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Endothelium dependent vasodilatation following brief ischaemia and reperfusion in anaesthetised swine

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Abstract

Study objective **— The aim as to compare the responses of intracoronary infusions of ATP, an endothelium dependent vasodilator, with adenosine following brief ischaemia (10 min) and reperfusion in a model of myocardial stunning.**

Design **— In group 1 (n=6), coronary blood flow and endocardial (endo) and epicardial (epi) percent segment length shortening were measured in the distribution of the left anterior descending coronary artery before and during maximal intracoronary infusions of either adenosine or ATP (20 $\mu g \cdot kg^{-1} \cdot min^{-1}$). Measurements were obtained before and after myocardial stunning both at control heart rate and during atrial pacing (150 $beats \cdot min^{-1}$). In group 2 (n=6), myocardial blood flows by microspheres and arterial-venous lactate and oxygen differences were determined following the same ischaemia-reperfusion protocol to characterise transmural changes in blood flow and metabolism in this model of stunning.**

Experimental material **— The experiments were done on 12 anaesthetised swine, weight 25–39 kg.**

Measurements and main results **— In group 1, baseline endo and epi segment length shortening were 16(SD 3)% and 14(6)% and following reperfusion were reduced to 10(4)% and 8(6)% respectively ($p<0.05$). Prior to stunning, minimal coronary resistances during adenosine and ATP were 0.81(0.40) and 0.76(0.25) mm $Hg \cdot min \cdot ml^{-1}$ respectively and following reperfusion were 0.86(0.31) (NS) and 0.85(0.23) (NS) mm $Hg \cdot min \cdot ml^{-1}$ respectively. Infusion of either vasodilator enchanced function by 30% following reperfusion whereas no such effect was observed prior to ischaemia. In group 2, no maldistribution of blood flow was observed following the same ischaemia-reperfusion protocol to account for this vasodilator enhancement in function. Percent lactate extraction values were 29(11)% and 25(14)% at preischaemic control and paced heart rates respectively, and following reperfusion were lowered to 0(12)% without pacing ($p<0.05$) and −1(34)% during pacing ($p<0.05$).**

Conclusions **— Brief ischaemia and reperfusion in swine induces myocardial stunning without altering the vasodilator responses of either ATP, an endothelium dependent vasodilator, or adenosine. Recruitment in postischaemic segment length shortening was observed during infusions of both vasodilators at a time when maldistribution of flow was not observed. Possible mechanisms include either enhanced washout of lactate from the reperfused myocardium or greater utilisation of substrates during higher blood flows.**

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It has now become widely accepted that regional myocardial function can be reversibly depressed following brief periods of ischaemia and reperfusion.[1] Although sustained abnormalities in transmural blood flow as measured by radioactive microspheres are not the cause of this entity called "myocardial stunning", evidence is accumulating that coronary perfusion during the recovery phase is important. Several groups, for instance, have demonstrated that infusion of vasodilators such as adenosine can improve postischaemic function during late reperfusion.[2–7] The mechanism of this vasodilator enhancement in function may be a result of either improved oxygen delivery to heterogeneously reperfused regions or a washout of certain metabolic byproducts from the ischaemic period.[8 9] The coronary vasculature in the distribution of regionally stunned myocardium may also be altered, both structurally and functionally. One hour of occlusion and reperfusion in dogs, for instance, induces morphological changes in the endothelial cells including focal injury and partial detachment from the underlying cell matrix.[10] The same degree of ischaemia and reperfusion has been shown to alter the vasodilator response of several endothelium dependent vasodilators including acetylcholine, bradykinin, arachidonic acid, and ATP.[11–14]

These findings, often referred to as vascular "stunning", have been induced after long periods of ischaemia, which may also cause more permanent alterations. Such irreversible changes as necrosis and "no reflow" have been documented after 20 minutes of coronary flow reduction.[15] In this study, we wished to determine whether myocardial and vascular stunning could be dissociated following a shorter period of ischaemia. Vascular function prior to and following ischaemia-reperfusion was tested by comparing the vasodilator responses of intracoronary ATP, an endothelium dependent vasodilator, with adenosine.[13 16] Because the effect of increased work load or shear stress could enhance any differences in endothelium dependent mechanisms, the agents were infused both at baseline heart rates and during atrial pacing. In a second group of animals, the same protocol was repeated to determine the effects on transmural coronary blood flow and regional arterial-venous oxygen and lactate differences.

