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Acute effects of nitric oxide blockade with L-NAME on arterial haemodynamics in the rat

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1 We employed the technique of impedance spectral analysis to investigate the role of endogenous nitric oxide (NO) in the regulation of steady and pulsatile haemodynamics in Wistar Kyoto rat (WKY).

2 A total of 12 WKYs was anaesthetized with pentobarbitol sodium (40 mg kg^{-1}, i.p.) and artificially ventilated with an animal respirator. The aortic pressure wave was monitored with a high fidelity Millar sensor, and aortic flow wave with an electromagnetic flow probe. The pressure and flow waves were subjected to Fourier transform for the analysis of impedance spectra.

3 The baseline cardiovascular parameters were mean arterial pressure (APm) 95 ± 9 mmHg, heart rate (HR) 338 ± 9 b.p.m., stroke volume (SV) 0.23 ± 0.01 ml, cardiac output (CO) 77.8 ± 1.6 ml min^{-1}, total peripheral resistance (TPR) 98 ± 11 ($\times 10^3$) dyne s cm^{-5}, characteristic impedance (Zc) 2046 ± 141 dyne s cm^{-5}, arterial compliance at mean AP (Cm) 3.78 ± 0.22 μl $mmHg^{-1}$ and backward pulse wave (P_b) 12.9 ± 0.6 mmHg.

4 An NO synthase inhibitor, N^G-nitro-L-arginine monomethyl ester (L-NAME) was administered at graded intravenous doses. This agent caused dose-dependent increases in AP and TPR with decreases in HR. At an accumulative dose of 10 mg kg^{-1}, APm was increased by 29 ± 3 mmHg (+31%) and TPR by 49 ± 6 ($\times 10^3$) dyne s cm^{-5} (+50%), while HR was reduced by 37 ± 5 b.p.m. (−11%) and CO by 10.4 ± 0.8 ml min^{-1} (−14%). The pulsatile haemodynamics including Zc and P_b were slightly increased by 14–15%. Cm was decreased by 1.09 μl $mmHg^{-1}$ (−29%). L-NAME also did not significantly affect the ventricular work including the steady, oscillatory and total work.

5 Aminoguanidine, a specific inhibitor for inducible NO synthase (iNOS), in dose 10–60 mg kg^{-1} i.v. did not alter the AP, HR and other parameters. The result indicated that blockade of constitutive NOS, but not iNOS is involved in these changes.

6 Angiotensin II (Ang) in various infusion doses was used to produce a profile of AP increase similar to that caused by L-NAME. Ang remarkably increased Zc, while TPR was moderately elevated. The pattern of haemodynamic changes was different from that following L-NAME.

7 The results suggest that blockade of the endogenous NO affects predominantly the arterial pressure and peripheral resistance. The Windkessel functions such as arterial impedance and pulse wave reflection are slightly increased. Ventricular works are not significantly altered.

Keywords: Nitric oxide; NO blockade; L-NAME; arterial haemodynamics; arterial impedance; vascular resistance; arterial hypertension

Introduction

Endothelium-derived factors play an important role in the relaxation of vascular smooth muscle (Furchgott & Zawadzki, 1980; Furchgott, 1983). Endothelium-derived relaxing factor (EDRF) has been identified as nitric oxide (NO) or a related substance (Ignarro *et al*., 1987; Palmer *et al*., 1987). This discovery led to extensive studies on the biosynthesis, physiology and pharmacology of NO in endothelial cells and other organs (Moncada *et al*., 1988, 1991). Several chemical analogues of L-arginine, the precursor of NO, were developed and used to block NO synthase (NOS) and the release of NO (Moncada *et al*., 1988; Rees *et al*., 1989, 1990a,b). In the cardiovascular system, these NOS inhibitors can create a 'biochemical denudation', resulting in vasoconstriction, increase in vascular resistance and elevation of arterial pressure (Moncada *et al*., 1991). The effect of NOS blockade with N^G-monomethyl-L-arginine (L-NMMA) and/or N^G-nitro-L-arginine methyl ester (L-NAME) on vascular tone indicates that a continuous release of NO maintains a dilator tone in vascular tissues (Rees *et al*., 1989, 1990a; Moncada *et al*., 1991). This NO-dependent tonic vasodilation has been demonstrated not only in isolated aortic rings (Palmer *et al*., 1988; Thomas *et al*., 1989; Giuliani *et al*., 1990; Katusic *et al*., 1990), but also in perfused vascular beds (Amezcua *et al*., 1989; Levi *et al*., 1990; Ward & Angus, 1993; Wang *et al*., 1995).

