N-nitro L-arginine causes coronary vasoconstriction and inhibits endothelium-dependent vasodilatation in anaesthetized greyhounds

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1 The effect of N-nitro-L-arginine (L-NNA), an inhibitor of nitric oxide biosynthesis, on large coronary artery diameter and coronary blood flow was examined in anaesthetized greyhounds. The effects of L-NNA on the coronary vascular responses to acetylcholine (ACh), glyceryl trinitrate (GTN) and 5-hydroxytryptamine (5-HT) were also assessed.

2 L-NNA (5 mg kg⁻¹), infused into the left circumflex coronary artery, increased systemic mean arterial pressure and decreased the external diameter of the artery. Infusion of L-NNA decreased coronary blood flow in 5 of the 7 dogs tested and increased mean coronary resistance but neither of these effects was statistically significant. There was no change in heart rate.

3 Intra-arterial injection of both ACh (0.01–0.05 µg kg⁻¹) and GTN (0.1–0.5 µg kg⁻¹) increased large coronary artery diameter and coronary blood flow. Coronary vascular responses to the endothelium-dependent vasodilator ACh were significantly reduced by L-NNA, whereas the responses to the endothelium-independent vasodilator GTN were not significantly affected.

4 5-HT (0.1 µg kg⁻¹, injected into the left circumflex coronary artery) decreased coronary artery diameter but increased coronary blood flow. After the administration of L-NNA the 5-HT-induced dilatation of the coronary resistance vessels was significantly attenuated whereas the constriction of the circumflex coronary artery was increased in 3 out of 3 dogs in which diameter could be measured, although the latter effect was not statistically significant.

5 These data indicate that L-NNA causes coronary and systemic vasoconstriction and selectively inhibits endothelium-dependent vasodilatation in the coronary circulation of the anaesthetized greyhound. Therefore endothelium-derived NO has an important role in the regulation of coronary vascular tone in the large arteries and the resistance vessels.

Keywords: Acetylcholine; coronary artery; glyceryl trinitrate, 5-hydroxytryptamine; vasoconstriction; vasodilatation; endothelium; N-nitro-L-arginine

Introduction

Vascular endothelial cells release a factor, closely related to nitric oxide (NO) that accounts for the vasodilator action of acetylcholine and other endogenous substances (Furchgott, 1984; Moncada *et al.*, 1989; Dusting *et al.*, 1989). It has recently been reported that a loss of endothelium-dependent coronary vasodilatation in man can be correlated with the presence of a number of risk factors for coronary artery disease (Vita *et al.*, 1990; Yasue *et al.*, 1990). Whilst this provides circumstantial evidence that endothelium-derived NO may play an important role in the regulation of the coronary circulation there have been few studies that have directly addressed this question. The finding that NO is synthesized in the endothelium from L-arginine (Palmer *et al.*, 1988) led to the discovery of L-arginine analogues which potently and specifically inhibit the synthesis of endothelium-derived NO both *in vitro* and *in vivo*. NO biosynthesis can be inhibited by N^G-monomethyl-L-arginine (L-NMMA, Palmer *et al.*, 1988) and N-nitro-L-arginine (L-NNA, Moore *et al.*, 1990; Dubbin *et al.*, 1990). Both of these agents increase arterial pressure in rats (Gardiner *et al.*, 1990; Zambetis *et al.*, 1990) and L-NMMA reduces forearm blood flow in human volunteers (Vallance *et al.*, 1989), constricts a variety of vascular beds in the conscious rat (Gardiner *et al.*, 1990) and causes coronary vasoconstriction in rabbit isolated hearts (Amezcuca *et al.*, 1989). These studies suggest that endothelium-derived NO has an important role in the regulation of vascular tone and arterial pressure. The aim of this study is to examine the effect of L-NNA on coronary vascular tone and on vascular responses to

acetylcholine (ACh), glyceryl trinitrate (GTN) and 5-hydroxytryptamine (5-HT) in the anaesthetized greyhound. Large coronary artery diameter and coronary blood flow were monitored in order to assess responses in both conduit and resistance vessels.

Methods

Greyhound dogs of either sex, weighing 25–38 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 PO_2 , PCO_2 and pH within the normal ranges (PO_2 : 85–110 mmHg; PCO_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 Druck pressure transducer. A continuous measurement of heart rate was obtained with a cardiachometer coupler triggered by the ECG.

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 coronary artery. The external diameter of the artery was measured with an ultrasonic transit-time dimension gauge (Triton Technology Inc.). Blood flow through the artery was measured with an electromagnetic flow probe (Statham) placed on the artery. A needle tipped catheter was placed in the artery proximal to the transducers for the administration of drugs. Care was taken during the placement of the transducers to limit dissection and damage of any visible nerves.

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Experimental protocol

ACh (0.01 – $0.05 \mu\text{g kg}^{-1}$), GTN (0.1 – $0.5 \mu\text{g kg}^{-1}$) and 5-HT ($0.1 \mu\text{g kg}^{-1}$) were injected as a bolus of less than 0.5 ml directly into the left circumflex coronary artery. After control responses to these agents had been obtained L-NNA (5 mg kg^{-1}) was infused into the left circumflex coronary artery over a period of 30 to 40 min and 15 min was allowed after completing the infusion to permit the arterial pressure, coronary artery diameter and coronary blood flow to stabilize before re-examining the responses to ACh, GTN and 5-HT.