Methods

GENERAL PREPARATION

Following an overnight fast, 12 cross bred Landrace-Yorkshire pigs of either sex (25-39 kg) were sedated with intramuscular ketamine (20 $mg \cdot kg^{-1}$) and intravenous metomidate (5 $mg \cdot kg^{-1}$). Animals were intubated and connected to a ventilator for intermittent positive pressure ventilation with a mixture of oxygen and nitrous oxide (1:2). Ventilator settings were adjusted during the experiments to maintain normal arterial pH (7.35-7.45), P_{CO_2} (4.7-6.0 kPa) and P_{O_2} (>20 kPa). The external jugular vein was cannulated with two 7F gauge catheters for administration of anaesthetics and saline. The anaesthetic regimen consisted of a bolus of intravenous α chloralose (150 $mg \cdot kg^{-1}$) dissolved in boric acid solution and a continuous infusion of low dose sodium pentobarbitone (5 $mg \cdot kg^{-1} \cdot h^{-1}$). The femoral arteries were cannulated with 7F catheters and used for either aortic blood pressure measurements, arterial blood gas collections, or reference sampling for microsphere determination. Rectal temperature was monitored throughout the experiment and maintained near 37°C with external heating pads. Pancuronium bromide (4 mg) was given intravenously, and following a midline thoracotomy the heart was suspended in a pericardial cradle. The left mammary vessels were ligated and the second left rib was removed for ease of further instrumentation. The left anterior descending coronary artery was dissected free of its adventitia, and an electromagnetic flow probe (2.25 or 2.50 mm; Skalar, Delft, The Netherlands) and hydraulic occluder were placed proximally. Meticulous attention was paid to the positioning and stability of the flow probe and zero flows were checked regularly throughout the experimental protocol. Pacing leads were attached to the left atrial appendage and connected to a pacing stimulator.

SPECIFIC INSTRUMENTATION

Group 1 (n=6). The purpose of this group was to test the vasodilator responses to intracoronary adenosine and ATP (Boehringer, Mannheim, Germany), prior to and following myocardial stunning at two different heart rates (baseline and during atrial pacing at 150 $beats \cdot min^{-1}$). Immediately distal to the probe and occluder, a small cannula (0.8 mm outer diameter) was inserted into the left anterior descending artery for administration of the vasodilators. A microtipped catheter (7F Millar) was advanced into the left ventricle via the left carotid artery and used to monitor left ventricular pressure and its first derivative (dP/dt). To measure regional segment length shortening in the distribution of the left anterior descending artery, pairs of ultrasonic crystals (Triton, Technology Inc, San Diego, CA, USA) were placed in the endocardial and epicardial layers, approximately 10–15 mm apart in each region. Systolic shortening in the two layers was calculated from the difference between lengths at end diastole (time of onset of positive dP/dt) and end systole (time of peak negative dP/dt) and expressed as a percent of end diastolic length.[17]

Group 2 (n=6). The purpose of this group was to determine transmural coronary blood flow at baseline heart rate and during atrial pacing (150 $beats \cdot min^{-1}$), before and after the same ischaemia-reperfusion protocol as in group 1. In addition, arterial and left anterior descending coronary venous samples were obtained for oxygen and lactate concentrations during each of the interventions. Instrumentation in this group was limited, with only a small cannula inserted into the left anterior descending coronary vein for sampling and a catheter inserted into the left atrium for administration of radiolabelled microspheres.

Studies were performed in accordance with the position of the American Heart Association on research animal use, and under the regulations of the Animal Care Committees of the VA Medical Center, Minneapolis, and Erasmus University Rotterdam.

