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The effect of ischaemia on endothelium-dependent vasodilatation and adrenoceptor-mediated vasoconstriction in rat isolated hearts

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1 The aim of this study was to investigate whether global ischaemia and reperfusion in rat isolated hearts affects endothelium-dependent vasodilatation and adrenoceptor-mediated vasoconstriction. In addition, it was first determined whether inhibition of the actions of nitric oxide (NO) influenced the responses to α-adrenoceptor agonists in the rat coronary vasculature.

2 In rat isolated, Langendorff perfused hearts, inhibition of NO with haemoglobin (Hb, 6 μM) significantly inhibited the vasodilator responses to the endothelium-dependent vasodilators, acetylcholine (ACh, 3-100 pmol), carbachol (CCh, 10-300 pmol), bradykinin (Bk, 1-30 pmol) and histamine (0.3-10 nmol) but did not affect responses to the endothelium-independent vasodilator, sodium nitroprusside (SNP, 0.01-1 nmol).

3 Inhibition of the action of NO by Hb significantly enhanced the vasoconstrictor response to the non-selective α-adrenoceptor agonist, noradrenaline (NA, 0.1-10 nmol) and the α_2-adrenoceptor agonist, B-HT 920 (0.001-1 μmol) but had no effect on the vascular response to the α_1-adrenoceptor agonist, methoxamine (MTX, 10-300 nmol).

4 In the perfused hearts ischaemia, induced by 30 min perfusion at 5% of the normal rate of flow, followed by 15 min of reperfusion (ischaemia/reperfusion) selectively impaired the vasodilator responses to ACh and CCh which act by muscarinic receptor stimulation but did not affect responses to the other endothelium-dependent vasodilators Bk and histamine or to the endothelium-independent dilator SNP.

5 After ischaemia/reperfusion the coronary vasoconstrictor responses to B-HT 920 were slightly but significantly enhanced whereas the responses to NA and MTX were unaffected.

6 Thus, in the rat isolated heart, low flow induced-ischaemia and reperfusion causes a selective impairment of muscarinic receptor-mediated vasodilatation but does not impair responses to all endothelium-dependent vasodilators. Enhanced constrictor responses to noradrenaline and B-HT 920 in the presence of Hb indicates that endogenous NO modulates the constriction of coronary resistance vessels in response to stimulation of α_2-adrenoceptors. Ischaemia and reperfusion in this isolated vascular bed caused only a small increase in the coronary vasoconstrictor response to α_2-adrenoceptor stimulation. It appears that in the rat isolated heart the degree of endothelial dysfunction caused by ischaemia/reperfusion is insufficient to cause a functionally significant change in α-adrenoceptor-mediated constriction.

Keywords: Adrenoceptors; constriction; dilatation; ischaemia; nitric oxide; rat heart

Introduction

Previous studies have demonstrated that adrenoceptor-mediated constriction is modulated by endothelium-derived nitric oxide (Cocks & Angus, 1983; Jones *et al.*, 1993; Woodman & Pannangpetch, 1994). Such observations suggest that endothelial injury may lead to enhanced constriction, perhaps contributing to coronary vasospasm, but there is little direct evidence of endothelial dysfunction leading to potentiated adrenergic coronary vasoconstriction (Woodman, 1995). Myocardial ischaemia and reperfusion is one such pathological condition that can lead to endothelial dysfunction and impaired endothelium-dependent dilatation of the coronary vasculature (Hearse *et al.*, 1993; Sobey & Woodman, 1993); however, little information is available regarding the effect of myocardial ischaemia on vasoconstriction. Previous examination of responses to α-adrenoceptor agonists has indicated that ischaemia does not affect coronary constrictor responses to noradrenaline (Gutterman *et al.*, 1992) or selective α_1- and α_2-adrenoceptor agonists (Ehring *et al.*, 1995) even though endothelial dysfunction was confirmed in the latter study. It is puzzling that even though ischaemia impaired endothelium-dependent dilatation there was no enhancement of constriction, given several reports that inhibition of NO synthase enhances adrenoceptor-mediated constriction of resistance vessels (Bauknight *et al.*, 1992; Ohyanagi *et al.*, 1992; Du *et al.*, 1992; Pannangpetch & Woodman, 1992). However, we have recently reported that inhibition of NO synthase in the dog coronary vasculature enhanced noradrenergic constriction of the epicardial but not the resistance coronary vasculature (Woodman & Pannangpetch, 1994). This suggests that the ability of NO to inhibit adrenoceptor-mediated constriction varies in different vascular beds or that perhaps metabolic or neurohumoral control mechanisms are able to compensate for the loss of NO-induced dilatation in the coronary resistance vasculature. The present investigation therefore had two aims. Firstly, to determine whether inhibition of the actions of NO enhances adrenoceptor-mediated constriction of coronary resistance vessels in the rat isolated perfused heart where neurohumoral influences have been eliminated and changes in metabolic demand can be controlled. Secondly, if responses to α-adrenoceptor agonists are modulated by NO, to determine whether ischaemia impairs endothelium-dependent vasodilatation in the same system and if so, whether that impairment results in enhanced adrenoceptor-mediated vasoconstriction.

