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# Contribution of the renin-angiotensin and kallikrein-kinin systems to short-term variability of blood pressure in two-kidney, one-clip hypertensive rats

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**Abstract**

Spectral analysis was recently chosen to characterize the fast oscillations, depending on the autonomic nervous system, in heart rate and blood pressure variabilities. Humoral stimuli could impinge on the low-frequency domain of blood pressure variability since the time lag to humoral system activation is greater. This study was designed to analyse low-frequency components of short-term variability of blood pressure of conscious rats in conditions where humoral systems were activated. We studied rats with two-kidney, one-clip Goldblatt hypertension in which the blood pressure level was dependent upon the renin-angiotensin and kallikrein-kinin systems. Spectral powers of the systolic and diastolic blood pressure and heart rate were computed in the high (respiratory)-, mid (0.2–0.6 Hz)- and low (0.02–0.2 Hz)-frequency bands, as detected by the fast Fourier transform technique in consecutive 102-s stationary periods. Hypertensive rats exhibited a marked low-frequency component of systolic (+261%) and diastolic (+169%) blood pressure variabilities when compared to sham-operated animals. First, losartan, a selective non-peptide angiotensin $AT_1$ receptor antagonist, reduced this low-frequency component (−44% and −25% for systolic and diastolic blood pressure). In a second series of hypertensive rats, HOE 140, D-Arg-[Hyp$^3$,Thi$^5$,D-Tic$^7$,Oic$^8$]bradykinin, a bradykinin $B_2$ receptor antagonist, decreased the low-frequency component of systolic (−28%) and diastolic (−40%) blood pressure. Losartan, added after HOE 140, induced a supplementary decrease of the low-frequency component (−60% and −42% for systolic and diastolic blood pressure). After the combined blockade, the low-frequency components of systolic and diastolic blood pressure variabilities of the hypertensive rats were equivalent to those of the control rats. Two-kidney, one-clip hypertension was also associated with an elevation of the mid-frequency component of the systolic blood pressure (+55%). The administration of HOE 140 did not change this component while losartan, alone or added after HOE 140, led to an increase (around +100%) in mid-frequency oscillations of systolic blood pressure. The high-frequency oscillations of systolic blood pressure were increased by losartan in the two series of hypertensive rats. Losartan increased the mid-frequency component of heart rate variability in sham-operated rats while the heart rate variability was not modified during any of the treatment periods in two-kidney, one-clip rats. In conclusion, an increase in the low-frequency component of blood pressure variability was observed in a model of hypertension where the blood pressure is dependent upon humoral activities. The reduction of the slow fluctuations following the combined blockade of the kallikrein-kinin and the renin-angiotensin systems suggested the contribution of these humoral systems to this low-frequency component of blood pressure variability.

*Keywords:* Blood pressure variability; HOE 140; Losartan; Renal hypertension; Spectral analysis

## 1. Introduction

The autonomic nervous system generates oscillations of blood pressure and heart rate located in the mid (0.2–0.6 Hz)- and high (respiratory)-frequency domains as assessed by spectral analysis (Akselrod et al., 1987; Japundzic et al., 1990; Cerutti et al., 1991; Daffonchio et al., 1991; Persson et al., 1992; Brown et al., 1994). Briefly, the vagus impinges on both mid-frequency and high-frequency components of heart rate variability, whereas the sympathetic nervous system mainly influences mid-frequency oscillations of blood pressure and heart rate.

Recently, two studies reported a significant increase in the low-frequency domain (0.02–0.2 Hz) of systolic

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blood pressure variability after sympathectomy (Cerutti et al., 1991; Daffonchio et al., 1991). Daffonchio et al. (1991) suggested that this effect depends on 'as yet unidentified factors potentially capable of promoting low-frequency blood pressure oscillations'. It is interesting to notice that, after sino-aortic deafferentation, the increased blood pressure lability, taken as the standard deviation of the mean blood pressure, is dependent on the interaction of the sympathetic nervous system and the renin-angiotensin system (Rodrigues-de-Oliveira and Machado, 1993). Considering the relatively large time lag to renin-angiotensin system activation (Kirchheim et al., 1985), one can hypothesize that renin stimuli impinge on the low-frequency domain.

This study was designed to analyse the low-frequency component of short-term variability of blood pressure in conscious rats with two-kidney, one-clip hypertension where the renin was activated without inhibition of the sympathetic nervous system. Furthermore, several studies have pointed to a role of endogenous bradykinin in the acute effects of angiotensin converting enzyme inhibitor in hypertensive rats (Carretero et al., 1981; Benetos et al., 1986; Carbonnel et al., 1988; Bao et al., 1992a). Previously, we had shown that bradykinin was able to change the blood pressure variability profile in normotensive rats (Ponchon et al., 1995). Therefore, it is conceivable that the interaction of the renin-angiotensin and kallikrein-kinin systems to the blood pressure regulation in this model of hypertension generates slow fluctuations of blood pressure.

The contributions of the renin-angiotensin and kallikrein-kinin systems to the slow fluctuations of blood pressure were tested using losartan, a selective non-peptide angiotensin II $AT_1$ receptor antagonist, and HOE 140, a bradykinin $B_2$ receptor antagonist.

Some of these data were presented at the 6th Scientific Meeting of the European Society of Hypertension, Milan, Italy, June 1993 (Ponchon et al., 1993).

