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Enhancement of noradrenergic constriction of large coronary arteries by inhibition of nitric oxide synthesis in anaesthetized dogs

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1 Coronary vascular responses to bilateral carotid occlusion (BCO) and the intravenous infusion of tyramine (Tyr, 20 $\mu g\,kg^{-1}\,min^{-1}$) and noradrenaline (NA, 0.5 $\mu g\,kg^{-1}\,min^{-1}$) were examined after bilateral vagotomy and antagonism of β-adrenoceptors. BCO, Tyr and NA decreased large coronary artery diameter and increased mean coronary resistance and systemic arterial pressure without affecting heart rate.

2 Inhibition of nitric oxide (NO) synthase with N^G-nitro-L-arginine (L-NNA, 5 and 15 $mg\,kg^{-1}$) significantly increased mean arterial pressure and decreased heart rate and large coronary artery diameter. Mean coronary resistance was unaffected by either dose of L-NNA. L-NNA significantly reduced depressor and coronary vasodilator responses to the endothelium-dependent vasodilator acetylcholine (ACh, 10 $\mu g\,kg^{-1}$, i.v.). Systemic and coronary vasodilator responses to sodium nitroprusside (SNP, 5 $\mu g\,kg^{-1}$) were unaffected by L-NNA with the exception that the dilatation of the large coronary artery was significantly enhanced by the higher dose.

3 L-NNA significantly enhanced constriction of the large coronary arteries caused by BCO, Tyr and NA but did not affect the increases in mean coronary resistance or systemic arterial pressure.

4 Inhibition of NO synthesis enhances adrenergic constriction of large coronary arteries caused by both neuronally released and exogenous noradrenaline. In contrast, L-NNA did not affect adrenergic constriction of coronary or systemic resistance vessels. Endothelium-derived NO may play an important role in the modulation of noradrenergic vasoconstriction in coronary conductance arteries.

Keywords: Bilateral carotid occlusion; large coronary arteries; N^G-nitro-L-arginine; noradrenaline; tyramine; vasoconstriction

Introduction

There is considerable evidence that the endothelium can modulate vasoconstriction produced by a variety of stimuli. In isolated blood vessels, removal of the endothelium enhances contraction to exogenous noradrenaline (Cocks & Angus, 1983; Miller & Vanhoutte, 1985; Martin *et al.*, 1986; McGrath *et al.*, 1990) and to electrical field stimulation (Tesfamariam *et al.*, 1987; Hynes *et al.*, 1988; Gonzalez *et al.*, 1990; Urabe *et al.*, 1991). The ability of the endothelium to inhibit vasoconstriction appears to be related to the release of the potent vasodilator, nitric oxide (NO). The endothelium synthesizes NO, or a closely related molecule (Palmer *et al.*, 1987), from the terminal guanidino nitrogen atom(s) of L-arginine (Palmer *et al.*, 1988). Analogues of L-arginine such as N^G-nitro-L-arginine (L-NNA) and N -monomethyl-L-arginine (L-NMMA) impair NO synthesis by inhibition of the catalysing enzyme NO synthase (Rees *et al.*, 1989). We (Du *et al.*, 1992; Pannangpetch & Woodman, 1992) and others (Tresize *et al.*, 1992; Vo *et al.*, 1992) have shown that inhibition of NO synthesis enhances adrenergic constriction in both isolated arteries and in intact vascular beds. These studies have led to suggestions that in various cardiovascular disease states, such as atherosclerosis and myocardial ischaemia, endothelial damage could contribute to enhanced vasoconstriction and in the coronary circulation perhaps lead to vasospasm.

The aim of this study was to examine the influence that inhibition of NO synthesis has on coronary vasoconstrictor responses to exogenous and neuronally released NA. Two different methods were used to stimulate the neuronal release of NA. Firstly, bilateral carotid occlusion was applied to stimulate the baroreceptor reflex to low carotid sinus pressure and secondly, tyramine (Tyr), an indirectly acting sympathomimetic, was infused to displace NA from intraneuronal stores. Responses to these stimuli were compared to the effects of the intravenous infusion of noradrenaline. All experiments were performed in anaesthetized dogs after bilateral vagotomy and antagonism of β-adrenoceptors to prevent any changes in cardiac contractility which would have confounded the effects on coronary vascular tone. Large coronary artery diameter and coronary blood flow were measured in order to allow comparison of effects in conduit and resistance arteries.