In the whole body, blockade of NO release with L-NMMA or L-NAME inevitably cause an increase in arterial pressure (Rees *et al*., 1989, 1990a; Ward & Angus, 1993). The increase in arterial pressure is accompanied by an increase in vascular resistance and decrease in blood flow in various vascular beds (Gardiner *et al*., 1990a, b; Fozard & Part, 1991). There is little doubt that endothelium-derived NO formation participates in the regulation of blood pressure and blood flow. However, little information is available with respect to the influence of NO on complete arterial haemodynamics, using the method of artieral impedance analysis.

The technique of arterial impedance analysis has been developed for the complete assessment of arterial haemodynamics and quantitation of ventricular afterload (O'Rourke & Taylor, 1967; Milnor, 1975; Nicholas *et al*., 1977; O'Rourke, 1982) and utilized extensively in recent years (Zuckerman & Yin, 1989; Chang *et al*., 1990, 1994; Hu *et al*., 1994, 1996; Chen *et al*., 1996). Its purpose is to analyse the

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instaneous relation between aortic pressure and flow through Fourier transform (frequency analysis) of the phasic pressure and flow waves. The spectral analysis derives many haemodynamic parameters including characteristic impedance, pulse wave reflection and ventricular work. These parameters are considered to be pulsatile and frequency-dependent and reflect mainly the viscoelastic properties of the aorta and large arteries or the 'Windkessel' functions of the arterial system. Furthermore, the steady components of arterial haemodynamics such as arterial pressure, cardiac output and total peripheral resistance can also be obtained from the measurement.

In the present experiment, we employed the technique of arterial impedance analysis to study the effects of NO blockade with L-NAME on arterial pressure, vascular resistance, cardiac output, arterial impedance, compliance, pulse wave reflection and ventricular work. This complete haemodynamic analysis was designed to provide more information with respect to the influences of NO release on the Windkessel and resistance vessels as well as on ventricular loading.

Methods

Experimental preparation

Twenty-two male rats of Wistar Kyoto strain (WKY) weighing 360–390 g (20–26 weeks of age) were used in this study. Each animal was anaesthetized with an intraperitoneal injection of sodium pentobarbitol 40 mg kg^{-1}. After anaesthesia, the trachea was cannulated to provide artificial ventilation with a tidal volume of 3–5 ml and respiratory rate of 50–70 breaths/min. The femoral artery was cannulated for the recording of femoral arterial pressure, and the femoral vein for the administration of supplemental anaesthetics and drugs. Measurements of aortic flow and pressure in rats were made according to the procedures described in previous studies (Hu *et al*., 1994; Chen *et al*., 1996). The chest was opened through the left third intercostal space. An electromagnetic flow probe (Carolina Medical Electronics Inc., Model 100 series, internal circumference 7–10 mm) was placed around the ascending aorta to measure aortic blood flow. A Millar catheter with one high-fidelity pressure sensor (Millar Instruments Co., Model SPR-407, Size 2F) was used to measure aortic pressure. To minimize baseline drift, the catheter was soaked in saline at room temperature for at least 1 h before insertion. The Millar catheter was inserted via the isolated right carotid artery into the ascending aorta until the catheter tip reached a position just distal to the flow probe.

Aortic pressure, flow waves and ECG were continuously displayed on a polygraph recorder (Gould, Model 2800S) and also recorded on a tape recorder (TEAC, Model MR-30) at a recording speed of 4.8 cm s^{-1} for off-line analysis. All data were registered after the pressure and flow signals had been stable for 3–5 min.

Calculations and data analysis

The pressure and flow signals were digitized at 1 ms intervals using a 12-bit analog-to-digital converter (Microstar Laboratories Inc., Model DAP 1200/4) interfaced to a personal computer. Four consecutive beats at stable state were selected for analysis. Zero flow was taken at the level of flow in the middle to late diastole. The largest modulus of this portion of the flow was considered to be the noise level. The calibration of the flow velocity signal was performed after the experiment. The flowmeter (Carolina Medical Electronics Inc., Model 501D) had a frequency response that was decreased by 3 dB at ~100 Hz. The phase lag was almost linear with frequency (1.2 degrees/Hz). Appropriate corrections were applied at each impedance harmonic to take the phase delay into account. All haemodynamic parameters were calculated beat by beat. The average value of four beats was obtained for an individual data point.