EXPERIMENTAL PROTOCOL

Group 1. Following a 30 min stabilisation period, baseline measurements were taken of mean aortic and left ventricular pressures, coronary blood flow, left ventricular dP/dt, and endocardial and epicardial segment length changes. Maximum vasodilator responses for both adenosine and ATP were determined prior to each experiment and those doses used for the remainder of the protocol (20-40 $\mu g \cdot kg^{-1} \cdot min^{-1}$). The maximal dose of intracoronary adenosine was infused continuously and recordings obtained after 5 min of steady state. The infusion was discontinued and following a 5 min washout period, the maximal dose of ATP was begun and measurements obtained after 5 min. The protocol was

then repeated during atrial pacing at 150 beats·min^{-1}. Ten minutes of ischaemia were then induced by adjusting the hydraulic occluder so that coronary blood flow was reduced by greater than 80% of the baseline value. We have previously found that this degree of partial coronary blood flow reduction induces myocardial stunning with less ventricular fibrillation compared with that of complete coronary occlusions.[18] The heart was reperfused for 30 min, and recordings were repeated prior to and following intracoronary infusions of adenosine and ATP, without and during atrial pacing.

Group 2. In this protocol, transmural blood flow was determined at baseline heart rate and during atrial pacing, both before and after the same ischaemia-reperfusion protocol as in group 1. Microspheres were injected prior to and during each pacing intervention as well as during the partial coronary occlusion. In addition, simultaneous atrial and left anterior descending coronary venous samples were obtained for oxygen saturation and lactate concentrations during the same sampling periods.

REGIONAL MYOCARDIAL BLOOD FLOW

For each flow measurement in group 2, 1-2 million microspheres (15 microns) of either ^{141}Ce, ^{113}Sn, ^{103}Ru, ^{95}Nb, or ^{46}Sc (NEN-TRAC, New England Nuclear, Boston, MA, USA) were injected into the left atrium. Reference arterial blood samples were withdrawn from the femoral artery catheter at a fixed rate of 10 ml·min^{-1}, from 15 s prior to until 1 min after microsphere injection. At the conclusion of the experiment, the animal was killed and the heart excised. The distribution of the postischaemic myocardium was identified by injecting patent violet dye into the left anterior descending coronary artery at the level of the occluder. Hearts were fixed in 10% formalin for 48 h, and separated into left anterior descending and non-left-anterior-descending regions. Each was then divided into three equal layers (inner, mid, and outer) and placed in 1-2 g samples. Myocardial and reference blood samples were counted in a multichannel analyser and regional blood flows determined.[19]

OXYGEN AND LACTATE CONTENTS

In group 2 animals, oxygen saturation was determined from arterial and left anterior descending cardiac venous samples by an OSM2 system (Radiometer; Copenhagen, Denmark). Blood for lactate determinations was collected in iced syringes containing heparin, and promptly centrifuged for later analysis by the enzymatic technique.[20] Oxygen content was determined from the formula (1.34×Hb×% oxygen saturation). Oxygen consumption in the distribution of the reperfused myocardium was then calculated by the product of microsphere flow and the arterial-coronary venous oxygen difference. Percent lactate extraction was calculated by the arterial-coronary venous lactate difference divided by arterial lactate ×100.

STATISTICS

Results are expressed as arithmetic means (SD). Pre- and post-ischaemia-reperfusion differences were tested for significance at the $p<0.05$ level by analysis of variance with repeated measurements (Fisher's PLSD and F test). In addition, microsphere flows during each intervention in group 2 were compared in the left anterior descending and non-left-anterior-descending coronary artery regions by unpaired *t* testing.

Results

SYSTEMIC HAEMODYNAMICS (Group 1)

Table I summarises the systemic haemodynamics during all interventions in group 1. Except for the increase in heart rate, there were no changes in any of the variables following reperfusion compared with preischaemic values.

CORONARY HAEMODYNAMICS AND REGIONAL FUNCTION (Group 1)

Coronary blood flows and endocardial and epicardial segment length changes are summarised in table II.

