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Contribution of the renin-angiotensin system to short-term blood pressure variability during blockade of nitric oxide synthesis in the rat

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1 The aim of this study was to investigate, by use of spectral analysis, (1) the blood pressure (BP) variability changes in the conscious rat during blockade of nitric oxide (NO) synthesis by the L-arginine analogue N^G-nitro-L-arginine methyl ester (L-NAME); (2) the involvement of the renin-angiotensin system in these modifications, by use of the angiotensin II AT_1-receptor antagonist losartan.

2 Blockade of NO synthesis was achieved by infusion for 1 h of a low-dose (10 $\mu g\ kg^{-1}\ min^{-1}$, i.v., $n=10$) and high-dose (100 $\mu g\ kg^{-1}\ min^{-1}$, i.v., $n=10$) of L-NAME. The same treatment was applied in two further groups ($2 \times n=10$) after a bolus dose of losartan (10 mg kg^{-1}, i.v.).

3 Thirty minutes after the start of the infusion of low-dose L-NAME, systolic BP (SBP) increased ($+10 \pm 3$ mmHg, $P<0.01$), with the effect being more pronounced 5 min after the end of L-NAME administration ($+20 \pm 4$ mmHg, $P<0.001$). With high-dose L-NAME, SBP increased immediately (5 min: $+8 \pm 2$ mmHg, $P<0.05$) and reached a maximum after 40 min ($+53 \pm 4$ mmHg, $P<0.001$); a bradycardia was observed (60 min: -44 ± 13 beats min^{-1}, $P<0.01$).

4 Low-dose L-NAME increased the low-frequency component (LF : 0.02-0.2 Hz) of SBP variability (50 min: 6.7 ± 1.7 $mmHg^2$ vs 3.4 ± 0.5 $mmHg^2$, $P<0.05$), whereas the high dose of L-NAME not only increased the LF component (40 min: 11.7 ± 2 $mmHg^2$ vs 2.7 ± 0.5 $mmHg^2$, $P<0.001$) but also decreased the mid frequency (MF : 0.2-0.6 Hz) component (60 min: 1.14 ± 0.3 $mmHg^2$ vs 1.7 ± 0.1 $mmHg^2$, $P<0.05$) of SBP.

5 Losartan did not modify BP levels but had a tachycardic effect ($+45$ beats min^{-1}). Moreover, losartan increased MF oscillations of SBP (4.26 ± 0.49 $mmHg^2$ vs 2.43 ± 0.25 $mmHg^2$, $P<0.001$), prevented the BP rise provoked by the low-dose of L-NAME and delayed the BP rise provoked by the high-dose of L-NAME. Losartan also prevented the amplification of the LF oscillations of SBP induced by L-NAME; the decrease of the MF oscillations of SBP induced by L-NAME was reinforced after losartan.

6 We conclude that the renin-angiotensin system is involved in the increase in variability of SBP in the LF range which resulted from the withdrawal of the vasodilating influence of NO. We propose that NO may counterbalance LF oscillations provoked by the activity of the renin-angiotensin system.

Keywords: Spectral analysis; N^G-nitro-L-arginine methyl ester (L-NAME); losartan; arterial pressure

Introduction

The utilization of nitric oxide (NO) synthase inhibitors, N^G-substituted arginine (Arg) analogues, including N^G-monomethyl-L-arginine (L-NMMA), N^G-nitro-L-arginine (L-NOARG) and its methyl ester (L-NAME), has led to the suggestion that a permanent synthesis of NO may be responsible for an endothelium-dependent vasodilating effect (Moncada *et al.*, 1991). Withdrawal of the vasodilator influence of NO by acute administration of NO synthase inhibitors elicits a marked arterial hypertension accompanied by a bradycardia, with increased total peripheral resistance and reduced cardiac output (Gardiner *et al.*, 1990; Wang *et al.*, 1992; 1995). In addition to its tonic regulation of blood pressure (BP), NO plays an important role in controlling the fluctuations of BP around a mean level (Persson, 1996). In the conscious dog, inhibition of the NO synthesis increased the overall variability of mean arterial BP over 24 hours, resulting in impaired buffering capacity of the circulation (Persson *et al.*, 1992a; Just *et al.*, 1994). These authors proposed that NO acts as a physiological buffer of BP fluctuations. In the conscious rat, NO was shown to buffer slow oscillations of BP (Cordero *et al.*, 1994).

Short-term fluctuations of BP and heart rate (HR) have been characterized in rats by use of a spectral analysis technique which provides a measure of the variability of BP and HR within distinct frequency domains (Akselrod *et al.*, 1987; Japundzic *et al.*, 1990; Cerutti *et al.*, 1991; Persson *et al.*, 1992b): (i) a high-frequency (HF) domain corresponding to fast oscillations which include a peak around 1.5 Hz, linked to respiration, (ii) a mid-frequency domain (MF: 0.2-0.6 Hz) corresponding to the Mayer waves is located around 0.4 Hz and related to the activity of the sympathetic nervous system (Japundzic *et al.*, 1990; Cerutti *et al.*, 1991; Persson *et al.*, 1992b; Brown *et al.*, 1994), (iii) low-frequency fluctuations (LF: 0.02-0.2 Hz) of BP have recently been demonstrated as being partly dependent upon the renin-angiotensin system (RAS) in normotensive rats (Gaudet *et al.*, 1995) and in renovascular hypertension (Ponchon & Elghozi, 1996).

Although the hypertension following the acute inhibition of NO synthesis is not generally considered to be driven by changes in the RAS (Lahera *et al.*, 1991; Baylis *et al.*, 1993; Nafrialdi *et al.*, 1994), the accompanying increase of BP variability occurs in a frequency area which is partly dependent upon this humoral system (Ponchon & Elghozi, 1996). To investigate the contribution of the RAS to BP variability changes

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encountered during acute NO synthesis inhibition, we infused two doses of L-NAME and assessed the effects on BP and HR variabilities after blockade of the RAS by losartan.