The calculations of the haemodynamic components are essentially the same as our previous reports (Hu *et al*., 1994; Chen *et al*., 1996). The aortic pressure and flow waves are subjected to Fourier transform to derive the pressure and flow harmonic:

$$P(k) = \sum_{n=0}^{N-1} p(n)\omega_N^{k_n} \tag{1}$$

$$\dot{Q}(k) = \sum_{n=0}^{N-1} q(n)\omega_N^{k_n} \tag{2}$$

where $k = 0, 1, 2, 3, ..., N-1$; $p(n)$ is the sampled sequence of pressure wave; $q(n)$ the sampled sequence of flow wave; $P(k)$ the modulus of pressure at kth harmonic; $\dot{Q}(k)$ the modulus of flow at kth harmonic. $P(k)$ and $\dot{Q}(k)$ can be rewritten as:

$$P(k) = |P(k)|e^{j\phi(k)} \tag{3}$$

$$\dot{Q}(k) = |\dot{Q}(k)|e^{j\phi(k)} \tag{4}$$

For each beat, the impedance modulus is the ratio of aortic pressure harmonic to flow harmonic:

$$Z(k) = \frac{|P(k)|}{|\dot{Q}(k)|} \tag{5}$$

The flow phase is subtracted from the pressure phase at each harmonic to yield the impedance phase angle:

$$\theta(k) = \varphi(k) - \phi(k) \tag{6}$$

Any flow harmonic with a modulus <1.5 times the noise was not used for impedance calculation. The characteristic impedance (Zc) was the average of impedance modulus in the frequency range of 15–45 Hz with coefficients of variation $<10\%$. First zero-crossing of impedance phase angle (fo) was evaluated by linear interpolation method from the data. Systolic, diastolic, mean aortic pressure (APs, APd, APm), heart rate (HR), stroke volume (SV) and total peripheral resistance (TPR) were also determined for each beat. Cardiac output (CO) was the product of SV and HR. Because of a curvilinear relation between pressure and intravascular volume in the arterial tree, an acute increase in pressure was associated with reduction in arterial compliance. The arterial compliance at pressure P (systolic, diastolic or mean) was obtained from the equation according to Liu *et al*. (1986) for an exponential pressure-volume relationship:

$$C(P) = \frac{SV}{K}\frac{b\exp^{bP}}{\exp^{bP_s^*} - \exp^{bP_d^*}} \tag{7}$$

where SV is the stroke volume, K the ratio of total area under the aortic pressure curve to the diastolic area, b the coefficient in the pressure/volume relation [-0.0131 in the aortic arch], P_s^* the pressure at the time of incisura, and P_d the end-diastolic pressure.

Total, pulsatile and steady external power (Wt, Wo, Ws) consisting of pressure and flow terms were also calculated (Milnor, 1989):

$$\mathrm{Ws} = \bar{P} \cdot \dot{\bar{Q}} \tag{8}$$

$$\mathrm{Wo} = \frac{1}{2}\sum \left|\dot{Q}(k)^2\right| |Z(k)| \cos\theta(k) \tag{9}$$

$$\mathrm{Wt} = \mathrm{Ws} + \mathrm{Wo} \tag{1 }$$

where $\bar{P}$ is the mean pressure; $\dot{\bar{Q}}$ the mean flow. The ratio of oscillatory to total power (Wo/Wt) was also calculated as an index for the efficiency with which the pulsatile energy was converted into forward flow. Finally, we decomposed the

measured pressure and flow waves into forward and backward components (Westerhof *et al*., 1972):

$$P_m = P_f + P_b \quad (11)$$

$$\dot{Q}_m = \dot{Q}_f + \dot{Q}_b \quad (1\ )$$

$$P_f = Z_c \cdot \dot{Q}_f \quad (1\ )$$

$$P_b = -Z_c \cdot \dot{Q}_b \quad (1\ )$$

where P is the pressure wave, $\dot{Q}$ the flow wave, m the measured wave, f the forward wave, b the backward wave. Thus the measured pressure and flow waves are equal to the sum of a forward wave and a backward wave. The forward pressure and flow waves are related by Z_c. The magnitudes (pulse pressure) of the *Pf* and *Pb* components, along with the ration of the backward to the forward magnitude (P_b/P_f) were used to characterize the wave reflection properties. All the data and derived haemodynamic parameters were analysed by computer programs developed in our laboratory.

Experimental protocol and statistical analysis

L-NAME (Sigma) was dissolved in saline at a concentration of 20 mg ml^{-1} and delivered intravenously by a slow bolus injection. Each animal reveived a vehicle injection (0.2–0.5 ml saline) followed by four doses (1, 4, 5 and 10 mg kg^{-1} of L-NAME. Six to ten minutes were allowed to obtain steady states following each dose. Because of the relatively long duration of action, the accumulative doses (1, 5, 10 and 20 mg kg^{-1}) of L-NAME were taken to determine the dose–response relation. As L-NAME is non-specific inhibitor that blocks both constitutive and inducible NO synthase (cNOS and iNOS), a selective iNOS inhibitor, aminoguanidine (Griffiths *et al*., 1993; Joly *et al*., 1994) was used to determine whether the inducible NO synthase was involved. This agent was dissolved in saline at a concentration of 30 mg ml^{-1} and a bolus injection (10–60 mg kg^{-1}, i.v.) administered. Angiotensin II (Ang) was tested in another group of animals, to allow a comparison to be made with the haemodynamic changes caused by L-NAME. Ang II was also dissolved in saline solution and administered by slow infusion at doses of 0.2, 0.4 and 0.8 μg kg^{-1} min^{-1}. Each dose was infused for a period of 5 min.