Methods

Mongrel and greyhound dogs of either sex weighing 13–31 kg were anaesthetized with thiopentone (25–30 $mg\,kg^{-1}$, i.v.) followed by α-chloralose (70 $mg\,kg^{-1}$, i.v., supplemented as necessary). The dogs were ventilated with room air plus additional oxygen as necessary to maintain arterial blood P_{O_2}, P_{CO_2} and pH within the normal range (P_{O_2}: 90–110 mmHg; P_{CO_2} 30–35 mmHg; pH: 7.25–7.35). Pressure in the thoracic aorta was measured by passing a catheter from the right femoral artery and connecting the catheter to a pressure transducer (Spectromed). The ECG was recorded from standard limb leads and was used to trigger a cardiotachometer to provide a continuous measurement of heart rate.

A thoracotomy was performed at the left fifth intercostal space and the pericardium was opened. A pair of 7 MHz piezoelectric transducers were sutured to opposing surfaces of the left circumflex artery. The external diameter of the artery was measured with an ultrasonic transit-time dimension gauge (Triton). Blood flow through the artery was measured with a cuff type electromagnetic flow probe (Skalar) placed on the artery. Care was taken during the placement of the transducers to limit dissection and damage to any visible

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nerves. Arterial pressure, coronary blood flow, coronary artery diameter and heart rate were recorded on a Grass (Model 7D) polygraph. Mean coronary resistance was calculated as the quotient of mean arterial pressure and mean coronary blood flow.

Experimental protocol

All experiments were performed after bilateral cervical vagotomy and antagonism of β-adrenoceptors with propranolol (1 mg kg^{-1}, i.v. plus 0.5 mg kg^{-1} h^{-1}) to prevent any reflex or β-adrenoceptor-mediated changes in heart rate. Responses to three stimuli, applied in random order, were compared: clamping of both common carotid arteries for 2 min to induce the baroreceptor reflex to low carotid sinus pressure and infusion of NA (0.5 μg kg^{-1} min^{-1}) or Tyr (20 μg kg^{-1} min^{-1}) intravenously for 5 min. We have previously found that these three stimuli produced similar pressor responses in anaesthetized dogs (Woodman, 1987) and that observation was confirmed in this study. Responses to these stimuli were also examined after inhibition of NO synthesis with two doses of N^G-nitro-L-arginine (L-NNA, 5 and 15 mg kg^{-1}, i.v.). L-NNA was infused intravenously over 15 to 20 min and a further 15 min was allowed after completing the infusion to allow arterial pressure, coronary artery diameter and coronary blood flow to stabilize before examination of further responses. Selective inhibition of NO synthesis was confirmed by examining responses to bolus injections of the endothelium-dependent vasodilator, ACh (10 μg kg^{-1}, i.v.) and the endothelium-independent dilator, sodium nitroprusside (SNP, 5 μg kg^{-1}, i.v.) at the end of each experiment. In some experiments the effect of ACh was also tested 15 min after L-NNA infusion, and before the constrictor stimuli, and the responses compared to those observed at the end of the experiment to ensure that L-NNA had a stable effect over the duration of these experiments. To test whether