Table I Systemic haemodynamic variables before ischaemia and 30 min postreperfusion (group 1). Values are means (SD)

	Preischaemia			*30 min reperfusion*		
	Baseline-1	*Adenosine*	*ATP*	*Baseline-2*	*Adenosine*	*ATP*
Control HR:						
MAP	78(17)	75(17)	74(21)	78(12)	78(13)	71(13)
HR	102(6)	101(8)	100(12)	116(8)*	116(9)*	117(13)*
LVEDP	7(1)	7(2)	7(3)	7(2)	7(2)	6(1)
LVdP/dt	1579(394)	1481(337)	1434(366)	1392(416)	1430(390)	1480(446)
Pacing HR:						
MAP	76(16)	76(18)	75(18)	76(13)	81(12)	76(12)
HR	150	150	150	150	150	150
LVEDP	8(2)	8(2)	8(2)	6(2)	5(3)	5(1)
LVdP/dt	1516(351)	1377(402)	1479(284)	1548(218)	1610(429)	1645(192)

MAP=mean arterial pressure (mm Hg); HR=heart rate (beats·min^{-1}); LVEDP=left ventricular end diastolic pressure (mm Hg); LVdP/dt=first derivative of left ventricular pressure (mm Hg·s^{-1})
*$p<0.05$ *v* baseline-1.

Table II Coronary haemodynamic variables and regional function before ischaemia and 30 min postreperfusion (group 1). Values are means (SD)

	Preischaemia			*30 min reperfusion*		
	Baseline-1	*Adenosine*	*ATP*	*Baseline-2*	*Adenosine*	*ATP*
Control HR						
LAD coronary artery						
Blood flow ($ml \cdot min^{-1}$)	30(13)	114(59)*	111(54)*	21(8)	98(32)†	90(33)†
Resistance (mm Hg·min·ml^{-1})	3.06(1.14)	0.81(0.40)*	0.76(0.25)*	4.11(1.12)*	0.86(0.31)†	0.85(0.23)†
Segment lengths (mm)						
Endo ES	9.1(1.5)	9.1(1.4)	9.1(1.6)	9.5(1.4)*	9.3(1.3)†	9.4(1.5)†
Endo ED	10.8(2.0)	10.8(2.0)	11.0(2.1)	10.6(1.9)	10.7(1.8)	10.8(2.0)
Endo shortening (%)	16(3)	16(3)	17(3)	10(4)*	13(4)†	13(4)
Epi ES	9.5(1.0)	9.5(1.0)	9.3(1.1)	10.1(1.1)*	9.9(1.1)†	9.7(1.1)†
Epi ED	11.1(1.6)	11.0(1.6)	10.9(1.8)	11.0(1.4)	11.0(1.5)	10.9(1.6)
Epi shortening (%)	14(6)	14(6)	14(5)	8(6)*	9(7)†	10(7)†
Pacing HR						
LAD coronary artery						
Blood flow ($ml \cdot min^{-1}$)	30(11)	106(56)*	97(47)*	23(5)	102(22)†	87(32)†
Resistance (mm Hg·min·ml^{-1})	2.88(1.28)	0.86(0.38)*	0.90(0.39)*	3.55(0.29)*	0.81(0.15)†	0.95(0.29)†
Segment lengths (mm)						
Endo ES	9.2(1.3)	9.6(1.1)	9.5(1.2)	9.8(1.2)*	9.3(1.4)†	9.4(1.4)†
Endo ED	10.6(1.5)	10.8(1.5)	10.7(1.5)	10.8(1.5)	10.6(1.4)	10.6(1.6)
Endo shortening (%)	13(2)	12(4)	11(3)	9(4.5)*	11(4)†	11(4)†
Epi ES	9.7(0.6)	10.2(0.8)	10.1(0.8)	10.4(0.8)*	9.5(0.9)†	9.6(0.7)†
Epi ED	11.0(0.9)	11.1(0.8)	11.1(0.8)	11.2(0.9)	10.7(1.1)†	10.7(1.0)†
Epi shortening (%)	10(5)	9(4)	8(4)	6(6)*	8(6)†	8(6)†