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Methods

Isolated perfused heart

Sprague-Dawley rats of either sex weighing 250–300 g were killed by a blow to the head and cervical dislocation. The hearts were rapidly excised and placed in ice-cold Krebs solution composed of (in mM): NaCl 118, KCl 4.7, KH_2PO_4 1.2, $Mg_2SO_4.7H_2O$ 1.2, (+)-glucose 11.0, $NaHCO_3$ 25.0 and $CaCl_2.2H_2O$ 2.54. A modified Langendorff procedure was used: the hearts were transferred to a perfusion apparatus and perfused via the aorta with oxygenated Krebs solution (95% O_2:5% CO_2) kept at 37°C. The hearts were perfused at a constant flow of 7–9 ml min^{-1} with a roller pump (Minipuls 3, Gilson) and allowed to beat spontaneously. Coronary perfusion pressure (CPP) was measured via a catheter placed just above the aortic perfusion line connected to a pressure transducer (CDX-111, Cobe) and polygraph (Model 7D, Grass). In this constant flow system changes in CPP represent changes in coronary resistance vessel tone. To measure the developed tension (DT), a hook was placed in the apex of the heart and attached to a force displacement transducer (FT 03, Grass). The baseline tension was adjusted to 4 g before any intervention. Heart rate was also monitored using the signal from the DT recording to trigger a cardiotachometer. Drugs were given through an injection port proximal to the aorta.

Experimental protocols

The effect of haemoglobin (Hb) on vasodilator responses After allowing a stabilization period of 20 min the resting tone in the coronary vascular bed was increased by infusion of the thromboxane-mimetic, U46619 (10^{-7}–10^{-6} M, 0.1–0.2 ml min^{-1}) to achieve a perfusion pressure of approximately 100 mmHg. Responses to the vasodilators, acetylcholine (ACh, 3–100 pmol), carbachol (CCh, 10–300 pmol), bradykinin (Bk, 1–30 pmol), histamine (0.3–30 nmol) or sodium nitroprusside (SNP, 0.01–1 nmol) were examined when the perfusion pressure had stabilised. Each heart was used to investigate responses to only one drug. In separate hearts Hb (6 μM, final concentration in the perfusate), an inhibitor of the actions of NO, was infused at the same time as U46619 and maintained for the duration of the experiments whilst the responses to each of the vasodilators listed above were examined. Hb was chosen to inhibit the actions of NO at it was found in preliminary experiments that the NO synthesis inhibitor N^G-nitro-L-arginine caused marked coronary vasoconstriction leading to ischaemia which resulted in decreased contractility and arrhythmias.

