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Increase by N^G-nitro-L-arginine methyl ester (L-NAME) of resistance to venous return in rats

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1 The effects of the nitric oxide (NO) synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME), on mean circulatory filling pressure (MCFP), total peripheral resistance (TPR), cardiac output (CO) and resistance to venous return (R_v) were studied in rats.

2 In conscious, unrestrained rats, L-NAME (0.5–16 mg kg^{-1}) dose-dependently increased mean arterial pressure (MAP) but not MCFP, an inverse index of venous compliance, either in the absence or presence of the ganglionic blocker mecamylamine (10 mg kg^{-1}).

3 In pentobarbitone-anaesthetized rats, L-NAME (2, 4, 8 mg kg^{-1}) increased MAP and reduced CO in a dose-related manner but did not change MCFP. TPR (+84, +140 and +192%) as well as R_v (+62, +72, +110%) were dose-dependently increased by L-NAME.

4 Our results show that L-NAME reduces CO by increasing arterial as well as venous resistances. L-NAME does not affect MCFP.

Keywords: N^G-nitro-L-arginine methyl ester (L-NAME); nitric oxide; mean circulatory filling pressure (MCFP); cardiac output, total peripheral resistance, venous resistance, capacitance vessels

Introduction

N^G-substituted arginine (Arg) analogues, which include N^G-monomethyl-L-arginine (L-NMMA), N^G-nitro-L-Arg (L-NOARG) and its methyl ester (L-NAME), are nitric oxide (NO) synthase inhibitors which suppress endothelium-dependent relaxation of arterial preparations *in vitro* and raise blood pressure *in vivo* (Aisaka *et al.*, 1989; Rees *et al.*, 1989; 1990; Wang & Pang, 1991; Wang *et al.*, 1993a,b,c; see Moncada *et al.*, 1991). The N^G-substituted Arg analogues also inhibit endothelium-dependent relaxations or cause contractions in venous smooth muscles which include the bovine pulmonary vein (Gold *et al.*, 1990), canine and rat femoral veins (Miller, 1991; Nagao & Vanhoutte, 1991), canine renal vein (Pawloski & Chapnick, 1991), rabbit external jugular vein (Martin *et al.*, 1992) and canine saphenous vein (Elmore *et al.*, 1992) *in vitro*.

There are few studies which investigate the effect of NO synthase inhibitors in the control of venomotor tone. The i.v. bolus injection of a single dose (10 mg kg^{-1}) of L-NAME into anaesthetized rabbits increased arterial pressure and reduced the cross-sectional area of the inferior vena cava suggesting venoconstriction (Schwarzacher *et al.*, 1992). However, it is unlear if the constriction was active or passive, due to a reduction in cardiac output. Intravenous bolus injection of L-NAME (37 μmol kg^{-1}) into ganglion-blocked, artificially-ventilated and anaesthetized cats receiving a continuous infusion of noradrenaline to maintain vascular tone, caused large increases in arterial and venous resistances but only a small rise in mean circulatory filling pressure (MCFP), an index of body venous tone (Bower & Law, 1993). In conscious, partially-restrained rats, i.v. injection of L-NMMA (25 mg kg^{-1}) caused a large increase in arterial pressure but only a small rise in MCFP (Glick *et al.*, 1993).

There is, as yet, no published study which concurrently investigates the dose-response effects of NO synthase inhibitors on MCFP and venous resistance in the intact animal. The aim of this study was to examine the dose-response effects of L-NAME on MCFP and resistance to venous return in rats. Since anaesthesia and surgical stress may affect the equilibration of central venous pressure and portal venous pressure, thereby altering the contribution of venous pressure from the splanchnic capacitance vessels (see Tabrizchi & Pang, 1992; Tabrizchi *et al.*, 1993; Pang, 1994), MCFP measurements were made in anaesthetized as well as conscious rats.