LAD=left anterior descending coronary artery; Endo=endocardial; Epi=epicardial; ED=end diastolic; ES=end systolic
*$p<0.05$ *v* baseline-1; †$p<0.05$ *v* baseline-2

Figure 1 shows a representative tracing in one animal. Following reperfusion (baseline-2), coronary resistances (mean arterial pressure/mean coronary blood flow) were higher compared with preischaemic values at both heart rates. Minimum resistances achieved with either adenosine or ATP, however, were unchanged compared with preischaemic values.

Following reperfusion, endocardial and epicardial segment length shortening was lower than preischaemic values at each heart rate. Figure 2 shows the effect of infusions of adenosine and ATP on (A) endocardial and (B) epicardial function prior to and following myocardial stunning at control heart rate. As shown, both vasodilators enhanced percent segment length shortening in the two layers following reperfusion but had no effect prior to ischaemia-reperfusion. The effect was the same at both heart rates. Because end diastolic lengths and systolic pressures were similar during these interventions, it is unlikely that the functional changes were a result of

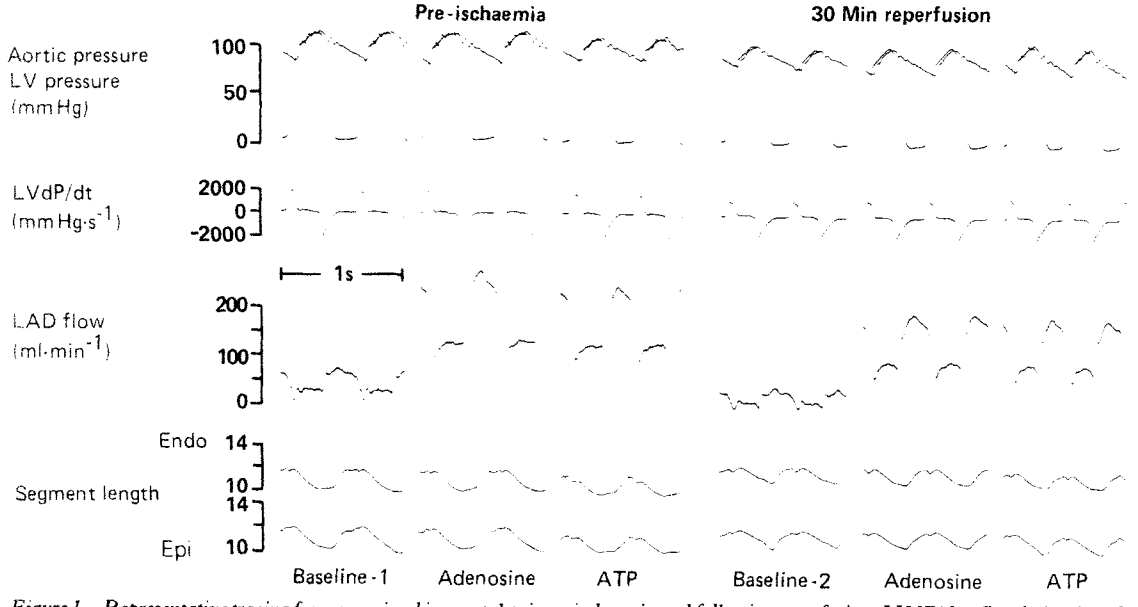

Figure 1 Representative tracing from an animal in group 1 prior to ischaemia and following reperfusion. LVdP/dt=first derivative of left ventricular pressure; LAD=left anterior descending coronary artery; Endo=endocardial; Epi=epicardial