The effect Hb on adrenoceptor-mediated vasoconstrictor responses

In this set of experiments all hearts were perfused with Krebs solution containing propranolol (1 μM) to prevent any β-adrenoceptor-mediated increases in rate or force of cardiac contraction which could lead to metabolic vasodilatation. The responses to the vasoconstrictors, noradrenaline (NA, 0.1–10 nmol), a non-selective α-adrenoceptor agonist, methoxamine (MTX, 10–300 nmol), an α-adrenoceptor agonist, and B-HT 920 (0.001–1 μmol), an α_2-adrenoceptor agonist were examined. Separate hearts were used for examination of the responses to each vasoconstrictor in the absence or in the presence of Hb (6 μM).

The effect of low flow ischaemia and reperfusion on vasodilator responses

After 20 min of stabilization, hearts were subjected to a 30 min period of ischaemia by reducing the level of perfusion to 5% of the control flow rate, followed by 15 min of reperfusion at the normal flow rate (ischaemia/reperfusion). The infusion of U46619 was started after 10 min of the 15 min period of reperfusion and the responses to vasodilators were then examined as described above. In a separate group of hearts control experiments were performed where the rate of perfusion was maintained at the normal level for 45 min before examining responses to the same agonists. In these control hearts infusion of U46619 was started 5 min before responses to the vasodilators were examined.

The effect of ischaemia/reperfusion on vasoconstrictor responses

The hearts were subjected to 30 min of low flow ischaemia and 15 min of reperfusion as described above or for control experiments, normally perfused for 45 min before examining the responses to the vasoconstrictors. The responses to only one of the α-adrenoceptor agonists were examined in each control or ischaemia/reperfused heart.

Drugs

Drugs used were: acetylcholine perchlorate (BDH), B-HT 920 (6-allyl-2-amino-5,6,7,8-tetrahydro-4H, thiazolo-(4,5-d)azepine dihydrochloride; Boehringer), bradykinin acetate (Sigma), carbachol (carbamylcholine chloride, Sigma), haemoglobin (bovine, Sigma), histamine diphosphate (Sigma), methoxamine hydrochloride (Sigma), (−)-noradrenaline bitartrate (Sigma), propranolol hydrochloride (Sigma), sodium nitroprusside (Sigma) and U46619(9,11-didoexy-9α,11α-epoxymethanoprostaglandin $F_2\alpha$, Sigma). All drugs were dissolved in saline except propranol which was dissolved in distilled water and U46619 which was dissolved in ethanol and diluted in saline. The final concentration of ethanol in the perfusate was 10^{-8} M or less.

Statistical analysis

Responses are presented as the mean ± s.e.mean of n experiments. Analysis of variance was used to test for any difference between the mean responses recorded in treated and control hearts. Baseline CPP represents an average of values of resting perfusion pressure measured whenever a drug was injected. Baseline DT and HR were also obtained by this procedure. Comparison of basal haemodynamic values was performed using Student's unpaired t test. In each case a P value of less than 0.05 was considered to represent a significant difference.

Results

Effect of haemoglobin (Hb) on responses to vasodilators

In hearts where the coronary vasculature was constricted with U46619 all of the vasodilators decreased coronary perfusion pressure (CPP) in a dose-dependent manner (Figures 1 and 2) without having any effect on rate or force of cardiac contraction (data not shown). The infusion of Hb (6 μM) significantly decreased the vasodilator responses to the endothelium-dependent vasodilators acetylcholine (ACh), carbachol (CCh), bradykinin (Bk) and histamine (Figure 1, $P < 0.05$) but had no effect on the responses to the endothelium-independent vasodilator sodium nitroprusside (SNP, Figure 2). There were no differences in the baseline values of CPP, developed tension (DT) and heart rate (HR) between the Hb-treated hearts and the control hearts (data not shown).

Effect of Hb on α-adrenoceptor-mediated vasoconstriction

The non-selective α-adrenoceptor agonist, noradrenaline (NA), the α_1-adrenoceptor agonist, methoxamine (MTX) and the α_2-adrenoceptor agonist, B-HT 920 all increased CPP in a dose-related manner (Figure 3) without affecting heart rate or force of contraction (data not shown). In the presence of Hb (6 μM)

the constrictor responses to NA and B-HT 920 were significantly enhanced whereas the responses to MTX were not affected (Figure 3).