Drugs

Drugs used were: acetylcholine perchlorate (BDH); glyceryl trinitrate (Boots); 5-hydroxytryptamine creatinine sulphate (Sigma); N-nitro-L-arginine (Sigma). ACh was dissolved in saline; 5-HT and L-NNA were dissolved in distilled water. Further dilutions of all drugs were made in saline.

Statistics

Baseline values of the haemodynamic variables and the responses to 5-HT measured before and after infusion of L-NNA were compared by a Wilcoxon signed rank test. Statistical comparison of the responses to ACh and GTN recorded before and after treatment with L-NNA was achieved by a two-way analysis of variance the two factors being the dose of the agonist and the treatment. This test treated all doses as a group to reveal whether a treatment had any significant effect on a response.

Results

Effect of the intra-coronary infusion of N-nitro-L-arginine on baseline parameters

L-NNA (5 mg kg^{-1}) was slowly infused into the left circumflex coronary artery of seven anaesthetized greyhounds over a period of 30 to 40 min and the effects on baseline haemodynamic values measured are shown in Table 1. L-NNA significantly increased mean arterial pressure and decreased large coronary artery diameter. In two dogs the constriction of the large coronary artery was such that the baseline diameter was less than the minimum measurable level of 2 mm and these dogs have been excluded from any data concerning large artery diameter. There was a tendency for coronary blood flow to decrease and mean coronary resistance to increase but neither of these changes was statistically significant. There was no change in heart rate.

Table 1 Baseline levels of the haemodynamic variables measured in 7 anaesthetized greyhounds before and after infusion of N-nitro-L-arginine (L-NNA) (5 mg kg^{-1}) into the left circumflex coronary artery

	Control	L-NNA
Mean arterial pressure (mmHg)	114 ± 7	$125 \pm 7^{**}$
Mean external coronary artery diameter (mm)	3.0 ± 0.4	$2.7 \pm 0.4^*$
Mean coronary blood flow (ml min^{-1})	43 ± 8	35 ± 6
Mean coronary resistance (mmHg min ml^{-1})	3.2 ± 0.6	5.1 ± 1.7
Heart rate (beats min^{-1})	130 ± 8	123 ± 8

Values are presented as the mean $\pm$ s.e.mean of data obtained from seven animals. * $P < 0.05$; ** $P < 0.01$ compared to control, Wilcoxon's signed rank test.

Figure 1 The effect of the intra-coronary injection of acetylcholine (ACh, $0.05 \mu\text{g kg}^{-1}$) on arterial pressure (AP), left circumflex coronary blood flow (CBF), mean coronary artery external diameter (CD) and heart rate (HR) in an anaesthetized greyhound. Responses are shown under control conditions and following the intra-coronary infusion of N-nitro-L-arginine (L-NNA, 5 mg kg^{-1}). The time bar represents 0.4 s at fast chart speed and 1 min at slow chart speed.

Effect of N-nitro-L-arginine on coronary dilator responses to acetylcholine and glyceryl trinitrate

Intra-coronary bolus injections of ACh (0.01 – $0.05 \mu\text{g kg}^{-1}$) and GTN (0.1 – $0.5 \mu\text{g kg}^{-1}$) increased large coronary artery

Figure 2 Increases in left circumflex coronary artery diameter (CD) and coronary blood flow (CBF) produced by the intra-coronary injection of acetylcholine are shown before (○) and after (●) treatment with N-nitro-L-arginine (L-NNA, 5 mg kg^{-1} , i.c.) in seven dogs. The values shown are the mean and vertical lines indicate the s.e.mean. Analysis of variance demonstrated that L-NNA treatment significantly ($P < 0.001$) attenuated the acetylcholine-induced dilatation of both the large coronary artery and the resistance vessels.

Figure 3 Increases in left circumflex coronary artery diameter (CD) and coronary blood flow (CBF) produced by the intra-coronary injection of glyceryl trinitrate are shown before (○) and after (●) treatment with N-nitro-L-arginine (L-NNA, 5 mg kg⁻¹ i.c.) in seven dogs. The values shown are the mean and vertical lines indicate the s.e.mean. L-NNA had no significant effect on the dilator responses to glyceryl trinitrate.

diameter and coronary blood flow (Figures 1–3). Neither ACh nor GTN had any effect on mean arterial pressure or heart rate. An experiment demonstrating the response to ACh before and after L-NNA is shown in Figure 1. After infusion of L-NNA the injection of ACh had no significant effect on large coronary artery diameter and the resistance vessel vasodilator responses were significantly less than the control responses

Figure 4 Decreases in left circumflex coronary artery diameter (CD) and increases in coronary blood flow (CBF) produced by the intra-coronary injection of 5-hydroxytryptamine (0.1 μg kg⁻¹) are shown before and after treatment with N-nitro-L-arginine (L-NNA, 5 mg kg⁻¹). The effect of 5-hydroxytryptamine on CBF is from 5 dogs but the effect on CD is from 3 dogs as in the 2 remaining dogs the artery constricted below the minimum level for detection of diameter. The values shown are the mean and the error bars indicate the s.e.mean. * $P < 0.05$, Wilcoxon's signed rank test.

