ELSEVIER

European Journal of Pharmacology 317 (1996) 285–291

ejp

In vivo inhibition of nitric oxide synthesis does not depend on renin-angiotensin system activation

Aldete Zappellini [a,*], Simone A. Teixeira [a], Marcelo N. Muscará [a], Roberto Zatz [b], Edson Antunes [a], Gilberto De Nucci [a]

[a] *Department of Pharmacology, Faculty of Medical Sciences, State University of Campinas, P.O. Box 6111, UNICAMP, 13081-970 Campinas, Brazil*
[b] *Department of Nephrology, Faculty of Medicine, University of São Paulo, São Paulo, Brazil*

Received 6 June 1996; revised 5 September 1996; accepted 10 September 1996

Abstract

The role of the renin-angiotensin system in the haemodynamic changes induced by acute administration of N^{ω}-nitro-L-arginine methyl ester in anaesthetised dogs was investigated. The left femoral artery and vein were cannulated for blood pressure measurement and drug administration, respectively. A Swan-Ganz catheter was introduced through the right femoral vein and advanced to the pulmonary artery. Pulmonary arterial pressure, right atrial pressure and cardiac output were also determined. N^{ω}-Nitro-L-arginine methyl ester (0.01–10.0 mg/kg) was administered alone (control animals, $n = 18$) or in the presence of the angiotensin-converting enzyme inhibitors, captopril (2 mg/kg, $n = 9$) or enalapril (2 mg/kg, $n = 7$) or of the bradykinin B_2 receptor antagonist D-[Arg-Hyp^3,Thi^5,D-Tic^7,Oic^8]bradykinin (Hoe 140, 0.1 mg/kg, $n = 6$). Cerebellum nitric oxide synthase and serum angiotensin-converting enzyme activities were also measured. N^{ω}-Nitro-L-arginine methyl ester induced dose-dependent increases in blood pressure and systemic vascular resistance and decreases in heart rate and cardiac output. Nitric oxide synthase activity was inhibited 58% by N^{ω}-nitro-L-arginine methyl ester (from 3.37 ± 0.30 to 1.40 ± 0.24 pmol/min per mg protein, $P < 0.05$, $n = 5$). Both enalapril and captopril potentiated the cardiovascular changes induced by bradykinin (300 ng/kg, bolus). Moreover, enalapril inhibited angiotensin-converting enzyme activity from 12.8 ± 1.2 to 1.1 ± 0.2 nmol/ml per min ($P < 0.05$, $n = 6$). Under these conditions, N^{ω}-nitro-L-arginine methyl ester administration elicited the same haemodynamic changes as those observed in non-treated animals, except for preventing the decrease in systolic index. Hoe 140 had no effect on the cardiovascular responses to N^{ω}-nitro-L-arginine methyl ester. These results indicate that the renin-angiotensin system does not modulate these haemodynamic changes.

Keywords: Nitric oxide (NO); Renin-angiotensin system; N^{ω}-Nitro-L-arginine methyl ester (L-NAME); Hoe 140; Enalapril; Blood pressure

1. Introduction

Endothelium-derived nitric oxide plays a fundamental role in the control of blood pressure by maintaining an active state of vasodilatation (Moncada and Higgs, 1993). Indeed, the acute administration of nitric oxide synthase inhibitors, such as N^{ω}-nitro-L-arginine methyl ester, increases systemic vascular resistance and blood pressure (Rees et al., 1989; Gardiner et al., 1990; Van Gelderen et al., 1993). In addition, these inhibitors commonly cause a decrease in both cardiac output and heart rate (Stamler et al., 1994; Zappellini et al., 1996). The chronic inhibition of nitric oxide synthase in rats increases arterial blood pressure (Baylis et al., 1992; Ribeiro et al., 1992) and represents a useful model of endothelium-dependent hypertension. Both angiotensin-converting enzyme inhibitors (Pollock et al., 1993; Moreno et al., 1995) and angiotensin II receptor antagonists can markedly reverse this hypertensive state (Ribeiro et al., 1992). In this paper, we have investigated the involvement of the renin-angiotensin system in the N^{ω}-nitro-L-arginine methyl ester-induced haemodynamic changes in anaesthetised dogs using two angiotensin-converting enzyme inhibitors (enalapril and captopril) and the bradykinin B_2 receptor antagonist D-[Arg-Hyp^3,Thi^5,D-Tic^7,Oic^8]bradykinin (Hoe 140, Wirth et al., 1991).

* Corresponding author. Fax: (55-19) 252-1516.