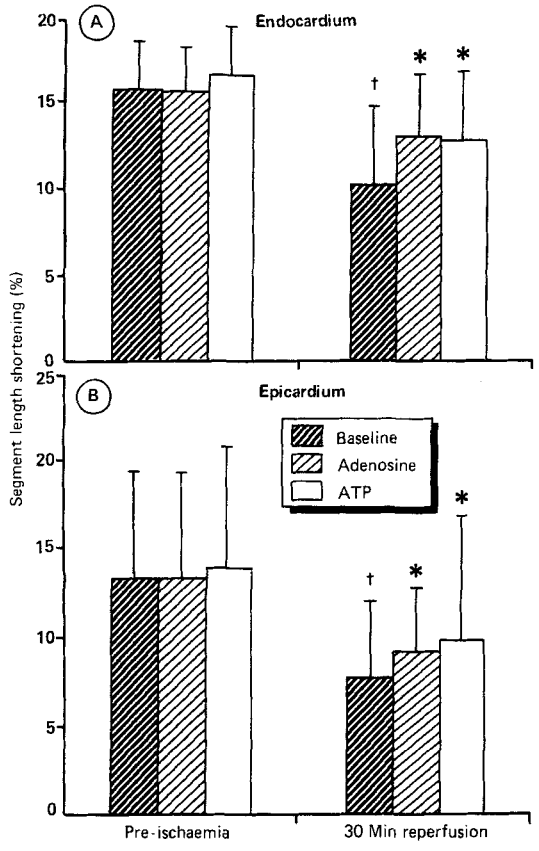

*Figure 2 (A) Endocardial systolic segment length changes (%) preischaemia and postreperfusion at control heart rate in group 1. (B) Epicardial systolic segment length changes (%) during the same interventions. Columns are means, bars=SD, n=6. *p<0.05 v baseline postischaemia; †p<0.05 v baseline preischaemia*

changes in the loading conditions.

MYOCARDIAL BLOOD FLOW, OXYGEN AND LACTATE (Group 2)

Heart rate was 75(20) beats·min^{-1} prior to ischaemia and increased to 81(29) ($p<0.05$) following reperfusion. Mean arterial pressure decreased slightly but insignificantly from 87(16) to 79(16) mm Hg during the same interval. During initial atrial pacing, mean arterial pressure was 79(10) mm Hg and it was 76(11) mm Hg following ischaemia-reperfusion (NS).

Myocardial blood flows in the left anterior descending and non-left-anterior-descending coronary artery regions are shown in table III. During ischaemia, blood flow in the left anterior descending region was reduced to 20% of baseline ($p<0.05$), while in the non-left-anterior-descending region, the reduction was not statistically significant. Following 10 min of ischaemia and 30 min of reperfusion, transmural flows returned to preischaemic baseline values in all layers. During pacing, transmural flow in the reperfused regions did not increase to the same degree as in the normal region and was significantly lower in the subendocardial layer.

Myocardial oxygen consumption in the left anterior descending region is also shown in table III. Although values tended to increase with pacing and decrease following reperfusion, the differences were not statistically significant. Lactate extraction during each intervention was measured from the reperfused bed and is shown in fig 3. Following 30 min of reperfusion, lactate production was greater at both heart rates compared with preischaemic baseline. It is interesting to note that lactate extraction following reperfusion remained unchanged at the two heart rates despite relative subendocardial hypoperfusion in the left anterior descending region. This suggests that the lower flows were not associated with ischaemia.

Table III Myocardial blood flow and oxygen consumption before ischaemia and 30 min postreperfusion (group 2). Values are means (SD)

