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Haemodynamic changes and acetylcholine-induced hypotensive responses after N^G-nitro-L-arginine methyl ester in rats and cats

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1 The haemodynamic effects of N^G-nitro-L-arginine methylester (L-NAME; 1, 3, 10 and 30 mg kg^{-1}) and its potential ability to attenuate the hypotensive responses to acetylcholine (0.03, 0.1, 1.0 and 3.0 μg kg^{-1}) have been investigated in anaesthetized rats and cats.

2 In the rat, L-NAME elicited a dose-dependent pressor effect increasing mean arterial blood pressure from the baseline value of 116 ± 4 mmHg to a maximum of 156 ± 6 mmHg with 30 mg kg^{-1}. This increase in blood pressure could be only partly reversed by L-arginine (300 mg kg^{-1}). However, the increase in blood pressure by lower doses (up to 10 mg kg^{-1}) of L-NAME was effectively reversed by L-arginine (1000 mg kg^{-1}).

3 In the cat, L-NAME did not significantly modify systemic haemodynamic variables (heart rate, mean arterial blood pressure, cardiac output, stroke volume or total peripheral resistance), when compared to the changes in saline-treated animals. Administration of L-arginine did not cause any significant effect in cats treated with L-NAME, but some decrease in heart rate and increases in cardiac output and stroke volume were observed in the saline-treated group.

4 With the lowest dose (1 mg kg^{-1}), L-NAME did not affect tissue blood flows in the cat, but higher doses (3 and 30 mg kg^{-1}) significantly reduced blood flows to the mesentery, stomach, spleen, intestines, lungs and the total liver. L-Arginine (300 mg kg^{-1}) injected into the control (saline-treated) animals resulted in a significant increase in blood flow to the heart, mesentery, lungs as well as the total liver, particularly its portal fraction. L-Arginine-induced increases in tissue blood flows (mesentery, kidneys, spleen, lungs, total liver and portal blood flow) in saline-treated animals were attenuated in animals treated with L-NAME.

5 The acetylcholine-induced peak hypotensive response was not reduced in rats or cats by L-NAME. The duration of acetylcholine response was, however, attenuated in both species by L-NAME. Treatment with L-arginine (10–100 mg kg^{-1}) did not change the acetylcholine-induced hypotension.

6 The above results reveal a marked difference between the haemodynamic effects of L-NAME in rats and cats and suggest that in cats, unlike rats, the role of the L-arginine-NO pathway in the regulation of blood pressure is rather limited, although such a pathway may exist in several tissues. Furthermore, the hypotensive response to acetylcholine in both species seems to be mediated largely by NO-independent pathways.

Keywords: Acetylcholine; L-arginine; endothelium; nitric oxide; N^G-nitro-L-arginine methyl ester.

Introduction

The vasodilator action of acetylcholine in a number of isolated blood vessels depends largely on the release of an endothelium-derived-relaxing factor (EDRF; Furchgott & Vanhoutte, 1989). EDRF has been characterized as being identical to nitric oxide (NO), which is cleaved from L-arginine by the action of the enzyme NO-synthase, present in endothelial cells (Palmer *et al.*, 1987; 1988). Once released, NO stimulates soluble guanylate cyclase to increase guanosine 3':5'-cyclic monophosphate (cyclic GMP) levels (Boulanger *et al.*, 1990; Kelm & Schräder, 1990). The formation of NO can be inhibited *in vitro* by the arginine analogues, such as N^G-monomethyl-L-arginine (L-NMMA) and N^G-nitro-L-arginine methylester (L-NAME) (Palmer *et al.*, 1988; Mülsch & Busse, 1990).

The local vasodilatation induced by acetylcholine is reported to be inhibited by L-NAME in the rat perfused mesentery (Moore *et al.*, 1990) and by L-NMMA in anaesthetized rats as well as in the human forearm arterial bed (Whittle *et al.*, 1989; Vallance *et al.*, 1989). In contrast, L-NAME has not been found to affect vasodepressor action in conscious rats (Gardiner *et al.*, 1990b). Since most experiments using arginine-analogues have been performed *in vitro*, we studied the systemic haemodynamic effects of L-NAME as well as its ability to modify the hypotensive responses to acetylcholine in rats and cats. In the latter animal species, tissue blood flow changes following the administration of L-NAME were also investigated.

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Methods

General

Rats Thirty-seven male Wistar-Kyoto rats (body weight 300–350 g) were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.). A trachea canula was inserted to facilitate ventilation. Catheters were placed in both jugular veins and the right carotid artery for, respectively, drug administration and blood pressure measurement by a pressure transducer (model P23Ac, Statham Laboratories, Hato Rey, Puerto Rico). Blood pressure was continuously recorded on a polygraph (model 7, Grass Instrument Company, Quincey, MA). Heart rate was derived from the blood pressure recordings. Temperature was maintained at 37°C with an electric blanket.

