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Possible dependence of pressor and heart rate effects of N^G-nitro-L-arginine on autonomic nerve activity

Yong-Xiang Wang & [1]Catherine C.Y. Pang

Department of Pharmacology & Therapeutics, Faculty of Medicine, The University of British Columbia, 2176 Health Sciences Mall, Vancouver, B.C. V6T 1Z3, Canada

1 The effects of N^G-nitro-L-arginine (L-NNA) on mean arterial pressure (MAP) and heart rate (HR) were investigated in conscious rats.

2 Intravenous bolus cumulative doses of L-NNA (1–32 mg kg^{-1}) dose-dependently increased MAP. Both mecamylamine and phentolamine increased MAP responses to L-NNA, angiotensin II and methoxamine. Propranolol, reserpine, atropine and captopril did not affect MAP response to L-NNA.

3 A significant negative correlation of HR and MAP responses to L-NNA was obtained in control rats but not in rats pretreated with reserpine or mecamylamine. Significant negative correlations also occurred in the presence of atropine, propranolol, phentolamine or captopril.

4 A single i.v. bolus dose of L-NNA (32 mg kg^{-1}) raised MAP to a peak value of 53 ± 3 mmHg and the effect lasted more than 2 h; the rise and recovery of MAP were accompanied by significant decrease and increase in HR, respectively. While both phentolamine and mecamylamine increased peak MAP response to L-NNA, mecamylamine abolished the biphasic HR response and phentolamine potentiated the bradycardic component of HR.

5 Blockade of the autonomic nervous and renin-angiotensin systems did not attenuate the pressor effects of L-NNA. However, the biphasic HR response to L-NNA is mediated via modulation of autonomic nerve activities.

Keywords: N^G-nitro-L-arginine (L-NNA); vasopressor; autonomic ganglion; sympathetic and parasympathetic nervous system; renin-angiotensin system

Introduction

There is evidence that endothelium-derived relaxing factor (EDRF) released by vascular endothelial cells is nitric oxide (NO) (Palmer *et al.*, 1987; Ignarro *et al.*, 1987) which is formed from the precursor L-arginine (L-Arg) (Palmer *et al.*, 1988; Sakuma *et al.*, 1988). It has been shown that NO synthase and endothelium-dependent vascular relaxation responses in isolated arteries are inhibited by N^G-substituted L-Arg analogues which include N^G-monomethyl-L-arginine (L-MMA) (Palmer *et al.*, 1988; Rees *et al.*, 1989a; 1990), N^G-nitro-L-arginine methyl ester (Moore *et al.*, 1990; Rees *et al.*, 1990), N-iminoethyl-L-ornithine (Rees *et al.*, 1990) and N^G-nitro-L-arginine (L-NNA) (Moore *et al.*, 1990; Mülsch & Busse, 1990; Kobayashi & Hattori, 1990; Ishii *et al.*, 1990). *In vivo* studies show that L-MMA (Rees *et al.*, 1989b; 1990; Aisaka *et al.*, 1989; Whittle *et al.*, 1989; Gardiner *et al.*, 1990b,c), N^G-nitro-L-arginine methyl ester (Gardiner *et al.*, 1990a,c,d), N-iminoethyl-L-ornithine (Rees *et al.*, 1990) and L-NNA (Wang & Pang, 1990) cause pressor responses and bradycardia.

Although it is likely that the pressor effects of L-Arg analogues are caused by the inhibition of NO production from vascular endothelial cells (Aisaka *et al.*, 1989; Rees *et al.*, 1989b; Wang & Pang, 1990), other vasopressor systems may contribute to the response. It has been shown that vascular endothelium inhibits the release of noradrenaline from sympathetic nerves which innervate canine pulmonary artery and vein suggesting that the endothelium, in part via endothelium-derived relaxing factor (EDRF) release, acted as an endogenous inhibitor of sympathetic transmitter release (Greenberg *et al.*, 1990). Togashi *et al.* (1990) showed that L-MMA increased postganglionic sympathetic nerve activities in intact and bilateral sino-aortic- and vagal-denervated rats and preganglionic adrenal nerve activity in sino-aortic and vagal-denervated rats. It has also been reported that EDRF inhibited renin release (Vidal *et al.*, 1988). Therefore it is logical to postulate that the haemodynamic effects of L-Arg analogues are partially mediated via potentiation of activities of the autonomic nervous and/or renin-angiotensin system(s).