## 2. Materials and methods

Two-kidney, one-clip hypertensive rats were prepared under pentobarbitone anaesthesia (60 mg/kg intraperitoneally, i.p.). A U-shaped silver clip with a preset internal gap of 0.2 mm (Amengual Frères, Lyon, France) was placed around the right renal artery of male Wistar rats weighing 210–260 g (Iffa-Credo, Les Oncins, France). Over the next 3–5 weeks, the rats were given free access to regular laboratory diet chow (A04, UAR, Epinay sur Orge, France) and tap water. The animals were maintained under controlled housing conditions (20 ± 1°C – lighting 8 a.m. to 8 p.m.). Only rats with systolic blood pressure (plethysmography tail cuff method) over 140 mm Hg were selected for the subsequent study i.e. catheterizations followed by blood pressure recordings. Sham-operated animals were taken as controls.

### 2.1. Experiments

The animals were prepared surgically as previously described (Grichois et al., 1992).

The experiments were started approximately 1 h after the rats had been connected to a pressure transducer and injection syringe.

A first group of sham-operated and hypertensive rats ($n = 9$ for each strain) received an i.v. injection of physiological saline (300 μl/kg). A 5-min recording session was initiated 20 min after the injection. The rats were then treated i.v. with the selective non-peptide angiotensin $AT_1$ receptor antagonist, losartan (Wong et al., 1991) (10 mg/100 μl saline/kg body weight), 20 min before the second 5-min recording session. Finally, angiotensin II blockade was assessed by measuring the blood pressure change secondary to an i.v. angiotensin II injection (100 ng/100 μl/kg).

A second group of rats ($n = 10$ for sham and hypertensive) was treated with a long-acting bradykinin $B_2$ receptor antagonist, HOE 140 (Bao et al., 1991; Wirth et al., 1991) (30 μg/300 μl per kg), secondary to an i.v. control saline injection (300 μl/kg). In pilot experiments, this dose of HOE 140 was able to abolish the marked hypotensive effects (−25 mm Hg) of an infusion of bradykinin 100 μg/min. Two recording sessions were started 5 min after the injections of saline and HOE 140. Lastly, losartan (10 mg/100 μl per kg) was injected i.v. 20 min prior to the third recording session. The recording sessions lasted 5 min.

### 2.2. Signal processing and spectrum analysis

Blood pressure signal processing and spectrum analysis have been detailed elsewhere (Grichois et al., 1992). The evenly spaced sampling allowed direct spectral analysis, using a fast Fourier transform algorithm, of a stationary period in a 1024-point time 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 heart rate or blood pressure spectra (ordinates) had units of $bpm^2$ or $mm\ Hg^2$. The sum of the values of consecutive bands (without the first band) represents the variance of heart rate or blood pressure. Integrated spectra of the systolic and diastolic blood pressure and heart rate 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 of the distribution of the variables of the 102.4-s files (1024 values) used for the spectral analysis were computed.

### 2.3. Plasma renin activity

Plasma renin activity was measured in additional groups of rats. We measured plasma renin activity in 5 two-kidney Goldblatt hypertensive rats compared to 10 sham-operated animals.

Blood was drawn from conscious rats from a right atrial catheter (No. 3 from Biotrol, Paris) implanted 2 days prior to the collection. Plasma renin activity was measured by radioimmunoassay according to a previously described method (Menard and Catt, 1972) using a SB-REN-2 kit (CIS Bio International, Gif sur Yvette, France).

### 2.4. Drugs

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, potassium salt) was generously given by Merck-Sharp and Dohme (Rahway, NJ, USA). Angiotensin II was obtained from Sigma chemical Co. (St Louis, Missouri, USA). HOE 140 (D-Arg-[Hyp$^3$,Thi$^5$,D-Tic$^7$,Oic$^8$]bradykinin) was kindly donated by Hoechst (Frankfurt/Main, Germany). All drugs were dissolved in saline.

### 2.5. Statistical analysis

The data are presented as the means ± standard error of the mean (S.E.M.).

Comparisons between the baseline values for sham-operated animals and hypertensive rats were made using Student's *t*-test.

Statistical analysis of plasma renin activity was performed using Student's-t *t*est. A logarithmic transformation of plasma renin activity was done before comparisons.

Comparisons during the two protocols were assessed by a two-factor analysis of variance (factor 1 being clip and factor 2 being one treatment) with repeated measures. Protected *t*-tests were done when the *F*-ratio for the between groups (clip versus sham, treatment versus saline) was significant ($P < 0.05$). The significance of the interaction factor (clip × treatment) was considered as indicating an action of one treatment depending on the blood pressure level status.