Methods

General

Six groups of male Wistar rats (Janvier, Le Genest-Saint-Isle, France) weighing 280–300 g were studied. Animals were maintained in controlled housing conditions (20 ± 1°C, lighting 08.00–20.00 h) and received tap water *ad libitum* and a standard rat chow diet (A 04, UAR, Epinay sur Orge, France). Experiments were performed in conscious, unrestrained animals.

Surgery

Animals were surgically prepared with catheters under anaesthesia (Pentobarbitone, 60 mg kg^{-1}, i.p.). Catheters were inserted into the right femoral artery to measure arterial BP and into the right jugular vein to allow the intravenous bolus injections and the infusions as previously described (Grichois *et al.*, 1992). Both catheters were tunneled subcutaneously to exit from the neck. Each animal received penicillin G (100,000 UI, i.p.) and was placed in an individual cage. After 2 days of recovery, the exteriorized venous catheter was connected to an electrical microsyringe (Perfusor EDL 2 Braun, Roucaire, Vélizy, France) for saline or L-NAME infusions and the arterial catheter was connected to a pressure transducer (Spectramed P 10EZ, Bilthoven, The Netherlands) for recording of pulsatile arterial pressure. The transducer was connected to a Gould RS 3400 Polygraph (Ballainvilliers, France). The output from the pulsatile arterial pressure preamplifier was connected to an A/D converter to permit data acquisition, storage and analysis by a 486 DX computer from Fujikama (Toronto, Canada).

Signal processing and spectrum analysis

Experiments were started approximately 1 h after the rats had been connected to the pressure transducer and injection syringe. The cages were housed in quiet surroundings. The BP signal processing and spectrum analysis have been detailed elsewhere (Grichois *et al.*, 1992). Briefly, the evenly spaced sampling allowed direct spectral analysis with a fast Fourier transform (FFT) algorithm of a stationary period in a 1024-point series. This corresponded to a 102.4-s period at the 10-Hz sampling rate. Thus, each spectral component (band) corresponded to a harmonic of 1/1024 Hz i.e. 0.00098 Hz. The first spectral component corresponded to the mean value of the variable. The power of the HR or BP spectra (ordinates) had units of (beats min^{-1})^2 and $mmHg^2$ respectively. The sum of the whole values of consecutive bands (without the first band) represents the variance of HR or BP. Integrated spectra of the systolic and diastolic BP and HR were computed in the high (respiratory), mid (0.2–0.6 Hz) and low (0.02–0.2 Hz) frequency bands. Finally, simple statistics i.e. mean and standard deviations (s.d.) of the distribution of the variables of the 102.4-s files (1024 values) used for the spectral analysis were computed.

Experimental protocol

The first series of experiments was designed to assess the modification of BP and HR variabilities induced by acute inhibition of NO synthesis. Two doses of N^G-nitro-L-arginine methyl ester (L-NAME) were used in an attempt to dissociate the effects on BP levels and the effects on BP variability. The first group of animals ($n=7$) received vehicle infusion (saline: 100 $\mu l\ kg^{-1}\ min^{-1}$, i.v.) for 100 min. The second group ($n=11$) was infused with low-dose of L-NAME (10 $\mu g\ kg^{-1}\ min^{-1}$, i.v.) and the third group ($n=10$) was infused with high-dose (100 $\mu g\ kg^{-1}\ min^{-1}$, i.v.) L-NAME. Infusions of L-NAME lasted 60 min and were followed by a 40 min vehicle infusion.

The second series of experiments was performed to estimate the involvement of the RAS in the modifications of BP and HR variabilities induced by the acute inhibition of NO synthesis. The RAS activity was therefore blocked by an i.v. bolus of losartan (10 mg kg^{-1}, 100 $\mu l\ kg^{-1}$) before the infusions of either saline (group 4, $n=7$), low-dose L-NAME (group 5, $n=10$) or high-dose L-NAME (group 6, $n=10$). Finally, the absence of a pressor response secondary to an angiotensin II injection (100 ng kg^{-1}, 100 $\mu l\ kg^{-1}$) indicated the completeness of angiotensin II (AII) receptor blockade by losartan.

In all studies, baseline BP and HR were recorded before any administration of saline or drug. In the first series of experiments, further recordings were made at different times for 100 min after the onset of the infusion. The data were compared to the corresponding baseline (pre-infusion) values. In the second series of experiments, the effects of losartan were examined 20 min after its injection by comparison of the losartan data to the corresponding baseline values. These losartan data then became the control values to which further data, obtained at different times for 100 min after the onset of the infusion, were compared. For each condition, a 102.4-s file was used from a five min recording session.

Drugs

Sodium pentobarbitone (6 g 100 ml^{-1}) was purchased from Sanofi (Libourne, France), penicillin G was from Diamant (Puteaux, France) and L-NAME, (N^{ω}-nitro-L-arginine methyl ester) and angiotensin II were obtained from Sigma chemical Co. (St Louis, Missouri, U.S.A.). Losartan (DuP 753, potassium salt of 2-*n*-butyl-4-chloro-5-hydroxymethyl-1-[(2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl)methyl]imidazole) was generously donated by Merck-Sharp and Dohme (Rahway, NJ, U.S.A.). Penicillin, L-NAME, losartan and angiotensin II were dissolved in saline.

Statistical analysis

Data are presented as mean ± s.e.mean. Variables were analysed following a normalization procedure where the pre-infusion data were considered as 100%. When the variance ratios were significant ($F>0.05$), a logarithmic transformation was applied. Statistical analysis was performed by use of Student's paired t test. Statistical significance was taken as $P<0.05$.

Results

The average values of systolic BP (SBP), diastolic BP (DBP), HR and their related s.d. were similar in the six groups of rats used in this study.