Cats Twelve male or female cats (body weight between 2.8 and 5.0 kg) were anaesthetized with ketamine (12 mg kg^{-1}, i.p.). A trachea canula was inserted for artificial ventilation by a respiratory pump (Loosco, Amsterdam, The Netherlands).

Both femoral veins were catheterized for i.v. administration of drugs and blood sampling. Anaesthesia was maintained with sodium pentobarbitone (6 mg kg^{-1}, i.v. bolus, followed by 3 mg kg^{-1} h^{-1}, i.v. infusion). Both femoral arteries were cannulated for blood sampling and measurement of arterial blood pressure with a pressure transducer (model P23Ac, Statham Laboratories, Hato Rey, Puerto Rico). Blood pressure was continuously recorded on a polygraph (model 7, Grass Instrument Company, Quincy, MA). The right atrium was catheterized for microsphere administration. Finally, a flow probe was placed around the ascending aorta: cardiac output was calculated by adding ascending aorta blood flow and myocardial blood flow (see below). Body temperature was maintained at 37°C with an electric blanket and pH and blood gases were kept between normal limits (Po_2, 90–120 mmHg; Pco_2, 25–35 mmHg; pH, 7.35–7.45) by adjusting respiratory rate and tidal volume. A stabilization period of 30–60 min was allowed.

Regional tissue blood flows were measured with the radioactive microsphere technique, by use of the reference blood sample method (Heymann *et al.*, 1977; Saxena *et al.*, 1980). For each measurement, a suspension of about 200,000 microspheres (15 μm diameter, NEN Company, Dreieich, West Germany), labelled with one of the isotopes (^{114}Ce, ^{113}Sn, ^{103}Ru, ^{95}Nb, or ^{46}Sc), was mixed and injected into the left atrium. At the end of the experiment the animals were killed and various tissues were dissected out, weighed and put in vials. The radioactivity in these vials was counted for 5–10 min in a γ-scintillation counter (Packard, Minaxi Autogamma 5000) using suitable windows for discriminating the different isotopes. Regional blood flows and cardiac output were calculated with a set of computer programmes especially designed for the radioactive microsphere technique (Saxena *et al.*, 1980).

Experimental protocol

Rats After a stabilization period each rat received five increasing i.v. doses of acetylcholine (0.03, 0.1, 0.3, 1.0 and 3.0 μg kg^{-1}) at intervals of 3–5 min. Peak hypotensive responses to acetylcholine and its duration of action, i.e. the time needed for 50% recovery of the effect, were recorded. Due to the short-lasting action of acetylcholine, duration of action could only be assessed for the three highest doses. The rats were then divided into 4 groups which received either saline (4 times 1 ml kg^{-1}; $n = 9$), L-NAME (1, 3, 10 and 30 mg kg^{-1}; $n = 12$), L-arginine (10, 30 and 100 mg kg^{-1}; $n = 7$) or phenylephrine (40, 120, 220 and 330 μg kg^{-1}; $n = 9$). In most of the animals (for n, see results), the different doses of saline or drugs, administered every 15–20 min, were followed by the five doses of acetylcholine. The animals which had received L-NAME were administered L-arginine (300 mg kg^{-1}) after the last injection of acetylcholine. An additional group of animals ($n = 6$) was treated with L-NAME (1, 3 and 10 mg kg^{-1}) followed by L-arginine (100 mg kg^{-1}).

The dose range of phenylephrine was chosen to induce sustained increases in blood pressure similar to that induced with L-NAME. The magnitude and duration of hypotensive responses to acetylcholine after the different treatments were compared to the respective controls.

Cats A similar protocol was followed in cats as in rats for L-NAME (1, 3, 10 and 30 mg kg^{-1}) and saline. After the first series of acetylcholine injections (0.03, 0.1, 0.3, 1.0 and 3.0 μg kg^{-1}) at baseline, the first batch of microspheres was injected in each animal to determine baseline values of cardiac output and regional haemodynamic variables. The animals then received either four doses of L-NAME (1, 3, 10 and 30 mg kg^{-1}, $n = 7$) or saline (1 ml kg^{-1}, $n = 5$) at intervals of 20 min. Ten minutes after each dose (except after 10 mg kg^{-1} L-NAME and the third dose of saline), a batch of microspheres was injected. Finally, a bolus injection of 300 mg kg^{-1} L-arginine was administered, followed 10 min later by the last batch of microspheres for tissue blood flow measurements.