Effect of ischaemia/reperfusion on vasodilator responses

Vasodilator responses to the endothelium-dependent vasodilators ACh, CCh, Bk and histamine were evaluated in rat isolated perfused hearts subjected to 30 min of low flow perfusion (ischaemia) followed by 15 min of reperfusion. Ischaemia and reperfusion decreased the force of contraction and arrhythmias were apparent during the reperfusion period; however, normal contractility recovered during the reperfusion period such that there were no significant differences between the control and ischaemia groups regarding CPP and contractility at the time of drug administration (Table 1). The dilator responses to ACh and CCh were significantly impaired by ischaemia/reperfusion compared to responses in control hearts (Figure 4). In contrast, the decreases in CPP in response to Bk and histamine were not affected by ischaemia (Figure 4). Increasing the period of low flow ischaemia to 60 min followed by 30 min reperfusion impaired the vascular response to ACh to a similar extent but still did not affect dilator responses to Bk (data not shown).

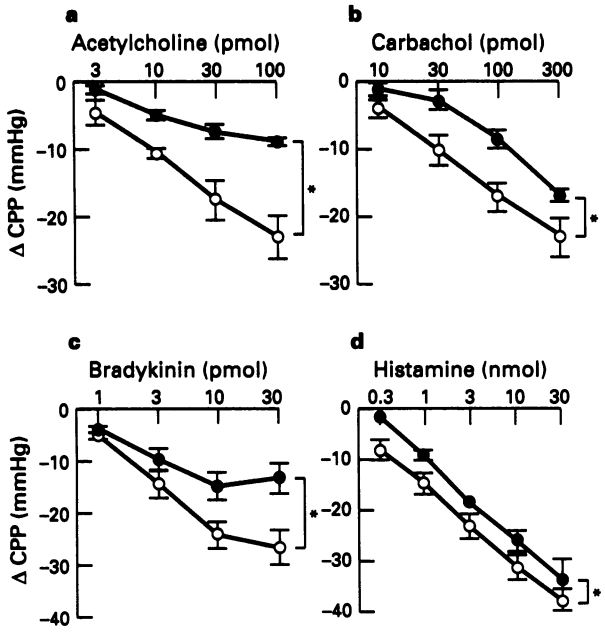

Figure 1 The effect of haemoglobin (Hb) on the decrease in coronary perfusion pressure (CPP) in response to endothelium-dependent vasodilators. The responses of the U46619 preconstricted vasculature in rat isolated hearts to endothelium-dependent vasodilators are shown as follows: acetylcholine (a, control $n=4$, Hb-treated $n=4$), carbachol (b, control $n=4$, Hb-treated $n=5$), bradykinin (c, control $n=5$, Hb-treated $n=5$) and histamine (d, control $n=5$, Hb-treated $n=4$). The infusion of haemoglobin (6 μM, ●) significantly inhibited the vasodilatation to each of these agents as compared to the responses in control hearts (○, $*P<0.05$, ANOVA).

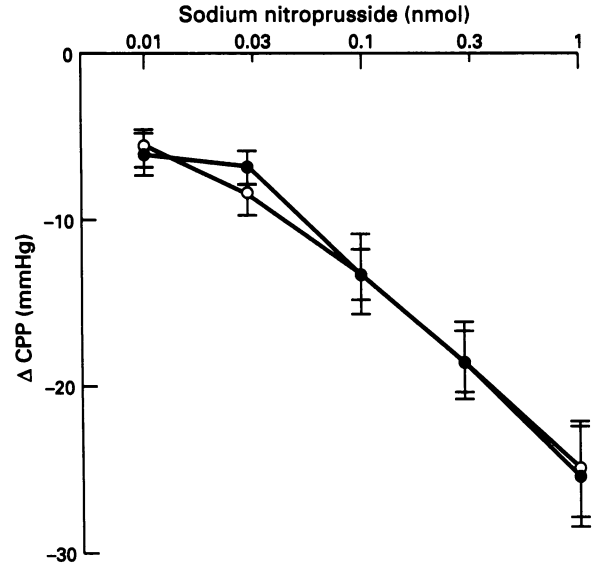

Figure 2 Effect of haemoglobin (Hb) on the decrease in coronary perfusion pressure (CPP) in response to the endothelium-independent vasodilator, sodium nitroprusside. The infusion of Hb 6 μM (●, $n=5$) did not affect the dilatation in response to sodium nitroprusside compared to the responses in control hearts (○, $n=5$).