Effects of L-NAME on blood pressure and heart rate levels and their standard deviations

Figures 1 and 2 show the changes in SBP and HR levels produced by infusions of low (10 $\mu g\ kg^{-1}\ min^{-1}$) and high-dose (100 $\mu g\ kg^{-1}\ min^{-1}$) of L-NAME, respectively. The infusion of vehicle for 100 min did not modify the SBP and HR levels, nor their respective s.d. (not shown). With low-dose L-NAME (Figure 1a), SBP increased significantly from the 30th min of infusion (+10 ± 3 mmHg, $P<0.01$). The maximum increase of SBP was reached 5 min after stopping L-NAME administration (+20 ± 4 mmHg, $P<0.001$). Conversely, there was no change in HR levels. The s.d. of SBP was increased by infusion of low-dose L-NAME and significant increases were seen 20 and 30 min into the infusion (20 min: 3.5 ± 0.3 mmHg *versus* 2.7 ± 0.2 mmHg, $P<0.05$). High-dose L-NAME (Figure 2a) increased SBP from the 5th min (+8 ± 2 mmHg, $P<0.05$), with a maximum effect at 40 min (+53 ± 4 mmHg, $P<0.001$).

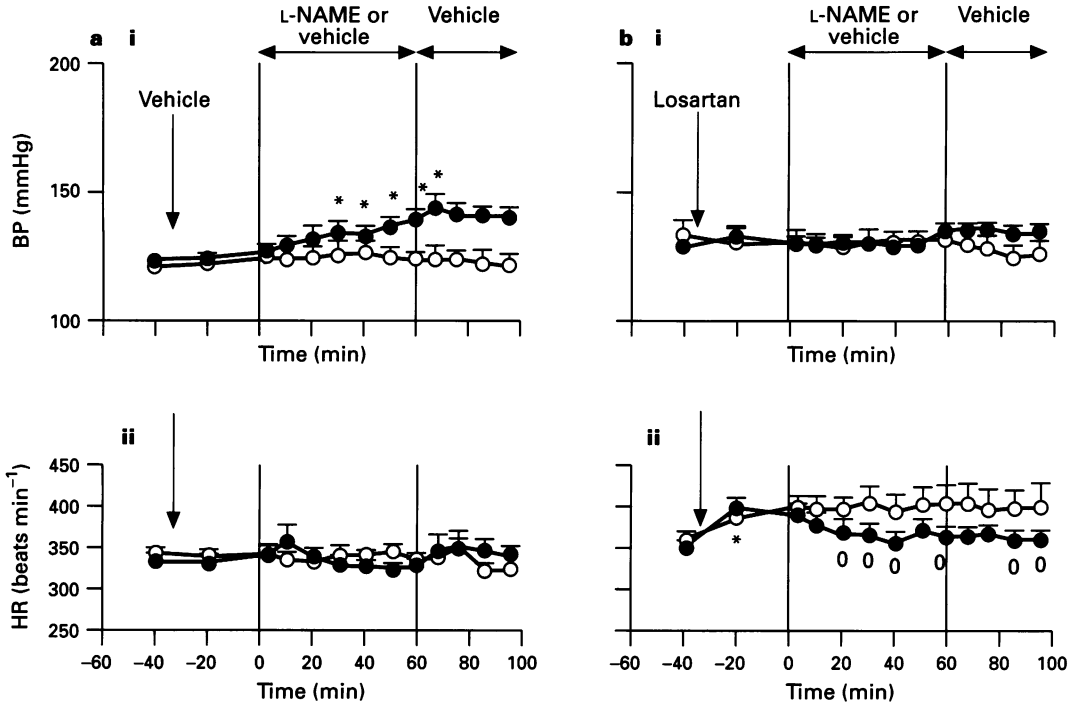

Figure 1 Effects (mean±s.e.mean) of infusions of N^G-nitro-L-arginine methyl ester (L-NAME, $10\,\mu g\,kg^{-1}\,min^{-1}$, i.v., ●) or vehicle (0.9% NaCl, $100\,\mu l\,kg^{-1}\,min^{-1}$, i.v., ○) on systolic blood pressure (BP, i) and on heart rate (HR, ii) in (a) vehicle-treated rats (i.v. bolus of $100\,\mu l\,kg^{-1}$, $n=7$ for controls, $n=11$ for L-NAME-infused rats) and (b) losartan-treated rats (i.v. bolus of $10\,mg\,100\,\mu l^{-1}\,kg^{-1}$, $n=7$ for controls, $n=10$ for L-NAME infused rats). $^*P<0.05$ for change relative to the corresponding baseline pre-administration resting value. $^\circ P<0.05$ for change relative to the corresponding losartan value.