Data presentation and analysis

All data in the text are mentioned as mean ± s.e.mean. The values after the different treatments were compared to the baseline values by use of Duncan' new multiple range test once a parameteric two-way analysis of variance (randomized block design) had revealed that the samples represented different populations. The significance of the changes induced by L-NAME, L-arginine or phenylephrine as compared to the respective saline treatment was tested by an unpaired Student's t test. A P value of 0.05 or less was considered statistically significant.

Drugs

The drugs used in this study were: L-arginine hydrochloride, N^G-nitro-L-arginine methylester (both drugs: Sigma, St Louis, U.S.A.), ketamine hydrochloride (A.U.V., Cuyk, The Netherlands), acetylcholine chloride, phenylephrine hydrochloride (both from the Department of Pharmacy, Erasmus University, Rotterdam, The Netherlands), sodium pentobarbitone (Sanofi BV, Maassluis, The Netherlands). All drugs were dissolved in sterile saline. The doses mentioned in the text refer to the respective salts.

Results

Rats

Haemodynamic effects Both mean arterial blood pressure and heart rate remained relatively stable in the rats receiving either saline or L-arginine injections. Both L-NAME and phenylephrine elicited pressor responses; from the baseline values of 116 ± 4 and 93 ± 4 mmHg, respectively, blood pressure increased dose-dependently to a maximum of 156 ± 6 and 135 ± 5 mmHg, respectively, with the highest doses. Heart rate decreased significantly compared to baseline values with L-NAME, but the decreases were not different from those seen in saline-treated animals. Phenylephrine did not change heart rate (Figure 1). The increase in blood pressure induced by L-NAME (30 mg kg^{-1}) could be partly reversed by the administration of L-arginine (300 mg kg^{-1}) (Figure 1).

In an additional group of 6 animals which received L-NAME up to 10 mg kg^{-1}, blood pressure increased significantly from 93 ± 2 to a maximum of 142 ± 4 mmHg and heart rate decreased significantly from 336 ± 11 to 282 ± 13 beats min^{-1}. L-Arginine reversed the L-NAME-induced increase in blood pressure, which returned to a value of 78 ± 9 mmHg ($P < 0.05$, versus L-NAME 10 mg kg^{-1}). However, the bradycardia induced by L-NAME remained unchanged after L-arginine (288 ± 13 beats min^{-1}).

Acetylcholine responses Acetylcholine (0.03–3.0 μg kg^{-1}) elicited a dose-dependent fall in mean arterial blood pressure in rats; before any treatment the highest dose induced a fall in blood pressure of 52 ± 11 mmHg ($n = 25$). The calculated dose inducing a 40 mmHg decrease in mean arterial blood pressure (D_{40}) was 0.8 ± 0.1 μg kg^{-1} ($n = 25$). Treatment of rats with the four doses of saline did not influence the hypotensive responses to acetylcholine (Figure 2). Compared to the rats treated with saline, L-NAME (3 and 10 mg kg^{-1}) increased the hypotensive effects of 0.3 to 3.0 μg kg^{-1} acetylcholine. The vasodilator response to acetylcholine was also potentiated during infusions of phenylephrine (220 and 330 μg kg^{-1} h^{-1}). Pretreatment with L-arginine (10–100 mg kg^{-1}) did not change the acetylcholine-induced hypotension (Figure 2).

The duration of the depressor response to acetylcholine, assessed as the time needed for 50% recovery, remained unchanged after treatment with either saline, L-arginine or phenylephrine. There was, however, some significant reduction