The data were expressed as mean $\pm$ s.e. Statistical evaluation of the dose–response relation was done with analysis of variance (ANOVA) and Scheffe test. A paired t test was used for comparisons of haemodynamic parameters between the control and experimental values. Differences were considered significant at a P value <0.05.

Results

Gross observation of the effects on AP and HR

Injection of drug vehicle produced no discernible changes. L-NAME 1 mg kg^{-1} increased the mean AP by 9 ± 3 mmHg, and decreased the HR by 3 ± 2 beats/min. When the accumulated dose reached 5 mg kg^{-1} (+4 mg kg^{-1}), the AP was increased by 16 ± 4 mmHg and HR decreased by 20 ± 4 beats/min. At the third additional dose (±5 mg kg^{-1}, accumulated dose 10 mg kg^{-1}), the AP increase and HR decrease were 29 ± 3 mmHg and 37 ± 5 beats/min, respectively. Thus, until first three doses caused dose-dependent changes in AP and HR ($P<0.01$). Responses appeared to be maximal at a cumulative dose of 10 mg kg^{-1} for the changes in AP ($+31\pm5$ mmHg) and HR (-44 ± 5 beats/min) following the fourth dose of L-NAME ($+10$ mg kg^{-1}, accumulated dose 20 mg kg^{-1}) were not different from the previous dose ($P>0.01$).

Aminoguanidine (10–60 mg kg^{-1} caused no discernible changes in AP and HR. For example, at 60 mg kg^{-1} the AP increased by 4 ± 3 mmHg and HR decreased by 2 ± 1 beats/min ($n=4$), which were not significant changes ($P>0.1$). Ang caused dose-dependent changes in AP and HR ($n=6$). At a dose of 0.2 μg kg^{-1} min^{-1}, the AP increase and HR decrease were $+16\pm2$ mmHg and -24 ± 3 beats/min, respectively. When the dose was increased to 0.4 μg kg^{-1} min^{-1}, AP was increased by $+38\pm4$ mmHg, HR was only slightly decreased by -7 beats/min. At a higher dose (0.8 μg kg^{-1} min^{-1}), AP was elevated by $+43\pm5$ mmHg while HR increased by $+22\pm5$ beats/min. The magnitude of AP increase at a dose of 0.4 μg kg^{-1} min^{-1} was close to that caused by L-NAME at doses of 10–20 mg kg^{-1}.

Analysis of the arterial haemodynamics

Figure 1 illustrates the recordings of aortic pressure and flow signals in one rat during steady state conditions after the injection of vehicle or L-NAME at an accumulative dose of 10 mg kg^{-1}. The aortic pressure level was significantly elevated. There was a slight reduction in the magnitude of the flow wave of each beat. However, the width of the flow wave was slightly increased. As a result, the stroke volume (the area under the flow wave) was not much altered. Spectral analysis of the pressure and flow waves was performed to obtain the impedance modulus and impedance phase (Figure 2). In this representative animal, L-NAME slightly increased the values of impedance modulus above 10 Hz. It did not appear to affect the impedance phase.

Because of the complexity of haemodynamics parameters derived from the impedance spectral analysis, only the changes after an accumulative dose at 10 mg kg^{-1} were compared with the vehicle control (Tables 1 and 2). Several important parameters were then selected to determine accumulative dose–response relationships (Figure 3). L-NAME significantly increased the AP (APs, APm and APd) by 29–31 mmHg (Table 1). The pulse pressure (PP) was not appreciably changed. L-NAME decreased the HR by 37 ± 5 beats/min. SV was not altered and CO was slightly decreased. The TPR was significantly elevated by 49 ± 6 ($\times10^3$) dyne s cm^{-5}. Table 2 shows the haemodynamic data of pulsatile components. Zc was increased from 2046 ± 141 to 2352 ± 160