2. Material and methods

2.1. Experimental procedure

Mongrel dogs of either sex (11.2 ± 0.7 kg) were initially anaesthetised with sodium pentobarbital (Sagatal, 30 mg/kg, i.v.). The animals were then intubated and artificially ventilated with pure oxygen. Anaesthesia was maintained with a combination of fentanyl citrate (Fentanil, 0.01 mg/kg per h), diazepam (Dienpax, 0.25 mg/kg per h) and pancuronium bromide (Pavulon, 0.10 mg/kg per h). The left femoral artery was cannulated for mean arterial blood pressure measurement (pressure transducer MX-860, Medex, USA) and the left femoral vein, for drug administration. A 7F-Swan-Ganz catheter (Edwards Laboratories, USA) was introduced through the right femoral vein and advanced to the pulmonary artery. The location of the catheter was confirmed by detection of the typical pressure wave of this artery. The catheter was connected to two MX-860 pressure transducers for the measurement of pulmonary arterial and right atrial pressures. The cardiac output was measured by the thermodilution technique (Fegler, 1954; Ganz et al., 1971). Briefly, 10 ml of cold saline (4–6°C) were injected through the proximal lumen of the Swan-Ganz catheter. The injection of this saline led to a change in blood temperature which was detected by a thermistor located 4 cm from the catheter tip, and transmitted to a computer (SDM 2000, Dixtal, Brazil) which calculated cardiac output. The measurement of cardiac output was performed at least in duplicate. All catheters were filled with heparinised saline (10 IU/ml) to prevent clotting. The heart rate was measured via a surface electrocardiogram (lead I). The haemodynamic parameters were allowed to stabilise for 20 min before any drug administration.

2.2. Experimental design

2.2.1. Control animals ($n = 18$)

In these animals, we assessed the effects of N^{ω}-nitro-L-arginine methyl ester on the haemodynamic parameters described above. After basal measurements had been obtained, N^{ω}-nitro-L-arginine methyl ester (0.01–10.0 mg/kg) was infused at a flow rate of 0.5 ml/min during 8 min. At the end of each infusion, the resulting haemodynamic changes were recorded. At the end of the entire protocol, the total amount of N^{ω}-nitro-L-arginine methyl ester which had been administered to each animal was 14.44 mg/kg.

2.2.2. Enalapril-treated animals ($n = 7$)

After stabilisation and measurement of the haemodynamic variables, bradykinin was injected (300 ng/kg, 0.5 ml, bolus) and at the maximum hypotensive response, the haemodynamic changes were measured. Following recovery, the haemodynamic variables were again measured and enalapril (2 mg/kg) was infused at a flow rate of 0.5 ml/min during 8 min. To check the efficacy of enalapril, at the end of the infusion, we repeated the bradykinin injection in order to assess whether there was any potentiation of the cardiovascular changes. Upon complete recovery from the effects of bradykinin, we obtained new haemodynamic measurements and then initiated the dose-response curve to N^{ω}-nitro-L-arginine methyl ester as described above. This entire protocol was repeated using captopril (2 mg/kg at 0.5 ml/min during 8 min) instead of enalapril.

2.2.3. Hoe 140-treated animals ($n = 6$)

After stabilisation and measurement of basal haemodynamic parameters, bradykinin (300 ng/kg, 0.5 ml, bolus) was injected and the maximum hypotensive effect measured. When the haemodynamic parameters returned to basal values, the bradykinin antagonist Hoe 140 (0.1 mg/kg) was infused at a flow rate of 0.5 ml/min, during 8 min. The effects of the antagonist on the haemodynamic parameters were measured at the end of this infusion. To assess the efficacy of receptor blockade, the injection of bradykinin was repeated. Subsequently, N^{ω}-nitro-L-arginine methyl ester was administered as described for the control animals (see above).

2.3. Calculation of haemodynamic parameters

All haemodynamic events, including mean arterial blood pressure, pulmonary arterial pressure, right atrial pressure, heart rate and cardiac output, were continuously displayed on a computer monitor and were recorded on a printer coupled to this system (SDM 2000, Dixtal, Brazil). Systemic vascular resistance was calculated as systemic vascular resistance = ((mean arterial blood pressure − right atrial pressure)/cardiac output) × 80, where 80 is the constant that converts mmHg/l per min to dyn · s/cm^5.

Systemic vascular resistance, systolic volume and cardiac output were corrected for body surface area (expressed in m^2) and were expressed as the index of systemic vascular resistance (index of systemic vascular resistance = systemic vascular resistance × body surface area), the systolic index (systolic index = systolic volume/body surface area) and the cardiac index (cardiac index = cardiac output/body surface area).

2.4. Determination of cerebellum nitric oxide synthase activity

In some of the control animals, we examined the inhibition of brain nitric oxide synthase as described by Förstermann et al. (1990). This assay is based on the conversion of [^{3}H]L-arginine to [^{3}H]L-citrulline. At the end of the dose-response curve to N^{ω}-nitro-L-arginine methyl ester the cerebellum was rapidly removed, weighed and homogenised in 5 volumes of cold incubation buffer (Tris-HCl

50 mM, pH 7.4) containing phenylmethylsulphonyl fluoride (1 mM) and 1 mM of L-citrulline. The homogenates were incubated at room temperature for 30 min in the presence of 1 mM NADPH, 2 mM $CaCl_2$ and 10 μM of L-[2,3,4,5-^{3}H]arginine monohydrochloride (100 000 dpm). Nitric oxide synthase activity was also measured in the absence of Ca^{2+} (omission of $CaCl_2$ and addition of 1 mM EGTA) and in the presence of 1 mM N^{ω}-nitro-L-arginine methyl ester in the incubation medium. The protein content of the samples was determined by the method of Peterson (1977) and the enzyme activity was expressed as pmol L-citrulline/min per mg protein. Nitric oxide synthase activity was also assayed in the cerebella of animals which received no drug, except for anaesthetic.

