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The effects of N$^{\omega}$-nitro-L-arginine methyl ester, sodium nitroprusside and noradrenaline on venous return in the anaesthetized cat

[1]E.A. Bower & [2]A.C.K. Law

Physiological Laboratory, University of Cambridge, Downing Street, Cambridge CB2 3EG

1 The vascular actions of N$^{\omega}$-nitro-L-arginine methyl ester (L-NAME), sodium nitroprusside and noradrenaline were investigated in cats under chloralose anaesthesia with controlled vascular tone and ventilation. Cardiac output, heart rate, vascular pressures and mean circulatory filling pressure (MCFP) were measured. Total peripheral resistance (TPR) and resistance to venous return (R_{vr}) were calculated from steady-state readings.

2 L-NAME (37 μmol kg^{-1}, i.v.) administered to ten cats receiving noradrenaline (6 nmol kg^{-1} min^{-1}, i.v.) increased aortic pressure by 47.5 ± 7.1 mmHg from 106 mmHg, and MCFP by 1.56 ± 0.36 mmHg from 10.0 mmHg (means ± s.e.means). Mean changes in portal venous pressure, RAP and heart rate were not significant. Cardiac output fell by 29.7 ± 3.3% from 130 ml min^{-1} kg^{-1}. TPR rose by 108 ± 7.2% from 796 mmHg l^{-1} min kg and R_{vr} by 58.4 ± 4.5% from 64 mmHg l^{-1} min kg.

3 Infusion of sodium nitroprusside into cats receiving noradrenaline evoked dose-related falls in aortic pressure, MCFP, TPR and R_{vr}. Changes in portal venous pressure, RAP and heart rate were not significant, and cardiac output fell slightly. After L-NAME, sensitivity to nitroprusside was increased by 139 ± 34% for MCFP, 176 ± 19% for TPR and 351 ± 39% for R_{vr}, and cardiac output rose slightly. The nitroprusside infusion required to restore TPR after L-NAME was estimated to be $5.8 \times 10^{\pm 0.41}$ nmol kg^{-1} min^{-1}, which was approximately three times more than that required to restore MCFP.

4 Infusion of noradrenaline evoked dose-related increases in aortic and portal venous pressures, heart rate, cardiac output, MCFP, TPR and R_{vr}. After L-NAME and nitroprusside (4.4 nmol kg^{-1} min^{-1}, i.v.), TPR and R_{vr} were not significantly different, but MCFP was reduced by 1.76 ± 0.24 mmHg, and cardiac output by 22 ± 1.9%. After subsequent expansion of the circulating blood volume (5–7.5 ml kg^{-1} dextran-saline), mean values for all parameters were restored to their previous levels. Sensitivity to noradrenaline was not significantly altered for heart rate, TPR and R_{vr}, but was reduced by 31.8 ± 12% for MCFP and by 66.5 ± 18% for cardiac output.

5 The depression of cardiac output by L-NAME is attributed to the increase in R_{vr}, partly compensated by the rise in MCFP. For a given rise in MCFP, the increase in R_{vr} was seven times greater after L-NAME than after noradrenaline, and the difference in the relative actions of the two drugs on resistance and capacitance vessels largely accounts for their contrasting effects on venous return. A procedure is suggested for replacement of vascular nitric oxide by nitroprusside infusion and blood volume expansion.

Keywords: Cardiac output; venous return; haemodynamics; vascular capacitance; vascular resistance; nitric oxide; N$^{\omega}$-nitro-L-arginine methyl ester (L-NAME); nitroprusside; noradrenaline

Introduction

Vascular endothelial cells have been shown to release several vasoactive agents either spontaneously or in response to chemical or mechanical stimuli, and an important endothelium-derived relaxing factor is nitric oxide (NO) derived from L-arginine (Ignarro, 1989; Moncada *et al.*, 1991a). Synthesis of NO can be blocked by analogues of L-arginine, such as N$^{\omega}$-nitro-L-arginine methyl ester (L-NAME; Moore *et al.*, 1990; Rees *et al.*, 1990). L-Arginine analogues administered *in vivo* increase peripheral resistance and aortic pressure and reduce cardiac output, evidently by preventing a normal, continuous release of NO from endothelial cells (Aisaka *et al.*, 1989; Rees *et al.*, 1989; Gardiner *et al.*, 1990a,b; Vargas *et al.*, 1991; Klabunde *et al.*, 1991; van Gelderen *et al.*, 1991). Subsequent administration of nitrodilators reduces resistance and aortic pressure but does not restore cardiac output, and the reduction in cardiac output has not yet been explained (Gardiner *et al.*, 1990a; Klabunde *et al.*, 1991; van Gelderen *et al.*, 1991; Widdop *et al.*, 1992).