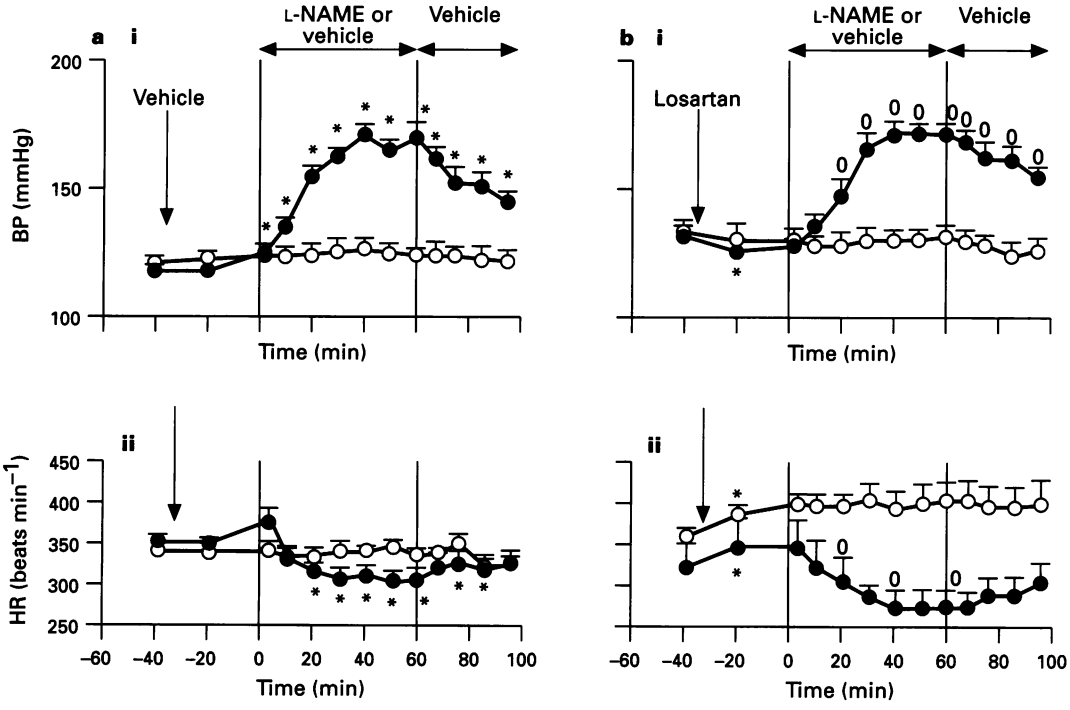

Figure 2 Effects (mean±s.e.mean) of infusions of N^G-nitro-L-arginine methyl ester (L-NAME, $100\,\mu g\,kg^{-1}\,min^{-1}$, i.v., ●) or vehicle (0.9% NaCl, $100\,\mu l\,kg^{-1}\,min^{-1}$, i.v., ○) on systolic blood pressure (BP, i) and on heart rate (HR, ii) in (a) vehicle-treated rats (i.v. bolus of $100\,\mu l\,kg^{-1}$, $n=7$ for controls, $n=10$ for L-NAME infused rats) and (b) losartan-treated rats (i.v. bolus of $10\,mg\,100\,\mu l^{-1}\,kg^{-1}$, $n=7$ for controls, $n=10$ for L-NAME infused rats). $^*P<0.05$ for change relative to the corresponding baseline pre-administration resting value. $^\circ P<0.05$ for change relative to the corresponding losartan value.

Concomitantly with the SBP increase, HR decreased. The largest bradycardic effect was seen after 60 min of the infusion (-44 ± 13 beats min^{-1}). The s.d. of SBP was increased all along the infusion of the high-dose of L-NAME (for example, at 40 min, s.d. was 4.3 ± 0.6 mmHg compared to control s.d. 2.5 ± 0.1 mmHg, $P<0.01$). This result corresponded to a 3 fold increase in the values of the variances by the high-dose of L-NAME.

Table 1 Changes in average values and standard deviations (s.d.) of systolic blood pressure (BP) and heart rate, 40 minutes after the onset of infusions of vehicle or L-NAME 10 μg kg^{-1} min^{-1} or 100 μg kg^{-1} min^{-1}

	Vehicle			*Losartan*		
	Group 1 (Vehicle)	*Group 2 (L-NAME 10)*	*Group 3 (L-NAME 100)*	*Group 4 (Vehicle)*	*Group 5 (L-NAME 10)*	*Group 6 (L-NAME 100)*
Rats (*n*)	7	11	10	7	10	10
Systolic BP change (mmHg)						
Average	4±2	9±3*	54±4***	0±2.9	−4±4	45±7***
s.d.	0.4±0.2	0.6±0.4	2.0±0.5**	0.0±0.4	−0.3±0.2	−0.4±0.3
Heart rate change (beats min^{-1})						
Average	3±9	−3±7	−39±12*	6±18	−40±10**	−88±12*
s.d.	1.0±1.4	0±1.2	5.1±1.9	−0.6±2.7	0.7±0.9	−2.7±2.1

An i.v. bolus of vehicle (groups 1, 2 and 3) or of losartan (10 mg kg^{-1}, 100 μl kg^{-1}; groups 4, 5 and 6) was given prior starting infusion. The values obtained 20 min after these bolus serve as reference to calculate the changes. Values are mean±s.e. mean. *$P<0.05$; **$P<0.01$; ***$P<0.001$

Effects of acute losartan on responses of blood pressure and heart rate levels and their standard deviations during L-NAME infusion

The effect of acute administration of losartan (10 mg kg^{-1}, 100 μl kg^{-1}, i.v.) was examined 20 min after its administration. We found no vehicle effect. Losartan slightly reduced SBP in group 6 (126±4 mmHg *versus* 131±3 mmHg, $P<0.01$), but did not affect the SBP of the other groups (group 4 : 130±6 mmHg *versus* 133±6 mmHg; group 5 : 133±3 mmHg *versus* 129±2 mmHg). Its main effect was to increase HR levels by 45 beats min^{-1} (mean change calculated for groups 4+5+6, $P<0.01$). On the other hand, the s.ds of SBP were enhanced after losartan administration (group 4 : 3.1±0.2 mmHg *versus* 2.6±0.2 mmHg, $P<0.05$; group 5 : 3.1±0.2 mmHg *versus* 2.6±0.1 mmHg, $P<0.01$; group 6 : 3.3±0.2 mmHg *versus* 2.7±0.1 mmHg, $P<0.05$). Losartan increased the s.ds of HR in all groups with a significant effect in group 6 (11.6±1.4 beats min^{-1} *versus* 7.0±0.5 beats min^{-1}, $P<0.05$).

