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# Endogenous renin and related short-term blood pressure variability in the conscious rat

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**Abstract**

This study was designed to investigate, by use of spectral analysis, the blood pressure variability changes induced in the conscious rat by activation of plasmatic renin activity. Rats were surgically prepared with a supra-renal catheter inserted via the left carotid artery to perform the infusions, and with a femoral artery catheter to measure blood pressure and heart rate. Secretion of renin was induced using β-adrenoceptor stimulation produced by isoprenaline. A first group ($n = 8$) was infused with isoprenaline: 0.003, 10, 100 and 300 ng/kg/min, at a rate of 20 μl/min. A second group ($n = 8$) was given a bolus injection of the angiotensin $AT_1$ receptor antagonist, valsartan (2 mg/kg, i.a.), prior to isoprenaline infusions. The lack of effect of infusion per se was checked in additional animals ($n = 8$) infused with saline only (20 μl/min). Five other groups of animals were prepared with arterial catheters as mentioned previously. Each group received one concentration of infused isoprenaline and samples of blood were collected for further determinations of plasma renin activity and catecholamine concentrations. Blood pressure recordings were analysed using the fast Fourier transform on 2048 points time series (204.8 s). Isoprenaline increased plasma renin activity and did not modify plasma catecholamine concentrations. The low-frequency (0.02–0.2 Hz) component of the systolic blood pressure variability was amplified by isoprenaline (10 ng/kg/min isoprenaline: $4.16 \pm 0.62$ mm $Hg^2$ vs. $2.90 \pm 0.44$ mm $Hg^2$ for control value, $P < 0.05$), a concentration that did not alter either blood pressure or heart rate levels. Isoprenaline lowered blood pressure and increased heart rate, starting at concentrations of 100 ng/kg/min. Valsartan, whose principal effect was generation of tachycardia (+25 bpm) modified neither blood pressure levels nor blood pressure variability. Valsartan prevented the amplification of the low-frequency oscillations of systolic blood pressure induced by isoprenaline (10 ng/kg/min isoprenaline: $2.53 \pm 0.38$ mm $Hg^2$ vs. $2.20 \pm 0.25$ mm $Hg^2$ for control value (valsartan, ns). We conclude that a moderate increase of plasma renin activity enhanced systolic blood pressure variability in the low-frequency range, without affecting blood pressure and heart rate levels. 

*Keywords:* Spectral analysis; Valsartan; Catecholamine; Isoprenaline; Arterial pressure

## 1. Introduction

Blood pressure and heart rate variabilities have been characterized in rats within distinct frequency domains using spectral analysis. The fast components of these short-term variabilities were clearly identified as the vagus impinging on both mid-frequency (0.2–0.6 Hz) and high-frequency (peak between 1 and 2 Hz) oscillations of heart rate, whereas the sympathetic nervous system mediates the mid-frequency oscillations of blood pressure and heart rate at the frequency (0.4 Hz) of the so-called Mayer waves (Akselrod et al., 1985; Japundzic et al., 1990; Cerutti et al., 1991; Brown et al., 1994). Significant increases in the low-frequency (0.02–0.2 Hz) domain of systolic blood pressure variability were described when the renin–angiotensin system was activated after sympathectomy (Cerutti et al., 1991; Daffonchio et al., 1991), during stress (Gaudet et al., 1994), after combined blockade of the autonomic nervous system and circulating vasopressin (Elghozi et al., 1995), in renovascular hypertension (Ponchon and Elghozi, 1996) and in hyperthyroidism (Safa-Tisseront et al., 1998). On the other hand, it has been hypothesized that the sympathetic nervous system could also contribute to low-frequency fluctuations. Previous reports mentioned that low-frequency fluctuations were dependent upon the activity of the autonomic nervous system (Akselrod et al., 1985; Cerutti et al., 1991) which was damped by renin–