Methods

Surgery

Male Sprague-Dawley rats (320–500 g) were anaesthetized with pentobarbitone (60 mg kg^{-1}) or halothane (2% in air). Body temperature was maintained at 37°C via a rectal thermometer and a heating pad connected to a Thermistemp Temperature Controller (Model 71; Yellow Spring Instrument Co Inc., OH, U.S.A.). A polyethylene cannula (PE5O) was inserted into the left iliac artery, for the recordings of mean arterial pressure (MAP), by a pressure transducer (P23DB, Gould Statham, CA, U.S.A.), and heart rate (HR), which was derived electronically from the upstroke of the arterial pulse pressure by a tachograph (Grass, Model 7P4G). PE50 cannulae were also inserted into the right iliac vein, for the administration of vehicle or drugs, and the inferior vena cava *via* the left iliac vein, for the measurement of central venous pressure (CVP) by another pressure transducer (P23DB, Gould Statham). A saline-filled, balloon-tipped catheter was inserted into the right atrium *via* the right external jugular vein. The proper location of the atrial balloon was tested by injecting saline into the balloon and obtaining a simultaneous decrease of MAP to 20–25 mmHg and an increase of CVP within 5 s of circulatory arrest. MAP, HR and CVP were continuously monitored and recorded by a Grass Polygraph (Model RPS 7C8).

In pentobarbitone-anaesthetized rats, an additional cannula was inserted via the right carotid artery into the left ventricle for the injection of radioactively-labelled microspheres, and into the left iliac artery for blood withdrawal, as described in detail elsewhere (Pang, 1983). These rats were given 30 min to stabilize before MAP, MCFP and cardiac output (CO) measurements were made.

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In halothane-anaesthetized rats, all cannulae were tunneled s.c. along the back, exteriorized at the back of the neck and secured. Bupivacaine (local anaesthetic, 0.25% solution) and cicatrin (antibiotic) were applied topically to the surgical wounds to alleviate pain and prevent infection, respectively. Halothane was withdrawn after the completion of surgery which took about 30 min. The rats were in the upright posture within 10 min of removing halothane and were used 6–8 h later. In our experience, 6 h is sufficient time for the recovery of the effects of brief halothane anaesthesia and minor surgery on haemodynamic measurements. Readings of MAP, HR and MCFP obtained in rats recovered from anaesthesia and surgery for 6 h were not different from those recovered from anaesthesia and surgery for 24 h. The conscious rats were allowed to wander freely in a small cage. The method for measuring MCFP in rats has been described in detail (see Tabrizchi & Pang, 1992). Briefly, steady-state readings of MAP and CVP were noted at 4 to 5 s after circulatory arrest via inflation of the implanted balloon. To avoid rapid equilibration of arterial and venous pressures during circulatory arrest, the arterial pressure contributed by the small amount of trapped arterial blood was corrected by the following equation: MCFP = VPP + 1/60 (FAP-VPP), where FAP and VPP represents the final arterial pressure and venous plateau pressure, respectively, obtained within 5 s of circulatory arrest, and 1/60 represents the ratio of arterial to venous compliance.

Microspheres studies

A well-stirred suspension (200 μl) containing 20,000–25,000 microspheres (15 μm diameter), labelled with ^{57}Co (Du Pont Canada Inc., Ontario, Canada), was injected and flushed over 10 s into the left ventricle in the control period and 20 min after the i.v. bolus injection of a drug or vehicle. At 10 s before the injection of each set of microspheres, blood was withdrawn (Harvard infusion/withdrawal pump) from the iliac arterial cannula into a heparinized syringe at 0.35 ml min^{-1} for 45 s. The blood removed was slowly injected back to the rats immediately after the counting of radioactivity at 80–160 kev using a 1185 Series Dual Channel Automatic Gamma Counter (Nuclear-Chicago, IL, U.S.A.) with a 3 inch NaI crystal.