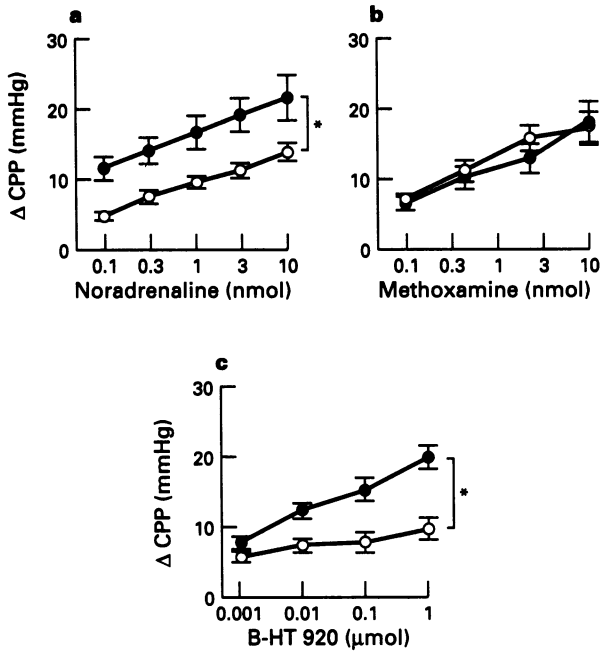

Figure 3 Effect of haemoglobin (Hb, 6 μM) on the increase in coronary perfusion pressure (CPP) in response to α-adrenoceptor agonists. Vasoconstriction in response to noradrenaline (control $n=7$, Hb-treated $n=7$) and the selective α_2-adrenoceptor agonist, B-HT 920 (control $n=6$, Hb-treated $n=6$) but not the α_1-adrenoceptor agonist, methoxamine (control $n=8$, Hb-treated $n=11$), was potentiated in the presence of Hb (●) as compared to the responses in control hearts (○). $*P<0.05$, ANOVA.

Table 1 The effect of 30 min low flow ischaemia and 15 min reperfusion on basal coronary perfusion pressure (CPP), developed tension (DT) and heart rate (HR) in rat isolated hearts

	Control	*30 min ischaemia*
*Vasodilator experiments**	$n=27$	$n=26$
CPP (mmHg)	101±3	96±3
DT (g)	8.7±0.4	8.0±0.4
HR (beats min^{-1})	224±9	245±11
Vasoconstrictor experiments	$n=15$	$n=15$
CPP (mmHg)	67±3	64±4
DT (g)	10.3±0.4	8.0±0.4
HR (beats min^{-1})	209±11	210±9

Data shown as mean±s.e.mean, n: total number of animals in each experimental group.
*The data shown for the vasodilator experiments were derived during infusion of the vasoconstrictor U46619 (10^{-7}–10^{-6} M).

In addition, the vasodilator response to SNP was not affected by ischaemia/reperfusion confirming that the function of the vascular smooth muscle was not affected (data not shown).

Effect of ischaemia/reperfusion on α-adrenoceptor-mediated vasoconstriction

Ischaemia and reperfusion did not affect the constrictor responses to NA or MTX whereas the responses to B-HT 920 were significantly but only slightly greater in ischaemic hearts in comparison to control hearts (Figure 5, $P<0.05$).