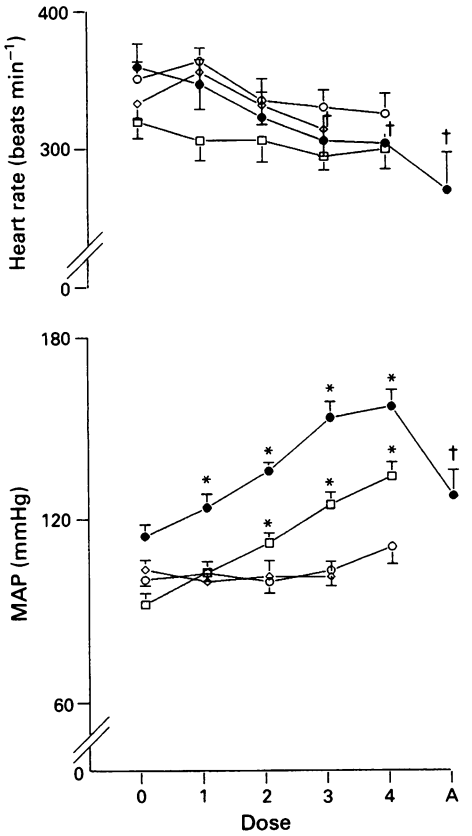

Figure 1 Mean arterial blood pressure (MAP) and heart rate in rats before (control; 0) and after treatment with four consecutive doses of saline (1 ml kg^{-1} each, $n = 9$; ○), N^G-nitro-L-arginine methylester (L-NAME, 1, 3, 10 and 30 mg kg^{-1}, $n = 12$; ●), phenylephrine (40, 120, 220 and 330 μg kg^{-1} h^{-1}, $n = 9$; □) or L-arginine (10, 30 and 100 mg kg^{-1}, $n = 7$; ◇). At A, L-arginine (300 mg kg^{-1}) was injected in the animals that had received L-NAME. Data represent mean with s.e.mean shown by vertical bars. Asterisks denote significant differences from the values in the saline experiments and crosses denote significant differences from baseline values, $P < 0.05$.

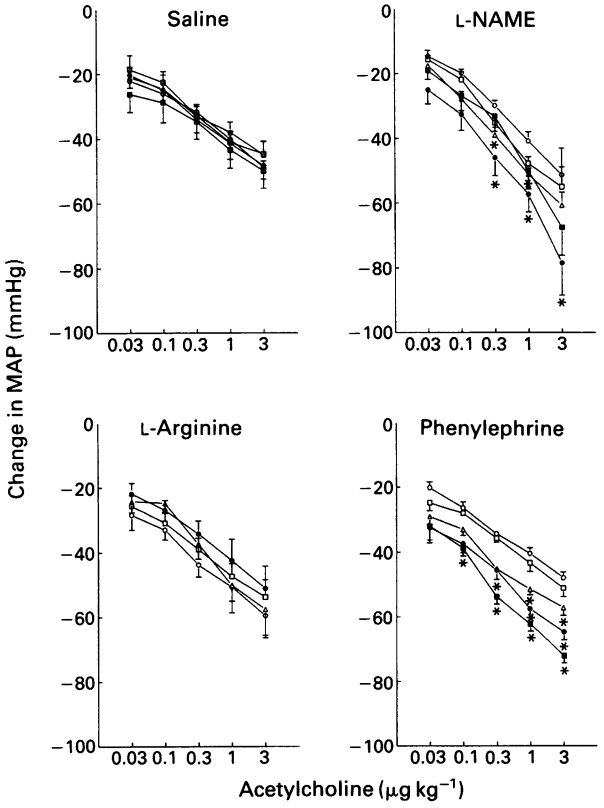

Figure 2 Decreases in mean arterial blood pressure (MAP) by acetylcholine (0.03–3.0 μg kg^{-1}) in rats before (control; ○) and after treatments with saline (1 ml kg^{-1} four times, $n = 8$), N^G-nitro-L-arginine methyl ester (L-NAME, 1, 3, 10 and 30 mg kg^{-1}, $n = 8$), phenylephrine (40, 120, 220 and 330 μg kg^{-1} h^{-1}, $n = 7$) or L-arginine (10, 30 and 100 mg kg^{-1}, $n = 7$). The increasing doses in each case are represented by the symbols (□), (△), (●) and (■), respectively. Data represent mean with s.e.mean shown by vertical bars. Asterisks denote significant differences from the values in the saline experiments, $P < 0.05$.

in the duration of action of acetylcholine (0.3 and 1.0 μg kg^{-1}) by L-NAME (Figure 3).

Cats

Haemodynamic effects The baseline values of the systemic and regional haemodynamic variables in the two groups of cats receiving, respectively, saline and L-NAME, both followed by L-arginine (300 mg kg^{-1}) are listed in Table 1. There were no significant differences between the two groups.

Figure 4 depicts the percentage changes in the systemic haemodynamic variables caused by saline (three injections) and L-NAME (1, 3 and 30 mg kg^{-1}). Treatment with saline did not cause any significant change other than a small decrease in heart rate. Similarly, the systemic haemodynamic effects of L-NAME in the cat were not marked. Although there was a small increase in total peripheral resistance (29 ± 5%) after 3 mg kg^{-1} L-NAME, these changes were only slightly more than those with the corresponding dose of saline (12 ± 5%). In the saline group, L-arginine decreased heart rate significantly (21 ± 3%), but cardiac output increased (12 ± 3%) due to an increase in stroke volume (42 ± 7%) (Figure 4). Administration of L-arginine did not cause any significant effect in cats treated with L-NAME, but the changes observed in the saline group appeared to be blunted (Figure 4).