2.5. Determination of serum nitrate and nitrite levels

Nitrate and nitrite levels were quantified by the method of Muscará and De Nucci (1996). Briefly, after separation on a strong anion-exchange column, nitrate anions were reduced to nitrite on a copper-plated cadmium-filled column and then detected by the Griess reaction. The sensitivity of the method was 30 pmol for both anions. For the assay, 3 ml blood samples were withdrawn before and after N^{ω}-nitro-L-arginine methyl ester administration from animals which had not been allowed to eat or drink for 12 h before the experiment in order to avoid nitrate ingestion. The blood was allowed to clot at room temperature and was then centrifuged (4200 rpm, 10 min) and the serum separated and stored at −20°C until analysed. The concentrations are expressed in μM.

2.6. Measurement of serum angiotensin-converting enzyme activity

Serum angiotensin-converting enzyme activity was assayed by measuring the amount of hippuric acid released from the synthetic substrate hippuryl-L-leucine at pH 8.3 and at 37°C (Cushman and Cheung, 1970). The released hippuric acid was quantified spectrophotometrically at 383 nm after reaction with cyanuric chloride (Hurst and Lovell-Smith, 1981). For the determination of angiotensin-converting enzyme activity in enalapril-treated animals, 3 ml blood samples were collected before and after administration of the drug and processed as described above. The sera were stored at −20°C until assayed for angiotensin-converting enzyme activity which was expressed as nmol/ml per min.

2.7. Drugs

Bradykinin and N^{ω}-nitro-L-arginine methyl ester were purchased from Sigma (USA) and pentobarbital sodium (Sagatal) was from May and Baker (UK). Diazepam (Diempax) was purchased from Sanofi (Brazil). Fentanyl citrate (Fentanil) was donated by Cristalia (Brazil) and pancuronium bromide (Pavulon) was obtained from the University Hospital Pharmacy. Hoe 140 was kindly provided by Hoechst (Germany), and enalapril by Biosintética (Brazil). L-[2,3,4,5-^{3}H]Arginine (specific activity 60 Ci/mmol) was supplied by Amersham (UK). The reagents used in the serum angiotensin-converting enzyme and cerebellum nitric oxide synthase assays were purchased from Sigma (USA).

2.8. Data and statistical analysis

All data are shown as the mean ± standard error of the mean (S.E.M.) and were analysed by analysis of variance for multiple comparisons followed by Duncan's test. In the case of nitric oxide synthase activity the data were analysed by analysis of variance followed by Newman-Keuls test. A P value < 0.05 was considered to be significant.

3. Results

3.1. Control animals

N^{ω}-Nitro-L-arginine methyl ester (0.01–10.0 mg/kg) induced a dose-dependent rise in mean arterial blood pressure (Fig. 1A). At 10 mg/kg, N^{ω}-nitro-L-arginine methyl ester increased blood pressure by 41.7% when compared to baseline values (from 91.3 ± 3.6 to 129.4 ± 4.0 mmHg, $P < 0.05$). N^{ω}-Nitro-L-arginine methyl ester also increased systemic vascular resistance (Fig. 1D). At the highest dose, N^{ω}-nitro-L-arginine methyl ester increased the index of systemic vascular resistance by 148.2% (from 1915.3 ± 177.6 to 4754.4 ± 363.5 dyn · s/cm^5 per m^2, $P < 0.05$). Parallel with these increases, the cardiac output (Fig. 1B) and the heart rate (Fig. 1C) decreased. At the highest dose of N^{ω}-nitro-L-arginine methyl ester, the cardiac index decreased by 44.3% (from 3.88 ± 0.25 to 2.16 ± 0.16 l/min per m^2, $P < 0.05$), the systolic index decreased 12.3% (Table 1) and the heart rate decreased by 38.5% (from 117 ± 6.7 to 72 ± 5.2 bpm, $P < 0.05$).

N^{ω}-Nitro-L-arginine methyl ester inhibited cerebellum nitric oxide synthase activity by 58% when compared to

Table 1
Effect of N^{ω}-nitro-L-arginine methyl ester (0.01–10.0 mg/kg) on the systolic index of control animals (L-NAME, $n = 18$) or in the animals pre-treated with captopril (2 mg/kg, $n = 9$), enalapril (2 mg/kg, $n = 7$) or Hoe 140 (0.1 mg/kg, $n = 6$)

		L-NAME	Captopril	Enalapril	Hoe 140
Basal		34.2 ± 2.8	28.3 ± 2.8	28.7 ± 1.8	37.3 ± 2.4
L-NAME	0.01	35.8 ± 2.5	29.0 ± 2.7	28.4 ± 2.9	36.7 ± 3.2
	0.03	36.9 ± 2.5	32.2 ± 3.2	30.4 ± 3.1	35.6 ± 2.3
	0.1	35.4 ± 2.7	32.0 ± 3.1	33.6 ± 4.2	37.8 ± 3.3
	0.3	34.2 ± 2.5	37.1 ± 4.5	33.7 ± 5.3	36.1 ± 4.0
	1.0	33.4 ± 2.3	35.2 ± 3.8	35.1 ± 5.1	34.7 ± 3.3
	3.0	30.6 ± 2.6 [a]	35.1 ± 4.6	34.6 ± 6.3	31.3 ± 2.4
	10.0	30.0 ± 1.9 [a]	34.5 ± 3.9	35.4 ± 6.1	27.9 ± 2.1 [a]

The values are mean ± S.E.M. and are expressed as ml/beat per m^2.
[a] $P < 0.05$ when compared to basal values.