The effects of losartan on SBP and HR are shown in Figures 1b and 2b. The infusion of vehicle *per se* did not change SBP and HR levels; s.d. of SBP and HR were not modified. Losartan prevented the rise in SBP usually produced by the infusion of low-dose L-NAME (Figure 1b). Heart rate, which was increased after losartan, decreased during the infusion of low-dose L-NAME. Losartan did not prevent the rise in SBP produced by the high-dose of L-NAME but delayed it (Figure 2b). The first significantly enhanced values were obtained after 20 min of infusion (+20±6 mmHg, $P<0.01$). The greatest change in SBP levels were seen after 50 min of infusion (+45±5 mmHg, $P<0.001$). The corresponding highest values of SBP were similar to those obtained during the infusion of high-dose L-NAME without losartan treatment (172±4 mmHg *versus* 171±4 mmHg).The bradycardic response produced by the infusion of high-dose L-NAME was not modified by the administration of losartan. Losartan prevented the rise of the s.d. of SBP produced by both the low and the high doses of L-NAME.

Table 1 shows the changes of the average SBP and HR values and their s.d. produced by the low and high doses of L-NAME after an administration of vehicle or losartan; the time 40 min of infusion was chosen because it corresponded to the greatest effect on SBP observed with the high-dose of L-NAME. In groups 1 and 4, the SBP and HR levels were not modified by infusions. The low-dose of L-NAME significantly enhanced SBP (group 2). This effect was totally suppressed when rats received losartan before starting the infusion (group 5). In this case, we observed a bradycardic response to L-NAME probably as a result of the former tachycardic effect of losartan. High-dose L-NAME greatly enhanced SBP and concomitantly decreased HR (group 6). The hypertensive effect of high-dose L-NAME was not changed by losartan and the bradycardic response was reinforced (−88±12 beats min^{-1} *versus* −39±12 beats min^{-1}).

Effects of L-NAME on blood pressure and heart rate variabilities: effects of losartan on these responses

Spectral components of the arterial pressure variability were not modified through all the experiments in the time control group 1.

Figure 3 illustrates the modifications of SBP variability produced by high-dose L-NAME, by losartan and by high-dose L-NAME after acute treatment with losartan. One hundred second recordings of SBP were presented because they corresponded to the time necessary to make the associated spectrum with FFT (exactly 102.4 s). In the saline spectrum we distinguish the HF oscillations represented by a peak around 1.4 Hz. This HF peak is located at the respiratory frequency of the animal. In the lower frequencies there is some power both in the MF area (0.2–0.4 Hz) and the LF area (0.02–0.2 Hz). The infusion of high-dose L-NAME not only increased the level of SBP but also strongly altered its oscillations. The changes in SBP variability by L-NAME showed modifications in the spectrum evidenced as enhancement of the power in the LF domain and a great reduction of the MF oscillations. Losartan, as previously shown in Table 1, did not modify the SBP level, but changed the oscillations, giving power in the MF component of the variability. Losartan did not change the hypertensive response produced by L-NAME infusion but strongly reduced the increase of power in the LF area produced by L-NAME. On the other hand, the increase of the MF oscillations after losartan alone was abolished by subsequent L-NAME infusion.

Figure 4 shows the effect of losartan on the power in the LF and MF frequency bands of the SBP spectra during infusions of low- and high- doses of L-NAME at different times corresponding to maximum effects. Blood pressure variability was not modified by saline infusion. During infusions of L-NAME, the LF fluctuations were progressively amplified. Low-dose L-NAME significantly increased the LF area at 50 min of infusion (6.66±1.68 mmHg^2 *versus* 3.43±0.50 mmHg^2, $P<0.05$). High-dose L-NAME significantly increased the LF area from the 30th min of infusion up to the 75th min, the greatest effect being observed at 40 min (11.7±2.39 mmHg^2 *versus* 2.69±0.50 mmHg^2, $P<0.001$). This LF fluctuation amplification was accompanied by a decrease in the MF area of SBP evident at 60 min (1.14±0.34 mmHg^2 *versus* 1.67±0.19 mmHg^2, $P<0.05$). In the DBP spectra, LF fluctuations decreased from the 10th min of infusion up to the end (at 40 min: 1.05±0.45 mmHg^2 *versus* 1.67±0.15 mmHg^2, $P<0.05$). Losartan prevented the amplification of the LF oscillations provoked by L-NAME. The MF oscillations pre-