Cardiac output depends in part on regulation of venous return by the balance between capacitance and resistance in the peripheral circulation (Guyton *et al.*, 1973). Changes in total vascular capacitance can be observed by measurement of mean circulatory filling pressure (MCFP; Bower & O'Donnell, 1991). Resistance to venous return (R_{vr}) is the combined resistance of all vessels up to the right atrium from the venous locations where the intravascular pressure is equal to MCFP, and it accounts for about 5% of the total peripheral resistance (TPR). It depends not only on venous calibre, but also on the positions of the MCFP values, which will migrate if blood volume is redistributed by changes in arterial resistance (Guyton *et al.*, 1959). Both MCFP and R_{vr} depend on the veins, and available evidence indicates that venous endothelium is less efficient than arterial endothelium as a source of relaxing factor. Isolated veins showed only weak responses to endothelium-dependent relaxing agents which were effective in arteries, although both types of vessel were relaxed by sodium nitroprusside and by relaxing factor released from arterial endothelium (De Mey & Vanhoutte,

[1] Author for correspondence.
[2] Present address: St. Louis University School of Medicine, 1402 South Grand Boulevard, St. Louis, Missouri 63104, U.S.A.

1982; Gruetter & Lemke, 1986; Seidel & La Rochelle, 1987; Ignarro *et al.*, 1988). Abolition of NO synthesis in a perfused cat hindlimb increased resistance mainly in large-bore resistance arteries, with much less effect on venous resistance (Ekelund & Mellander, 1990). However, changes in MCFP and R_{vr}, and hence in cardiac output, are responses of the whole body which cannot be extrapolated from behaviour of isolated vessels or perfused organs, and there are no reports on how they are affected by endogenous NO.

Investigation of the haemodynamic role of NO was complicated by the possibility that L-arginine analogues may increase vascular sensitivity to catecholamines (Seidel & La Rochelle, 1987; Moncada *et al.*, 1991b; Vo *et al.*, 1991) and may therefore modify responses to noradrenaline and to physiological changes in vasomotor tone. It is not certain whether the sensitivity increases because catecholamines stimulate NO release or whether the change is a secondary consequence of NO deficiency or altered vascular tone (Vargas *et al.*, 1990; Chyu *et al.*, 1992). Tests to detect modulation of NO release have not as yet been performed before and after replacement of the potentially variable endogenous secretion by equivalent controlled administration of a nitrodilator.

This paper has three main objectives. Firstly, to describe and relate the changes in MCFP, R_{vr} and cardiac output evoked by the direct vascular actions of L-NAME. Secondly, to measure the reversal of these changes by sodium nitroprusside, in order to devise a procedure for replacement of endogenous NO by a nitrodilator. Thirdly, to discover whether this replacement therapy alters the haemodynamic response to noradrenaline. In order to isolate the direct cardiovascular actions of the drugs, reflexes were suppressed, aortic pressure was maintained by infusion of noradrenaline, and positive pressure ventilation controlled intrathoracic pressure and blood gas tensions. The results obtained provide a basis for investigation of the more complex interactions in intact animals.

Methods

Male cats (3.5–5.3 kg) were used under anaesthesia induced with chloroform and maintained with chloralose (70 mg kg^{-1}, i.a.). Preliminary surgery took 2 h; spontaneous respiratory movements and autonomic reflexes were then suppressed by continuous i.v. infusion of a blocking solution described below, and positive pressure ventilation was applied at 50 breaths min^{-1}. Before starting the infusion, the level of anaesthesia was tested by forelimb toe pinch, corneal stimulation and sudden noise or vibration; a supplementary dose of chloralose (35–47 mg kg^{-1}) was given if responses were not absent or weak. Thereafter, reliance was placed on the well established stability of chloralose anaesthesia, and the experiment was terminated by overdose of anaesthetic at a maximum of 5 h after starting the infusion. Previous experiments on spontaneously breathing cats had shown that the anaesthetic regime maintained stable surgical anaesthesia for at least this period. In three cats, the blocking infusion was stopped at the end of the experiment; spontaneous respiration returned in 30–60 min, and it was confirmed that full surgical anaesthesia persisted at 5 h after commencement of the infusion.

The trachea was cannulated for artificial ventilation. Double-lumen catheters (4F; Vygon) passed down the left femoral and left jugular veins served respectively for continuous infusion of drugs and for injection of thermal indicator and measurement of right atrial pressure (RAP). Methods used for measurement of cardiac output by thermal dilution and of aortic, portal venous and right atrial pressures have been described elsewhere (Bower & Ead, 1976; Barnes *et al.*, 1980; 1986), as has the technique for MCFP measurement by circulatory arrest (Bower & O'Donnell, 1991). From analysis of variance of data from four cats in Figure 3, coefficients of variation were 0.044 for cardiac output and 0.029 for MCFP.

Apparatus

Heart rate and pressure signals were recorded by a four-channel penwriter (Multitrace 4; Lectromed Ltd.) and were transmitted to a computer (M290; Olivetti Ltd, with AS-1F interface; Cambridge Electronic Design) which sampled the inputs at 1000 Hz and displayed traces of the four signals. For measurement of MCFP, the heart rate trace was displaced off screen, the pressure traces were set to equal zero positions and sensitivities, and the display was set to stop after one sweep on closure of a switch at the moment of circulatory arrest. On demand, the computer calculated mean values of the input signals over a preset period (normally 8 s) and stored them in a disc file. Oxygen tension (Pa_{O_2}), carbon dioxide tension (Pa_{CO_2}) and pH in samples of arterial blood were measured by means of a blood micro system analyser (BMS3 Mk2; Radiometer). Drugs were infused by means of peristaltic pumps (Minipuls 3; Gilson).