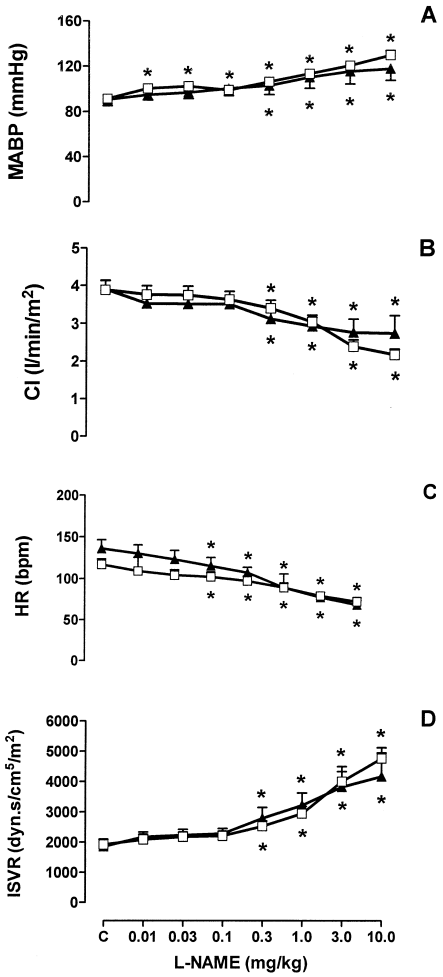

Fig. 1. Effects of N^{ω}-nitro-L-arginine methyl ester (L-NAME, 0.01–10.0 mg/kg) on the mean arterial blood pressure (MABP, panel A), cardiac index (CI, panel B), heart rate (HR, panel C) and index of systemic vascular resistance (ISVR, panel D) of anaesthetised dogs in the absence (□, $n = 18$) and in the presence (▲, $n = 7$) of enalapril (2 mg/kg). Each dose of N^{ω}-nitro-L-arginine methyl ester and enalapril was infused for 8 min at a rate of 0.5 ml/min. Values are the mean ± S.E.M. * $P < 0.05$ versus the respective control (C).

Table 2
Nitrate and nitrite levels and angiotensin-converting enzyme (ACE) activity in dog serum

	Treatment	Nitrate (μM)	Nitrite (μM)	ACE (nmol/ml per min)
Basal	–	10.2 ± 1.9	0.26 ± 0.12	13.0 ± 1.7
L-NAME	0.01	12.1 ± 2.5	0.42 ± 0.16	12.0 ± 1.9
	0.03	9.3 ± 1.5	0.54 ± 0.17	12.1 ± 1.3
	0.1	10.7 ± 1.2	0.58 ± 0.18	12.0 ± 1.5
	0.3	9.2 ± 2.0	0.28 ± 0.10	11.8 ± 1.7
	1.0	7.9 ± 1.5	0.60 ± 0.22	12.3 ± 1.4
	3.0	8.7 ± 1.5	0.48 ± 0.13	12.9 ± 2.3
	10.0	9.3 ± 2.5	0.50 ± 0.18	11.2 ± 1.7

Blood was withdrawn before (Basal) and after the infusion of each dose of N^{ω}-nitro-L-arginine methyl ester (0.01–10.0 mg/kg). Values represent the mean ± S.E.M. ($n = 5$).

Table 3
Effect of enalapril (2 mg/kg, $n = 9$) and Hoe 140 (0.1 mg/kg, $n = 6$) infusions on the basal mean arterial blood pressure (MABP, mmHg), cardiac index (CI, l/min per m^2), systolic index (SI, ml/beat per m^2), heart rate (HR, bpm) and index of systemic vascular resistance (ISVR, dyn·s/cm^5 per m^2) of anaesthetised dogs

	Enalapril		Hoe 140	
	Before	After	Before	After
MABP	94.4 ± 4.7	90.6 ± 3.4	97.5 ± 6.2	95.5 ± 8.6
CI	3.08 ± 0.47	3.48 ± 0.21 [a]	3.79 ± 0.31	3.63 ± 0.33
SI	26.7 ± 2.4	28.5 ± 2.1 [a]	36.6 ± 4.6	36.4 ± 4.5
HR	117 ± 7.2	124 ± 8.7	107 ± 8.9	103 ± 8.8
ISVR	2471.4 ± 187.0	2091.6 ± 122.3 [a]	2110.0 ± 187.7	2103.3 ± 101.8

Values represent the mean ± S.E.M. Results were analysed by analysis of variance followed by Duncan's test. [a] $P < 0.05$ when compared to values before drug administration.