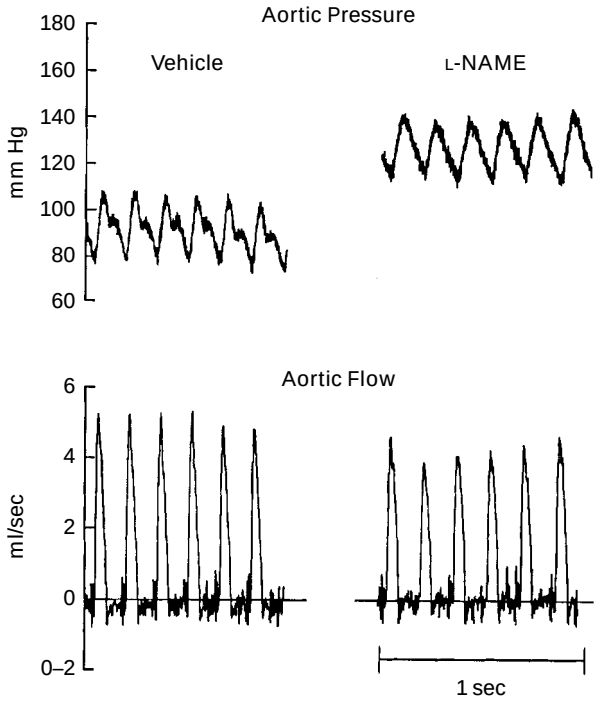

Figure 1 Recordings of aortic pressure and flow waves in one rat after vehicle injection (left) and after L-NAME (accumulative dose 10 mg kg^{-1} (right)).

($+306\pm38$ dyne s cm^{-5}). The values of arterial compliance (Cd, Cs, Cm) were decreased by 26–29%. Wave reflection (P_b) was increased by 14 ± 3% and P_b/P_f by 32 ± 4%. The other parameters including fo, external work of the heart (Wo and Ws) were not significantly affected by the administration of L-NAME.

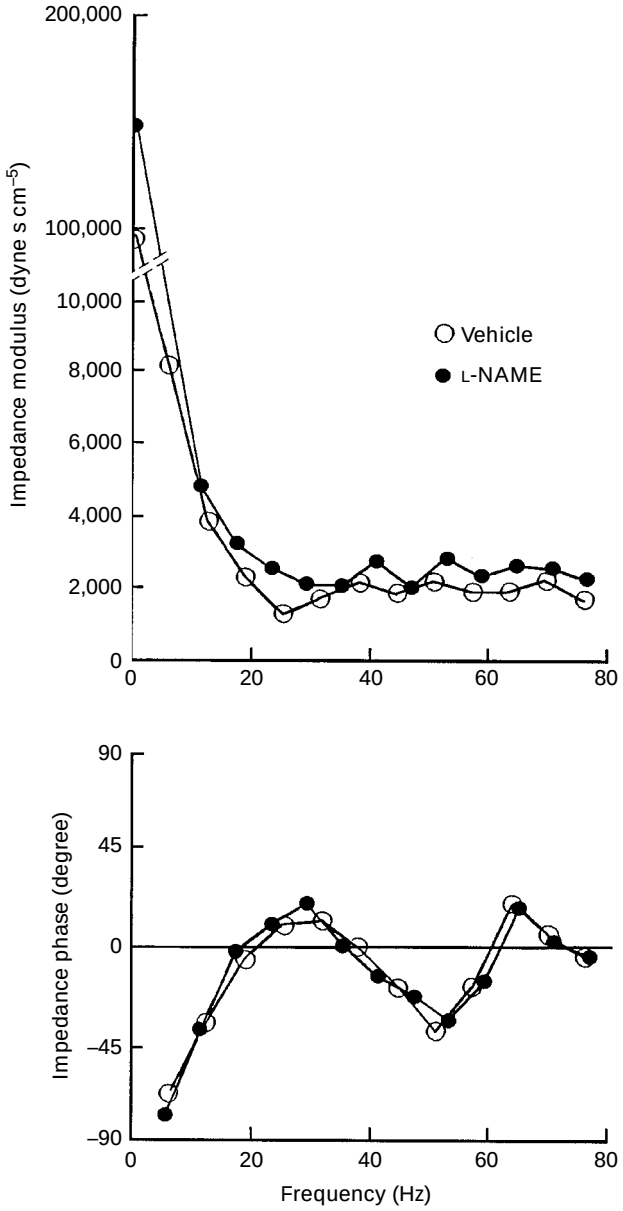

Figure 2 Impedance modulus and phase obtained after injection of vehicle (○) or L-NAME (●) (accumulative dose 10 mg kg^{-1}). Note the marked difference in the impedance modulus at 0 Hz (the value for total peripheral resistance). The impedance modulus after 10 Hz is slightly elevated following L-NAME. There is not much difference in the impedance phase between vehicle and L-NAME.

Figure 3 illustratees the changes in AP, HR, CO, TPR, *Zc* and Cm in response to graded doses of L-NAME. It appeared that maximal responses of these parameters occurred at an accumulative dose of L-NAME 10 mg kg^{-1}. Below this dose, responses in dose-dependent manner could be observed. The results demonstrated that L-NAME predominantly increased peripheral resistance and arterial pressure. The values of arterial compliance were moderately decreased. *Zc* and P_b were slightly increased. L-NAME did not discernibly affect the ventricular work.