Drugs and chemicals

The following drugs were used in the experiments: chloroform (Fisons Ltd.), α-chloralose (B.D.H. Ltd.), vecuronium bromide (Organon Teknika Ltd.), [β-mercapto-β,β-cyclopentamethylenepropionyl[1],-O-Me-Tyr[2],Arg[8]]-vasopressin (Manning compound; Sigma), captopril (Sigma), noradrenaline bitartrate (Sigma), sodium nitroprusside (B.D.H. Ltd.), N^{ω}-nitro-L-arginine methyl ester hydrochloride (Sigma), heparin (Sigma). Nitroprusside was dissolved in 3% Na citrate solution and was protected from light, noradrenaline was dissolved in a solution of 5% glucose and 1% $Na_2S_2O_5$ at pH 3.6, and other drugs were dissolved in 0.9% NaCl solution. Contrast medium for fluoroscopy was 54% Na iothalamate injection B.P. (Conray 325; May & Baker Ltd.). Blood volume was expanded with 6% dextran 150 in 0.9% NaCl solution (Dextraven 150; Fisons Ltd.).

The blocking solution used to suppress spontaneous respiratory movements, cardiovascular reflexes and the effects of circulating angiotensin I and vasopressin was based on that described by Gardiner *et al.* (1990b), and it contained vecuronium (0.5 mg ml^{-1}), hexamethonium (10 mg ml^{-1}), captopril (2 mg ml^{-1}) and Manning compound (20 μg ml^{-1}). The solution was administered by continuous infusion (0.5 ml kg^{-1} + 0.5 ml kg^{-1} h^{-1}, i.v.), and aortic pressure was restored to the middle of its safe range by infusion of noradrenaline (6 nmol kg^{-1} min^{-1}, i.v.). Table 1 shows the cardiovascular effects of this procedure. The immediate consequence of the positive pressure ventilation was a rise in RAP. Aortic pressure and TPR were reduced by 18%. MCFP was not significantly changed, and cardiac output was maintained by a 13% fall in R_{vr} helped by a 15% increase in heart rate.

Experimental procedure

The cat lay on its back on a heated table, and all manometers were zeroed by reference to a Marriotte's bottle filled with saline to the level of half the height of the cat's sternum. After completion of the surgery, heparin was administered (5 mg kg^{-1}, i.v.), anaesthesia was assessed and controlled ventilation was instituted. When circulatory conditions were steady at 5–15 min after any change, a computer sample of the heart rate and vascular pressures and a cardiac output measurement was taken, immediately followed by a measurement of MCFP. The measurements were repeated after 2–3 min to provide at least two samples at each steady state of the experiment. The computer derived TPR from:

(aortic pressure – RAP)/(cardiac output) mmHg l^{-1} min kg,

R_{vr} from:

Table 1 Cardiovascular parameters in eighteen cats under chloralose anaesthesia before and after infusion of blocking solution and noradrenaline and establishment of positive pressure ventilation as described in Methods

	Before blockade	*After blockade*	*Mean change*
Aortic pressure (mmHg)	140 ± 3.6	114 ± 4.3	−26.1 ± 3.3***
Portal venous pressure (mmHg)	6.58 ± 0.28	7.60 ± 0.32	+1.02 ± 0.24***
Right atrial pressure (mmHg)	0.563 ± 0.19	1.91 ± 0.24	+1.35 ± 0.24***
Mean circulatory filling pressure (mmHg)	10.64 ± 0.49	10.5 ± 0.50	−0.131 ± 0.25
Heart rate (beats min^{-1})	202 ± 7.0	232 ± 5.9	+29.9 ± 6.3***
Cardiac output ($ml\ min^{-1}\ kg^{-1}$)	147 ± 4.3	143 ± 4.1	−3.86 ± 3.2
Left ventricular stroke work ($mJ\ beat^{-1}\ kg^{-1}$)	13.8 ± 0.64	9.56 ± 0.59	−4.20 ± 0.41***
Total peripheral resistance ($mmHg\ l^{-1}\ min\ kg$)	962 ± 37	786 ± 28	−176 ± 33***
Resistance to venous return ($mmHg\ l^{-1}\ min\ kg$)	69.3 ± 3.2	60.4 ± 2.6	−8.9 ± 2.5**

Values are mean ± s.e.mean.
Probabilities of changes: $^{**}P<0.01$; $^{***}P<0.001$ (paired t test).

$$(\text{MCFP} - \text{RAP})/(\text{cardiac output})\ \text{mmHg}\ l^{-1}\ \text{min kg},$$

and left ventricular stroke work from:

$$(\text{aortic pressure} \times \text{cardiac output})/(\text{heart rate} \times 0.0075)\ \text{mJ beat}^{-1}\ \text{kg}^{-1},$$

where cardiac output is in $1\ min^{-1}$. Dose-response relationships for nitroprusside and noradrenaline were obtained by varying infusion from a middle to a minimum rate, then to a maximum, and back to the middle rate.