Table 4
Potentiation of the bradykinin-induced cardiovascular changes by enalapril

	Enalapril		
	Before	After	Potentiation (%)
MABP (mmHg)	−19.7 ± 2.8	−48.7 ± 2.6 [a]	146
CI (l/min per m^2)	−0.73 ± 0.10	+2.00 ± 0.41 [a]	174
HR (bpm)	+22 ± 6.1	+48 ± 16.0	118
ISVR (dyn·s/cm^5 per m^2)	−993.8 ± 132.5	−1466.1 ± 107.7 [a]	147

The haemodynamic parameters measured were mean arterial blood pressure (MABP), cardiac index (CI), heart rate (HR) and index of systemic vascular resistance (ISVR). Values are the mean ± S.E.M. ($n = 7$). [a] $P < 0.05$ when compared to values obtained before enalapril administration.

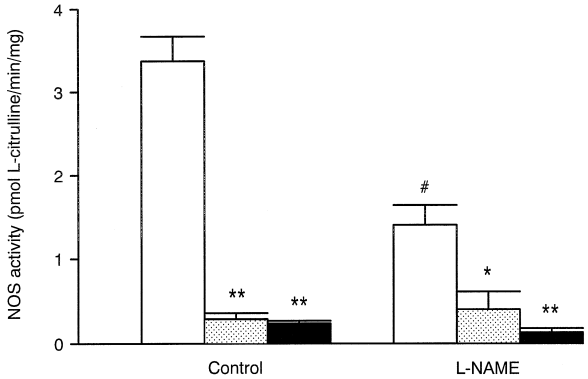

Fig. 2. Nitric oxide synthase (NOS) activity in cerebellum homogenates from control animals (no drug administered, $n = 5$, Control) and animals which received N^{ω}-nitro-L-arginine methyl ester (total dose of 14.44 mg/kg, $n = 5$, L-NAME). Nitric oxide synthase activity was assayed in normal Ca^{2+}-containing buffer (total activity, open columns), in the presence of 1 mM EGTA (Ca^{2+}-free buffer, stippled columns), and in the presence of 1 mM N^{ω}-nitro-L-arginine methyl ester (solid columns). The data represent the mean ± S.E.M. * $P < 0.05$ and ** $P < 0.01$ when compared to the corresponding activity in Ca^{2+}-containing buffer. $^{\#}$ $P < 0.01$ when compared to the activity in Ca^{2+}-containing buffer of the non-N^{ω}-nitro-L-arginine methyl ester-treated animals.

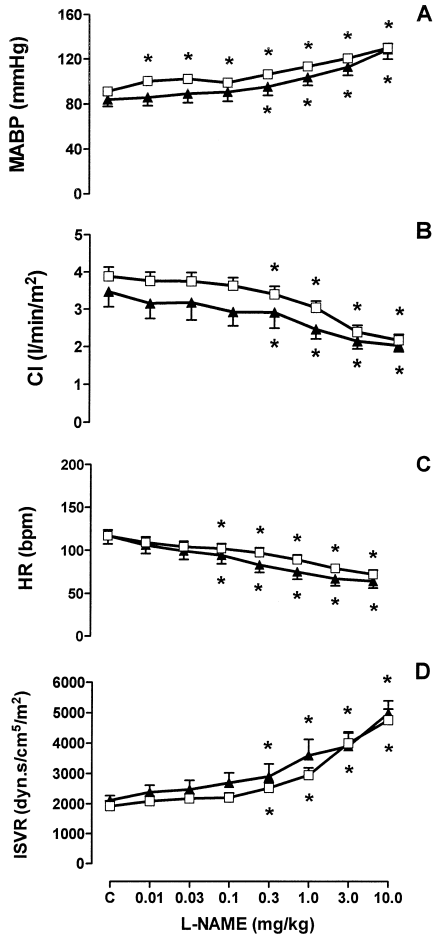

Fig. 3. Effects of N^{ω}-nitro-L-arginine methyl ester (L-NAME, 0.01–10.0 mg/kg) on the mean arterial blood pressure (MABP, panel A), cardiac index (CI, panel B), heart rate (HR, panel C) and index of systemic vascular resistance (ISVR, panel D) of anaesthetised dogs in the absence (□, $n = 18$) and presence (▲, $n = 9$) of captopril (2 mg/kg). Each dose of L-NAME and captopril was infused for 8 min at a flow rate of 0.5 ml/min. The values are the mean ± S.E.M. * $P < 0.05$ versus the respective control (C).

animals in which no drug was administered (Fig. 2). The in vitro conversion of [^{3}H]L-arginine to [^{3}H]L-citrulline was strongly inhibited (> 90%) when Ca^{2+} was omitted or N^{ω}-nitro-L-arginine methyl ester was added to the incubation medium, indicating that this conversion is due to the constitutive form of nitric oxide synthase. Despite the marked enzyme inhibition, neither the serum nitrate and nitrite levels nor the serum angiotensin-converting enzyme activity was significantly altered by N^{ω}-nitro-L-arginine methyl ester (Table 2).