Discussion

This study demonstrates that α_2-adrenoceptor-mediated vasoconstriction is modulated by endogenously released nitric oxide in the rat coronary vasculature. Although ischaemia/reperfusion caused a degree of endothelial dysfunction, indicated by the impaired responses to muscarinic receptor agonists, there was little or no effect on α-adrenoceptor-mediated vasoconstriction.

Acetylcholine, carbachol, bradykinin and histamine were all found to cause coronary vasodilatation which was sensitive to inhibition by haemoglobin confirming that each of these agonists stimulated the release of NO. Surprisingly however, ischaemia and reperfusion impaired responses to the muscarinic receptor agonists, acetylcholine and carbachol but did not affect responses to bradykinin or histamine. This suggests that, rather than causing general endothelial dysfunction, ischaemia in the rat isolated heart may have selectivity impaired muscarinic mechanisms. Ischaemia in rat isolated hearts has previously been reported to impair endothelium-dependent cholinergic (Tsao & Lefer, 1990; Yaghi & Watts, 1993) and 5-hydroxytryptaminergic dilatation (Saldanha & Hearse, 1989; Mankad *et al.*, 1992) but there are no previous reports of the effect of ischaemia on bradykinin or histamine in this preparation. However bradykinin-induced relaxation of isolated epicardial coronary arteries has been reported to be impaired by prior ischaemia and reperfusion *in vivo* (Headrick *et al.*, 1990; Piana *et al.*, 1994) whereas responses to histamine have not been examined in similar experiments. There have been reports indicating that dilators not acting on cholinoceptors may be resistant to attenuation by ischaemia. For example, Pearson *et al.* (1992) reported that dog coronary arterial rings previously exposed to ischaemia and reperfusion *in vivo* exhibited an impaired relaxation response to ACh but not to the calcium ionophore A23187 and Kim *et al.* (1992) found in the same preparation that 30 min of ischaemia abolished responses to ACh whereas bradykinin continued to cause some relaxation. In the present study extending the period of ischaemia from 30 to 60 min did not change the reactivity to ACh or bradykinin indicating that prolonging the period of ischaemia did not increase the impairment of vasodilator responses. As the mechanism of ischaemia-induced impairment of vasodilator responses to any of these endothelium-dependent agonists is uncertain, it remains a matter of speculation as to why responses to some agonists may be more affected than others.

Clearly in the current experiments, where the hearts are isolated and perfused with physiological saline, the process of ischaemia and reperfusion differs from that occurring *in vivo*. Blood-borne products such as leukocytes are well established as being involved in ischaemia-induced myocardial dysfunction and may also be involved in vascular injury (Sobey & Woodman, 1993). However, our observations that muscarinic agonists are more sensitive to endothelial dysfunction are consistent with the finding that atheroma-like lesions have a greater effect on the relaxant responses to ACh than other endothelium-dependent dilators (Arthur & Dusting, 1992). Furthermore, Seccombe *et al.* (1994) have reported that generation of oxygen radicals *in vitro* impairs endothelium-dependent relaxation to ACh and adenosine diphosphate but not