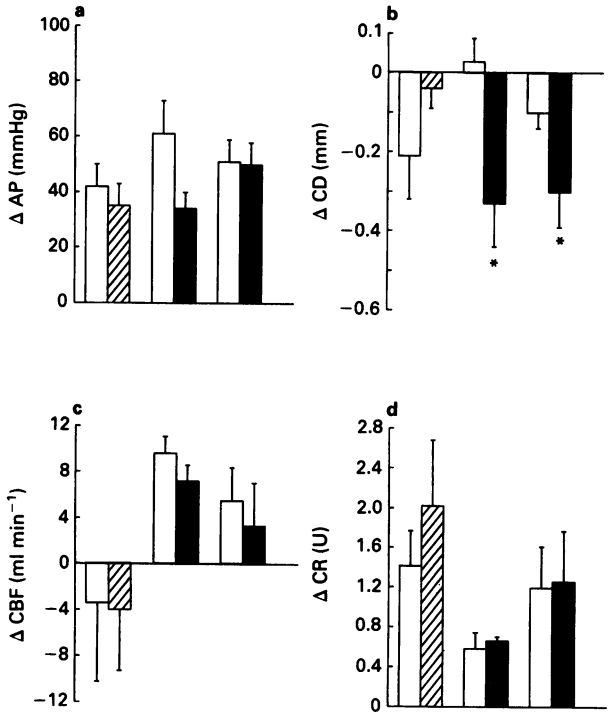

Figure 2 Effect of the 5 min intravenous infusion of tyramine (20 mg kg^{-1} min^{-1}) on (a) mean arterial pressure (ΔAP), (b) large coronary artery diameter (ΔCD), (c) mean coronary blood flow (ΔCBF) and (d) mean coronary resistance (ΔCR) before (open columns) and after infusion of saline (widely hatched columns, $n = 8$), N^G-nitro-L-arginine 5 mg kg^{-1} (closely hatched columns, $n = 6$) or 15 mg kg^{-1} (solid columns, $n = 11$). The values shown are the mean with s.e.mean. $^*P < 0.05$, Wilcoxon's matched pairs test.

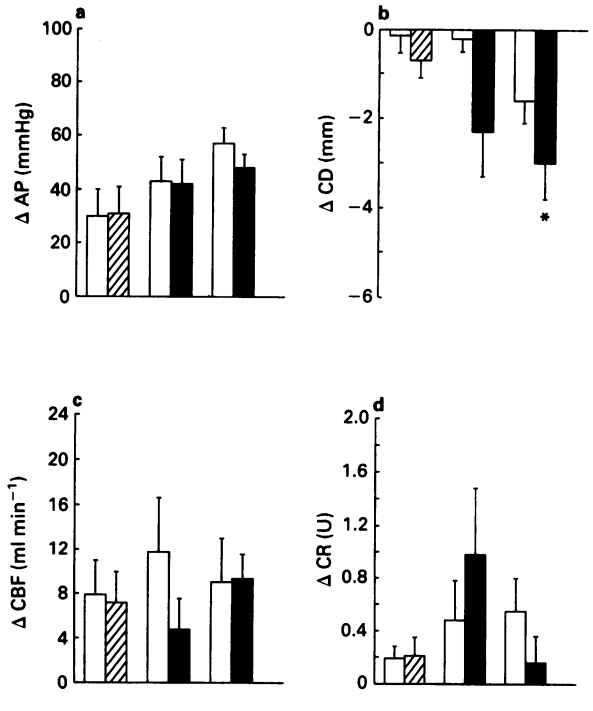

Figure 1 Effect of bilateral carotid occlusion for 2 min on (a) mean arterial pressure (ΔAP), (b) large coronary artery diameter (ΔCD), (c) mean coronary blood flow (ΔCBF) and mean coronary resistance (ΔCR) before (open columns) and after infusion of saline (widely hatched columns, $n = 7$), N^G-nitro-L-arginine 5 mg kg^{-1} (closely hatched columns, $n = 8$) or 15 mg kg^{-1} (solid columns, $n = 13$). The values shown are the mean with s.e.mean. $^*P < 0.05$, Wilcoxon's matched pairs test.