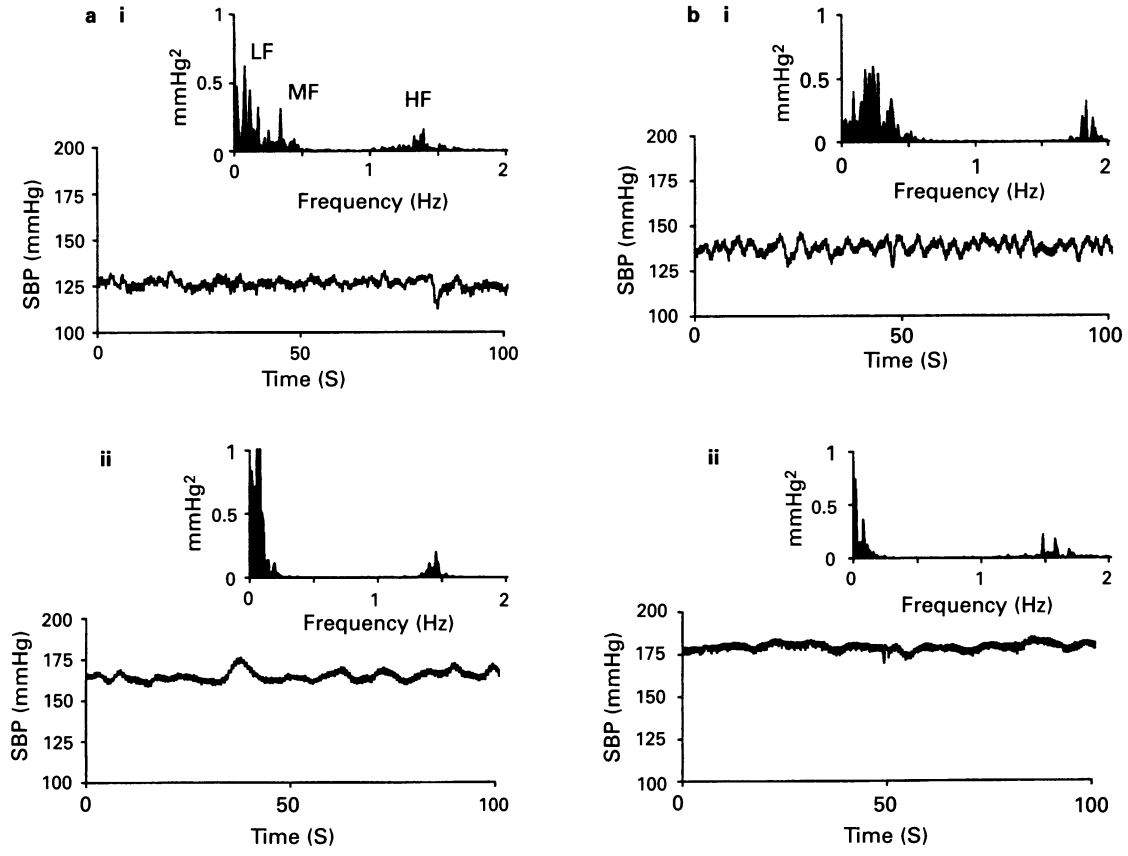

Figure 3 Examples of systolic blood pressure (SBP) digitized recordings for 100 s and their corresponding spectra of rats infused with vehicle after (a) saline or (b) losartan administration (i) and of rats infused with a high-dose of N^G-nitro-L-arginine methyl ester (L-NAME) ($100\,\mu g\,kg^{-1}\,min^{-1}$, i.v.) after saline or losartan administration (ii).

viously increased by losartan were diminished by high-dose L-NAME (0.37 ± 0.11 mmHg^2 *versus* 4.54 ± 0.79 mmHg^2, $P<0.05$).

Heart rate variability was not modified either by vehicle or by L-NAME infusions. Losartan did not change HR variability. After blockade by the losartan, the infusion of high-dose L-NAME decreased the MF oscillations of HR from the 20th min of infusion up to the end (at 40 min: 4.27 ± 1.06 (beats min^{-1})^2 *versus* 9.55 ± 3.99 (beats min^{-1})^2).

Discussion

The present study was designed to characterize the pattern of BP and HR variability during acute blockade of NO synthase activity by L-NAME. By use of a spectral analysis technique, we showed that the withdrawal of the vasodilating influence of NO resulted in hypertension combined with an amplification of the LF component of BP variability. Interestingly, the expression of these oscillations in the LF range was abolished when the RAS was previously blocked by the angiotensin II AT_1-receptor antagonist, losartan. We therefore propose that endogenous NO counteracts LF fluctuations generated by the activity of the RAS.

Acute administration of both doses of L-NAME increased BP in normotensive rats. The pressor effect induced by low-dose L-NAME was evident 30 min after the onset of the infusion while it was seen immediately with high-dose L-NAME. This latter dose produced the greatest BP increase. We showed that two different doses of L-NAME produced different latency periods for hypertension, as well as different maximum effects. These results confirm the dose-dependent effect of L-NAME (Gardiner *et al.*, 1990; Gardiner & Bennett, 1992; Wang *et al.*,

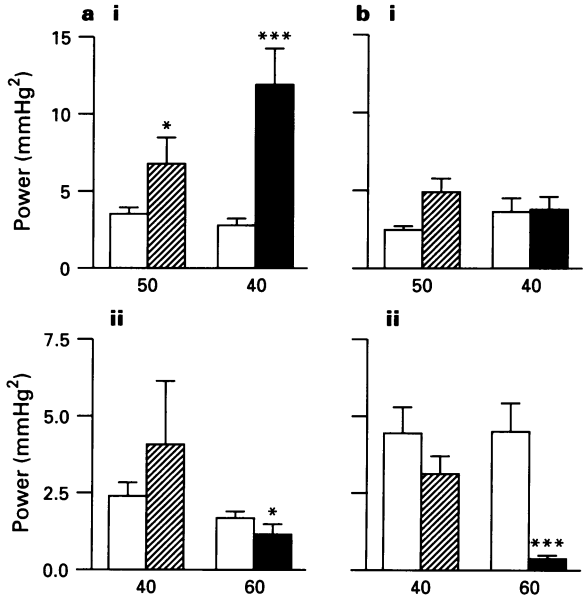

Figure 4 Areas of the (i) low-frequency (0.02–0.2 Hz) and (ii) mid-frequency (0.2–0.6 Hz) components of the systolic blood pressure spectra in (a) vehicle- or (b) losartan-treated rats after N^G-nitro-L-arginine methyl ester (L-NAME) $10\,\mu g\,kg^{-1}\,min^{-1}$, i.v. (control: open column; L-NAME : hatched columns; vehicle: $n=11$ and losartan: $n=10$) or L-NAME $100\,\mu g\,kg^{-1}\,min^{-1}$, i.v., (solid columns, vehicle: $n=10$ and losartan: $n=10$) infusions. Numbers under horizontal axis correspond to the time of infusion in min. Values are means±s.e.mean. $^*P<0.05$, $^{***}P<0.001$.