The aims of this study were: (1) to assess the contribution of the autonomic nervous and renin-angiotensin systems on pressor response to L-NNA; (2) to examine whether the bradycardia produced in response to L-NNA is mediated via reflex activation of the autonomic nervous system.

Methods

Surgical preparation

Sprague-Dawley rats (240–400 g) were anaesthetized with halothane (4% in air for induction, 2% in air for surgical preparation). A polyethylene cannula (PE50) was inserted into the left iliac artery to allow recordings of mean arterial pressure (MAP). PE50 cannulae were also inserted into the right (or both) iliac vein(s) for the administration of drugs. The cannulae were filled with heparinized saline (25 iu ml^{-1}) and tunnelled s.c. along the back, exteriorized at the back of the neck and secured. The rats were given 6–7 h recovery of the effects of halothane and surgery before further use.

Experimental protocol

The indwelling arterial catheter from each rat was connected to a pressure transducer (P23DB, Gould Statham, CA, U.S.A.) for the recordings of MAP and heart rate (HR) which was derived electronically from the upstroke of the arterial pulse pressure by a tachograph (Grass, Model 7P4G). The conscious rats were allowed to wander freely in a small cage for 1 h before the administrations of drugs. MAP and HR were continuously monitored. The rats were killed by an overdose of pentobarbitone at the end of each experiment. Two main studies were conducted:

Dose-response curves for N^G-nitro-L-arginine Rats, randomly divided into seven groups ($n = 6$ each), were pretreated with: (I) normal saline (0.9% NaCl); (II) phentolamine (i.v. infusion,

[1] Author for correspondence.

Table 1 Baseline values of mean arterial pressure (MAP) and heart rate (HR) (mean ± s.e.mean) in conscious rats prior to and 40 min after the administration of normal saline, phentolamine, propranolol, reserpine, mecamylamine, atropine and captopril

	MAP (mmHg)		*HR* (beats min^{-1})	
Antagonists	*Before*	*After*	*Before*	*After*
Protocol 1				
Normal saline	103 ± 5	104 ± 5	403 ± 10	410 ± 8
Phentolamine	106 ± 4	69 ± 7*	442 ± 12	518 ± 18*
Propranolol	104 ± 4	104 ± 4	364 ± 10	334 ± 8*
Reserpine	—	80 ± 5†	—	295 ± 11†
Mecamylamine	113 ± 3	88 ± 4*	377 ± 9	313 ± 13*
Atropine	116 ± 2	118 ± 3	370 ± 23	432 ± 18*
Captopril	105 ± 6	103 ± 5	372 ± 19	418 ± 16*
Protocol 2				
Normal saline	106 ± 3	104 ± 3	343 ± 13	344 ± 13
Mecamylamine	108 ± 4	69 ± 2*	431 ± 10	308 ± 8*
Phentolamine	101 ± 5	72 ± 3*	401 ± 13	443 ± 8*

* Denotes significant difference from corresponding control values within the same group ($P < 0.05$); † Denotes significant difference from normal saline group ($P < 0.05$). $n = 6$ per group.

300 μg kg^{-1} min^{-1}); (III) propranolol (i.v. bolus at 1 mg kg^{-1} followed by infusion at 1.6 μg kg^{-1} min^{-1}); (IV) reserpine (5 mg kg^{-1}, i.p., 24 h prior to the study); (V) mecamylamine (i.v. bolus at 10 mg kg^{-1} followed by infusion at 300 μg kg^{-1} min^{-1}); (VI) atropine (i.v. bolus at 10 mg kg^{-1} followed by infusion at 8 μg kg^{-1} min^{-1}) and (VII) captopril (20 mg kg^{-1}, i.v. bolus). With the exception of reserpine and captopril, all antagonists were continuously infused for approximately 160 min, i.e., to the end of the experiment. Cumulative doses of L-NNA (1–32 mg kg^{-1}, i.v. bolus) were given 40 min after the administration of the vehicle or blockers at dose-intervals of 15–20 min, the period required to obtain steady state MAP responses. A single dose of angiotensin I (1 μg kg^{-1}), methoxamine (20 or 30 μg kg^{-1}), acetylcholine (1 μg kg^{-1}) or isoprenaline (1 μg kg^{-1}) was injected as an i.v. bolus prior to and 20 min after the start of administration of captopril, phentolamine, atropine or propranolol, respectively, and again 2 h after giving L-NNA to assess the degrees of inhibition at the start and completion of the studies. In rats pretreated with reserpine and vehicle, tyramine (200 μg kg^{-1}) was injected as an i.v. bolus 20 min prior to and 2 h after giving L-NNA. Excluding the equilibration time, the duration of each study was approximately 3 h.