3.2. Enalapril-treated animals

Table 3 shows the effects of enalapril on basal haemodynamic variables. Enalapril potentiated the cardiovascular changes induced by bradykinin (300 ng/kg, Table 4) and inhibited serum angiotensin-converting enzyme activity by 91% (from 12.8 ± 1.2 to 1.1 ± 0.2 nmol/ml per min, $n = 6$, $P < 0.05$). Despite the enzyme inhibition, the N^{ω}-nitro-L-arginine methyl ester-induced haemodynamic changes were unaltered. At the highest dose, N^{ω}-nitro-L-arginine methyl ester increased mean arterial blood pressure by 29.6% when compared to basal values (from 90.6 ± 5.4 to 117.4 ± 10.1 mmHg, $P < 0.05$, Fig. 1A). N^{ω}-Nitro-L-arginine methyl ester also dose-dependently increased the systemic vascular resistance (Fig. 1D). At 10 mg/kg, the index of systemic vascular resistance increased 124.1% (from 1857.4 ± 88.4 to 4163.5 ± 785.4 dyn · s/cm^5 per m^2, $P < 0.05$). Finally, N^{ω}-nitro-L-arginine methyl ester produced a dose-dependent decrease in the cardiac output and heart rate (Fig. 1B and C, respectively). At the highest dose, the cardiac index decreased 30.1% (from 3.89 ± 0.17 to 2.72 ± 0.48 l/min per m^2, $P < 0.05$), while the heart rate decreased 50.0% (from 136 ± 10.7 to 68 ± 6.3 bpm, $P < 0.05$). However, in enalapril-treated animals, the systolic index did not decrease in response to N^{ω}-nitro-L-arginine methyl ester administration (Table 1).

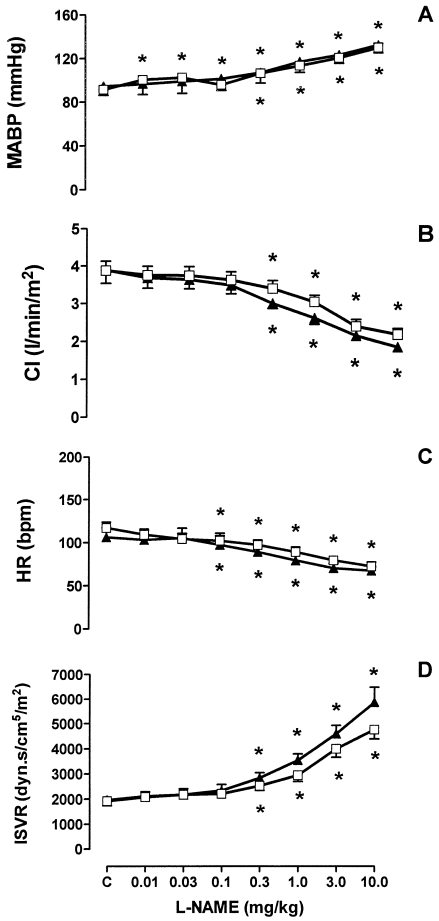

Fig. 4. Effects of N^{ω}-nitro-L-arginine methyl ester (L-NAME, 0.01–10.0 mg/kg) on the mean arterial blood pressure (MABP, panel A), cardiac index (CI, panel B), heart rate (HR, panel C) and index of systemic vascular resistance (ISVR, panel D) of anaesthetised dogs in the absence (□, $n = 18$) and presence (▲, $n = 6$) of the bradykinin B_2 receptor antagonist Hoe 140 (0.1 mg/kg). Each dose of N^{ω}-nitro-L-arginine methyl ester and Hoe 140 was infused for 8 min at a rate of 0.5 ml/min. Values are the mean ± S.E.M. * $P < 0.05$ compared to the respective control (C).

3.3. Captopril-treated animals

Captopril induced similar changes to enalapril in the basal haemodynamic parameters, i.e., a fall in mean arterial blood pressure (from 95.9 ± 5.0 to 90.1 ± 5.0 mmHg, $P < 0.05$). Captopril infusion also potentiated the bradykinin-induced cardiovascular changes (data not shown). As with enalapril, captopril failed to affect the N^{ω}-nitro-L-arginine methyl ester-induced haemodynamic changes (Fig. 3). Despite the fall in the cardiac index, N^{ω}-nitro-L-arginine methyl ester did not decrease the systolic index (Table 1).

3.4. Hoe 140-treated animals

Hoe 140 (0.1 mg/kg) did not significantly change the haemodynamic parameters of anaesthetised dogs (Table 3). The maximum hypotensive effect induced by bradykinin (300 ng/kg) was -42.8 ± 7.1 mmHg (from 98.4 ± 4.8 to 55.6 ± 7.3 mmHg) and was abolished by Hoe 140. In the presence of bradykinin B_2 receptor blockade, N^{ω}-nitro-L-arginine methyl ester dose-dependently affected all of the haemodynamic parameters. At 10.0 mg/kg, mean arterial blood pressure increased 39.8% (from 94.3 ± 8.2 to 131.8 ± 7.3 mmHg, $P < 0.05$ when compared to basal values, Fig. 4A). At the same dose, the index of systemic vascular resistance increased 199.8% (from 1956.8 ± 135.8 to 5867.3 ± 619.6 dyn · s/cm^5 per m^2, $P < 0.05$; Fig. 4D). Simultaneously, N^{ω}-nitro-L-arginine methyl ester decreased the cardiac output and caused bradycardia (Fig. 4B and C). At the highest dose, N^{ω}-nitro-L-arginine methyl ester reduced the cardiac index by 52.9% (from 3.89 ± 0.35 to 1.83 ± 0.11 l/min per m^2, $P < 0.05$) while the heart rate decreased 36.8% (from 106 ± 11.3 to 67 ± 5.1 bpm, $P < 0.05$). The decrease in the cardiac index was accompanied by a decrease in the systolic index (25.2%, Table 1).