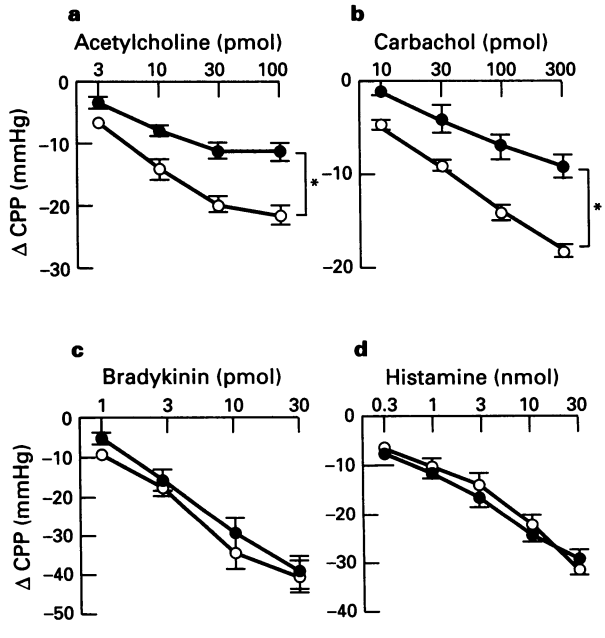

Figure 4 The effect of 30 min low flow-ischaemia and 15 min reperfusion on the responses to endothelium-dependent vasodilators in rat coronary resistance arteries preconstricted with U46619. The vasodilator responses to acetylcholine (a, control $n=9$, ischaemia $n=6$) and carbachol (b, control $n=4$, ischaemia $n=4$) but not to bradykinin (c, control $n=5$, ischaemia $n=6$) or histamine (d, control $n=5$, ischaemia $n=6$) were significantly impaired by 30 min low flow ischaemia-reperfusion (●) as compared to responses in control hearts (○). *$P<0.05$ compared to time control group, ANOVA.

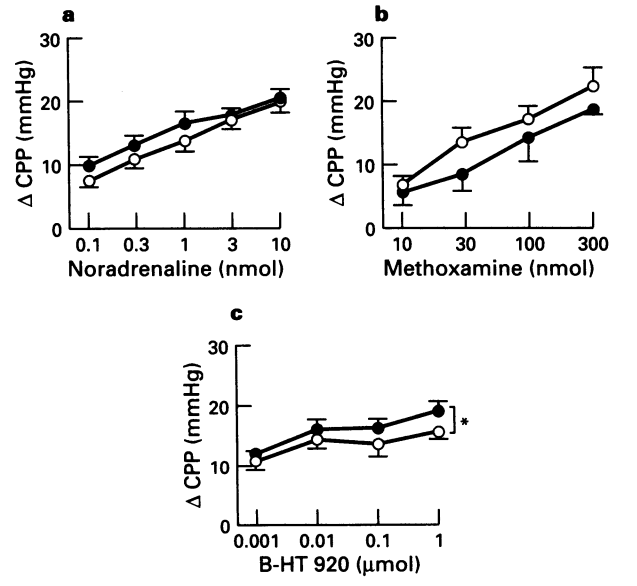

Figure 5 The effect of ischaemia on the coronary vascular responses to α-adrenoceptor agonists in rat isolated hearts perfused with Krebs solution containing propranolol (1 μM). After 30 min of low flow-ischaemia followed by 15 min of reperfusion (●), the constrictor responses to B-HT 920 (c) were significantly potentiated whilst the responses to noradrenaline (a) and methoxamine (b) were comparable to responses observed in time control hearts (○); 5 rats were used in each experiment. *$P<0.05$ compared to time control group, ANOVA.

to bradykinin or the calcium ionophore A23187 supporting the possibility of graded sensitivity to factors affecting endothelial function such as oxygen radicals. Perhaps in future studies the use of different endothelium-dependent dilators may be able to indicate the degree of endothelial dysfunction.

Inhibition of the actions of nitric oxide with haemoglobin enhanced the constrictor responses to noradrenaline and B-HT 920 but not to methoxamine suggesting that in the isolated hearts, α_2-adrenoceptor-mediated constriction is more sensitive to modulation by NO than is constriction in response to the stimulation of α_1-adrenoceptors. Although we have reported that adrenergic constriction of dog coronary resistance vessels *in vivo* is unaffected by inhibition of NO synthesis (Woodman & Pannangpetch, 1994) it is possible that in the intact circulation other endogenous dilators are able to compensate for the absence of NO. There have been relatively few studies examining the interaction of α-adrenoceptor subtypes and NO in resistance blood vessels. Most experiments have been performed in large arteries (Godfraind *et al.*, 1985; McGrath *et al.*, 1990; Kaneko & Sunano, 1993; Thompson & Weiner, 1993) generally demonstrating that NO inhibits α_2-adrenoceptor-mediated constriction. In the microvasculature of the rat cremaster muscle both α_1- and α_2-adrenoceptor-mediated responses were modulated by NO but α_2-adrenoceptor-mediated constriction was more sensitive than α_1-adrenoceptor-mediated constriction to that modulation (Ohyanagi *et al.*, 1992). In addition Jones *et al.* (1993), using intravital microscopy to measure the diameter of epicardial coronary microvessels, reported that selective stimulation of α_1- or α_2-adrenoceptors caused constriction only in the presence of NO synthesis inhibition, suggesting that in that preparation NO modulated constriction mediated by both receptor subtypes. The selective enhancement of α_2-adrenoceptor-mediated constriction by haemoglobin in the present experiments is consistent with the proposal, arising from experiments performed using conductance arteries, that α_2-adrenoceptor stimulation causes the release of endothelium-derived NO to modulate the direct vasoconstriction (Cocks & Angus, 1983; Angus *et al.*, 1986).