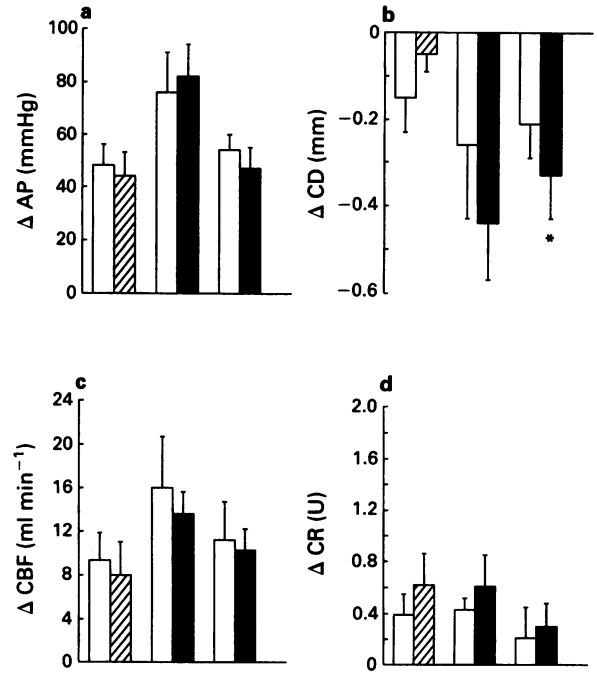

Figure 3 Effect of the 5 min intravenous infusion of noradrenaline (0.5 μg kg^{-1} min^{-1}) on (a) mean arterial pressure (ΔAP), (b) large coronary artery diameter (ΔCD), (c) mean coronary blood flow (ΔCBF) and (d) mean coronary resistance (ΔCR) before (open columns) and after infusion of saline (widely hatched columns, $n = 8$), N^G-nitro-L-arginine 5 mg kg^{-1} (closely hatched columns, $n = 6$) or 15 mg kg^{-1} (solid columns, $n = 11$). The values shown are the mean with s.e.mean. $^*P < 0.05$, Wilcoxon's matched pairs test.

there was any change in vascular reactivity over time in a separate group of dogs responses to BCO, Tyr and NA were tested before and after the infusion of saline.

Drugs

Drugs used were: acetylcholine perchlorate (BDH); (−)-noradrenaline bitartrate (Sigma); N^G-nitro-L-arginine (Sigma); (±)-propranolol hydrochloride (Sigma); sodium nitroprusside (Sigma); tyramine hydrochloride (Sigma). All drugs were dissolved in saline with the exception of L-NNA and propranolol which were dissolved in distilled water. Further dilutions of all drugs were made in saline.

Statistics

As it was found that there was some variability in the coronary vascular responses to BCO and Tyr between dogs, the experiments were designed so that each dog acted as its own control. Results are expressed as the mean ± s.e.mean of n experiments and only one experiment was performed in each dog. Baseline values of the haemodynamic variables and responses to vasodilator and vasoconstrictor stimuli were compared by Wilcoxon's matched pairs test.

Results

All of the data were collected after bilateral vagotomy and antagonism of β-adrenoceptors with propranolol.

Response to BCO, tyramine and noradrenaline

Control responses to BCO for 2 min and the intravenous infusion of Tyr (20 μg kg^{-1} min^{-1}) or NA (0.5 μg kg^{-1} min^{-1}) for 5 min are shown in Figures 1–3. Each of these stimuli increased mean arterial pressure and mean coronary resistance and decreased large coronary artery diameter without affecting heart rate (data not shown).

Effect of the intravenous infusion of N^G-nitro-L-arginine on baseline parameters

L-NNA (5 or 15 mg kg^{-1}) was infused intravenously over 15–20 min. The preparation was then allowed to stabilize for 15 min and the effects of L-NNA on the baseline haemodynamic values were measured (Tables 1 and 2). Both doses of L-NNA significantly increased mean arterial pressure and decreased large coronary artery diameter and heart rate. L-NNA had no significant effect on coronary blood flow. In

Table 1 The effect of intravenous administration of N^G-nitro-L-arginine (5 mg kg^{-1}) on baseline haemodynamic variables in each experiment

	Baseline haemodynamic variables									
	Mean arterial pressure (mmHg)		*Mean coronary blood flow* (ml min^{-1})		*Mean coronary resistance* (mmHg min ml^{-1})		*Mean coronary artery diameter* (mm)		*Heart rate* (beats min^{-1})	
Experiment number	*Control*	*L-NNA*	*Control*	*L-NNA*	*Control*	*L-NNA*	*Control*	*L-NNA*	*Control*	*L-NNA*
2391	100	110	54	50	1.85	2.2	3.93	3.89	118	114
2491	93	103	20	22	4.65	4.68	3.2	3.16	118	118
2591	108	140	20	28	5.4	5.0	–	–	144	140
192	105	100	–	–	–	–	2.88	2.79	148	140
292	93	105	48	52	1.94	2.02	6.43	6.36	126	120
392	120	125	42	40	2.86	3.13	3.32	3.2	120	116
293	115	115	44	44	2.61	2.61	3.78	3.7	132	130
593	115	128	30	32	3.83	4.0	4.92	4.86	126	128
993	125	125	48	40	2.6	3.13	3.5	3.52	112	108
Mean	108	117*	38	38	3.2	3.3	4.0	3.94*	127	124*
s.e.mean	4	4	5	4	0.4	0.4	0.41	0.41	4	4

*$P < 0.05$, Wilcoxon's matched pairs test.