Comparisons were made between experiment means based on at least two consecutive steady-state computer, cardiac output and MCFP samples at each stage, and estimates of variance for groups of experiments were derived by analysis of variance of the combined experiment means. Group means are quoted plus and minus the standard error of the mean unless otherwise stated; they were compared by a paired Student's t test. Mean linear regression coefficients for groups of experiments were derived from the combined sums of squares of errors calculated for each experiment, and were compared by t test (Snedecor & Cochran, 1967). Linearities of log dose-response relationships were tested by comparing the upper and lower segments of responses by paired and unpaired t tests and by t test of regression coefficients.

Results

The vascular response to N^{ω}-nitro-L-arginine methyl ester

Table 2 shows cardiovascular changes which followed administration of L-NAME ($37\ \mu mol\ kg^{-1}$, i.v.) in ten consecutive experiments, and Figure 1 shows the relationships between cardiac output, RAP and MCFP. Arterial blood parameters were: pH, 7.36 ± 0.01; Pa_{O_2}, 86.6 ± 3.5 mmHg; Pa_{CO_2}, 34.4 ± 1.4 mmHg; haematocrit, 40.6 ± 1.14%. When L-NAME was given, aortic pressure rose over 3–6 min to a transient peak in some cats or to a steady level in others. Measurements were taken when conditions were stable, after 10–20 min. In all cases aortic pressure and MCFP rose, and cardiac output fell by 29.7 ± 3.3%. The reduced output was associated with a rise in R_{vr} of 58.4 ± 4.5% (Figure 1) and the increased aortic pressure with a rise in TPR of 108 ± 7.2%. Changes in RAP, portal venous pressure, heart rate and left ventricular stroke work were usually small and inconsistent, but in one cat RAP rose by 4.5 mmHg and cardiac output and stroke work fell by 44% and 47% respectively. Four cats received second doses of L-NAME (37–370 $\mu mol\ kg^{-1}$) at times from 20 min to 170 min later; none showed any further significant increase in MCFP, TPR or R_{vr}.

Table 2 Cardiovascular parameters in ten cats before and after i.v. administration of N^{ω}-nitro-L-arginine methyl ester (L-NAME, $37\ \mu mol\ kg^{-1}$)

	Before L-NAME	*After L-NAME*	*Mean change*
Aortic pressure (mmHg)	106 ± 7.7	153 ± 6.9	+47.5 ± 7.1***
Portal venous pressure (mmHg)	7.72 ± 0.51	7.88 ± 0.57	+0.153 ± 0.38
Right atrial pressure (mmHg)	1.70 ± 0.30	2.28 ± 0.63	+0.572 ± 0.46
Mean circulatory filling pressure (mmHg)	10.0 ± 0.58	11.6 ± 0.72	+1.56 ± 0.36**
Heart rate (beats min^{-1})	230 ± 5.9	229 ± 7.4	−0.626 ± 6.5
Cardiac output ($ml\ min^{-1}\ kg^{-1}$)	130 ± 5.4	91.8 ± 3.5	−38.6 ± 4.3***
Left ventricular stroke work ($mJ\ beat^{-1}\ kg^{-1}$)	8.28 ± 0.99	8.33 ± 0.62	+0.055 ± 0.82
Total peripheral resistance ($mmHg\ l^{-1}\ min\ kg$)	796 ± 45	1660 ± 73	+860 ± 57***
Resistance to venous return ($mmHg\ l^{-1}\ min\ kg$)	64 ± 3.3	101 ± 5.5	+37.4 ± 2.9***

Reflexes were suppressed as described in Methods.
Values are mean ± s.e.mean.
For changes evoked by L-NAME: $^{**}P<0.01$; $^{***}P<0.001$ (paired t test).

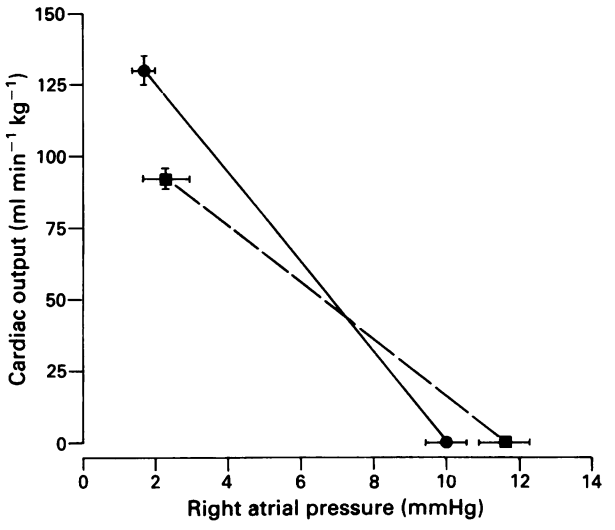

Figure 1 The relationship of cardiac output and right atrial pressure to mean circulatory filling pressure (MCFP) before (●) and after (■) i.v. administration of N^{ω}-nitro-L-arginine methyl ester (L-NAME, 37 μmol kg^{-1}). Values are mean ± s.e.mean; lines join related output and MCFP values, and their slopes are inversely proportional to R_{vr}. Reflexes were suppressed as described in Methods.