Although there is considerable evidence from studies performed both *in vivo* and *in vitro* that endothelium-derived nitric oxide opposes adrenoceptor-mediated constriction (Cocks & Angus, 1983; Godfraind *et al.*, 1985; McGrath *et al.*, 1990; Du *et al.*, 1992) there is little evidence that ischaemia-induced endothelial dysfunction leads to any enhancement of adrenoceptor-mediated constriction (Woodman, 1995). In the present study, where it was confirmed that the constrictor responses to both noradrenaline and B-HT 920 were modulated by NO, only the responses to B-HT 920 were slightly enhanced by ischaemia. This small effect may be consistent with a level of ischaemia-induced endothelial dysfunction only sufficient to affect weaker stimulators of NO release; however, the slight enhancement of constriction observed in response to the α_2-adrenoceptor agonist is unlikely to represent an effect of any functional significance. Two previous *in vivo* studies also failed to demonstrate any effect of ischaemia and reperfusion on adrenoceptor-mediated constriction. Gutterman *et al.* (1992) reported that a brief (15 min) period of occlusion of the left anterior descending artery in dogs did not affect the constrictor response to noradrenaline. However endothelial function was not assessed in that study and the period of occlusion may have been too brief to cause any change in vasodilator reactivity. Recently Ehring *et al.* (1995) assessed the effect of myocardial ischaemia and reperfusion in the dog on both endothelium-dependent dilatation and adrenoceptor-mediated coronary constriction. It was reported that, after 1 h occlusion and 3 h reperfusion of the circumflex coronary artery, there was significant impairment of ACh-induced dilatation but no change in constrictor responses to either α_1- or α_2-adrenoceptor agonists when assessed *in vivo*. As discussed earlier it appears that responses to ACh are relatively more readily impaired by ischaemia and the use of other agents such as bradykinin might be useful to assess the level of endothelial dysfunction. However, a further complicating factor is that ischaemia/reperfusion significantly impairs dilator responses to both endothelium-dependent and endothelium-independent agonists *in vivo* (Sobey & Woodman, 1993), perhaps as a result of capillary plugging. This makes it difficult to assess the level of endothelial function after ischaemia in the intact circulation. Furthermore in this intact preparation, other endogenous vasodilators may be able to compensate for any ischaemia-induced loss of NO activity as we have previously reported that inhibition of NO synthesis does not affect adrenoceptor-mediated constriction in the dog coronary resistance vasculature (Woodman & Pannangpetch, 1994).

In conclusion, in the rat isolated perfused heart, responses to endothelium-dependent dilators are not uniformly impaired by low-flow ischaemia. Muscarinic receptor agonists are particularly sensitive to the effects of ischaemia whereas responses to bradykinin and histamine were unaffected. The use of a variety of agonists may be useful in the assessment of the level of endothelial dysfunction in different models of ischaemia and care should be taken before assuming that loss of muscarinic dilatation implies general endothelial dysfunction. In the rat coronary resistance vasculature, α_2-adrenoceptor-mediated constriction is modulated by NO. Although ischaemia caused a slight enhancement of that constriction it is doubtful whether the change in reactivity is sufficient to be of functional significance. Thus, despite causing some endothelial dysfunction ischaemia does not affect adrenoceptor-mediated constriction in the rat coronary vasculature.

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