Time course of responses to a single dose of N^G-nitro-L-arginine Another three groups of rats ($n = 6$ each) were pretreated with: (VIII) normal saline; (IX) mecamylamine; (X) phentolamine, at the same doses as those described previously. In phentolamine and mecamylamine groups, angiotensin II was injected as an i.v. bolus prior to and 20 min after the administration of a blocker. In Groups VIII, IX and X, a single dose of L-NNA (32 mg kg^{-1}) was injected as an i.v. bolus 40 min after the start of administration of a blocker. MAP and HR were continuously monitored for 2 h.

Drugs

The following drugs were obtained from Sigma Chemical Co. (MO, U.S.A.): N^G-nitro-L-arginine (L-NNA), mecamylamine hydrochloride, atropine sulphate, Des-Asp^1-angiotensin I acetate, angiotensin II acetate, acetylcholine hydrochloride, (±)-propranolol hydrochloride, (−)-isopropylnoradrenaline hydrochloride and tyramine hydrochloride. The following drugs were also used: phentolamine hydrochloride (Ciba Pharmaceutical Co., NJ, U.S.A.), methoxamine hydrochloride (B.W. & Co. Ltd., Quebec, Canada), captopril (E.R. Squibb & Sons Inc., NJ, U.S.A.) and reserpine (Ciba Pharmaceutical Co., Quebec, Canada). All drugs were dissolved in normal saline.

Calculation and statistical analysis

The ED_{50} and maximum response (E_{max}) values of L-NNA were obtained from individual dose-response curves. Correlation coefficient (r), slope and intercept were calculated from individual HR versus MAP curves at various doses of L-NNA. Rise phase $t_{1/2}$ of L-NNA were obtained from time-course curves. To obtain normal distribution of rise phase $t_{1/2}$, the data were logarithmically-transformed prior to statistical analysis. All data were analyzed by the analysis of variance followed by Duncan's multiple range test with $P < 0.05$ selected as the criterion for statistical significance. All results are expressed as mean ± standard error (s.e.mean) except for rise phase $t_{1/2}$ which is expressed as geometric mean and 95% confidence range.

Results

Effects of antagonists on mean arterial pressure and heart rate

Table 1 shows baseline MAP and HR (protocol 1: Groups I to VII; protocol 2: Groups VIII to X) in rats prior to and 40 min after pretreatment with normal saline, phentolamine, propranolol, reserpine, mecamylamine, atropine or captopril. Normal saline (protocols 1 and 2) affected neither MAP nor HR. MAP was not affected by propranolol, atropine or captopril but significantly decreased by phentolamine, reserpine and mecamylamine. HR was decreased by reserpine, mecamylamine and propranolol and increased by phentolamine, atropine and captopril.

Table 2 shows the effects of the antagonists on responses to several agonists. Phentolamine (Group II) completely blocked

Table 2 The effects of antagonists on mean arterial pressure (MAP) and heart rate (HR) responses to several agonists in conscious rats

		Change in MAP (mmHg)			*Change in HR* (beats min^{-1})		
Blocker	*Agonist*	a	b	c	a	b	c
Phent	Mtx1	40 ± 6	0	0	−106 ± 14	0	0
Reserp	Tyram	46 ± 3[d]	17 ± 5	17 ± 5	−108 ± 11*	−16 ± 10	−21 ± 9
Prop	Isop	−37 ± 3	1 ± 1	17 ± 8	116 ± 13	2 ± 2	27 ± 9
Mecam	Mtx2	55 ± 2	101 ± 5	43 ± 8	−169 ± 17	0	0
Atrop	ACh	−44 ± 2	0	0	47 ± 2	0	0
Capt	AI	43 ± 4	0	15 ± 1	−83 ± 13	0	−28 ± 7