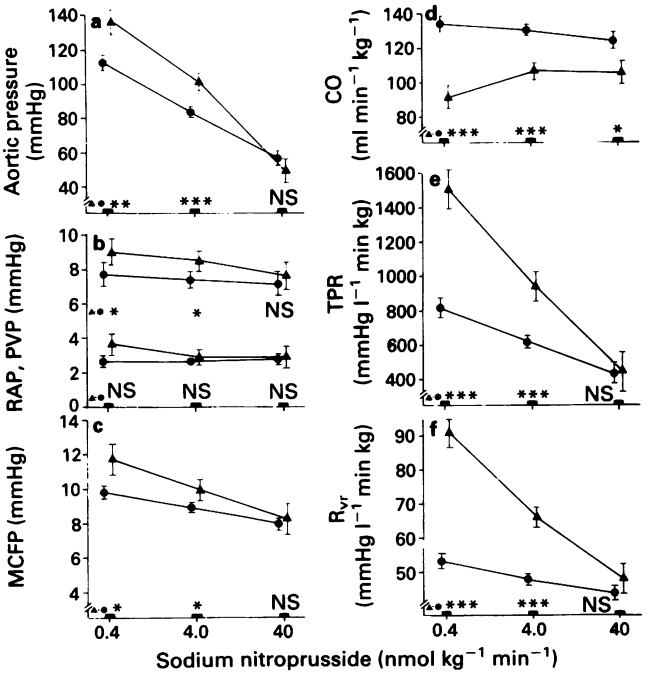

Figure 2 Cardiovascular parameters during i.v. infusion of sodium nitroprusside in four cats before (●) and after (▲) administration of N^{ω}-nitro-L-arginine methyl ester (L-NAME, 37 μmol kg^{-1}): (a) aortic pressure; (b) portal venous pressure (PVP; upper lines) and right atrial pressure (RAP; lower lines); (c) mean circulatory filling pressure (MCFP); (d) cardiac output (CO); (e) total peripheral resistance (TPR); (f) resistance to venous return (R_{vr}). Reflexes were suppressed as described in Methods. Values are mean ± s.e.mean. For differences between pairs of points: $*P<0.05$; $**P<0.01$; $***P<0.001$ (paired t test).

The vascular response to sodium nitroprusside

The response to sodium nitroprusside infusion over the range 0.4–40 nmol kg^{-1} min^{-1} was explored in four cats before and after administration of L-NAME. Throughout this series, heart rate was 253 ± 3.9 beats min^{-1} and did not vary significantly. Other results are shown in Figure 2 and Table 3.

With increasing nitroprusside dose before L-NAME, aortic pressure, MCFP, TPR and R_{vr} fell progressively (Figure 2a, c,e,f). Variations in portal venous pressure and RAP were small and inconsistent (Figure 2b). Mean cardiac output was 130 ± 2.3 ml min^{-1} kg^{-1} and changed little (Figure 2d), but the mean regression coefficient indicated a significant fall (Table 3). The falls in MCFP, TPR and R_{vr} were linearly related to the logarithm of the nitroprusside dose (correlation coefficient $r>0.73$, $P<0.01$), and their mean regression coefficients were all significant (Table 3; $P<0.001$).

After L-NAME (37 μmol kg^{-1}), aortic pressure, MCFP, TPR and R_{vr} at the lower nitroprusside doses were higher and changed more with increasing infusion rate (Figure 2a, c,e,f). Portal venous pressure and RAP still showed little variation (Figure 2b). Mean cardiac output fell to 102 ± 3.6 ml min^{-1} kg^{-1} after L-NAME, and changes were small and variable (Figure 2d), but the mean regression coefficient indicated a rise in output which differed significantly from the fall observed previously (Table 3). The enhanced sensitivities of MCFP, TPR and R_{vr} to nitroprusside were reflected in their regression coefficients (Table 3), which were increased by 139 ± 34%, 176 ± 19% and 351 ± 39% respectively.

In the absence of nitroprusside before L-NAME, the mean value for TPR was 891 ± 87 mmHg l^{-1} min kg and for MCFP was 10.4 ± 0.67 mmHg. The nitroprusside infusion required after L-NAME to reduce TPR to its previous value was calculated from the regression data to be $5.8 \times 10^{\pm 0.41}$ nmol kg^{-1} min^{-1}. Comparison of nitroprusside doses calculated for each cat indicated that the dose required for restoration of TPR was $3.3 \times 10^{\pm 0.51}$ times greater than that for MCFP.