The effects of L-NAME (1, 3 and 30 mg kg^{-1}) on tissue blood flows in the cat are shown in Figure 5. With the lowest dose no changes were observed, but the higher doses of L-NAME significantly reduced blood flows to several tissues including the mesentery, stomach, spleen, intestines, lungs and the total liver (Figure 5). L-Arginine (300 mg kg^{-1}) injected into the control (saline-treated) animals resulted in a significant increase in blood flow to the heart, mesentery, lungs as well as the total liver, particularly its portal fraction (Figure

Table 1 Baseline values of systemic haemodynamic variables and organ blood flow in anaesthetized cats treated with saline ($n = 5$) or N^G-nitro-L-arginine methyl ester (L-NAME) ($n = 7$)

	Saline	*L-NAME*
Systemic haemodynamic variables		
Mean arterial blood pressure (mmHg)	101.2 ± 6.1	95.1 ± 5.6
Heart rate (beats min^{-1})	153.0 ± 4.9	143.3 ± 14.6
Stroke volume (ml)	3.3 ± 0.3	3.9 ± 0.8
Cardiac output (ml min^{-1})	503.8 ± 42.1	512.7 ± 76.9
Total peripheral resistance (mmHg l^{-1} min^{-1})	203.5 ± 11.4	214.4 ± 29.0
Organ blood flow (ml min^{-1})		
Heart	13.5 ± 5.0	10.1 ± 1.5
Brain	8.5 ± 1.8	7.1 ± 1.1
Lungs	39.9 ± 5.7	39.8 ± 5.2
Stomach	8.0 ± 2.1	7.2 ± 1.7
Intestine	27.8 ± 6.0	25.6 ± 3.3
Spleen	14.0 ± 4.6	7.1 ± 1.6
Mesentery	7.6 ± 2.6	5.2 ± 1.1
Portal	68.7 ± 16.4	58.4 ± 9.5
Total liver	86.2 ± 23.7	71.0 ± 8.9
Kidneys	39.2 ± 5.9	38.5 ± 4.4
Muscle	3.8 ± 0.9	3.4 ± 0.4
Skin	1.4 ± 0.2	1.5 ± 0.4

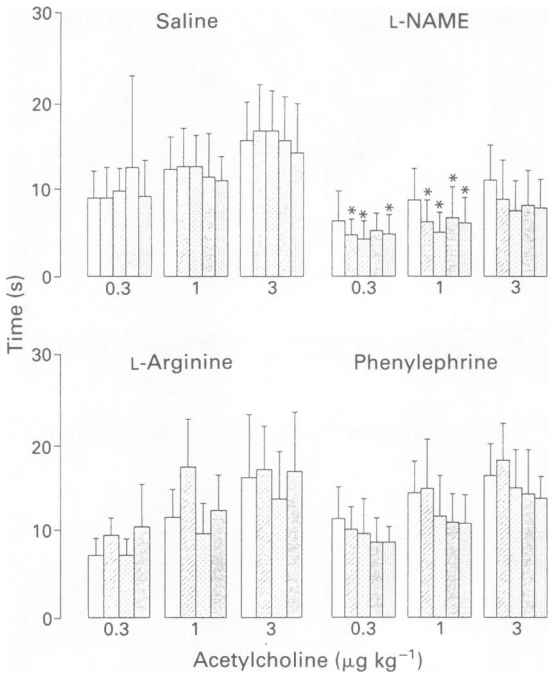

Figure 3 Time needed for 50% recovery in the hypotensive action of acetylcholine (0.3, 1 and 3 $\mu g\,kg^{-1}$) in rats treated with saline (1 $ml\,kg^{-1}$ four times, $n = 8$), N^G-nitro-L-arginine methyl ester (L-NAME, 1, 3, 10 and 30 $mg\,kg^{-1}$, $n = 8$), L-arginine (10, 30 and 100 $mg\,kg^{-1}$, $n = 7$) or phenylephrine (40, 120, 220 and 330 $\mu g\,kg^{-1}\,h^{-1}$, $n = 7$). In each set of panels, the 5 columns (4 in case of L-arginine) represent, sequentially, the responses before and after the increasing doses of saline or drugs. Data represent mean with s.e.mean shown by vertical bars. Asterisks denote significant differences from the values in the saline experiments, $P < 0.05$.