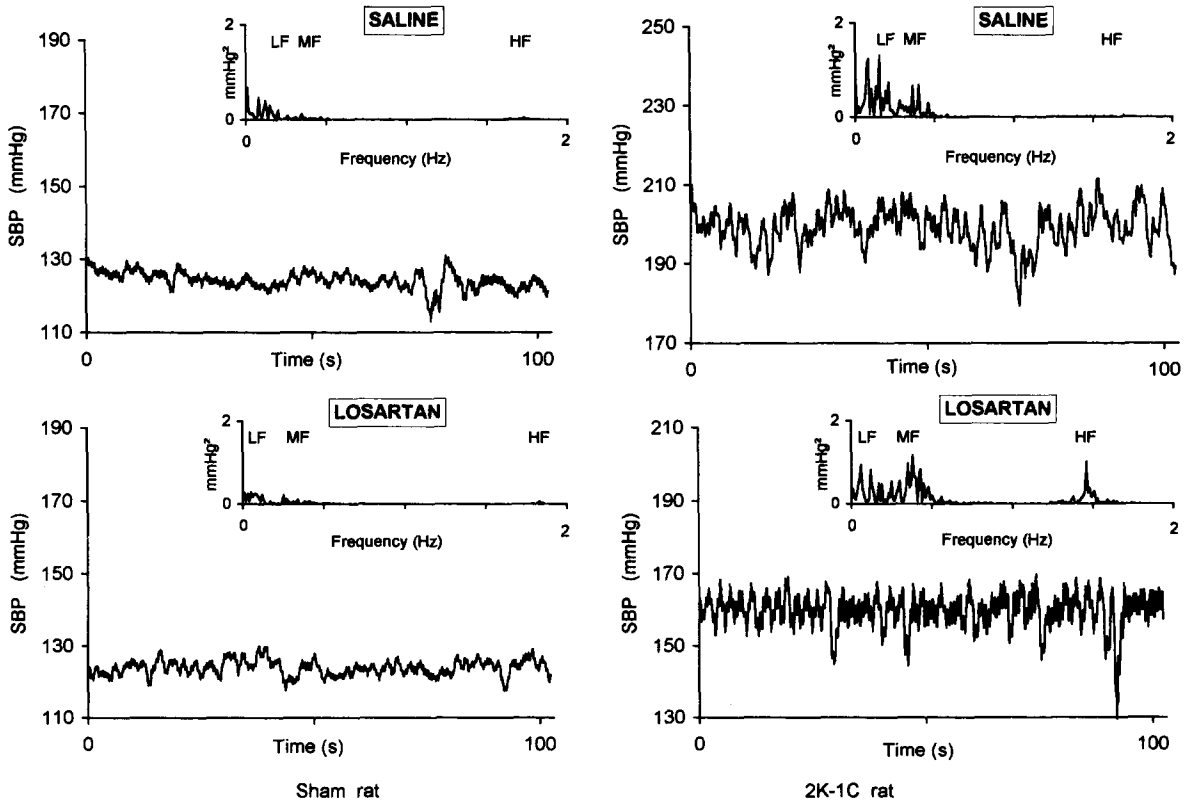


Fig. 1. Examples of 100-s systolic blood pressure digitized recordings, with corresponding spectra, of a sham-operated animal (left) and of a two-kidney, one-clip hypertensive rat (right) following saline injection and after losartan.

Table 1
Baseline levels of sham-operated and two-kidney, one-clip renovascular hypertensive rats

| | Sham-operated rats ($n = 19$) | Two-kidney one-clip rats ($n = 19$) |
|---|---|---|
| *SBP* | | |
| Average (mm Hg) | 125 ± 1 | 172 ± 4 [b] |
| SD (mm Hg) | 2.8 ± 0.1 | 4.1 ± 0.2 [b] |
| LF (mm Hg$^2$) | 2.18 ± 0.27 | 7.88 ± 1.15 [b] |
| MF (mm Hg$^2$) | 3.49 ± 0.45 | 5.41 ± 0.82 [a] |
| HF (mm Hg$^2$) | 0.39 ± 0.06 | 0.69 ± 0.21 |
| *DBP* | | |
| Average (mm Hg) | 93 ± 1 | 128 ± 3 [b] |
| SD (mm Hg) | 2.6 ± 0.1 | 3.4 ± 0.2 [b] |
| LF (mm Hg$^2$) | 2.08 ± 0.27 | 5.6 ± 0.93 [b] |
| MF (mm Hg$^2$) | 3.55 ± 0.44 | 4.11 ± 0.6 |
| HF (mm Hg$^2$) | 0.1 ± 0.02 | 0.2 ± 0.06 |
| *HR* | | |
| Average (bpm) | 358 ± 5 | 352 ± 7 |
| SD (bpm) | 9.1 ± 0.8 | 9.2 ± 0.7 |
| LF (bpm$^2$) | 38.23 ± 5.83 | 45.45 ± 8.6 |
| MF (bpm$^2$) | 10.35 ± 2.35 | 4.79 ± 0.8 [a] |
| HF (bpm$^2$) | 2.23 ± 0.51 | 2.06 ± 0.39 |

Systolic (SBP) and diastolic (DBP) blood pressure; heart rate (HR); standard deviation (SD). LF, MF and HF are low-, mid- and high-frequency, respectively. Values are expressed as means ± S.E.M. [a] $P < 0.05$, [b] $P < 0.01$, versus sham-operated rats; unpaired Student's $t$-test.

## 3. Results

### 3.1. Baseline systolic and diastolic blood pressure, heart rate and their respective spectra in sham-operated and hypertensive rats

Renal hypertension produced by a two-kidney procedure corresponded to a marked rise in systolic blood pressure (+47 mm Hg, $P < 0.001$) and diastolic blood pressure (+35 mm Hg, $P < 0.001$) and no significant change in heart rate (−6 bpm) when average levels in rats with a clip were compared to levels observed in sham-operated animals as shown in Table 1. The standard deviations of systolic blood pressure and diastolic blood pressure were increased in hypertensive rats (+48% and +31%, $P < 0.001$). The standard deviation of heart rate was unaffected by the stenosis. Examples of blood pressure digitized recordings of one normotensive and one hypertensive rat are shown in Fig. 1. The blood pressure short-term variability of the sham-operated rat was mainly composed of 2.5-s period oscillations (approximately four cycles within 10 s, corresponding to the mid-frequency component) and high-frequency (respiratory) fluctuations. The corresponding spectrum exhibited a peak around 1.5 Hz representing the high-frequency oscillations, the power in the mid-frequency component was concentrated in a peak occurring at roughly 0.4 Hz. The blood pressure of the rat with one-clip was elevated and slow fluctuations (low-frequency; approximately 20-s period) were present, in addition to an increased amplitude of the mid-frequency oscillations. The occurrence of these slow fluctuations of blood pressure was illustrated by the peak located below 0.2 Hz in the corresponding spectrum.