1992; 1995). A HR decrease mirrored the hypertension produced by the high-dose of L-NAME throughout the whole period of infusion. This bradycardic response probably was a consequence of the baroreflex action in order to attenuate hypertension. A direct muscarinic antagonist effect of L-NAME has been described by Buxton *et al.* (1993). In the present work, the cardiac parasympathetic reflex activation, subsequent to increased BP, would have overcome this antagonist effect, resulting in a bradycardia. We cannot exclude a possible intervention of a cardiac sympathetic inhibition in this bradycardia. Hypertension with bradycardia has been obtained with other acute NO synthase inhibitors (Rees *et al.*, 1989; Nafrialdi *et al.*, 1994; Richard *et al.*, 1995). L-NAME was shown to increase BP by elevating total peripheral resistance rather than cardiac output, the latter being dose-dependently decreased by L-NAME (Gardiner *et al.*, 1990; Wang *et al.*, 1993; 1995). Furthermore, acute administration of L-arginine analogues were shown to increase splanchnic (Gardiner *et al.*, 1990) and renal vascular resistances (Baylis *et al.*, 1990; Zatz & De Nucci, 1991).

At the present, interactions between NO and the RAS are not clear (Reid & Chiu, 1995). The large number of conflicting results prevents clear statements to be made. In an attempt to assess an involvement of the RAS in the modification of both the level and the variability of BP and HR during an acute blockade of NO synthesis, we administered the AII AT1-receptor antagonist losartan, before starting the L-NAME infusions. We showed that losartan given acutely did not modify BP levels and increased HR. No change in BP indicated that in the normotensive rat, the RAS is not the primary pressor system contributing to resting BP. Additionally, the level of BP is probably maintained by both vascular and cardiac sympathetic reflex activation in response to a vasodilatation provoked by losartan. Such a result has previously been shown with losartan, in the WKY rat strain (Gaudet *et al.*, 1995), with an angiotensin converting enzyme inhibitor in rats of the Lyon strain (Lo *et al.*, 1991) and in the dog (Hasser & Bishop, 1988). The increased BP produced by the infusion of low-dose L-NAME appeared belatedly and remained after L-NAME administration had ceased. The high-dose of L-NAME rapidly increased BP which returned back to the lower level after administration had ended. Acute losartan totally prevented the increase of BP produced by the low-dose of L-NAME and delayed the BP rise produced by the high-dose of L-NAME, without changing the pattern of response. We presume that the low-dose of L-NAME was not sufficient to increase BP immediately by a systemic haemodynamic effect, but could have activated the RAS which in turn would be responsible for the delayed and persistent BP increase. Activation of the RAS could not be attributed to decreased renal blood flow due to L-NAME, since it was shown that when the changes in renal perfusion pressure and β-adrenoceptor-mediated activity are controlled, inhibition of NO synthesis by L-NAME still resulted in increased plasma renin activity (Sigmon *et al.*, 1992). On the other hand, the high-dose of L-NAME induced an early BP increase which was delayed after blockade of the AII receptors by losartan. This indicated that the RAS is necessary for increasing BP by the low-dose of L-NAME but plays a minor role in the development of the hypertension due to the acute high-dose of L-NAME. In this latter case, intervention of the RAS could be relayed by other pressor systems. This result is consistent with the study of Nafrialdi and colleagues (1994) who showed that previous blockade of the RAS by losartan or enalapril did not alter acute L-NAME-induced changes in BP, when a high-dose of L-NAME was used. Furthermore, Gardiner & Bennett (1992) showed that the RAS was not involved indispensibly in the systemic pressor or regional haemodynamic responses to L-NAME in rats. Another effect of L-NAME could be to increase sensitivity to AII, without activation of the RAS since it was shown that acute administration of pressor doses of L-NAME was associated with decreased plasma renin activity (Sigmon *et al.*, 1992; Johnson & Freeman, 1992). Sensitivity to vasoactive drugs has been shown to be modified by blockade of the NO synthase activity. Thus, the NO synthesis inhibition was associated with an increased vasoconstrictor effect of noradrenaline, arginine-vasopressin and angiotensin II (Conrad & Whittemore, 1992) and with an enhanced vasodilator response to sodium nitroprusside (Bryant *et al.*, 1995). Besides these modifications of sensitivity to vasoactive substances, the hypertension produced by the high-dose of L-NAME could be explained by the activation of some pressor systems other than the RAS. The involvement of the sympathetic nervous system is controversial. Thus, Sakuma and colleagues (1992) showed that acute L-NMMA increased BP together with the renal nervous sympathetic activity. On the other hand, previous blockade of the α_1-adrenoceptors by prazosin did not modify the pressor response to acute L-NMMA (Nafrialdi *et al.*, 1994). Blockade of NO synthesis with L-NAME induced powerful pressor and vasoconstrictor effects in sympathectomized rats, indicating that the basal synthesis and release of NO did not require integrity of the sympathetic vascular innervation (Zhang *et al.*, 1992). Finally, the hypertension could be partly due to unmasking of the vasopressive response to endothelin since antagonizing the ET_A and ET_B endothelin receptors by bosentan reduced the pressor response to L-NAME (Richard *et al.*, 1995).

A common estimate of BP variability is given by the s.d. of the frequency distribution of the pressure values. When L-NAME was administered, the increased mean s.d. of systolic BP associated with elevated BP levels reflected an overall increased BP variability. The theorem of Parseval stated that there is a conservation of energy between temporal and frequential domains and leads by a calculation to an equivalence between the total power of a spectrum (except the first band) and the variance. Blocking the NO system increased BP power (variance) three fold. A similar effect was shown by Just *et al.* (1994) and Nafz *et al.* (1996) who found, in dogs, that following blockade of NO formation by N^G-nitro-L-arginine (L-NNA) the total power of BP was elevated more than two fold. Augmented s.d. of BP could have been due to the occurrence of marked slow fluctuations since the s.d. was predominantly influenced by fluctuations of the lowest frequencies. Losartan increased the s.d. of the BP and prevented the further rise normally induced by L-NAME.

In contrast to this time domain of pressure variability, spectral analysis of BP fluctuations provides a tool to determine the variability in definite frequency ranges. We showed that acute L-NAME increased BP dose-dependently and enhanced the LF component of SBP variability. Inhibition of NO synthesis by the high-dose of L-NAME greatly amplified the LF fluctuations of the SBP. Losartan prevented the amplification of the LF fluctuations, without changing the pressor response to the high-dose of L-NAME.