The vascular response to noradrenaline

The response to noradrenaline infusion over the range 2–18 nmol kg^{-1} min^{-1} was explored in four cats before and after administration of L-NAME and institution of replacement therapy (Figure 3). After L-NAME (37 μmol kg^{-1}),

Table 3 Linear regression coefficients of cardiovascular parameters on log dose of sodium nitroprusside in four cats before and after i.v. administration of N^{ω}-nitro-L-arginine methyl ester (L-NAME, 37 μmol kg^{-1})

	Before L-NAME	*After L-NAME*
Cardiac output (ml min^{-1} kg^{-1} per log unit)	−4.79 ± 1.8	+7.05 ± 3.3**
Mean circulatory filling pressure (mmHg per log unit)	−0.788 ± 0.12	−1.88 ± 0.24***
Total peripheral resistance (mmHg l^{-1} min kg per log unit)	−193 ± 14	−532 ± 34***
Resistance to venous return (mmHg l^{-1} min kg per log unit)	−4.75 ± 0.92	−21.4 ± 1.6***

Reflexes were suppressed as described in Methods.
Values are mean ± s.e.mean.
For all coefficients, $P<0.05$; for changes evoked by L-NAME: $**P<0.01$; $***P<0.001$ (t test).

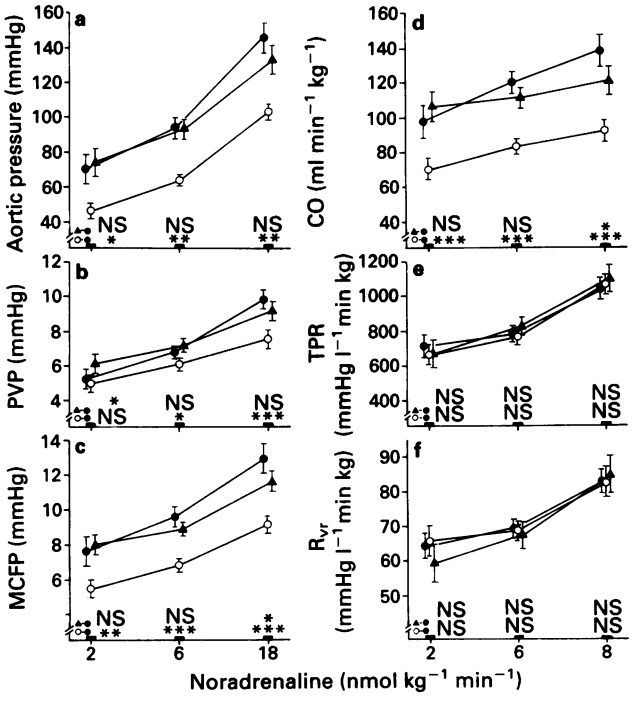

Figure 3 Cardiovascular parameters during i.v. infusion of noradrenaline in four cats: (●) before N$^{\omega}$-nitro-L-arginine methyl ester (L-NAME); (○) during continuous infusion of sodium nitroprusside (4.4 nmol kg^{-1} min^{-1}) after administration of L-NAME (37 μmol kg^{-1}); (▲) during nitroprusside infusion after L-NAME and after administration of dextran-saline solution (6.9 ± 0.6 ml kg^{-1}). (a) Aortic pressure; (b) portal venous pressure (PVP); (c) mean circulatory filling pressure (MCFP); (d) cardiac ouput (CO); (e) total peripheral resistance (TPR); (f) resistance to venous return (R_{vr}). Reflexes were suppressed as described in Methods. Values are mean ± s.e.mean. For differences between pairs of points: $*P<0.05$; $**P<0.01$; $***P<0.001$ (paired t test).

sodium nitroprusside (4.4 nmol kg^{-1} min^{-1}) was infused continuously for the rest of the experiment and the series of tests was repeated. The circulating blood volume was then expanded with a volume of dextran-saline solution judged sufficient to restore MCFP to its previous value (5–7.5 ml kg^{-1}; mean 6.9 ml kg^{-1}), and the tests were repeated again.

With increasing noradrenaline before L-NAME, heart rate rose by 53.5 ± 11 beats min^{-1} (25.6 ± 5.3%) from 209 beats min^{-1}. All vascular pressures except RAP rose progressively, and the increases were associated with rises in cardiac output, TPR and R_{vr} (Figure 3). Mean RAP was 1.35 ± 0.16 mmHg and did not vary significantly.

After administration of L-NAME and commencement of nitroprusside infusion, heart rate values and changes were not significantly altered, but vascular pressures and cardiac output were depressed. At a noradrenaline dose of 6 nmol kg^{-1} min^{-1}, L-NAME plus nitroprusside reduced MCFP by 1.76 ± 0.24 mmHg from 8.69 ± 0.65 mmHg and cardiac output by 22.0 ± 1.9% from 108 ± 5.3 ml min^{-1} kg^{-1} ($P<0.01$; paired t test). All parameters except RAP varied with noradrenaline (Figure 3); mean RAP was 1.16 ± 0.21 mmHg and did not vary significantly. Values for TPR and R_{vr} did not differ significantly from those observed before L-NAME (Figure 3e,f).

After expansion of the circulating blood volume, heart rate values and changes were not significantly altered, but vascular pressures and cardiac output were restored to values close to those observed before L-NAME. All parameters except RAP varied with noradrenaline (Figure 3); mean RAP was 1.59 ± 0.12 mmHg and did not vary significantly. Changes in cardiac output were small, but the mean regression coefficient indicated a significant rise (Table 4). Values for TPR and R_{vr} did not differ significantly from those observed before L-NAME (Figure 3e,f).