(Figure 2). In contrast, GTN tended to cause greater increases in large coronary artery diameter in the presence of L-NNA although there was no significant difference between the two groups of responses (Figure 3). GTN-induced dilatation of the coronary resistance vessels was unaffected by the presence of L-NNA (Figure 3).

Effect of N-nitro-L-arginine on coronary responses to 5-hydroxytryptamine

In five dogs, intra-coronary artery injection of 5-HT (0.1 μg kg⁻¹) increased coronary blood flow but decreased large coronary artery diameter (Figure 4) without having any effect on mean arterial pressure or heart rate. Pretreatment with L-NNA significantly reduced the 5-HT-induced increase in coronary blood flow (Figure 4). The effect of 5-HT on large coronary artery diameter could only be measured in three of the dogs in the presence of L-NNA due to the reduction in baseline diameter. In each of these dogs the 5-HT-induced large artery constriction was potentiated in the presence of L-NNA but overall there was no statistically significant difference (Figure 4).

Discussion

L-NNA inhibits the biosynthesis of NO in endothelial cells (Moore *et al.*, 1990; Dubbin *et al.*, 1990) and increases arterial pressure in anaesthetized rats (Zambetis *et al.*, 1990). We have now shown that in the anaesthetized greyhound L-NNA increases arterial pressure and causes constriction of the left circumflex coronary artery despite the increase in distending pressure. Coronary blood flow declined and coronary resistance increased in five out of seven dogs treated with L-NNA, indicating constriction of resistance vessels. These effects are consistent with those of L-NMMA which increases arterial pressure in the rat (Whittle *et al.*, 1989; Gardiner *et al.*, 1990) and rabbit (Rees *et al.*, 1989), and constricts the carotid, renal, mesenteric and hindlimb vascular beds of conscious rats (Gardiner *et al.*, 1990). Our observations suggest that a basal release of NO from the arterial endothelium has an important dilator influence on the coronary vasculature.

In addition to its vasoconstrictor action, L-NNA inhibited the coronary vascular responses to the endothelium-dependent vasodilator ACh in both the large coronary artery and the coronary resistance vessels. This was not a non-specific effect resulting from the enhanced tone in those vessels after L-NNA treatment for the dilator responses to the endothelium-independent vasodilator GTN were unaffected by L-NNA. In fact GTN tended to cause greater dilatation of the large coronary arteries after treatment with L-NNA, perhaps due to the greater tone in that vessel and hence increased vasodilator reserve. The selective inhibitory action of L-NNA on endothelium dilatation further supports the hypothesis that the coronary vasoconstriction is due to inhibition of NO release from the endothelium.

L-NNA also attenuated increases in coronary blood flow induced by 5-HT. The coronary vascular response to 5-HT in the dog varies with vessel size. In this and in earlier studies (Brum *et al.*, 1984; Lamping *et al.*, 1985; Woodman, 1990) 5-HT has been shown to constrict the large coronary arteries but to dilate the resistance vessels. In isolated coronary arteries 5-HT causes the release of endothelium-derived relaxing factor (EDRF, Cocks & Angus, 1983; Cohen *et al.*, 1983) and removal of the endothelium in dogs enhances the constrictor response to 5-HT in the epicardial coronary arteries (Brum *et al.*, 1984; Lamping *et al.*, 1985), suggesting that in large coronary arteries release of endothelium-derived NO normally opposes the direct vasoconstrictor action of 5-HT. In our study the large artery constriction induced by 5-HT was increased after L-NNA, but the number of observations was limited by the marked constriction of the arteries to below the minimal detectable diameter. Importantly, L-NNA

significantly attenuated the increase in coronary blood flow produced by 5-HT indicating for the first time that the dilatation of the resistance vessels is endothelium-dependent.

In conclusion, our study demonstrates that L-NNA, an inhibitor of NO biosynthesis in endothelial cells, selectively inhibits endothelium-dependent vasodilatation in the coronary circulation of the dog. In addition L-NNA causes coronary vasoconstriction, particularly of the large coronary arteries, suggesting that endothelium-derived NO has an important role in the regulation of coronary vascular tone. It is known that endothelium-dependent vasodilatation is impaired by advanced atherosclerosis (Habib *et al.*, 1986; Verbeuren *et al.*, 1986; Bossaller *et al.*, 1987), and we have recently found that impairment of endothelial cell function

precedes the development of intimal thickening in early atheroma-like lesions (Dusting *et al.*, 1990). Moreover, the loss of endothelium-dependent coronary vasodilatation in man correlates with the presence of risk factors for coronary artery disease (Vita *et al.*, 1990; Yasue *et al.*, 1990). Therefore our current findings support suggestions that endothelium-derived NO has an important role in the maintenance of coronary blood flow and that endothelial damage might provoke coronary vasospasm and myocardial ischaemia.

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