Experimental protocol

Pentobarbitone-anaesthetized rats Rats were randomly divided into 2 groups ($n = 6$ each). At 20 min after a baseline measurement of CO followed by MCFP, one group received i.v. bolus injections of cumulative doses of L-NAME (2, 4, 8 mg kg^{-1} or 7, 15, 30 μmol kg^{-1}) whereas in the other group, injections of equal volumes of the vehicle (0.9% NaCl, 0.2 to 0.5 ml kg^{-1}) were made, at dose-intervals of 20 min. CO followed by MCFP measurements were made after the injection of a drug or vehicle, at the plateau phase of the response to L-NAME. A blood sample was removed at the end of the stabilization period and after the completion of the study to monitor any changes in haematocrit.

Conscious rats Rats were randomly divided into four groups ($n = 6$ each). In two groups of intact rats a cumulative dose-response curve was obtained to i.v. bolus injections of L-NAME (0.5–16 mg kg^{-1} or 1.9–59.0 μmol kg^{-1}) or to equal volumes (0.06–1 mg kg^{-1}) of normal saline. MCFP measurements were made at 10 min after the injection of a drug or vehicle. Mecamylamine (10 mg kg^{-1}) was given as an i.v. bolus injection to another two groups of rats. This dose of mecamylamine was found to block ganglionic transmission effectively for more than 2 h (Wang & Pang, 1991). Ten minutes later, i.v. bolus injections of L-NAME or vehicle were also given to each group.

Drugs

L-NAME hydrochloride and mecamylamine hydrochloride were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.) and dissolved in normal saline (0.9% NaCl solution). Cicatrin was from Burroughs Wellcome Inc., (Que., Canada) and bupivacaine HCl was from Sanofi Winthrop (Ont, Canada).

Calculations and statistics analysis

CO (ml min^{-1}), total peripheral resistance (TPR, mmHg min ml^{-1}) and venous resistance (R_v; mmHg min ml^{-1}) were calculated as follows:

$$CO = \frac{\text{rate of withdrawal of blood} \times \text{total injected c.p.m.}}{\text{c.p.m. in withdrawn blood}}$$

$$TPR = \frac{BP}{CO}$$

$$R_v = \frac{MCFP - CVP}{CO}$$

Due to the technical difficulty of measuring right atrial pressure and MCFP concurrently in small animals, CVP rather than right atrial pressure was used to estimate pressure gradient to venous return (MCFP-right atrial pressure). This is legitimate since mean CVP is nearly identical to mean right atrial pressure (see Rothe, 1993).

All results were expressed as mean ± s.e.mean and analysed by the analysis of variance/covariance followed by Duncan's multiple range test, with $P < 0.05$ selected as the criterion for statistical significance. Profile/trend analysis was used to compare dose-dependency of responses using the statistical package, SYSTAT v. 5.03 (SYSTAT Inc., IL, U.S.A.).

Results

Anaesthetized rats

The vehicle did not alter significantly MAP, HR, R_v, TPR or MCFP (Figure 1) but slightly increased CO, which, at the highest dose, was significantly different from baseline CO (Figure 1e). There was slight blood loss during surgery, as haematocrit was insignificantly reduced from 55 ± 3 to 50 ± 2% from the beginning to the end of the study ($P > 0.05$). L-NAME significantly and dose-dependently ($P < 0.05$) increased MAP (Figure 1a), R_v and TPR (Figure 1c,d) and reduced CO (Figure 1e) but did not alter MCFP (Figure 1f), when the values were compared with the corresponding readings in the vehicle group. HR was insignificantly decreased (Figure 1b). The haematocrit was also insignificantly decreased ($P > 0.05$) in L-NAME-treated rats, from a baseline value of 57 ± 1 to 51 ± 1% from the beginning to the end of the study.

Conscious rats

Circulatory arrest for <6 s did not cause any distress or visible behavioural disturbances in the rats. The four groups of rats had similar baseline readings of MAP, HR and MCFP (Table 1). In two groups of rats given mecamylamine, MAP, HR and MCFP were decreased by 35%, 7% and 23% of control readings (pooled values), respectively (Table 1).