The effects (mean ± s.e.mean) of isoprenaline (Isop, 1 μg kg^{-1}), tyramine (Tyram, 200 μg kg^{-1}), methoxamine (Mtx1 20 μg kg^{-1}; Mtx2, 30 μg kg^{-1}), acetylcholine (ACh, 1 μg kg^{-1}) and angiotensin I (AI, 1 μg kg^{-1}) on MAP and HR were obtained before (a), 20 min after (b) the administrations of: phentolamine (Phent), propranolol (Prop), reserpine (Reserp), mecamylamine (Mecam), atropine (Atrop) or captopril (Capt) and, 2 h after (c) an i.v. bolus injection of N^G-nitro-L-arginine.
All results in (b) and (c) are significantly different from the corresponding control values (a) within the same group ($P < 0.05$). [d] The data were from the vehicle-treated rat group. $n = 6$ per group.

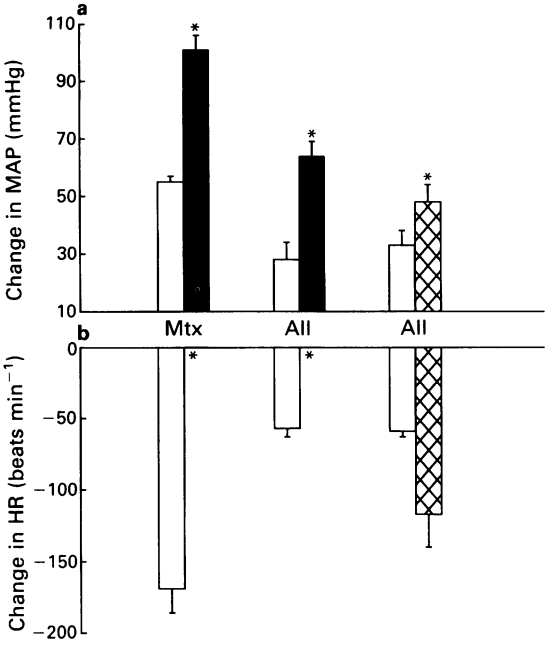

Figure 1 Effects of methoxamine (Mtx) and angiotensin II (AII) on mean arterial pressure (MAP) (a) and heart rate (HR) (b) before (open columns) and 20 min after the administrations of mecamylamine (filled columns) and phentolamine (cross hatched column). Values are means with s.e.mean shown by vertical bars; $n = 6$ in each group. * Represents significant difference from corresponding control values prior to the administration of an antagonist ($P < 0.05$).

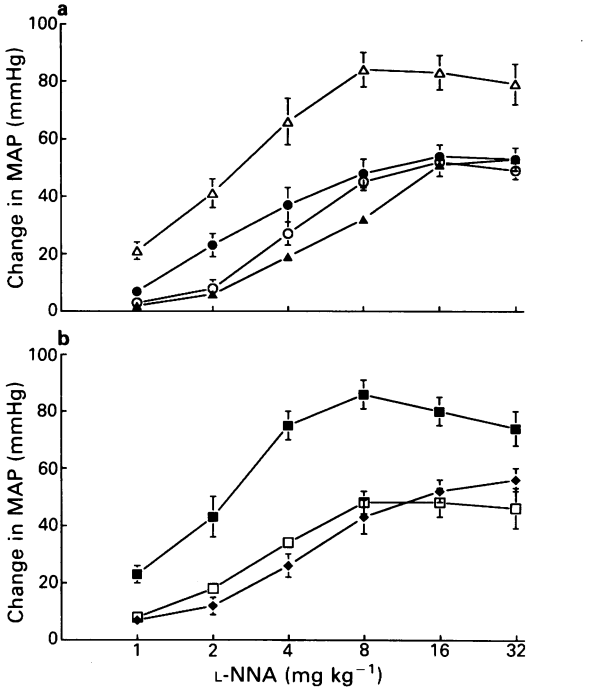

Figure 2 Dose-response curves (mean ± s.e.mean) of the effects of i.v. bolus doses of N^G-nitro-L-arginine (L-NNA) on mean arterial pressure (MAP) in groups ($n = 6$ each) of conscious rats pretreated with normal saline (○ in a), reserpine (● in a), phentolamine (△ in a), propranolol (▲ in a), atropine (□ in b), mecamylamine (■ in b) and captopril (◆ in b).