4. Discussion

The present results clearly demonstrate that the angiotensin-converting enzyme inhibitors enalapril and captopril failed to affect the haemodynamic changes induced by acute N^{ω}-nitro-L-arginine methyl ester administration in the anaesthetised dog. The bradykinin B_2 antagonist Hoe 140 also had no significant effect on these haemodynamic changes. These results are unlikely to reflect inadequate doses of the compounds used, since enalapril and captopril strongly potentiated, while Hoe 140 abolished, the cardiovascular changes induced by bradykinin. Furthermore, at the dose used, enalapril markedly inhibited serum angiotensin-converting enzyme activity. Together, these findings indicate that the renin-angiotensin system does not modulate the haemodynamic changes induced by acute N^{ω}-nitro-L-arginine methyl ester administration in the anaesthetised dog. Similar results were obtained by Nafrialdi and Mimran (1993) who verified that angiotensin II receptor blockade by losartan did not prevent the development of either arterial hypertension or bradycardia induced by acute N^{ω}-nitro-L-arginine methyl ester administration. Moreover, losartan did not alter the decrease in cardiac output induced by N^{ω}-nitro-L-arginine methyl ester in two-kidney, one-clip hypertensive rats (Sigmon and Beierwaltes, 1993).

In rats, chronic inhibition of nitric oxide synthesis by N^{ω}-nitro-L-arginine methyl ester leads to hypertension (Baylis et al., 1992; Ribeiro et al., 1992). In contrast to the acute situation, daily co-administration of N^{ω}-nitro-L-arginine methyl ester and losartan (Ribeiro et al., 1992) or N^{ω}-nitro-L-arginine methyl ester and enalapril (Pollock et al., 1993; Moreno et al., 1995) prevents the development of arterial hypertension. These observations indicate that chronic, but not acute N^{ω}-nitro-L-arginine methyl ester-induced hypertension is modulated by renin-angiotensin system activation.

One possible explanation for this discrepancy would reflect the action of nitric oxide synthase inhibitors on renin release. Acute nitric oxide blockade induces renal vasoconstriction (Gardiner et al., 1990; Zatz and De Nucci, 1991) and hence could evoke the release of renin. However, acute nitric oxide blockade diminishes renin secretion (Johnson and Freeman, 1992; Naess et al., 1993; Deng et al., 1994; Schricker et al., 1995) further indicating that the haemodynamic effects induced by acute N^{ω}-nitro-L-arginine methyl ester administration are independent of the renin-angiotensin system activation. Indeed, chronic administration of nitric oxide synthesis inhibitors is associated with hyporeninemia (Dananberg et al., 1993; Navarro et al., 1994). Considering that tissue renin-angiotensin system seems to play a more important role in controlling blood pressure than circulating renin-angiotensin system (Dzau, 1986; Campbell, 1987; Falkenhahn et al., 1994), these studies may reflect this difference since they have measured plasma, and not tissue renin activity.

Pretreatment of the animals with either enalapril or captopril prevented the decrease in the systolic index induced by N^{ω}-nitro-L-arginine methyl ester. Since angiotensin II exerts a positive inotropic effect (Falkenhahn et al., 1994), this protection suggests that bradykinin, rather than angiotensin II, modulates canine myocardial contractility. The finding that the decrease in cardiac output persisted even in the animals treated with angiotensin-converting enzyme inhibitors indicates that this effect is mainly due to bradycardia.

Acknowledgements

A.Z. is a fellow of FAPESP (92/0469-0) and S.A.T. of CAPES. We thank Aírton Fernando de Paula and Flávio do Rego de Araújo Faro for technical assistance.

References

Baylis, C., B. Mitruka and A. Deng, 1992, Chronic blockade of nitric oxide synthesis in the rat produces systemic hypertension and glomerular damage, J. Clin. Invest. 90, 278.

Campbell, D.Z., 1987, Circulating and tissue angiotensin systems, J. Clin. Invest. 79, 1.

Cushman, D.W. and H.S. Cheung, 1970, Spectrophotometric assay and properties of the ACE of the rabbit lung, Biochem. Pharmacol. 20, 1637.

Dananberg, J., R.S. Sider and R.J. Grekin, 1993, Sustained hypertension induced by orally administered nitro-L-arginine, Hypertension 21, 359.

Deng, X.L., W.J. Welch and C.S. Wilcox, 1994, Renal vasoconstriction during inhibition of NO synthase: effect of dietary salt, Kidney Int. 46, 639.

Dzau, V.J., 1986, Significance of the vascular renin-angiotensin pathway, Hypertension 8, 553.

Falkenhahn, M., P. Gohlke, M. Paul, M. Stoll and T. Unger, 1994, The renin-angiotensin system in the heart and vascular wall: new therapeutic aspects, J. Cardiovasc. Pharmacol. 24 (Suppl. 2), S6.