The average power of the three components of systolic blood pressure, diastolic blood pressure and heart rate variabilities of the two strains of rats are given in Table 1. Two-kidney, one-clip hypertension selectively and markedly increased the low-frequency component of systolic and diastolic blood pressure spectra when hypertensive rats were compared to sham-operated rats (+261% and +169%, $P < 0.001$). Elevation of the mid-frequency component of the systolic blood pressure spectrum (+55%, $P < 0.05$) was associated with this effect. The mid-frequency component of the heart rate spectrum was decreased (−53%, $P < 0.05$) by the stenosis.

The position of the high-frequency peak (respiratory rate) was not affected by renal artery stenosis. The position of the systolic blood pressure peak of rats with two-kidney, one-clip hypertension was around 1.4 Hz, corresponding to an average respiratory rate of 84 cycles/min.

### 3.2. Plasma renin activity

A significant increase in plasma renin activity ($P < 0.01$) was observed following two-kidney, one-clip hypertension. The values obtained for sham-operated animals were 3.3 ± 0.4 ng of angiotensin I/ml per h ($n = 10$) and for rats with two-kidney, one-clip hypertension the plasma renin activity was 9.0 ± 4.6 ng of angiotensin I/ml per h ($n = 5$).

Table 2
The average values and standard deviations (SD) of the systolic (SBP) and diastolic (DBP) blood pressures and heart rate (HR) following saline and losartan injections in the groups of sham-operated and two-kidney, one-clip rats

| | Sham-operated rats ($n = 9$) | | Two-kidney, one-clip rats ($n = 9$) | |
|---|---|---|---|---|
| | Saline | Losartan | Saline | Losartan |
| *SBP* | | | | |
| Average (mm Hg) | 128 ± 2 | 120 ± 4 [a] | 175 ± 6 | 152 ± 5 [a] |
| SD (mm Hg) | 2.8 ± 0.2 | 2.9 ± 0.1 | 4.0 ± 0.2 | 4.5 ± 0.6 |
| *DBP* | | | | |
| Average (mm Hg) | 94 ± 2 | 83 ± 3 [a] | 130 ± 5 | 110 ± 3 [a] |
| SD (mm Hg) | 2.6 ± 0.2 | 2.6 ± 0.1 | 3.3 ± 0.2 | 3.9 ± 0.4 |
| *HR* | | | | |
| Average (bpm) | 369 ± 6 | 416 ± 11 [a] | 366 ± 8 | 415 ± 14 [a] |
| SD (bpm) | 10.3 ± 0.9 | 10.9 ± 1.2 | 9.0 ± 0.6 | 11.7 ± 1.5 |

Values are expressed as means ± S.E.M. [a] $P < 0.01$, versus saline; two-factor analysis of variance with repeated measures.

### 3.3. Effects of angiotensin $AT_1$ receptor blockade

The average values and standard deviations of systolic blood pressure, diastolic blood pressure and heart rate of the two strains of rats following saline and losartan injections are given in Table 2.

Losartan administration to sham-operated rats resulted in a moderate systolic and diastolic blood pressure decrease (−8 mm Hg and −11 mm Hg, $P < 0.01$). A significant tachycardia was observed (+47 bpm, $P < 0.01$). Losartan did not significantly affect the standard deviations of systolic and diastolic blood pressure and heart rate. In the example shown in Fig. 1, losartan did not change the blood pressure oscillations in this group of rats.

In the hypertensive group, losartan produced a marked fall in systolic blood pressure and diastolic blood pressure (−23 mm Hg and −20 mm Hg, $P < 0.01$). The significance of the interaction factor indicated an effect of losartan of greater amplitude in hypertensive rats than in sham-operated rats. A significant tachycardia was observed (+49 bpm, $P < 0.01$). The standard deviations of systolic and diastolic blood pressure and heart rate were not significantly modified. In the example shown in Fig. 1, it is apparent that the hypertensive rat treated with losartan exhibits a lower blood pressure level together with a reduced low-frequency component of blood pressure variability, illustrated by the decrease of the peak located below 0.2 Hz in the corresponding spectrum. The blood pressure variability profile after losartan combines marked high-frequency (respiratory) and mid-frequency oscillations as shown in the systolic blood pressure spectrum.

Fig. 2 shows the effects of losartan on the average areas of the three components of systolic and diastolic blood pressure and heart rate spectra. Losartan administered to sham-operated rats was without any effect on the low- or mid-frequency components of blood pressure and heart rate spectra. A minor increase in the high-frequency component of systolic blood pressure was observed ($P < 0.05$). Losartan caused a significant reduction of the low-frequency component of the systolic blood pressure spectrum of hypertensive rats (−44%, $P < 0.01$). The reduction of the low-frequency component of the diastolic blood pressure spectrum (−25%) did not reach significance. However, in spite of the administration of losartan, the area of the low-frequency domain stayed greater in the hypertensive rats than in the normotensive rats (+74%). In contrast with this reduction in the low-frequency component, we observed a significant increase in the mid-frequency components of systolic and diastolic blood pressure