pressor effects and bradycardia induced by methoxamine throughout the study period. The pressor response to methoxamine was enhanced by mecamylamine (Group V) at 20 min but not altered at 2 h following the administration of L-NNA. The reflex bradycardia induced by methoxamine was abolished by mecamylamine throughout the experiments. Intravenous bolus doses of tyramine in vehicle-treated rats (Group I) increased MAP and decreased HR; in Group IV rats pretreated with reserpine tyramine caused markedly less pressor and bradycardic responses than in control rats. Isoprenaline (Group III) caused depressor and tachycardic responses; both were almost totally abolished at 20 min after the injection of propranolol and remained markedly attenuated 2 h after the administration of L-NNA. Atropine (Group VI) completely abolished the depressor and reflex tachycardic response of acetylcholine throughout the study period. At 20 min after the injection of captopril (Group VII), the pressure and bradycardic effects of angiotensin I were abolished. Both responses to angiotensin I remained attenuated 2 h after the injections of L-NNA.

Pressor responses to angiotensin II (Groups IX and X) and methoxamine (Group V) were potentiated by mecamylamine or phentolamine (Figure 1). Reflex bradycardia in response to angiotensin II and methoxamine was totally blocked by mecamylamine but reflex bradycardia to angiotensin II was unaffected by phentolamine.

Dose-response curves for N^G-nitro-L-arginine

Intravenous bolus doses of L-NNA in vehicle-treated rats dose-dependently increased MAP (Figure 2). Pretreatment with either mecamylamine or phentolamine potentiated the pressor response to L-NNA by reducing ED_{50} and increasing E_{max} values (Figure 2, Table 3). Pretreatment with the other antagonists used in this study did not significantly alter the dose-MAP response curves for L-NNA (Figure 2, Table 3).

Figure 3 shows the relationships between HR and MAP for rats in Groups I to VII. In vehicle-treated rats (Group I), MAP after L-NNA was negatively correlated with HR. Significant correlations of MAP with HR were also obtained in rats pretreated with phentolamine, propranolol, atropine or captopril but not with reserpine or mecamylamine (Table 4). The slope of the curve was not significantly altered by atropine, propranolol or captopril but was significantly increased by phentolamine. The intercept was decreased by propranolol,

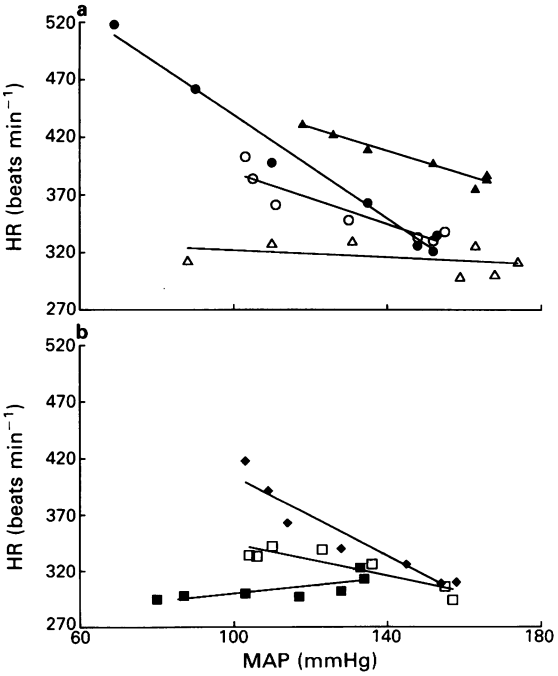

Figure 3 Relationship of heart rate (HR) (a) to mean arterial pressure (MAP) (b) after injection of N^G-nitro-L-arginine (L-NNA, 1–32 mg kg^{-1}, i.v. bolus) in conscious rats pretreated with normal saline (○ in a), phentolamine (● in a), mecamylamine (△ in a), atropine (▲ in a), propranolol (□ in b), reserpine (■ in b) and captopril (◆ in b). Each point represents mean values from six rats given the same dose of L-NNA.