Aminoguanidine affected neither AP and HR nor the other haemodynamic parameters. The changes in AP, HR, CO, TPR, *Zc* and Cm following graded doses of Ang are shown in Figure 4. As these changes were compared with those by L-NAME (Figure 3), a quite similar profile of pressor response (APm increase) was observed between these two agents. However, it appeared that Ang caused greater increases in *Zc* ($+35\pm6$%, $+56\pm8$% and $+64\pm10$% at each dose) than TPR. TPR increased by 19% following the low dose of Ang and did not rise further with increasing doses. In addition, L-NAME decreased HR and CO at all tested doses, while Ang slightly increased the CO at higher doses. Cm was decreased similarly by L-NAME and Ang at all doses.

Discussion

The analysis of arterial impedance spectra through Fourier transform of the aortic pressure and flow waves provides a useful tool for a complete assessment of steady and pulsatile arterial haemodynamics (O'Rourke, 1982; Chang *et al*., 1990; Hu *et al*., 1994; Chen *et al*., 1996). Recently, we have reported the importance of pulsatile haemodynamics for the development of ventricular hypertrophy in animals with long-term hypertension (Hu *et al*., 1994). In the present study, we employed the technique of impedance spectral analysis to evaluate the arterial haemodynamic changes following NO blockade with L-NAME. The major findings were as follows: (1) acute NO blockade greatly increased arterial pressure and peripheral resistance, and (2) the actions were relatively weak on the arterial impedance modulus, wave reflection and ventricular work. The results indicate that systemic administration of L-NAME predominantly affects resistance functions, while exerting smaller effects on the Windkessel functions.

It has been well documented that the release of NO maintains a dilator tone in small arterioles as well as in aortic segments and large arteries (Moncada *et al*., 1988, 1991; Giuliani *et al*., 1990; Katusic *et al*., 1990; Amezcua *et al*., 1989; Levi *et al*., 1990). With respect to its effects on arterial resistance, several studies determining regional blood flows or employing direct arterial perfusion have demonstrated that acute NO blockade causes a decrease in blood flow and an increase in resistance in various vascular beds (Amezcua *et al*., 1989; Gardiner *et al*., 1990a, b; Forzard & Part, 1991). Here, we found that the hypertension caused by L-NAME was accompanied by a marked increase in total peripheral resistance and a slight decrease in cardiac output. The reduction in cardiac output was in turn due to a slight bradycardia without a

Table 1 Acute effect of L-NAME (10 mg^{-1} kg) on haemodynamics of steady component

	Aortic pressure (mmHg)			*PP*	*HR*	*SV*	*CO*	*TPR* ($\times10^3$)
	APs	*APm*	*APd*	mmHg	beats/min	ml	ml^{-1} min	dyne s cm^{-5}
Vehicle	111 ± 9	95 ± 9	80 ± 9	30 ± 2	338 ± 10	0.23 ± 0.01	77.8 ± 1.6	98 ± 11
L-NAME	142 ± 9	124 ± 5	110 ± 6	32 ± 3	301 ± 8	0.22 ± 0.02	67.4 ± 1.4	147 ± 16
P value	<0.01	<0.01	<0.01	NS	<0.05	NS	<0.05	<0.01

Values are mean $\pm$ s.e. ($n=12$). APs, APm and APd = aortic pressure corresponding to peak systolic, mean and end diastolic pressure; PP = pulse pressure; HR = heart rate; SV = stroke volume; CO = cardiac output; TPR = peripheral resistance.

Table 2 Acute effects of L-NAME (10 mg^{-1} kg) on pulsatile haemodynamics

	Zc	*Cd*	*Cs*	*Cm*	*fo*	*Wo*	*Ws*	*Wo/Wt*	*Pf*	*Pb*	*Pb/Pf*
	dyne s cm^{-5}	μl^{-1} mmHg			Hz	mW		%	mmHg		%
Vehicle	2046±141	4.04±0.24	3.42±0.22	3.78±0.22	27±3	1.04±0.08	16.12±1.51	6.2±0.4	18.8±0.6	12.9±0.6	69±8
L-NAME	2340±173	3.01±0.18	2.52±0.16	2.69±0.20	23±3	1.12±0.04	17.68±1.12	6.1±0.3	16.1±0.5	14.7±0.6	91±6
P value	NS	<0.05	<0.05	<0.05	NS	NS	NS	NS	<0.01	<0.05	<0.01

Values are mean±s.e. (n=12). Zc=characteristic impedance; Cd, Cs and Cm=arterial compliance corresponding to end diastolic, peak systolic and mean pressure; fo=first zero crossing frequency of impedance phase angle; Ws, Wo and Wt=external power corresponding to steady, oscillatory and total power; Pb, Pf=magnitude of backward and forward components of pressure wave.