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angiotensin system activity (Akselrod et al., 1985). Activation of the sympathetic nervous system could increase the low-frequency component of systolic blood pressure and oppose the decrease of the low-frequency component after blockade of the renin–angiotensin system (Gaudet et al., 1995). Besides the interactions between the sympathetic nervous system and the renin–angiotensin system which influenced the low-frequency domain, opposite effects of nitric oxide and renin–angiotensin system have been described. Nitric oxide has been shown to be an effective blood pressure buffer (Just et al., 1994) and its acute withdrawal leads to hypertension and increased low-frequency fluctuations of systolic blood pressure (Cordero et al., 1994; Gouédard et al., 1996). Increased variability of systolic blood pressure in the low-frequency range, resulting from the withdrawal of the vasodilating influence of nitric oxide only occurred when the renin–angiotensin system was active (Gouédard et al., 1996), suggesting that nitric oxide might counterbalance low-frequency oscillations provoked by the renin–angiotensin system activity. Finally, after severe hemorrhage, the spontaneous blood pressure recovery was characterized by the occurrence of slow fluctuations of systolic and diastolic blood pressure (Ponchon and Elghozi, 1997). The individual contribution of the sympathetic nervous system, the renin–angiotensin system and arginine vasopressin to low-frequency fluctuations, assessed by sequential blockade of each system, led these authors to conclude that there was a hierarchy between humoral systems in the genesis of low-frequency fluctuations of blood pressure, with the slow oscillations being generated by the main pressor system and being dampened by the other systems.

Three main mechanisms regulating renin secretion have been described (Hackenthal et al., 1990): barosensitivity or pressure dependent secretion, chemosensitivity or salt dependent secretion, and the secretions influenced by hormones and neurotransmitters. The most important factors are the sympathetic nervous system, the negative feedback of angiotensin II on renin secretion and the endothelial nitric oxide synthesis.

The aim of the present study was to examine the effects of endogenous renin on blood pressure variability in the conscious normotensive rat. We induced a graded rise of plasma renin activity, using β-adrenoceptor stimulation produced by isoprenaline (Leenen and Mcdonald, 1974). Isoprenaline can activate both cardiac $\beta_1$-adrenoceptors, leading to tachycardia, and $\beta_2$-adrenoceptors, located in the peripheral vasculature to provoke vasodilation of the skeletal muscle vasculature, inducing hypotension. In order to avoid these two peripheral effects, isoprenaline was infused via the left carotid artery into the abdominal aorta above the renal arteries. The plasma levels of renin and catecholamines were assayed and the related changes in blood pressure oscillations were studied using spectral analysis. Valsartan was used to evaluate the renin dependence of the generated oscillations of blood pressure.

## 2. Methods

### 2.1. General

Eleven groups of male Wistar rats (Janvier, Le Genest-Saint-Isle, France) weighing 280–300 g were studied. The animals were maintained under controlled housing conditions ($20 \pm 1$°C, lighting 8 am–8 pm) and received tap water ad libitum and a standard rat chow diet (A O4, UAR, Epinay sur Orge, France). Experiments were performed in conscious, unrestrained animals. All experiments conformed to the relevant guidelines of the French Ministry of Agriculture for scientific experimentation on animals and with European Communities Council Directive (86/609/EEC). Our laboratory and personnel are authorized to conduct such an investigation according to the Ministry's Executive Order No. 89-02683.

### 2.2. Surgery

The animals were surgically prepared with catheters under pentobarbital sodium anesthesia (60 mg/kg, i.p.). Catheters of polyethylene tubing [(no. 800 from Portex, Berck (0.28 mm i.d., 0.61 mm o.d.), heat-fused to no. 3 from Biotrol, Paris (0.58 mm i.d., 0.96 mm o.d.)] were inserted into the right femoral artery to measure arterial blood pressure and via the left carotid artery into the abdominal aorta, above the renal arteries, to allow the local infusion of the non-selective β-adrenoceptor agonist, isoprenaline. 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 carotid catheter was connected to an electric microsyringe (Harvard Apparatus 22 pump, Les Ulis, France) for saline or isoprenaline infusions, and the femoral catheter was connected to a pressure transducer (Spectramed P 10EZ, Bilthoven, The Netherlands) for the 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 using a 486 DX computer from Fujikama (Toronto, Canada). At the end of the experiment, the correct position of the supra-renal catheter was checked in each rat by autopsy following a lethal administration of pentobarbital sodium (180 mg/kg, i.p.).