Table 3 ED_{50} values and maximum effects (E_{max}) of N^G-nitro-arginine (L-NNA) on mean arterial pressure in conscious rats pretreated with normal saline, phentolamine, propranolol, reserpine, mecamylamine, atropine or captopril

Antagonist	ED_{50} (mg kg^{-1})	E_{max} (mmHg)
Normal saline	4.3 ± 0.8	52 ± 2
Phentolamine	2.1 ± 0.2*	87 ± 6*
Propranolol	6.3 ± 0.6	54 ± 3
Reserpine	3.1 ± 0.6	56 ± 4
Mecamylamine	1.9 ± 0.2*	86 ± 5*
Atropine	2.7 ± 0.3	51 ± 5
Captopril	5.0 ± 1.1	56 ± 4

All values represent mean ± s.e.mean. $n = 6$ per group. * Denotes significant difference from normal saline-treated group ($P < 0.05$).

Table 4 Slope, intercept and correlation coefficient (r) of the heart rate vs mean artery pressure curves of N^G-nitro-L-arginine (L-NNA, 1–32 mg kg^{-1}, i.v. bolus) in conscious rats ($n = 6$ per group) pretreated with normal saline, phentolamine, propranolol, reserpine, mecamylamine, atropine or captopril

Group	r	*Slope*	*Intercept*
Normal saline	0.85 ± 0.03[a]	−1.17 ± 0.24	509 ± 32
Phentolamine	0.96 ± 0.01[a]	−2.29 ± 0.08*	669 ± 23*
Propranolol	0.80 ± 0.05[a]	−0.75 ± 0.20	419 ± 20*
Reserpine	0.72 ± 0.06	[b]	[b]
Mecamylamine	0.46 ± 0.12	[b]	[b]
Atropine	0.83 ± 0.04[a]	−1.16 ± 0.22	557 ± 51
Captopril	0.89 ± 0.02[a]	−1.68 ± 0.14	570 ± 17

Slope (beats min^{-1} mmHg^{-1}), intercept (beats min^{-1}) and r values represent mean ± s.e.mean. [a] Denotes significance of r ($P < 0.05$); [b] values were not obtained due to insignificant correlation coefficient; * denotes significant difference from respective values in normal saline group ($P < 0.05$).

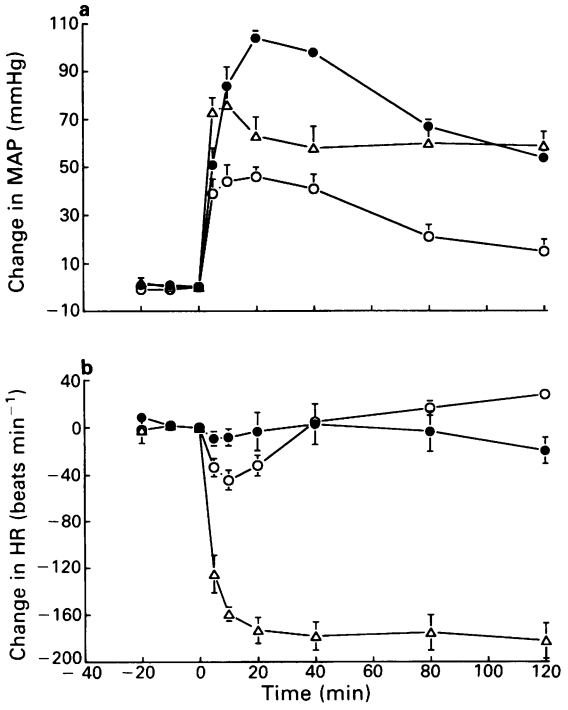

Figure 4 Time course of the effects of N^G-nitro-L-arginine (L-NNA, 32 mg kg^{-1}) on mean arterial pressure (MAP) (a) and heart rate (HR) (b) in groups ($n = 6$ each) of conscious rats pretreated with normal saline (○), mecamylamine (●) and phentolamine (△). Values are means with s.e.mean shown by vertical bars.

increased by phentolamine but not significantly altered by captopril and atropine.