Table 2 The effect of intravenous administration of N^G-nitro-L-arginine (15 mg kg^{-1}) on baseline haemodynamic variables in each experiment

	Baseline haemodynamic variables									
	Mean arterial pressure (mmHg)		*Mean coronary blood flow* (ml min^{-1})		*Mean coronary resistance* (mmHg min ml^{-1})		*Mean coronary artery diameter* (mm)		*Heart rate* (beats min^{-1})	
Experiment number	*Control*	*L-NNA*	*Control*	*L-NNA*	*Control*	*L-NNA*	*Control*	*L-NNA*	*Control*	*L-NNA*
1091	120	135	15	16	8.0	8.44	–	–	128	124
1191	103	120	34	48	3.03	2.5	3.6	3.52	160	152
1291	110	133	48	52	2.3	2.56	3.34	3.23	124	116
1391	93	105	28	32	3.32	3.28	2.48	2.37	136	128
1491	113	120	18	18	6.28	6.67	3.03	2.84	124	112
1591	95	113	62	64	1.53	1.77	4.24	4.2	140	132
1691	135	150	24	20	5.62	7.5	2.72	2.6	128	120
2091	93	115	32	40	2.91	2.88	3.53	3.46	110	106
2191	140	147	80	66	1.75	2.23	3.7	3.28	156	152
2291	125	145	80	82	1.56	1.77	4.39	4.3	128	128
293	115	130	44	36	2.61	3.61	3.8	3.52	132	124
593	115	160	26	44	5.96	3.64	4.92	4.61	126	126
993	125	125	48	36	2.6	3.47	3.5	3.46	112	104
2693	165	180	72	72	2.29	2.5	4.15	4.08	144	144
Mean	118	134*	44	45	3.5	3.8	3.7	3.5*	132	126*
s.e.mean	5	5	6	5	0.5	0.6	0.2	0.2	4	4

*$P < 0.05$, Wilcoxon's matched pairs test.

most experiments mean coronary resistance increased; however, in 2 dogs (2591, Table 1; 593, Table 2) coronary resistance decreased. In each of these experiments the pressor response was larger than that seen in any other members of the group and may have initiated reflex changes in coronary resistance not seen in the other experiments.

Effect of N^G-nitro-L-arginine on haemodynamic responses to BCO, tyramine and noradrenaline

Responses to BCO, tyramine and NA were examined before and after inhibition of NO synthesis with the 2 doses of L-NNA, 5 and 15 mg kg^{-1}, i.v. Increases in mean arterial pressure and mean coronary resistance caused by any of these stimuli were unaffected by either dose of L-NNA (Figures 1–3). In contrast, the Tyr-induced constriction of the large coronary artery was significantly enhanced by the lower dose of L-NNA and the large artery constrictor responses to each of the stimuli were enhanced by the higher dose of L-NNA (Figures 1–3). Responses to BCO, Tyr and NA were unaffected by treatment with saline (Figures 1–3).

Effect of N^G-nitro-L-arginine on haemodynamic responses to acetylcholine and sodium nitroprusside

Responses to bolus injections of the endothelium-dependent dilator, ACh (10 μg kg^{-1}, i.v.) and the endothelium-independent dilator, SNP (5 μg kg^{-1}, i.v.) were examined before and after inhibition of NO synthesis with the 2 doses of L-NNA, 5 and 15 mg kg^{-1}, i.v. When measured at the end of the experiment, ACh-induced systemic and coronary dilatation were significantly attenuated by both doses of L-NNA (Figure 4). To test whether L-NNA caused a stable level of inhibition, responses to ACh were also tested 15 min after L-NNA infusion in some dogs (L-NNA 5 mg kg^{-1}, $n = 5$; L-NNA 15 mg kg^{-1}, $n = 9$) and again at the end of the experiment. In those experiments there were no significant differences in the responses to the first and second administration of ACh after NO synthesis inhibition (data not shown). In contrast, L-NNA did not affect responses to SNP with the exception that there was a significant enhancement of the large artery dilatation in the presence of the higher dose of L-NNA (Figure 5).