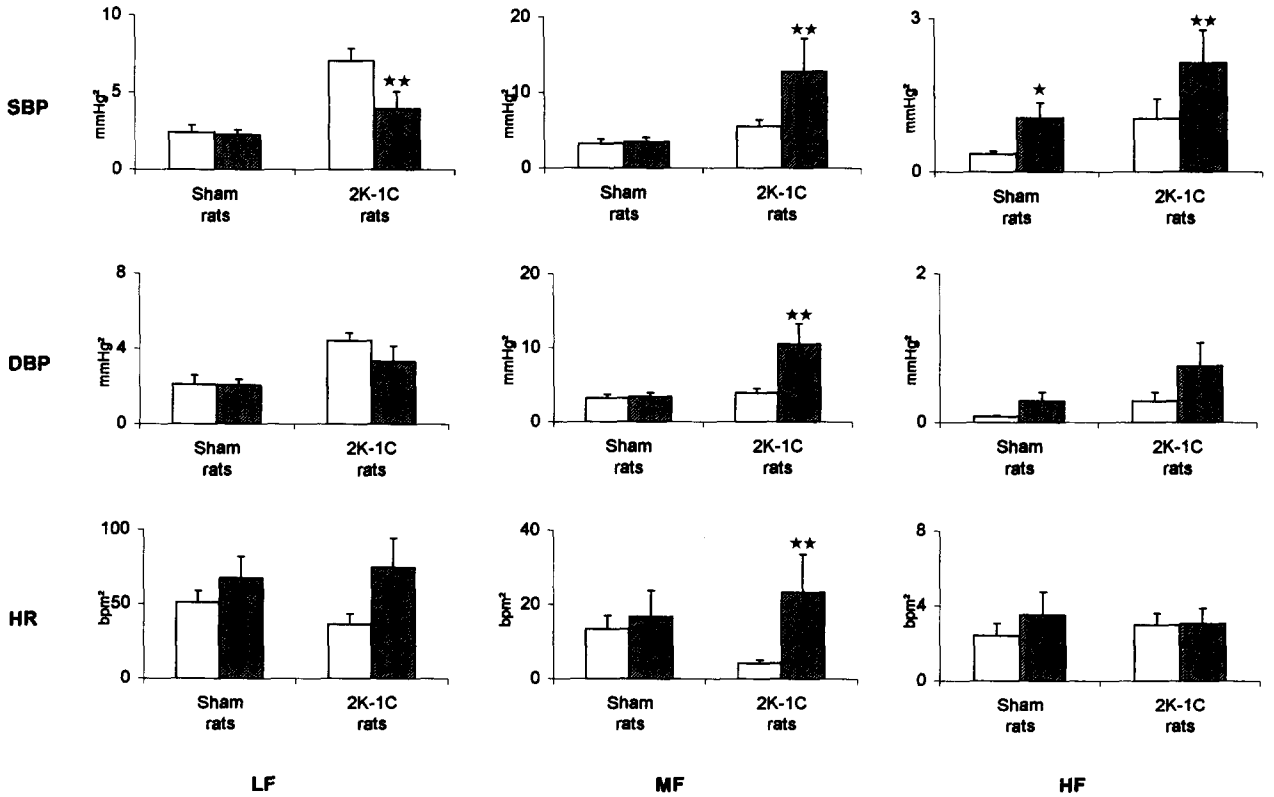


Fig. 2. Average areas under the curve of the low (LF)-, mid (MF)- and high (HF)-frequency components of the systolic (SBP), diastolic (DBP) blood pressure and heart rate (HR) spectra after saline (open bars) and losartan (hatched bars) in sham-operated rats ($n = 9$) and two-kidney, one-clip rats (2K-1C) ($n = 9$). Columns represent means of results, vertical line shows S.E.M. Significance refers to comparisons between baseline and treatment conditions using two-factor analysis of variance with repeated measures: * $P < 0.05$; ** $P < 0.01$.

Table 3
The average values and standard deviations (SD) of the systolic (SBP) and diastolic (DBP) blood pressures and heart rate (HR) following saline, HOE 140 and losartan injections in the groups of sham-operated and two-kidney, one-clip rats

| | Sham-operated rats ($n = 10$) | | | Two-kidney, one-clip rats ($n = 10$) | | |
|---|---|---|---|---|---|---|
| | Saline | HOE 140 | Losartan | Saline | HOE 140 | Losartan |
| *SBP* | | | | | | |
| Average (mm Hg) | 122 ± 1 | 124 ± 2 | 121 ± 2 | 173 ± 5 | 171 ± 6 | 154 ± 4 [a,b] |
| SD (mm Hg) | 2.7 ± 0.1 | 2.9 ± 0.1 | 3.2 ± 0.2 | 4.1 ± 0.4 | 3.6 ± 0.3 | 3.5 ± 0.4 |
| *DBP* | | | | | | |
| Average (mm Hg) | 92 ± 2 | 95 ± 2 | 91 ± 2 | 127 ± 4 | 126 ± 4 | 112 ± 4 [a,b] |
| SD (mm Hg) | 2.7 ± 0.2 | 2.8 ± 0.1 | 3.0 ± 0.2 | 3.6 ± 0.3 | 3.1 ± 0.2 | 3.2 ± 0.3 |
| *HR* | | | | | | |
| Average (bpm) | 348 ± 7 | 351 ± 10 | 375 ± 10 | 340 ± 9 | 333 ± 11 | 392 ± 19 [a,b] |
| SD (bpm) | 8.0 ± 1.1 | 9.0 ± 1 | 9.4 ± 0.9 | 9.5 ± 1.2 | 8.2 ± 0.7 | 9.2 ± 0.6 |

Values are expressed as means ± S.E.M. [a] $P < 0.01$, versus saline; [b] $P < 0.01$, versus HOE 140; two-factor analysis of variance with repeated measures.

variabilities (+132% and +164%, $P < 0.01$). Losartan also increased the high-frequency components of the systolic blood pressure spectrum ($P < 0.01$). The increase observed in the high-frequency components of the diastolic blood pressure variability was not significant. Losartan administration resulted in a significant rise of the mid-frequency component of the heart rate spectrum (+445%, $P < 0.01$).