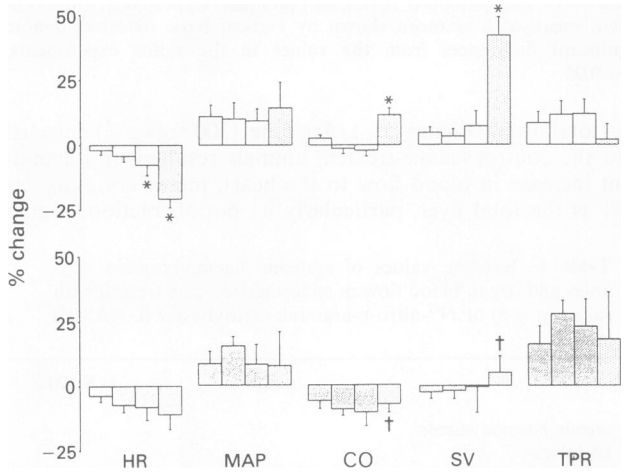

Figure 4 Percentage changes from baseline in systemic haemodynamic values in cats treated with saline (1 $ml\,kg^{-1}$ three times, $n = 5$) or N^G-nitro-L-arginine methyl ester (L-NAME, 1, 3 and 30 $mg\,kg^{-1}$, $n = 7$). Data after the 3rd dose of saline or L-NAME (10 $mg\,kg^{-1}$), where no microspheres were given (see Methods), were not collated. In each set of panels, the first three columns represent, sequentially, the increasing doses of saline or L-NAME, while the fourth column represents L-arginine (300 $mg\,kg^{-1}$). Abbreviations: heart rate (HR), mean arterial blood pressure (MAP), cardiac output (CO), stroke volume (SV) and total peripheral resistance (TPR). Data represent mean with s.e.mean shown by vertical bars. Asterisks denote significant changes compared to baseline values, crosses denote significant changes compared to saline experiments, $P < 0.05$.

5). The changes induced by L-arginine in animals treated with L-NAME were significantly different from the changes in saline-treated animals in the mesentery, kidneys, spleen, lungs as well as the total liver and portal blood flow (Figure 5).

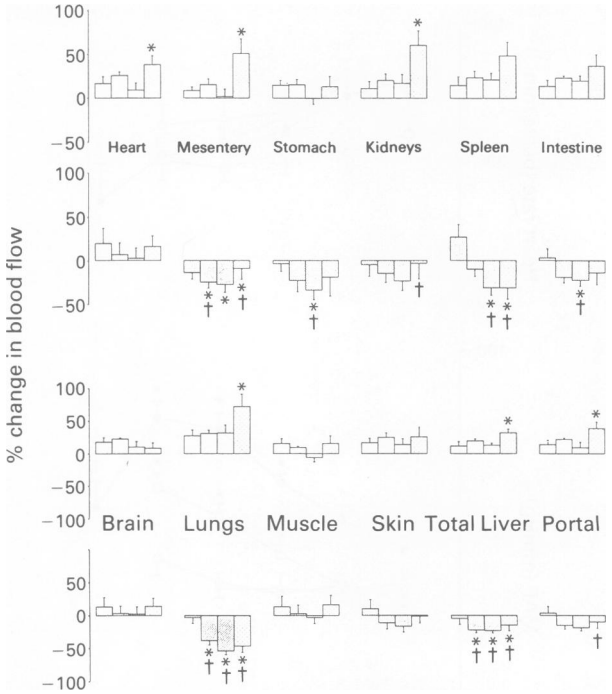

Figure 5 Percentage changes from baseline in tissue blood flows in cats treated with saline (1 $ml\,kg^{-1}$ three times, $n = 5$) or N^G nitro-L-arginine methyl ester (L-NAME, 1, 3 and 30 $mg\,kg^{-1}$, $n = 7$). As mentioned in Methods, tissue blood flows were not measured after the 3rd dose of saline or L-NAME (10 $mg\,kg^{-1}$). In each set of panels, the first three columns, sequentially, represent the increasing doses of saline or L-NAME, while the fourth column represents L-arginine (300 $mg\,kg^{-1}$). Data represent mean with s.e.mean shown by vertical bars. Asterisks denote significant changes compared to baseline values, crosses denote significant changes compared to saline experiments, $P < 0.05$.

Acetylcholine responses Acetylcholine induced a dose-dependent hypotensive effect in anaesthetized cats in doses ranging from 0.03 to 3.0 $\mu g\,kg^{-1}$. The fall in mean arterial blood pressure after the highest dose was 62 ± 5 mmHg ($n = 9$). The calculated dose of acetylcholine inducing a 40 mmHg decrease in mean blood pressure (D_{40}) was $0.11 \pm 0.04\,\mu g\,kg^{-1}$ ($n = 9$). L-NAME (1, 3, 10 and 30 $mg\,kg^{-1}$) did not significantly affect the fall in blood pressure induced by acetylcholine (Figure 6). However, L-NAME (from 3 $mg\,kg^{-1}$ and higher) was able to reduce the duration of action of acetylcholine; the time needed for 50% recovery in the acetylcholine responses was clearly reduced (Figure 7).