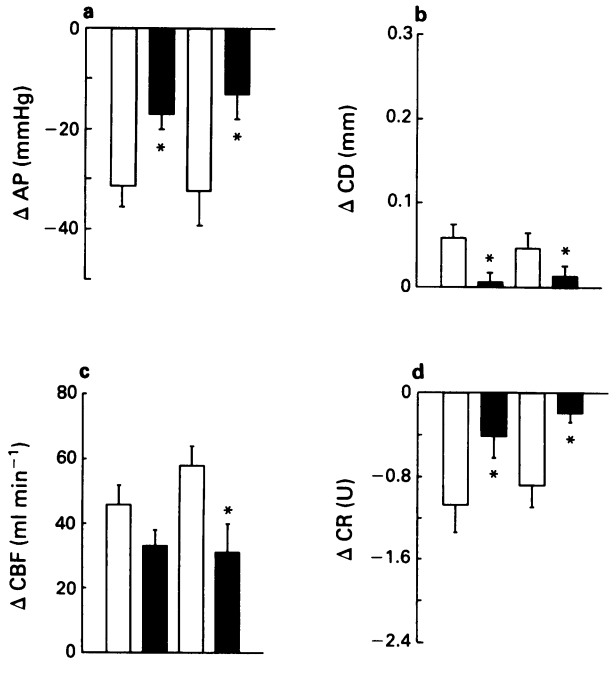

Figure 4 Effect of the bolus intravenous injection of acetylcholine 10 μg kg^{-1} (ACh) before (open columns) and after infusion of N^G-nitro-L-arginine 5 mg kg^{-1} (stippled columns, $n = 9$) or 15 mg kg^{-1} (solid columns, $n = 14$). The values shown are the mean with s.e.mean. *$P < 0.05$, Wilcoxon's matched pairs test.

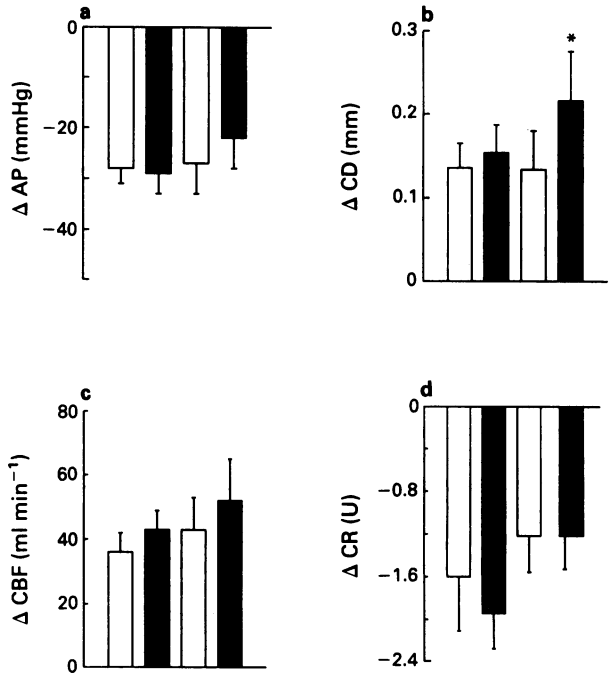

Figure 5 Effect of the bolus intravenous injection of sodium nitroprusside (SNP) before (open columns) and after infusion of N^G-nitro-L-arginine 5 mg kg^{-1} (stippled columns, $n = 9$) or 15 mg kg^{-1} (solid columns, $n = 14$). The values shown are the mean with s.e.mean. *$P < 0.05$, Wilcoxon's matched pairs test.

Discussion

This study demonstrated that in the anaesthetized dog, inhibition of NO synthesis with L-NNA enhanced adrenergic constriction of the large coronary arteries without affecting responses in the coronary resistance vessels or in the systemic circulation. L-NNA caused similar enhancement of responses to NA released from sympathetic nerves due to stimulation of the baroreceptor reflex by bilateral carotid occlusion or due to administration of the indirectly acting sympathomimetic tyramine. Large artery constrictor responses to the infusion of exogenous NA were also enhanced by L-NNA.