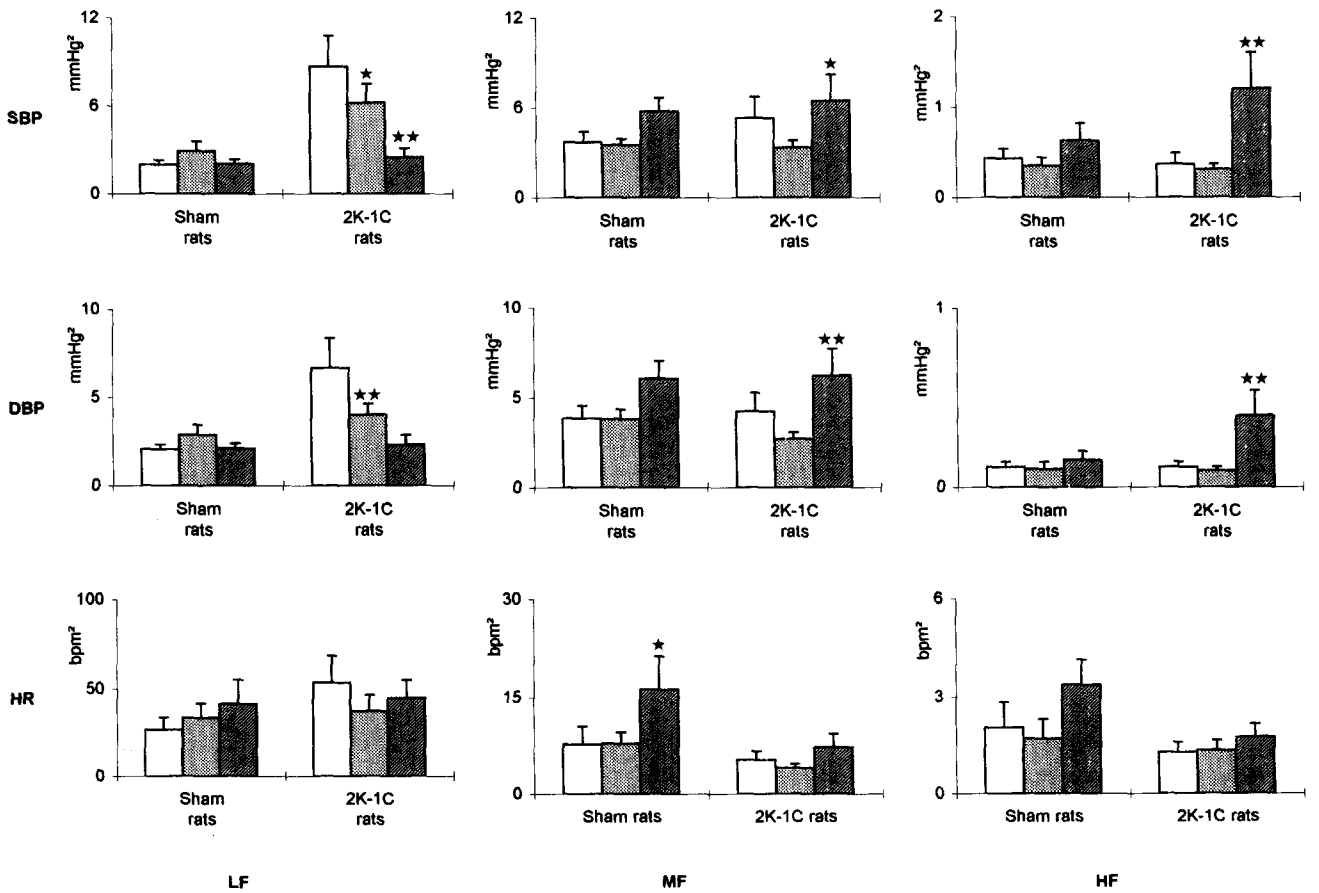


Fig. 3. Average areas under the curve of the low (LF)-, mid (MF)- and high (HF)-frequency components of the systolic (SBP), diastolic (DBP) blood pressure and heart rate (HR) spectra after saline (open bars), HOE 140 (dotted bars) and losartan (hatched bars) in sham-operated rats ($n = 10$) and two-kidney, one-clip (2K-1C) rats ($n = 10$). Columns represent means of results, vertical line shows S.E.M. Significance refers to comparisons to the preceding values: HOE 140 versus saline, losartan versus HOE 140, using two-factor analysis of variance with repeated measures. * $P < 0.05$; ** $P < 0.01$.

The positions of the high-frequency peaks of systolic and diastolic blood pressure were not affected by losartan (results not shown).

### 3.4. Effects of bradykinin $B_2$ and angiotensin $AT_1$ receptor blockade

HOE 140 did not determine significant changes of the average blood pressure and heart rate levels in normotensive and hypertensive rats as shown in Table 3. Losartan, injected 15 min after HOE 140, did not affect blood pressure or heart rate of the sham-operated rats. In contrast, losartan resulted in significant systolic (−17 mm Hg, $P < 0.01$) and diastolic (−14 mm Hg, $P < 0.01$) blood pressure falls associated with a marked tachycardia (+52 bpm, $P < 0.01$) in the hypertensive group. We only observed a slight decrease of the systolic blood pressure standard deviation of hypertensive rats after HOE 140 during this sequential blockade.