The baroreceptor reflex has been shown to influence the BP variability in different frequency domains. During the chronic phase of a sinoaortic denervation, LF oscillations were increased as MF oscillations were diminished in cats (DiRienzo *et al.*, 1991) and in rats (Cerutti *et al.*, 1994). In our study, the high-dose of L-NAME produced hypertension and activation of the baroreceptor reflex function as shown by the long-lasting bradycardia associated with the arterial pressure increase. Decreased sensitivity of cardiac baroreflex (Lantelme *et al.*, 1994) together with decreased baroreceptor reflex control of renal nerve activity (Scrogin *et al.*, 1994) were described in conscious hypertensive rats chronically treated by L-NAME. Conversely, acute blockade of NO synthesis by L-NMMA which produced a rise in BP associated with sustained bradycardia, increased the maximum gain of the baroreceptor reflex control, both of renal nerve activity and of HR in SHR (Kumagai *et al.*, 1993). If we speculate that in our study baroreceptor reflex function was impaired by L-NAME, this change might have participated in the increase in LF oscillations and decrease in MF oscillations observed with high-dose L-NAME. Losartan would have restored a better control of arterial pressure variability since blockade of the RAS has been shown to produce a substantial improvement of the baroreceptor

reflex function through an AT_1-dependant mechanism (Kumagai *et al.*, 1993). On the other hand, among the systems that could be involved in generating LF fluctuations, humoral systems have been previously suggested. Indeed, spectral analysis of the BP variability of chronically sympathectomized rats revealed spectral power in the LF bands (Cerutti *et al.*, 1991; Daffonchio *et al.*, 1991). In these cases, withdrawal of regulation of the BP and its variability by the sympathetic nervous system led to the activation of humoral regulatory processes responsible for slow fluctuations of BP. Furthermore, Ponchon & Elghozi (1996) recently demonstrated reduced slow fluctuations of BP after losartan in renin-dependent hypertensive rats, demonstrating the involvement of the RAS in the LF domain of SBP variability. Persson *et al.* (1992a) previously demonstrated, in conscious dogs, that inhibition of NO production by L-NNA increased the power of the frequency oscillations of BP below 0.5 Hz. This was interpreted in favour of a proposed role of NO in buffering arterial BP (Just *et al.*, 1994). Similarly, it has been shown in rats that LF fluctuations of SBP were increased during an i.v. infusion of N^{ω}-monomethyl-L-arginine (L-NMMA) (Cordero *et al.*, 1994). In the present study, we showed that inhibition of NO synthesis produced slow oscillations of BP which could be interpreted as inhibition of a buffering effect of NO. We also suggest that the BP oscillations normally counterbalanced by NO were partly generated by activity of the RAS.

Another modification of SBP variability provoked by the high-dose of L-NAME concerned the MF oscillations. These 0.2–0.6 Hz oscillations in rats are under the control of the vascular sympathetic system. Thus, chronic destruction of the sympathetic nervous fibres by guanethidine (Cerutti *et al.*, 1991) or 6-hydroxydopamine (Daffonchio *et al.*, 1991) drastically reduced the MF oscillations of SBP. In addition, blockade of α_1-adrenoceptors by prazosin (Japundzic *et al.*, 1990), α_1- and α_2-adrenoceptors by phentolamine (Cerutti *et al.*, 1991) or blocking ganglionic transmission with hexamethonium (Japundzic *et al.*, 1990), provoked an important decrease of the MF component of SBP variability. Moreover, a direct link between the MF oscillations of BP and the vascular sympathetic nervous activity has been shown by Brown *et al.* (1994) who found that the MF oscillations of the BP correlated with the activity of the sympathetic renal nerves. In the present work, blockade of NO synthesis increased BP and revealed the expression of LF fluctuations of BP. This effect was particularly strong with the high-dose of L-NAME which produced hypertension with a marked increase of BP reaching 53 mmHg and a four fold increase of LF power. The associated decrease in the MF range, could be attributed to reflex sympathetic inhibition following vasoconstriction induced by L-NAME. This sympathetic inhibition could also be partly responsible for the bradycardia observed during L-NAME infusion. Thus we cannot rule out the participation of an altered baroreflex function in the observed decrease in MF oscillations. We showed that modifications of BP variability in the LF and MF range could be dissociated and displayed maximum effects which were not necessarily concomitant. Spectral decomposition of the BP signal showed that losartan increased the MF component of BP variability probably indicating a reflex sympathetic activation triggered by vasodilatation after losartan. An amplification of the MF oscillations of SBP after acute losartan has previously been shown in renovascular hypertensive rats (Ponchon & Elghozi, 1996) and in SHR (Gaudet *et al.*, 1995). Similarly, an acute blockade of the RAS by enalapril produced an increase of the MF oscillations of BP in normal rats (Grichois *et al.*, 1992). In addition, acute administration of losartan was shown to increase renal sympathetic activity in SHR (Kumagai *et al.*, 1993). After losartan, decrease of the MF oscillations of SBP by high-dose L-NAME remained and this effect was independent of modification of LF component since this was later unchanged. Therefore, we propose that decreased MF oscillations reflect a reduction in sympathetic tone in response to the vasoconstriction provoked by high-dose L-NAME.

In summary, acute blockade of NO synthesis leads to hypertension characterized by an overexpression of BP variability. Spectral analysis shows that this exaggerated BP variability is characterized by decreased MF oscillations and greatly enhanced LF fluctuations. Disappearance of the amplification of the LF fluctuations normally produced by L-NAME after blockade of the RAS suggests that this humoral system contributes to their genesis. We suggest that NO normally buffers LF fluctuations generated by the RAS.

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