In intact rats, there were slight decreases in MCFP but not MAP or HR with the passage of time (Figure 2a,b,c). L-NAME significantly and dose-dependently ($P < 0.05$) increased MAP and decreased HR but did not alter MCFP, when the values were compared with the corresponding time-control readings in rats given the vehicle (Figure 2a,b,c).

Table 1 Baseline values of mean arterial pressure (MAP), heart rate (HR) and mean circulatory filling pressure (MCFP) in four groups ($n = 6$ each) of conscious, unrestrained rats to be given i.v. bolus injections of N^G-nitro-L-arginine methyl ester (L-NAME) (I and III) or normal saline (II and IV)

	MAP (mmHg)		*HR* (beats min^{-1})		*MCFP* (mmHg)	
	Before	*After*	*Before*	*After*	*Before*	*After*
I	102 ± 3	–	407 ± 8	–	6.6 ± 0.3	–
II	100 ± 2	–	400 ± 7	–	6.8 ± 0.2	–
Pooled	101 ± 2	–	404 ± 6	–	6.7 ± 0.2	–
III	104 ± 4	72 ± 4[a]	378 ± 9	355 ± 11[a]	6.5 ± 0.3	5.1 ± 0.3[a]
IV	105 ± 2	65 ± 2[a]	398 ± 7	367 ± 14[a]	6.4 ± 0.2	4.9 ± 0.2[a]
Pooled	105 ± 2	68 ± 3[a]	390 ± 6	362 ± 10[a]	6.4 ± 0.2	5.0 ± 0.2[a]

Values are mean ± s.e.mean. [a]denotes significant difference from saline-treated control group ($P < 0.05$). Baseline readings of MAP, HR and MCFP in Groups III and IV were obtained before as well as 10 min after pretreatment with mecamylamine.

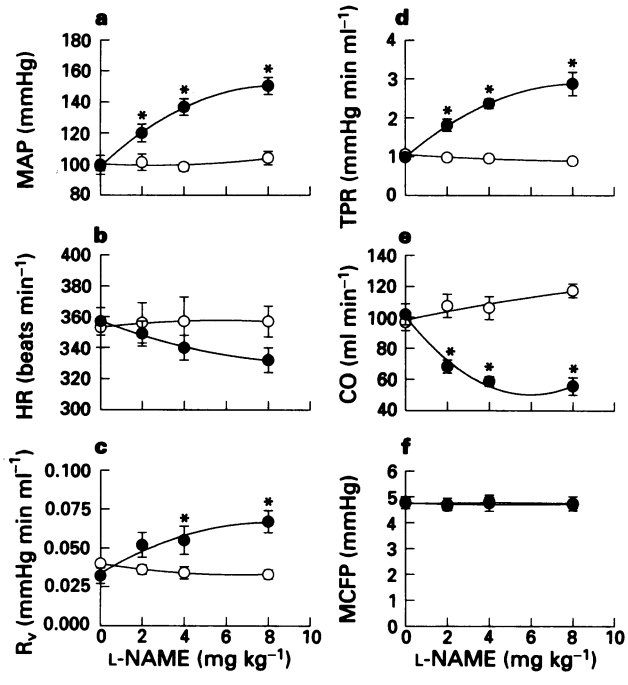

Figure 1 Effects (mean ± s.e.mean) of i.v. bolus injections of N^G-nitro-L-arginine methyl ester (L-NAME, ●) and equal volumes of vehicle (0.9% NaCl, ○) on mean arterial pressure (MAP, a), heart rate (HR, b), venous resistance (R_v, c), total peripheral resistance (TPR, d), cardiac output (CO, e) and mean circulatory filling pressure (MCFP, f) in pentobarbitone-anaesthetized rats ($n = 6$ each group). *Denotes significant difference from the corresponding reading in vehicle-treated rats ($P < 0.05$).