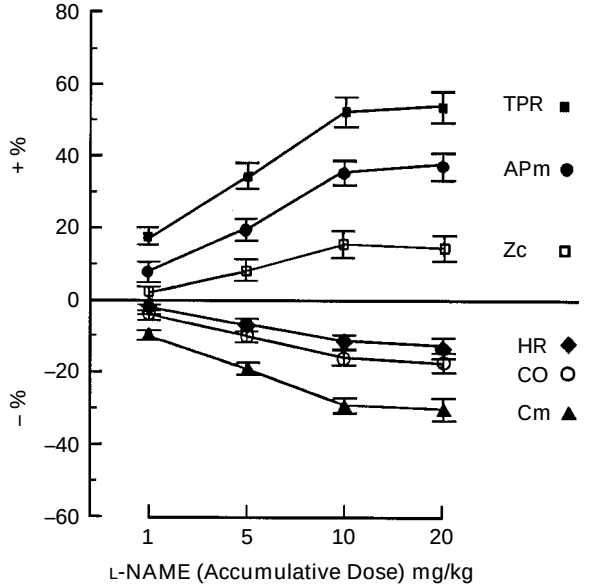

Figure 3 Percentage changes in total peripheral resistance (TPR), mean arterial pressure (APm), characteristic impedance (*Zc*), heart rate (HR), cardiac output (CO) and mean arterial compliance (Cm) in response to graded doses of L-NAME (in arithmetic scale). Statistical evaluation (ANOVA) indicates that the dose–response relations for all parameters were significant ($P<0.05$) below an accumulative dose of 10 mg kg^{-1}. Responses to 10 mg kg^{-1} and 20 mg kg^{-1} were not significantly different ($P>0.01$).

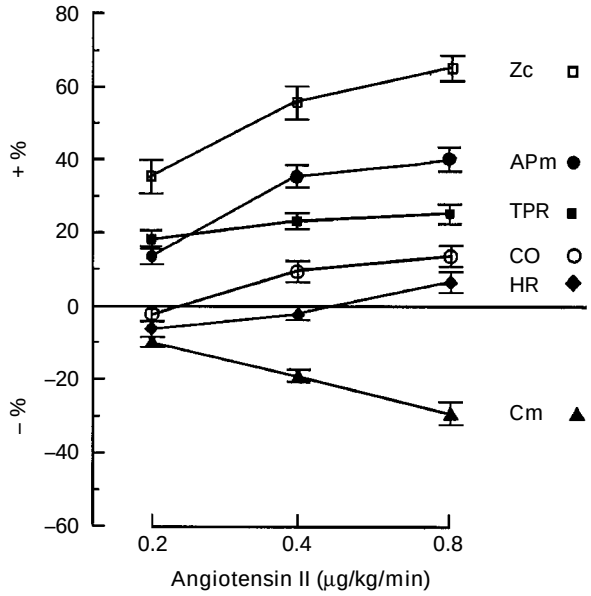

Figure 4 Percentage change in haemodynamic parameters (abbreviations as for Figure 3) in response to infusion of angiotensin (doses in arithmetic scale). Note the similar pattern of APm increase compared with that following L-NAME (Figure 3). The degree and pattern of changes in TPR, *Zc*, HR and CO are different between L-NAME and angiotensin.

significant change in stroke volume. To test whether iNOS was involved in these changes, aminoguanidine, a selective iNOS inhibitor was given. We found that this agent did not affect AP, HR or other haemodynamic parameters, indicating that blockade of cNOS activity underlay these cardiovascular changes. The vasoconstrictor induced by NOS inhibition may also be attributed to blockade of NO release from not only the endothelium, but also perivascular nitrergic nerves. Recent studies in dogs and monkeys (Yoshida *et al*., 1993; Okamura *et al*., 1996) have demonstrated the presence of nerve fibres containing NOS in the arterial wall. This nitrergic nervous system can be activated by ganglionic stimulants (e.g. nicotine) causing the release of NO and vasodilatation. On the other-hand, NOS inhibitors and ganglionic blocking agents produce nerve inhibition and vasoconstriction. The systemic administration of L-NAME might affect both endothelium- and nerve-derived NO. However, it has been suggested that vasodilatation mediated by neuronal NO in rats is not as significant as that in dogs and monkeys (Pegoraro *et al*., 1992; Okamura *et al*., 1996).