The sequential blockade of the two humoral systems affected only the mid-frequency component of the heart rate spectra in sham-operated rats (Fig. 3). In the hypertensive group, HOE 140 reduced the low-frequency components of systolic (−28%, $P < 0.05$) and diastolic (−40%, $P < 0.01$) blood pressure spectra. Neither mid- nor high-frequency components of systolic and diastolic blood pressure variabilities were changed by HOE 140. Losartan, following HOE 140, reduced the low-frequency domain of systolic blood pressure (−60%, $P < 0.01$). The decrease of the diastolic blood pressure low-frequency component (−42%) induced by losartan did not reach significance. After combined bradykinin $B_2$ and angiotensin $AT_1$ receptor blockade, as shown in Fig. 3, the low-frequency components of the systolic and diastolic blood pressure spectra of the hypertensive rats were equivalent to those of sham-operated animals. In addition to the decrease of the low-frequency domains, losartan induced a rise of the mid-frequency components of systolic (+93%, $P < 0.01$) and diastolic (+130%, $P < 0.01$) blood pressure. Furthermore, we observed an increase in the high-frequency components of systolic and diastolic blood pressure ($P < 0.01$) spectra. The three components of the heart rate spectrum were not modified during any of the treatment periods.

The position of the high-frequency peak was unaffected by the two treatments (result unshown)

## 4. Discussion

In the present study we characterized a low-frequency component of blood pressure variability using a spectral analysis technique in two-kidney, one-clip hypertension. Interestingly the underlying blood pressure fluctuations were abolished after the combined blockade of the kallikrein-kinin and renin-angiotensin systems. It is therefore tempting to associate hormonal systems with the mechanisms generating low-frequency fluctuations in blood pressure.

We analysed a model of exaggerated renin-angiotensin system activity associated with hypertension. It is admitted that angiotensin II is critically involved in the form of two-kidney, one-clip hypertension in which one renal artery is clamped and the contralateral kidney is left in place (Brunner et al., 1971). The activation of the renin-angiotensin system in two-kidney, one-clip hypertension was confirmed in the present study by the 3-fold increased plasma renin activity measured in renovascular rats. Furthermore, the fall in systemic blood pressure induced by acute administration of losartan to two-kidney, one-clip rats could be considered as reflecting the involvement of the renin-angiotensin system in the establishment of the blood pressure level in these animals.

The lack of increase in blood pressure following administration of HOE 140 shows that, in normotensive and hypertensive rats, bradykinin does not seem to participate in blood pressure regulation under basal conditions. While circulating bradykinin is not directly involved in blood pressure regulation of conscious normotensive rats (Aubert et al., 1988), several studies have pointed to a role of endogenous bradykinin in blood pressure regulation in two-kidney, one-clip hypertension (Carretero et al., 1981; Benetos et al., 1986; Carbonnel et al., 1988). The acute antihypertensive effects of angiotensin converting enzyme inhibitors could be attenuated or partially reversed by administration of unspecific bradykinin antagonists. However, the results from these studies were recently contested by Salgado and Name (1994) who used HOE 140, a specific bradykinin $B_2$ receptor antagonist. The discrepancy between findings of these studies might be related to differences between bradykinin antagonists. In contrast to acute administration, chronic treatment with HOE 140 attenuated the long-term antihypertensive action of ramipril (Bao et al., 1992b). Furthermore, Bao et al. (1992a) reported that chronic HOE 140, alone, induced a slight increase in blood pressure, supporting the hypothesis of a role of endogenous bradykinin in blood pressure regulation in two-kidney, one-clip hypertension. Thus, the role of bradykinin in blood pressure regulation in two-kidney, one-clip hypertension remains unclear. Carretero et al. (1981) suggested that the formation and destruction of bradykinin occur in an equilibrium situation, and that when steady state is altered, kinins become important in the regulation of blood pressure.

A common estimate of blood pressure variability is given by the standard deviation of the frequency distribution of the pressure values. The greater mean stan-

dard deviation of systolic blood pressure observed in two-kidney, one-clip hypertension reflected an overall increased blood pressure variability. In contrast to this time domain of pressure variability, spectral analysis of blood pressure fluctuations provides a tool to determine the variability in definite frequency ranges. By this means, we observed a selective overexpression of low-frequency and mid-frequency fluctuations in two-kidney, one-clip hypertension. The three components of blood pressure variability were affected by the specific antagonists of the receptors of angiotensin II or bradykinin. The greater standard deviation of blood pressure observed in hypertensive rats could have been due to the occurrence of marked slow fluctuations, since the standard deviation was predominantly influenced by fluctuations of the lowest frequencies. However, the reductions of the low-frequency component of systolic blood pressure variability induced by losartan or HOE 140 were not associated with a decrease of the standard deviation except for a slight reduction after HOE 140. It cannot be excluded that the absence of a significant decrease of standard deviation is due to opposite changes of the three components of blood pressure variability: the decrease of the low-frequency component of systolic blood pressure variability was counteracted by increases of the mid-frequency and high-frequency components.