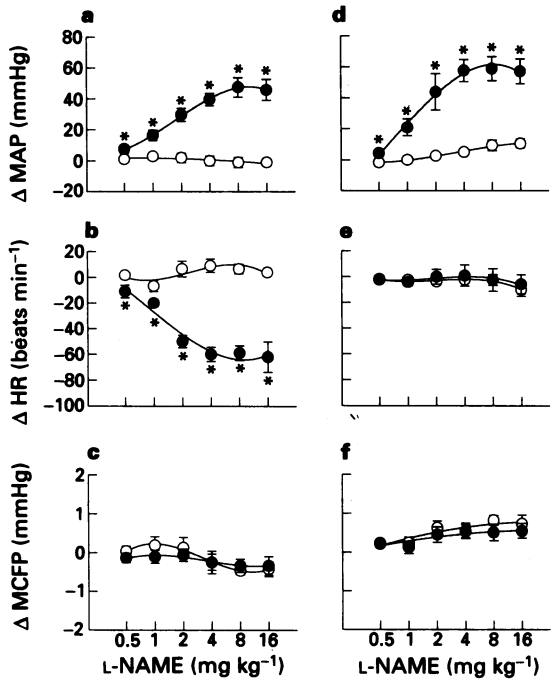

Figure 2 Effects (means ± s.e.mean) of i.v. bolus injections of N^G-nitro-L-arginine methyl ester (L-NAME, ●) and equal volumes of vehicle (0.9% NaCl, ○) on mean arterial pressure (MAP), heart rate (HR) and mean circulatory filling pressure (MCFP) in conscious, unrestrained rats ($n = 6$ each group) in the absence (a, b, c) and presence (d, e, f) of mecamylamine (10 mg kg^{-1}, i.v. bolus injection).

In mecamylamine-pretreated rats, the vehicle caused slight increases in MAP and MCFP but not HR (Figure 2d,e,f). L-NAME caused marked and dose-dependent ($P < 0.05$) increases in MAP but did not alter HR or MCFP when the readings were compared with the corresponding time-controls in rats given the vehicle (Figure 2d,e,f).

Discussion

Our results show that L-NAME raises MAP but not MCFP in anaesthetized rats. Since a combination of anaesthesia and surgical stress may interfere with the equilibration of CVP and portal venous pressure thereby reducing pressure contribution from the splanchnic venous bed (Tabrizchi & Pang, 1992; Tabrizchi *et al.*, 1993; Pang, 1994), MCFP measurements were also made in conscious, freely-moving rats. The results show that L-NAME also did not alter MCFP in either intact or ganglion-blocked conscious rats. Unlike L-NAME, noradrenaline, angiotensin II as well as angiotensin III (Pang & Tabrizchi, 1986; Tabrizchi & Pang, 1987; Tabrizchi *et al.*, 1992) were found in similar studies in our laboratory to raise MAP and MCFP dose-dependently. Therefore, the absence of a rise in MCFP in the present study is not due to a lack of sensitivity of the technique to detect changes. Our results are somewhat different from those of Bower & Law (1993) which show that a large dose of L-NAME caused a significant though small increase in MCFP in reflex-blocked but noradrenaline-infused, artificially-ventilated and anaesthetized cats. In another study using partially-restrained rats, a single dose of L-NMMA caused a large elevation of MAP but a small increase in MCFP in only one of the two groups of intact rats (Glick *et al.*, 1993). Since MCFP is inversely proportional to venous compliance in the absence of a change in stressed blood volume (see Tabrizchi & Pang, 1992; Pang, 1994), the small effect of L-NAME on MCFP in various studies suggests that L-NAME is unimportant in the control of venous compliance. It should be noted that MCFP measurements do not reveal whether or not unstressed blood volume is altered, since only stressed blood volume con-

tributes to pressure development (see Pang, 1994). MCFP measurements alone also do not reveal if venous resistance is altered since MCFP readings are obtained during circulatory arrest (hence, an absence of flow resistance).