Although MCFP and cardiac output values at 2–6 nmol kg^{-1} min^{-1} noradrenaline did not differ significantly from their corresponding values before L-NAME, at 18 nmol kg^{-1} min^{-1} noradrenaline both were lower than before (Figure 3c, d). Log dose-response relationships for heart rate, cardiac output, MCFP, TPR and R_{vr} before and after administration of L-NAME, nitroprusside and dextran-saline solution did not depart significantly from linearity, and changes were assessed by calculation of mean linear regression coefficients (Table 4). After treatment, sensitivity to noradrenaline was reduced by 31.8 ± 12% for MCFP and by 66.5 ± 18% for cardiac output. For heart rate, TPR and R_{vr}, the differences between the responses before and after treatment were not significant.

Discussion

The rise in MCFP of 1.5 mmHg evoked by L-NAME is modest but significant. For comparison, increments of up to 6 mmHg were elicited by strong reflex vasomotor stimulation in hypoxia (Bower & O'Donnell, 1991). Changes in MCFP are not proportional to their initial values and cannot be compared directly with resistance changes. However, if nitroprusside was infused at a rate sufficient to restore TPR to its level before L-NAME, MCFP was reduced by 1.8 mmHg below its previous value, and the circulating blood volume had to be expanded by 7 ml kg^{-1} to restore it. The estimated replacement dose of nitroprusside was about one third as great for MCFP as for TPR. These observations are consistent with reports that less NO is released by venous than by arterial endothelium.

The changes in TPR were comparable to those reported for the rat with intact and with suppressed reflexes (Gardiner

Table 4 Linear regression coefficients of cardiovascular parameters on log dose of noradrenaline in four cats before and after i.v. administration of N$^{\omega}$-nitro-L-arginine methyl ester (L-NAME, 37 μmol kg^{-1}), sodium nitroprusside (4.4 nmol kg^{-1} min^{-1}) and dextran-saline (6.9 ± 0.6 ml kg^{-1})

	Before L-NAME	*After L-NAME*
Heart rate (beats min^{-1} per log unit)	+55.7 ± 3.8	+53.0 ± 7.7
Cardiac output (ml min^{-1} kg^{-1} per log unit)	+46.3 ± 7.1	+15.5 ± 4.7***
Mean circulatory filling pressure (mmHg per log unit)	+5.56 ± 0.50	+3.79 ± 0.42**
Total peripheral resistance (mmHg l^{-1} min kg per log unit)	+321 ± 42	+415 ± 43
Resistance to venous return (mmHg l^{-1} min kg per log unit)	+17.6 ± 2.3	+23.1 ± 2.5

Reflexes were suppressed as described in Methods.
Values are mean ± s.e.mean.
For all coefficients, $P<0.05$; for changes evoked by L-NAME: $**P<0.01$; $***P<0.001$ (t test).

et al., 1990a,b; Van Gelderen *et al.*, 1991). In the cat, L-NAME has been reported to increase resistance to perfusion of limb tissues by more than 90% (Ekelund & Mellander, 1990; Bellan *et al.*, 1991). McMahon *et al.* (1992) observed rises in aortic pressure of 37% in reserpine-treated cats and 27% in normal cats. Van Gelderen *et al.* (1991) reported that in the cat, unlike the rat, changes in aortic pressure, cardiac output and peripheral resistance evoked by L-NAME were small; they gave L-NAME in progressively increasing doses to cats with intact reflexes, so changes may have been obscured by homeostatic adaptations.

The proportionate rise in R_{vr} was half as great as that in TPR, which is substantially more than would be expected from the relative effects of L-NAME on venous and arterial resistances in skeletal muscle (Ekelund & Mellander, 1990). There may therefore have been a contribution to the rise in R_{vr} from redistribution of blood volume or flow in addition to any increase in resistance due to actual venoconstriction. The lack of change in portal venous pressure argues against pooling of blood in the splanchnic vessels by specific constriction of hepatic vessels. Available information indicates that arterial constriction is general (Gardiner *et al.*, 1990a), and would lead to volume redistribution from venous to arterial compartments with migration of MCFP locations towards the sites of arteriolar constriction, thus lengthening the path for venous return.

The increase in R_{vr} evoked by L-NAME dominated venous return and was largely responsible for the 30% depression of cardiac output (Figure 1). The rise in MCFP partly countered the effect of the rise in resistance, and in its absence output would have fallen by about 40%. L-NAME is known to induce constriction of coronary vessels (Woodman & Dusting, 1991; Jones & Brody, 1992; Ueeda *et al.*, 1992; Lamontagne *et al.*, 1992). Coronary vasoconstriction would be partly compensated by the rise in aortic pressure, and Sarnoff *et al.* (1960) reported that if coronary flow was adequate, variation in flow had little effect on cardiac contractility. In the present experiments cardiac contractility was not specifically investigated, but heart rate did not change significantly and there was little evidence that the heart impeded venous return after L-NAME administration. In most cats, RAP rose or fell by less than 0.5 mmHg and changes in left ventricular stroke work were small and inconsistent, as the fall in venous return approximately matched the rise in aortic pressure. In the one cat in which RAP rose by 4.5 mmHg and cardiac output and stroke work fell substantially, ventricular performance may have been impaired by NO deficiency. Gardiner *et al.* (1990a) noted that the fall in output observed after L-NAME in the rat with intact reflexes was not associated with a rise in RAP, despite a substantial fall in heart rate, and Widdop *et al.* (1992) reported that atropine abolished the fall in heart rate but gave no lasting improvement in cardiac output.