It is well established that removal of the endothelium enhances noradrenaline-induced contraction in isolated conductance arteries, including coronary arteries, from dogs and other species (Cocks & Angus, 1983; Miller & Vanhoutte, 1985; Berkenboom *et al.*, 1991). The inhibitory effect of the endothelium appears to be mediated by nitric oxide as inhibitors of NO synthesis also enhance noradrenaline-induced contraction of dog isolated coronary arteries (Berkenboom *et al.*, 1991).

The increased vasoconstriction in response to BCO in the presence of L-NNA could involve a prejunctional action of NO as well as an effect on vascular smooth muscle reactivity. Tesfamariam *et al.* (1987) found that in rabbit isolated carotid arteries the stimulation-induced release of NA was reduced by the presence of the endothelium. In contrast other reports suggested that endothelium-derived NO does not influence NA release (Bucher *et al.*, 1992; Vo *et al.*, 1992). In the present study, neuronal release of NA was stimulated by two means, activation of the baroreceptor reflex to cause a release that would be subject to prejunctional modulation and infusion of Tyr that displaces NA from sympathetic nerves which is therefore not subject to prejunctional control.

Tyr-induced constriction was enhanced by L-NNA in a similar manner to constriction to BCO suggesting that if there is any influence of NO over NA release it is only a minor contributor to the enhanced responses.

It is not known whether the endothelium-mediated inhibition of adrenergic constriction is due to a basal or a stimulated release of NO. Observations that inhibition of NO synthesis increases coronary vascular tone (Benyo *et al.*, 1991; Humphries *et al.*, 1991; Woodman & Dusting, 1991; Sonntag *et al.*, 1992) indicate that there is a significant basal release of NO that could non-selectively oppose constrictor stimuli. In the present study, L-NNA significantly constricted the large coronary artery without significantly affecting coronary blood flow or coronary resistance, a finding consistent with our previous study with L-NNA (Woodman & Dusting, 1990) and the observations of Chu *et al.* (1990) who examined the effect of L-NMMA in conscious dogs. In contrast Richard *et al.* (1991) reported that L-NNA and L-NMMA caused significant increases in coronary resistance in anaesthetized dogs. However, it is interesting that in the same study myocardial tissue perfusion, measured with radioactive microspheres, was unaffected by the inhibitors of NO synthesis. Given that the inhibition of NO synthesis also causes systemic vasoconstriction and hypertension which will initiate metabolic and reflex modulation of coronary vascular tone, it is difficult to assess the level to which the basal release of NO modulates coronary vascular tone. In the present study L-NNA increased coronary resistance in the majority of experiments; however, in 2 experiments where the pressor response to L-NNA was large, coronary resistance surprisingly decreased. Consequently in the whole group there was no statistically significant change in coronary resistance in response to L-NNA. In addition NA has been reported to stimulate the release of EDRF (NO) through the stimulation of α_2-adrenoceptors on endothelial cells (Angus *et al.*, 1986). It is not possible to distinguish between the potential role of basal and stimulated release of NO in the modulation of noradrenergic coronary vasoconstriction in these experiments.

A surprising finding of this study was that although L-NNA enhanced constriction of the large coronary arteries in response to neuronally-released or exogenous NA, there was no enhancement of the constriction of coronary resistance vessels nor of the systemic pressor response. NO is an important regulator of resistance vessel tone and inhibitors of NO synthesis have been reported to enhance adrenergic constriction of resistance vessels. We have found that in the rat autoperfused mesentery, L-NNA enhances responses to both NA and stimulation of sympathetic nerves (Pannangpetch & Woodman, 1992). We have also observed that in conscious rabbits in the presence of autonomic blockade, L-NNA enhances hindquarters constriction caused by the infusion of NA and Tyr (Du *et al.*, 1992) although there is no enhancement of the systemic pressor response. Together with the present study this suggests that the extent to which NO influences adrenergic constriction of resistance vessels may vary in different vascular beds. The absence of an effect of L-NNA was not due to an inability to inhibit NO synthesis in resistance arteries as the ACh-induced increase in coronary blood flow and reduction in arterial pressure were both significantly attenuated.

In conclusion, impaired synthesis of NO enhanced sympathetic nervous constriction of the large coronary artery but not of the resistance vessels. In atherosclerotic arteries or after ischaemia and reperfusion, where NO-mediated dilatation is impaired (Sobey & Woodman, 1993), sympathetic vasoconstriction may be enhanced, resulting in impaired blood flow to the heart.

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