L-NAME significantly reduced HR in conscious rats but not anaesthetized rats. Attenuated reflex changes in HR are characteristic of anaesthetized rats. The bradycardic effect of L-NAME in intact rats was abolished by mecamylamine indicating the completeness of ganglionic blockade. The effectiveness of mecamylamine in suppressing the reflex bradycardic but not the pressor effect of L-NAME in conscious rats is consistent with our previous findings (Wang & Pang, 1991).

Our present and previous (Wang & Pang, 1991) results of the preservation of pressor responses to L-NOARG following ganglionic blockade are in accordance with those of others using pentolinium or chlorisondamine as the ganglionic blocker (Chyu *et al.*, 1992; Pucci *et al.*, 1992; Pegoraro *et al.*, 1992; Bower & Law, 1993). However, there are reports that ganglionic blockers (chlorisondamine or hexamethonium) abolish or attenuate pressor responses to L-NMMA or L-NOARG in anaesthetized rats or dogs (Vargas *et al.*, 1990; Lacolley *et al.*, 1991; Toda *et al.*, 1993), which suggest the existence of nitrergic, vasodilator nerves innervating the arterial wall (Toda *et al.*, 1993). To resolve these discrepancies, a systematic study of the pressor effects of N^G-nitro-L-Arg analogues using various ganglion blockers in conscious and anaesthetized animals is needed.

Our results also show that L-NAME increased MAP by elevating TPR rather than CO. In fact, CO was dose-dependently decreased by L-NAME. Similar observations of the effects of L-NAME and L-NOARG on CO and TPR have been reported (Gardiner *et al.*, 1990; Bower & Law, 1993; Wang *et al.*, 1993a; Herity *et al.*, 1994). L-NOARG was found to cause generalized peripheral vasoconstriction, as vascular conductances in all beds, which include the lungs, heart, liver, stomach, intestine, colon, kidneys, spleen, muscle skin, testes and brain, were reduced (Wang *et al.*, 1993a). The greatest vasoconstrictor influence was in the bronchial bed whereas the least was in the hepatic arterial bed. In addition to increasing arteriolar resistance (+84, +140, +192%), L-NAME also dose-dependently increased resistance to venous return (+62, +72 and +110%) at 7, 15, 30 μmol kg^{-1}, respectively). The L-NAME-induced increases in R_v are similar to, though larger in magnitude than, those in areflex, noradrenaline-infused cats, in which L-NAME (37 μmol kg^{-1}) increased R_v by 58%. These results show that the reduction in CO by L-NAME was a consequence of increased arteriolar and venous resistances.

Our results are supportive of an important role for N^G-substituted Arg analogues in the maintenance of venomotor tone. The prominent venous effect of L-NAME and related compounds is in elevating venous resistance, thereby reducing venous return. In contrast to VPP at circulatory arrest, CVP was not elevated by L-NAME in either conscious or anaesthetized rats (results not shown). The absence of a change in CVP by L-NAME is to be expected since CVP is downstream venous pressure (the resultant pressure after pressure drops at arterial and venous resistance sites) and is therefore a poor indicator of venomotor tone.

The pressor responses of N^G-substituted-L-Arg analogues are generally believed to be secondary to the inhibition of endogenous NO biosynthesis (Aisaka *et al.*, 1989; Rees *et al.*, 1989; see Moncada *et al.*, 1991). On the basis of this hypothesis, the marked effect of L-NAME on R_v suggests that endogenously synthesized NO lowers R_v.

To summarize, L-NAME dose-dependently increased MAP, TPR and R_v and reduced CO but did not alter MCFP in pentobarbitone-anaesthetized rats. L-NAME also dose-dependently raised MAP but not MCFP in conscious rats, both in the absence and presence of the ganglion blocker, mecamylamine. Our results show that L-NAME reduces CO by increasing resistance in arterioles and capacitance vessels.

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