It was surprising that our results revealed a relatively weak action of L-NAME on the arterial impedance and pulse wave reflection. These pulsatile haemodynamic components reflect mainly the changes in viscoelastic properties of the aorta and large arteries (O'Rourke, 1982; Chang *et al*., 1990; Hu *et al*., 1994; Chen *et al*., 1996). It appears that our findings are somehow not in agreement with the results of many studies in which NO blockade has been shown to cause pronounced constriction of aortic segments and/or large arteries (Rees *et al*., 1989, 1990a; Thomas *et al*., 1989; Faraci, 1991; Moncada *et al*., 1991). In particular, Faraci (1991) compared the acute effects of NO blockade on the constrictor responses of large arteries and small arterioles in the cerebral circulation of anaesthetized rats. In a cranial window preparation, he found that topical application of a NO synthase inhibitor caused constriction of large arteries by 10.4%. In contrast, the diameter of small arterioles was only reduced by 3.7%. These findings led to the conclusion that NO release in the cerebral circulation had a greater influence on basal tone in large arteries than small arterioles. This conclusion appears opposite to the results we present here and is subjected to discussion. First, whether observations made in the cerebral circulation can be applied to the other vascular beds is not certain. Rees *et al*. (1990c) observed the diameter changes in microcirculation of the hamster cheek pouch. They found that NO was a potent dilator, while NO blockade produced strong constriction of the small arterioles (8–35 μm). These findings coupled with studies into regional blood flow (Amezcua *et al*., 1989; Thomas *et al*., 1989; Giuliani *et al*., 1990; Katusic *et al*., 1990; Levi *et al*., 1990; Gardiner *et al*., 1990a,b; Fozard & Part, 1991) in-

dicate that NO exerts potent effects on arteriolar resistance vessels. Second, as the vascular resistance is inversely proportional to the fourth power of radius, a small constriction in the arterioles can produce a great increase in the vascular resistance. Finally, it should be noted that in the above experiments (Faraci, 1991) topical application NO synthase inhibitors were applied in different sections of the pial vessels. The results could be different from those obtained within intact vascular beds or the systemic circulation, particularly when NO synthase inhibitors are given systemically. In this connection, Griffith and coworkers (Griffith *et al*., 1987; Griffith & Edwards, 1990) used microangiographic techniques to measure arterial diameter changes in the perfused rabbit ear. An intra-arterial infusion instead of topical application was used to apply the NO synthase inhibitor. They found that constriction was more prominent in small arterioles than upstream large arteries. Their results indicate that interaction between various segments occurs in the arterial system. For example, an increase in perfusion pressure due to downstream vasoconstriction may cause passive dilation and offsets the possible vasoconstriction of the upstream large vessels.

Although we demonstrated in this study that the impedance spectra and wave reflection were slightly changed after NO blockade with L-NAME, the arterial compliance was moderately decreased. The observations made by Griffith and coworkers from their experimetns in the rabbit ear artery (Griffith *et al*., 1987; Griffith & Edwards, 1990) can be considered when looking for a possible explanation. Acute NO blockade might have produced vasoconstriction of the large Windkessel vessels, as reflected by the increased *Zc* and decreased arterial compliance. However, a possible larger increase in arterial impedance could be counterbalanced by an increase in the diameter of the aorta and large arteries due to a rise in arterial pressure. As *Zc* is inversely related to the aortic lumen (Levy *et al*., 1988; Chang *et al*., 1990), an increase in aortic diameter tends to reduce the value of *Zc*. A decreased arterial compliance together with a slight change in *Zc* could also be a passive effect resulting from an increase in arterial pressure. As the pressure–volume relation in the arterial tree is curvilinear in nature, a rise in the arterial pressure can cause a passive decrease in arterial compliance (Liu *et al*., 1986; Chang *et al*., 1990).

In the present study, we found that blocking the basal release of endogenous NO affected mainly the arterial pressure and vascular resistance. The impedance spectra was slightly changed. In this connection, it appears that exogenous nitrovasodilators may similarly have exterted their effects of the level of the resistance vessels. Gundel *et al*. (1981) reported that nitroprusside given in doses sufficient to lower the AP by 20% and TPR by 38% did not cuase significant change in aortic impedance spectra in patients with angina pectoris. Yin *et al*. (1983) also found that nitroprusside decreased the arterial pressure, but not the characteristic impedance in patients with heart failure. In order to determine whether the haemodynamic changes associated with a pressor response were specific for L-NAME, we used angiotensin for comparison. Comparison of the haemodynamic changes caused by these two pressor agents (Figures 3 and 4) demonstrated that although having similar profiles of AP increase, these two agents produced different patterns of changes in peripheral resistance and characteristic impedance.

Arnal *et al*. (1993) recently reported that after a period (4–8 weeks) of chronic administration of L-NAME, rats developed sustained hypertension. However, ventricular hypertrophy was not found in this model of hypertension. Previous studies from our laboratory (Hu *et al*., 1994; Chen *et al*., 1996) demonstrated that impedance factors were more important than the arterial pressure and peripheral resistance in the development of ventricular hypertrophy in spontaneously hypertensive rats. The relative weak effect of NO synthase inhibition on arterial impedance, wave reflection and ventricular work might be one of the reasons why ventricular hypertrophy did not develop in the hypertensive model of chronic NO blockade.

In summary, analysis of arterial haemodynamics indicates that acute NO blockade with L-NAME predominantly affects the resistance vessels. The Windkessel functions, pulse wave reflection and ventricular work are only slightly or little altered.

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