Time course of the effects of N^G-nitro-L-arginine

In control rats given normal saline the MAP response to a single dose of L-NNA started almost immediately and reached a plateau 10 min after injection (Figure 4a). The rise phase $t_{1/2}$ was 4.8 min (geometric mean, 95% confidence limit: 2.0–11.6); MAP at 40 min was not different from MAP at 10 min and remained elevated 2 h after injection. Mecamylamine and phentolamine potentiated the peak MAP response to L-NNA (Figure 4a). Mecamylamine did not alter the rise phase $t_{1/2}$ (5.5 min, 95% confidence limit: 3.2–9.4) but phentolamine reduced it (to 1.5 min, 95% confidence limit: 1.0–2.3).

The pressor response to L-NNA was accompanied by initial significant decreases of HR at 5, 10, 20 min after injection followed by a recovery of HR and continual significant increases of HR at 80 and 120 min even when MAP was still above the control level (Figure 4b). Mecamylamine abolished the biphasic effects of L-NNA on HR. Phentolamine, on the other hand, potentiated and prolonged the bradycardia.

Discussion

Our results show that L-NNA is a potent and long-lasting pressor agent in conscious rats. Captopril and blockers of the autonomic nervous system, namely, mecamylamine, phentolamine, reserpine, propranolol and atropine, did not attenuate the pressor responses to L-NNA. This indicates that the pressor effect of L-NNA does not rely on the integrity of these two vasopressor systems. It has been reported that the pressor effects of L-MMA (Rees *et al.*, 1989b; Aisaka *et al.*, 1989) and L-NNA (Wang & Pang, 1990) are antagonized by L-Arg suggesting that N^G-substituted L-Arg analogues raise MAP via inhibiting NO synthase.

Pretreatment with mecamylamine potentiated MAP responses to L-NNA, angiotensin II and methoxamine. Phentolamine increased pressor responses to L-NNA and angiotensin II. Phentolamine but not mecamylamine, however, reduced the rise time $t_{1/2}$. This non-specific enhancement of the pressor effects of vasopressor agents after ganglionic or α-adrenoceptor blockade is consistent with well-known observations that acute pressor responses in intact animals are buffered by the simultaneous withdrawal of sympathetic tone to vascular smooth muscles (Lum & Rashleigh, 1961; Mawji & Lockett, 1963; Minson *et al.*, 1989).

MAP response to the injection of a single dose of L-NNA was associated with significant initial bradycardia (0 to 40 min) followed by tachycardia. A biphasic HR response to L-NNA was also observed in pentobarbitone-anaesthetized rats (Wang & Pang, 1990). Biphasic HR responses to L-MMA in chloralose and urethane anaesthetized rats have also been described (Togashi *et al.*, 1990). Mecamylamine abolished HR responses to L-NNA, suggesting that the biphasic HR response is mediated via reflex changes in the activities of the autonomic nervous system.

The tachycardic component was not seen in rats given cumulative doses of L-NNA, presumably due to the shorter observation time given to each dose of L-NNA. The results from cumulative dose-response relationships to L-NNA in the absence of an antagonist, show that the MAP effects of L-NNA are negatively correlated with HR. Treatment with mecamylamine or reserpine abolished the reflex changes in HR following alterations in MAP. The slope of the HR-MAP curve was slightly reduced by propranolol but unaffected by atropine. The lack of a correlation of HR to MAP after treatment with reserpine suggests that in conscious rats, inhibition of sympathetic nerve activity rather than potentiation of parasympathetic nerve activity is involved in reflex changes in HR. These results are consistent with the observation that bradycardia induced by L-MMA was associated with reduced renal

sympathetic nerve activity (Togashi *et al.*, 1990). Our results also show that phentolamine increased the slope of the curve. Phentolamine has been shown to increase markedly plasma levels of adrenaline and noradrenaline (Tabrizchi *et al.*, 1988). Therefore, enhanced reflex bradycardia in response to L-NNA in the presence of phentolamine may have been a consequence of elevated background sympathetic nerve activities as baseline HR was elevated by phentolamine.

In conclusion, the pressor effect of L-NNA does not rely on the integrity of the autonomic nervous system or the renin-angiotensin system. The biphasic changes of HR induced by L-NNA are attributable to reflex alterations in the activities of the autonomic nervous system.

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