The depression of aortic pressure, MCFP, cardiac output and TPR observed during infusion of sodium nitroprusside is consistent with previous reports on the dog, cat and rat (Pouleur *et al.*, 1980; Ignarro *et al.*, 1981; Thomas *et al.*, 1988). The fall in cardiac output with no change in RAP indicates that the fall in MCFP here outweighed the benefit to venous return of the reduced R_{vr}. After administration of L-NAME, there was a marked increase in the sensitivity of resistance and MCFP to nitroprusside, which is consistent with up-regulation of soluble guanylate cyclase in the absence of NO (Shirasaki & Su, 1985; Moncada *et al.*, 1991b; Gardiner *et al.*, 1991). The sensitivity of resistance increased more than did that of MCFP, perhaps due to a greater basal release of NO in resistance vessels. A consequence for venous return was that the fall in R_{vr} now outweighed the depression of MCFP and cardiac output tended to rise slightly. Nevertheless, a nitroprusside infusion which restored TPR depressed MCFP below its previous value, so venous return did not recover fully until MCFP was restored by expansion of the circulating blood volume.

The MCFP increments evoked by noradrenaline were about half as great as those induced by similar doses of adrenaline in the dog under total spinal anaesthesia (Guyton *et al.*, 1958). Unlike adrenaline in the dog, noradrenaline also significantly increased R_{vr}, but the changes in MCFP were dominant and venous return rose by 41%. For a given rise in MCFP, the increase in R_{vr} was seven times greater after L-NAME than after noradrenaline, and the contrast between the effects of L-NAME and noradrenaline on venous return is due mainly to the difference in their relative actions on resistance and capacitance vessels. Cardiac stimulation by noradrenaline would have helped the heart to accept the augmented venous return without a rise in RAP, but it could not by itself have generated an output increase of the magnitude observed (Guyton *et al.*, 1958; Barnes *et al.*, 1980; 1986). Indeed, after replacement of NO by nitroprusside, the output increase evoked by noradrenaline was reduced by two thirds although the cardiac acceleration was unchanged (Table 4).

After NO replacement, MCFP responses to noradrenaline were depressed sufficiently to account for the smaller changes in venous return, and resistance responses were not significantly altered. The dose of L-NAME used was enough to suppress basal release of NO, as in the rat (Vargas *et al.*, 1991), and would be expected to reduce evoked release. A higher dose of L-NAME (370 μmol kg^{-1}) with replacement therapy was tested in one cat, but there was no evidence of increased noradrenaline sensitivity. Potentially, noradrenaline might release NO not only directly but also by increasing endothelial shear stress (Rubanyi *et al.*, 1986; Hutcheson & Griffith, 1991; Lamontagne *et al.*, 1992), and the aortic pressure and cardiac output increments evoked by the transition from low to high dose imply an increase of about 90%. The need to keep within safe levels of aortic pressure limited the range of noradrenaline doses used, compared to those possible *in vitro*. Higher local concentrations may perhaps be a more effective stimulus to NO release (Vo *et al.*, 1991), but local constriction reduces rather than increases shear stress unless flow is maintained by a rise in aortic pressure. The present results therefore imply that noradrenaline in doses consistent with physiological levels of aortic pressure has little effect on endothelial NO secretion either directly or through increased shear stress.

For replacement of endothelial NO, sodium nitroprusside provides a readily controllable source of NO (Feelisch & Noack, 1987; Bates *et al.*, 1991). At low RAP values, cardiac output varies with MCFP and is simpler to measure. A satisfactory replacement therapy appears to be to administer L-NAME, to infuse nitroprusside at a rate adjusted to restore TPR, and to expand circulating blood volume until cardiac output is restored as well. The responsiveness of capacitance vessels is then reduced by about one third, but in other respects the general haemodynamic conditions resemble those prevailing before L-NAME. Possible changes in cardiac output distribution remain to be studied. Subject to these limitations, the procedure is potentially useful for *in vivo* investigation of NO secretion. Preliminary tests in unblocked, chloralose-anaesthetized cats required nitroprusside doses similar to those reported here.

In conclusion, administration of L-NAME evoked a modest increase in MCFP opposed by a substantial increase in R_{vr} which accounted for the observed decrease in cardiac output. Infusion of nitroprusside reversed the increases, but the dose adequate to restore the resistances was excessive for MCFP and therefore left cardiac output depressed; MCFP and cardiac output could however be restored by expansion of the blood volume. Resistance responses to noradrenaline were not significantly changed and MCFP responses were slightly depressed by this replacement of endogenous NO.

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