	Baseline	*Pacing*	*Ischaemia*	*30 min reperfusion*	*30 min reperfusion +pacing*
		Myocardial blood flow (ml·g^{-1}·min^{-1})			
Transmural					
LAD	0.92(0.32)	1.17(0.69)	0.18(0.22)†	0.89(0.39)	0.89(0.32)
Non-LAD	1.00(0.29)	1.23(0.49)	0.82(0.34)	0.99(0.37)	1.10(0.25)
Endocardium					
LAD	0.88(0.26)	1.18(0.62)	0.23(0.30)†	0.89(0.32)	0.84(0.24)*
Non-LAD	1.06(0.31)	1.23(0.49)	0.87(0.37)	1.04(0.43)	1.13(0.19)
Mid-myocardium					
LAD	0.93(0.30)	1.25(0.75)	0.18(0.23)†	0.97(0.41)	0.96(0.32)
Non-LAD	1.10(0.37)	1.28(0.48)	0.88(0.43)	1.00(0.35)	1.13(0.23)
Epicardium					
LAD	0.96(0.43)	1.12(0.73)	0.16(0.18)†	0.86(0.48)	0.87(0.41)
Non-LAD	0.96(0.31)	1.16(0.54)	0.85(0.39)	0.95(0.40)	1.05(0.34)
		LAD myocardial oxygen consumption			
O_2-ex (ml·100 ml^{-1} blood)	7.94(2.55)	7.56(2.52)		6.64(2.66)	7.77(2.85)
MVo_2 (ml·100 g^{-1}·min^{-1})	6.46(1.80)	8.11(2.46)		5.04(3.02)	5.93(1.58)

LAD=left anterior descending coronary artery; O_2-ex=myocardial oxygen extraction; MVo_2=myocardial oxygen consumption
*p<0.05; †p<0.01 *v* non-LAD region (unpaired *t* test)

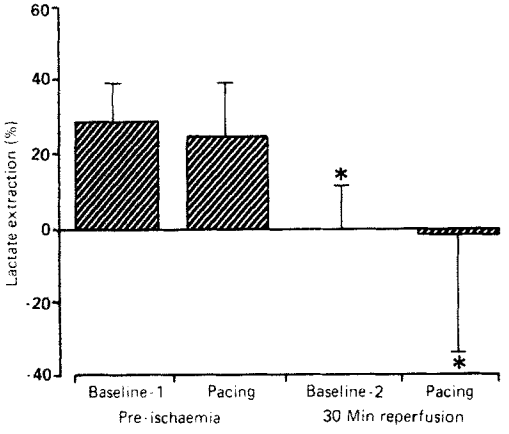

Figure 3 Lactate extraction (%) at control heart rate and during atrial pacing (150 beats·min^{-1}), prior to and following ischaemia-reperfusion in group 2. Columns are means, bars=SD, n=6.
**p<0.05 v baseline preischaemia*

Discussion

In this model of myocardial stunning, we have shown that the vasodilator responses of intracoronary infusions of ATP and adenosine are not altered following brief ischaemia and reperfusion. Unlike adenosine, ATP requires an intact endothelium for vasoactivity and thus has been used as a marker of altered endothelial cell function.[13 16] Other endothelial dependent vasodilators such as acetylcholine, prostacyclin, and bradykinin have also been studied in various models of ischaemia-reperfusion. In anaesthetised dogs for instance, their vasodilator capacity is attenuated following 45-60 min of complete coronary occlusion.[11-14] This "vascular stunning" has primarily been demonstrated after prolonged periods of ischaemia, which may also induce more permanent changes such as necrosis and "no-reflow".[15] Our results are consistent with a more recent study which shows no differences in vascular reactivity between ADP and adenosine following brief ischaemia.[21] We have extended these findings to ATP and adenosine and in addition have measured the effect on function during vasodilatation. The present study dissociates mechanical stunning during reperfusion from functional changes in the vasculature at a time when permanent changes are unlikely.

Following reperfusion, minimal coronary resistances during intracoronary infusions of either ATP or adenosine were not different from the preischaemic baseline values. This is consistent with the findings in both conscious and open chest dogs, where the vasodilator response to adenosine following 10 min of ischaemia and reperfusion were not altered.[22 23] In anaesthetised dogs, Bolli *et al*[6] have recently reported that coronary resistance was higher in regionally stunned myocardium 4 h after a 15 min occlusion. They also reported that minimal coronary resistances during intravenous infusions of adenosine or papaverine were higher in the reperfused regions compared with normally perfused myocardium and proposed that microvascular stunning existed. It is possible that our model of 10 minutes of partial coronary occlusion is not severe enough to achieve the differences that they found after 15 minutes of complete occlusion. Johnson *et al*,[24] however, have studied maximal flow-pressure lines during intracoronary adenosine infusions in anaesthetised swine and showed no change in slopes following 20 minutes of complete occlusion and 15-45 minutes of reperfusion. This would suggest that functional alterations in the vasculature are minimal when ischaemia is less than 20 minutes in duration.