As shown by Fig. 1, the two-kidney, one-clip hypertension added low-frequency fluctuations to the combination of mid-frequency and high-frequency oscillations found in sham-operated animals. In the first series of animals, losartan reduced the low-frequency component of blood pressure variability in addition to its hypotensive effects. However, the area of the low-frequency domain stayed greater in the hypertensive rats than in the normotensive animals. In spite of the lack of blood pressure change, HOE 140 decreased the low-frequency component of blood pressure variability in the second series of rats. Interestingly, the subsequent injection of losartan abolished the difference in the low-frequency fluctuations between the two strains of rats.

Previous reports mentioned that low-frequency fluctuations are dependent upon the activity of the autonomic nervous system (Akselrod et al., 1985; Cerutti et al., 1991) which is damped by renin-angiotensin system activity (Akselrod et al., 1985). Interestingly, spectral analysis of the blood pressure variability of chronically sympathectomized rats over about 100-s segments also revealed spectral power in low-frequency bands (Cerutti et al., 1991; Daffonchio et al., 1991). It is conceivable that the reduced activity of a neural regulatory mechanism led to the activation of a humoral regulatory process responsible for slow fluctuations of blood pressure. This was in agreement with the results of study of Rodrigues-de-Oliveira and Machado (1993) where the renin-angiotensin system was responsible, in association with the sympathetic nervous system, for the increased blood pressure lability in sino-aortic deafferented rats. In the present work where the activity of the arterial sympathetic nervous system was unaffected, the partial reduction of the slow fluctuations following angiotensin $AT_1$ receptor blockade by losartan demonstrated the involvement of the renin-angiotensin system in the low-frequency domain of systolic blood pressure variability. However, the residual low-frequency fluctuations after losartan suggested the dependence of this component upon other factors. In the present work, we also studied the share of the kallikrein-kinin system using a specific bradykinin $B_2$ receptor. Interestingly, the combined blockade of the kallikrein-kinin and the renin-angiotensin systems abolished the low-frequency component of systolic blood pressure variability in renovascular rats. This supports our hypothesis that these two humoral factors play a role in generating low-frequency fluctuations. The lack of blood pressure change after HOE 140 suggests that the involvement of the kallikrein-kinin system in blood pressure variability is not necessarily associated with a role in blood pressure regulation.

We cannot exclude the involvement in the slow fluctuations of other factors which were able to influence the variability of blood pressure and heart rate, for example adrenal catecholamines (Tulen et al., 1994), nitric oxide (Cordero et al., 1994; Just et al., 1994) or atrial natriuretic factor (Butler et al., 1994).

The increase of the mean standard deviation of systolic blood pressure in hypertensive rats was not confined to the low-frequency range. We also observed an overexpression of the mid-frequency oscillations of the systolic blood pressure of these animals.

These oscillations of blood pressure depend to a large extent on sympathetic activity in the resistance vessels (Pagani et al., 1986; Japundzic et al., 1990; Cerutti et al., 1991; Daffonchio et al., 1991; Persson et al., 1992). Recently, Brown et al. (1994), assessing directly the spectral relationships between sympathetic nervous system activity with blood pressure and heart rate, reported a close coupling between sympathetic nervous system and blood pressure at 0.4 Hz, raising the possibility that the blood pressure spectral power at 0.4 Hz reliably reflects sympathetic activity. The two kidney, one-clip hypertension was associated with overactivity of the sympathetic nervous system (Katholi et al., 1982), a decrease in the sensitivity of arterial baroreflexes (Edmunds et al., 1990) and significant modification of function and morphology of the vessels (Levy et al., 1988) which could be responsible for the pronounced mid-frequency oscillations of systolic blood pressure observed in this model of hypertension.

The present administration of losartan caused an increase in the mid-frequency component of blood

pressure variability in hypertensive rats while it did not change this component in normotensive rats. Akselrod et al. (1985) already reported that acute blockade of the renin-angiotensin system with teprotide resulted in increased blood pressure fluctuations in the mid-frequency range. We also reported such an increase in the 0.4 Hz component of blood pressure variability after an acute administration of enalapril (Grichois et al., 1992). Recently, a difference in the effects of losartan between normotensive and hypertensive rats was described by Gaudet et al. (1995) in spontaneously hypertensive rats. This increase in the mid-frequency range could be attributed to sympathetic reflex activation following the vasodilation induced by losartan, this sympathetic activation also being responsible for the tachycardia observed after losartan.

The opposite changes of low-frequency and mid-frequency domains observed during this study show that it is necessary to separate low-frequency fluctuations in the range of 0.02 – 0.2 Hz from mid-frequency oscillations of 0.4 Hz because these bands may represent different aspects of short-term cardiovascular control mechanisms. The decrease of the low-frequency domain induced by HOE 140 and losartan associated with an activation of the sympathetic nervous system, illustrated by the marked mid-frequency oscillations, was a supplementary argument to suggest that humoral factors are able to generate low-frequency fluctuations of blood pressure independently from the activity of the sympathetic nervous system.

Losartan also increased the amplitude of the high-frequency oscillations of blood pressure in the two series of hypertensive rats. The origin of this effect remains uncertain since losartan did not affect the factors influencing the high-frequency oscillations of blood pressure, i.e. the respiratory tidal volume (Laude et al., 1995) and the cardiac output variations (Toska and Eriksen, 1993).

In summary, stenosis of a renal artery leads to renin-dependent hypertension characterized by an overexpression of the blood pressure variability. Spectral analysis shows that this exaggerated blood pressure variability was partly confined to the increased low-frequency component resulting from the occurrence of slow blood pressure fluctuations. The reduction of these slow fluctuations following the combined blockade of the kallikrein-kinin and the renin-angiotensin systems suggests the contribution of these humoral systems in the low-frequency component of blood pressure variability.

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