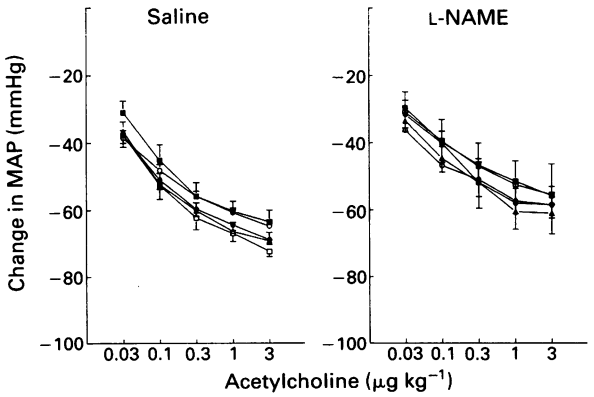

Figure 6 Decreases in mean arterial blood pressure (MAP) by acetylcholine (0.03–3.0 $\mu g\,kg^{-1}$) in cats before (control; ○) and after treatments with saline (1 $ml\,kg^{-1}$ four times, $n = 5$) or N^G-nitro-L-arginine methyl ester (L-NAME, 1, 3, 10 and 30 $mg\,kg^{-1}$, $n = 6$). The increasing doses in each case are represented by the symbols (□), (△), (●) and (■) respectively. Data represent mean with s.e.mean shown by vertical bars. No significant differences between saline and L-NAME experiments were observed.

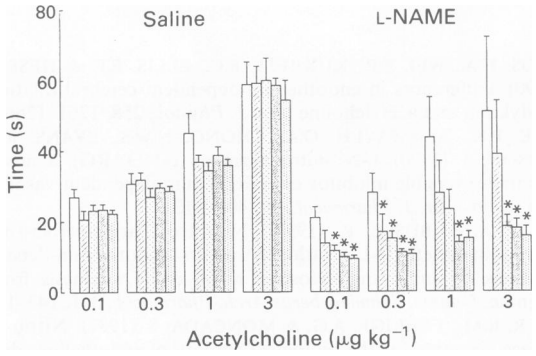

Figure 7 Time needed for 50% recovery in the hypotensive action of acetylcholine (0.3–3.0 $\mu g\,kg^{-1}$) in cats treated with saline (1 $ml\,kg^{-1}$ four times, $n = 5$) or N^G-nitro-L-arginine methyl ester (L-NAME, 1, 3, 10 and 30 $mg\,kg^{-1}$, $n = 6$). In each set of panels, the 5 columns represent, sequentially, the responses before and after increasing doses of saline or L-NAME. Data represent mean with s.e.mean shown by vertical bars. Asterisks denote significant differences from the values in the saline experiments, $P < 0.05$.

Discussion

Haemodynamic responses

Our findings that L-NAME, when injected in pentobarbitone-anaesthetized rats, induced a significant dose-dependent increase in blood pressure and reversal by L-arginine is consistent with results found in conscious as well as anaesthetized rats (Gardiner *et al.*, 1990a,b,c; Rees *et al.*, 1990). However, the concomitant fall in heart rate reported in anaesthetized rats by Rees *et al.* (1990) was comparatively less marked with L-NAME in our experiments as heart rate also decreased in animals treated with saline only. Administration of L-arginine, particularly when the dose was higher (100 fold) than that of L-NAME (see also Rees *et al.*, 1990), reversed the increases in blood pressure induced by L-NAME.

In contrast to rats, surprisingly, no significant changes in mean arterial blood pressure were found with four consecutive doses of L-NAME (1, 3, 10 and 30 $mg\,kg^{-1}$) in cats anaesthetized with pentobarbitone and ketamine (initially). This inability of L-NAME to elicit hypertension has also been observed in cats anaesthetized with α-chloralose and pentobarbitone (R. Saxena, E. Scheffers & R. Shukla, unpublished). Though L-NAME had little overall effect on haemodynamics, the drug did elicit a dose-dependent and significant decrease in blood flow to and, therefore, an increase in vascular resistance in several tissues (lungs, liver, stomach, small intestine and mesentery), suggesting an inhibition of the release of NO by L-NAME in these organs. Administration of L-arginine in cats, treated with L-NAME, did not reverse the decrease in blood flow induced by L-NAME. In saline-treated animals, however, L-arginine revealed a significant increase in blood flow in the lungs, mesentery, liver and several other organs, including the heart and the kidneys. The above results suggest that in cats there seems to be a difference in the basal release of NO; some organs, like the lungs, liver and mesentery, show more basal release than other tissues like the heart and kidneys. The findings that neither L-NAME nor L-arginine changed the mean arterial blood pressure and that L-NAME induced a decrease in blood flow in only a few organs suggest little contribution of the L-arginine-NO pathway to blood pressure regulation in the anaesthetized cat.