Microvascular stunning is particularly interesting in light of the observations that both vasodilators in group 1 enhanced segment length shortening in the reperfused myocardium. This flow dependent improvement in function was not observed prior to ischaemia-reperfusion and thus is different from the original observations made by Gregg.[25] In group 2 animals, transmural flow distribution returned to normal following the same ischaemia-reperfusion protocol as in group 1. Although pacing following reperfusion was associated with greater differences in flow between the two regions, lactate production did not increase and thus it seems unlikely that these changes in flow resulted in ischaemia. Others have also reported improved function with vasodilatation in models of myocardial stunning. Following 12 five minute ischaemic periods in dogs, Stahl *et al*[2] infused three different vasodilators, dipyridamole, papaverine, and glyceryl trinitrate, and showed a selective improvement in segment length following reperfusion. They postulated that the improved function resulted from an increase in flow to incompletely reperfused regions. With longer periods of ischaemia and reperfusion, others have shown that adenosine not only improves function but also limits reperfusion injury. The proposed mechanism is a result of the ability of adenosine to reduce microvascular plugging of leucocytes and thus prevent endothelial cell damage and "no reflow".[3-5]

Mechanisms other than microvascular "no reflow" have been investigated to elucidate this flow dependent recruitment in function following stunning. Although adenosine and ATP can provide substrate for ATP production during reperfusion, this does not seem to be the explanation for the improved function. In isolated rat hearts exposed to three hours of hypothermic arrest, functional recovery improved when reperfused with adenosine but ATP levels did not increase.[7] It is possible that increased flow during reperfusion improves function by the wash out of metabolic byproducts from the ischaemia. In isolated working rat hearts, intermittent reperfusion during 40 minutes of global ischaemia improved functional

recovery compared with hearts exposed to no reperfusion.[8] The authors hypothesised that intracellular calcium homeostasis was maintained in this model by preventing the build up of intracellular sodium. This might also explain why mitochondrial function is improved when dogs have been reperfused with adenosine following brief ischaemia.[9]

Our data show that lactate was continuously produced even 30 minutes after reperfusion. Because the distribution of coronary blood flow as measured by microspheres had returned to preischaemic values at that time, it is unlikely that the myocardium was still ischaemic. It is possible that the higher flows with ATP and adenosine improved contractile function by the wash out of lactate in the reperfused regions. Neely *et al*[26] have shown that lactate production during anaerobic glycolysis may be responsible for ventricular dysfunction. In isolated rabbit myocardium, tissue lactate levels have been shown to be raised following brief ischaemia and reperfusion and are lower when adenosine is included in the reperfusate.[27] An alternative explanation to these observations is that vasodilatation during reperfusion improves contractile function by increasing oxygen delivery and enhancing substrate utilisation.[7]

In summary, we have shown that alterations in regional function induced by 10 minutes of partial coronary occlusion and 30 minutes of reperfusion in swine are not associated with altered responses to the endothelium dependent vasodilator ATP or to adenosine. Postischaemic function is improved with both vasodilators, however, and may result from either enhanced washout of metabolic byproducts such as lactate or improved utilisation of substrates.

LIMITATIONS OF THE STUDY

A major criticism of this work is the lack of specifity of the vasodilator responses of either adenosine or ATP with regard to the endothelium. Although ATP relaxes preconstricted arteries via the release of endothelium dependent relaxing factor in vitro, its mechanism of vasodilatation in the intact animal may be more non-specific. Likewise, adenosine has been considered to be an endothelium independent vasodilator, but recent work suggests that its receptors may also be linked to guanylate cyclase.[28] We feel, however, that the inability to attenuate the vasodilator response of either agent in our model suggests that the endothelium remains functionally intact.

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