (Timmermans *et al*., 1993) which has been shown to reduce the incidence of electrical arrhythmia induced by IR through a mechanism independent of AT_1 receptor blockade (Lee *et al*., 1997; Thomas *et al*., 1996). Secondly, there is growing evidence that AT_1 and AT_2 receptors interact (Ford *et al*., 1996; Gyurko *et al*., 1992; Hein *et al*., 1995; Ichiki *et al*., 1995; Jalowy *et al*., 1998). Therefore, selective antagonism or anti-sense oligonucleotide treatment (Yang *et al*., 1998) targeted against one or other receptor subtype might reflect an uncoupling of an interaction between receptor subtypes or a reduction in deleterious dysrhythmia rather than inhibition of effects mediated by angiotensin II, *per se*, on IR injury.

Angiotensin II has a similar affinity for AT_1 and AT_2 receptors, determined by binding studies to be $\sim$1 nM (Dudley *et al*., 1991; Hunyady *et al*., 1996) and will thus occupy equal proportions of AT_1 and AT_2 receptors. However, it is known that AT_1 receptors desensitize upon exposure to angiotensin II (Kai *et al*., 1996; Tang *et al*., 1995; Thekkumkara *et al*., 1995) but little is known about AT_2 receptor desensitization. Although angiotensin II AT_1 receptors did appear to desensitize over the course of angiotensin II exposure in our protocol, a significant proportion of the response remained. The potential effects of exogenous angiotensin II on AT_2 receptor desensitization are harder to gauge. However, AT_2 receptors do not appear to desensitize in response to agonist treatment (Csikos *et al*., 1998; Hein *et al*., 1997).

Less severe IR injury can manifest itself as post-ischaemic contractile dysfunction in the absence of irreversible damage despite the restoration of coronary flow (Bolli & Marban, 1999). Isolated working rat hearts, as developed by Neely *et al*. (1967), provide a more sensitive index of post-ischaemic myocardial stunning than non-working hearts due to their higher and more physiological workloads (Galinanes & Hearse, 1990). Using isolated working rat hearts, subjected to 30 min global, no-flow ischaemia, we did not observe any

exacerbation of stunning in response to angiotensin II concentrations of up to 100 nM after 30 min reperfusion.

Previously we have demonstrated opposing effects of selective antagonism of AT_1 and AT_2 receptors on recovery of post-ischaemic contractile function (Ford *et al*., 1996). These findings suggest that balanced blockade, or conversely, stimulation of both angiotensin II receptor subtypes has no effect on stunning after 30 min reperfusion. Our finding that stunning after 30 min reperfusion was unaffected by angiotensin II at concentrations of 1 to 100 nM (50 to 99% receptor occupancy) supports the hypothesis that balanced stimulation of AT_1 and AT_2 receptors has no overall effect on post-ischaemic myocardial stunning.

In summary, we have used two different models of isolated heart perfusion to provide evidence that angiotensin II does not adversely affect the short-term outcome from IR injury. There is no evidence that angiotensin II itself (in the range of 1 – 100 nM) has any effect on contractile dysfunction during reperfusion. Together with previous studies using selective AT_1 and AT_2 receptor antagonists, our data suggest that AT_1 and AT_2 receptors have opposing effects on IR injury such that angiotensin II, *per se*, has no effect on contractile dysfunction associated with IR injury. However, the presence of angiotensin II during ischaemia and reperfusion leads to a marked (3 fold) reduction in infarct size.

These studies were funded in part by a grant from the Heart & Stroke Foundation of Alberta. Dr W.R. Ford is a Pharmacology Fellow in the University of Cambridge.

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(Received May 10, 2001
Accepted June 11, 2001)