Although L-arginine increased blood flow in several organs, the total peripheral resistance did not change. This can be due to a concomitant increase in cardiac output, following an enhanced stroke volume due to an improved cardiac filling. Gardiner *et al.* (1990c) suggest that the decrease in cardiac output induced by L-NAME in rats could be due to a direct negative inotropic action or due to a coronary vasoconstriction. Indeed, L-NAME can induce coronary vasoconstriction in conscious rabbits (Humphries *et al.*, 1991). Our experiments in cats, however, show that despite a significant increase in myocardial blood flow by L-arginine, L-NAME neither decreased coronary artery blood flow nor decreased cardiac output. This again suggests that, unlike the rat, there is little spontaneous release of NO from the cat heart.

Acetylcholine responses

There seems to be little doubt that the vascular smooth muscle relaxation *in vitro* or local vasodilatation in several vascular beds following acetylcholine administration in different species is largely dependent on the release of NO and is, consequently, inhibited by drugs like L-NAME (Aisaka *et al.*, 1989; Furchgott & Vanhoutte, 1989; Vallance *et al.*, 1989; Whittle *et al.*, 1989; Kontos *et al.*, 1990; Moore *et al.*, 1990). However, it has also been reported that the hypotensive response to acetylcholine in conscious rats is not amenable to blockade by L-NAME (Gardiner *et al.*, 1990b). Our results, both in anaesthetized rats and cats, are in agreement with the latter findings. Indeed, the hypotensive effect of acetylcholine was even potentiated in the rat after treatment with 10 $mg\,kg^{-1}$ L-NAME. This apparent potentiation was due to the increase in arterial pressure by L-NAME, since it was also observed after phenylephrine infusions in the rat, but not during L-NAME treatment in the cat where arterial pressure was not affected by the drug. Therefore, it seems likely that the magnitude of the hypotension elicited by acetylcholine *in vivo* is not dependent on the release of NO. It may, however, be noted that the highest dose of L-NAME (30 $mg\,kg^{-1}$) did not further increase the depressor responses to acetylcholine in rats; on the contrary the magnitude of the responses were not significantly different from those in saline-treated animals (see Figure 2), suggesting that there may be some inhibition of the acetylcholine depressor effects in rats at the highest dose of L-NAME.

Despite little change in the magnitude of the hypotensive response to acetylcholine, there was a significant reduction in its duration by L-NAME in both cats and rats; phenylephrine infusion in the rat had no effect. In contrast to experiments in guinea-pigs with L-NMMA (Aisaka *et al.*, 1989), L-arginine did not prolong the duration of the acetylcholine response in our experiments. Nevertheless, our findings do support the suggestions of Aisaka *et al.* (1989) that the first phase of the depressor response to acetylcholine is NO-independent. It is tempting to speculate that the second phase is, in part, due to a hyperpolarization of the vascular smooth muscle resulting from the release of an endothelium-dependent-hyperpolarizing-factor (EDHF). Indeed, in dog mesenteric arteries the hyperpolarization induced by acetylcholine, in contrast to the relaxation, seems to be NO-independent, since oxyhaemoglobin and methylene blue prevented only the latter (Komori *et al.*, 1988). However, in guinea-pig isolated uterine arteries L-NMMA blocked both hyperpolarization and relaxation responses to acetylcholine, suggesting that EDHF may be identical to NO in this species (Tare *et al.*, 1990).

Lastly, it may be mentioned that acetylcholine, when applied topically, induces cerebral dilatation in cats by releasing an EDRF-like substance (Kontos *et al.*, 1990). Our experiments, however showed no significant increase in brain blood flow by L-arginine or a decrease by L-NAME. These findings may indicate that, unlike large conducting vessels, there is only a limited contribution of EDRF/NO to cerebral blood flow regulation.

In conclusion, the present *in vivo* experiments reveal a marked difference between the effects of L-NAME in rats and cats; in contrast to rats, the contribution of the L-arginine-NO pathway to blood pressure regulation in the cat seems to be very limited, although such a pathway seems to exist in several tissues. In addition, L-NAME does not affect the magnitude of hypotensive response to acetylcholine in either species, but reduces its duration.

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