Fegler, G., 1954, Measurement of cardiac output in anesthetized animals by a thermodilution method, Q. J. Exp. Physiol. 39, 153.

Förstermann, U., L.D. Gorsky, J.S. Pollock, H.H.H.W. Schmidt, M. Heller and F. Murad, 1990, Regional distribution of EDRF/NO-synthesizing enzyme(s) in rat brain, Biochem. Biophys. Res. Commun. 168, 727.

Ganz, W., R. Donoso, H.S. Marcus, J.S. Forrestier and H.J.C. Swan, 1971, A new technique for measurement of cardiac output by thermodilution in man, Am. J. Cardiol. 27, 392.

Gardiner, S.M., A.M. Compton, P.A. Kemp and T. Bennett, 1990, Regional and cardiac effects of N^G-nitro-L-arginine methyl ester in conscious, Long Evans rats, Br. J. Pharmacol. 101, 625.

Hurst, P.L. and C.J. Lovell-Smith, 1981, Optimized assay for serum ACE activity, Clin. Chem. 27, 2048.

Johnson, R.A. and R.F. Freeman, 1992, Pressure natriuresis in rats during blockade of the L-arginine/nitric oxide pathway, Hypertension 19, 333.

Moncada, S. and A. Higgs, 1993, The L-arginine-nitric oxide pathway, New Engl. J. Med. 329, 2002.

Moreno Jr., H., L.P. Nathan, S.K.P. Costa, K. Metze, E. Antunes, R. Zatz and G. De Nucci, 1995, Enalapril does not prevent the myocardial ischaemia caused by the chronic inhibition of nitric oxide synthesis, Eur. J. Pharmacol. 287, 93.

Muscará, M.N. and G. De Nucci, 1996, Simultaneous determination of nitrite and nitrate anions in plasma, urine and cell culture supernatants by high-performance liquid chromatography with post-column reactions, J. Chromatogr. B 686, 157.

Naess, P.A., G. Christensen, K.A. Kirkeboen and F. Kiil, 1993, Effect on renin release of inhibiting renal nitric oxide synthesis in anaesthetized dogs, Acta Physiol. Scand. 148, 137.

Nafrialdi, B.J. and A. Mimran, 1993, Renin-angiotensin system in the pressor effect of acute *N*-omega-nitro-L-arginine methyl ester, J. Hypertens. 11 (Suppl. 5), S324.

Navarro, J., A. Sanchez, J. Saiz, L.M. Ruilope, J. Garciaestan, J.C. Romero, S. Moncada and V. Lahera, 1994, Hormonal, renal and metabolic alterations during hypertension induced by chronic inhibition of NO in rats, Am. J. Physiol. 36, R1516.

Peterson, G.L., 1977, A simplification of the protein assay method of Lowry et al. which is more generally applicable, Anal. Biochem. 83, 346.

Pollock, D.M., J.S. Polakowski, B.J. Divish and T.J. Opgenorth, 1993, Angiotensin blockade reverses hypertension during long-term nitric oxide synthase inhibition, Hypertension 21, 660.

Rees, D.D., R.M.J. Palmer and S. Moncada, 1989, Role of endothelium-derived nitric oxide in the regulation of blood pressure, Proc. Natl. Acad. Sci. USA 86, 3375.

Ribeiro, M.O., E. Antunes, G. De Nucci, S.M. Lovisolo and R. Zatz, 1992, Chronic inhibition of nitric oxide synthesis – a new model of arterial hypertension, Hypertension 20, 298.

Schricker, K., I. Hegyi, M. Hamann, B. Kaissling and A. Kurtz, 1995, Tonic stimulation of renin gene expression by nitric oxide is counteracted by tonic inhibition through angiotensin II, Proc. Natl. Acad. Sci. USA 92, 8006.

Sigmon, D.H. and W.H. Beierwaltes, 1993, Renal nitric oxide and angiotensin II interaction in renovascular hypertension, Hypertension 22, 237.

Stamler, J.S., E. Loh, M.-A. Roddy, K.E. Currie and M.A. Creager, 1994, Nitric oxide regulates basal systemic and pulmonary vascular resistance in healthy humans, Circulation 89, 2035.

Van Gelderen, E.M., M.D.B. Boer and P.R. Saxena, 1993, N^G-Nitro-L-arginine methyl ester: systemic and pulmonary haemodynamics, tissue blood flow and arteriovenous shunting in the pig, Naunyn-Schmiedeberg's Arch. Pharmacol. 348, 417.

Wirth, K., F.J. Hoek, U. Albus, W. Linz, H. Anagnostopoulos, S.T. Henke, G. Breipohl, W. Konig, J. Knolle and B.A. Scholkens, 1991, HOE 140, a new potent and long acting bradykinin-antagonist: in vivo studies, Br. J. Pharmacol. 102, 774.

Zappellini, A., H. Moreno Jr., E. Antunes and G. De Nucci, 1996, Evaluation of two different inspiratory fractions on the hemodynamic effects of N^{ω}-nitro-L-arginine methyl ester in anesthetized dogs, Braz. J. Med. Biol. Res. 29, 33.

Zatz, R. and G. De Nucci, 1991, Effects of acute nitric oxide inhibition on rat glomerular microcirculation, Am. J. Physiol, 261, F360.