### 2.3. Signal processing and spectrum analysis

Experiments were started approximately 1 h after the rats had been connected to the pressure transducer and the perfusor. The individual cages were housed in quiet surroundings and the animals had free access to tap water. The blood pressure signal processing and spectrum analy-

ses were modified from those previously detailed (Grichois et al., 1992). Briefly, the evenly spaced sampling allowed direct spectral analysis using a fast Fourier transform algorithm of a stationary period in a 2048-point series. This corresponded to a 204.8-s period at the 10-Hz sampling rate. Thus, each spectral component (band) corresponded to a harmonic of 1/2048 Hz, i.e. 0.00049 Hz. The first spectral component corresponded to the mean value of the variable. The power of the blood pressure spectra (ordinates) had units of mm $Hg^2$. The sum of the whole values of consecutive bands (without the first band) represents the variance of blood pressure. Integrated spectra of the systolic blood pressure 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 204.8-s files (2048 values) used for the spectral analysis were computed.

### 2.4. Experimental protocol

Experiments were conducted 2 days after the surgical procedure. Blood pressure recordings lasted 10 min.

The first series of experiments ($n = 8$) was designed to assess the modifications of blood pressure and heart rate variabilities induced by isoprenaline. Isoprenaline was infused in increasing concentrations in conscious rats [saline and 0.003, 10, 100 and 300 ng/kg/min isoprenaline, at a rate of 20 μl/min (Pfeifer et al., 1995)]. Each infusion rate was maintained for 15 min, which is a sufficient duration to allow steady-state increases in plasma renin concentrations. Five recordings of blood pressure were computed from each rat. The first recording started 15 min after the beginning of vehicle infusion. The lack of effect of an infusion on blood pressure oscillations per se, over the entire time period of experimentation was checked in additional animals ($n = 6$) infused with saline only.

Experiment 2 quantified the renin and catecholamine responses to isoprenaline. Five groups of animals were used. Within each group, the animals were infused with a single concentration of isoprenaline at a rate of 20 μl/min. Two-milliliter blood samples were collected: 1 ml was collected in a chilled Eppendorf tube containing $EDTA_{Na^{2+}}$ for plasma renin activity measurement, 1 ml was collected in a chilled Eppendorf tube containing an anticoagulant/antioxidant mixture of ethylene glycol and reduced glutathione for catechol assays. The samples were immediately centrifuged (3000 rpm for 3 min at 4°C) and the plasma was frozen at −80°C.

Plasma renin activity was measured by radioimmunoassay according to a previously described method (Menard and Catt, 1972) using a REN-CT2 kit (CIS bio international, Gif-sur-Yvette, France). Catechols were extracted from plasma by alumina adsorption, separated by high-performance liquid chromatography, and the amounts were quantified by electrochemical detection according to a previously described method (Medvedev et al., 1990). The chromatographic system consisted of a model 480 high-precision pump, Gina model autosampler, model STH 585 column oven, Chromeleon 3.03 chromatography data system (Gynkotek, Germering, Germany), model 5100A coulometric detector equipped with a model 5021 conditioning cell and a model 5011 analytical cell (Environmental Sciences Associates), and a 25-cm Altex Ultrasphere column (ODS 4.6 mm × 25 cm, 5-μm particle size, Beckman Instruments). Analysis was performed at 24°C with the operating potentials set at +0.35 V for the guard cell and −0.35 and +0.29 V for detectors 1 and 2, respectively. All measurements were made by using the oxidizing potential applied at detector 2, and compounds in plasma were identified by their retention behavior compared with that of authentic standard solutions. The intra-assay coefficients of variation were ±2% for norepinephrine, epinephrine, dihydroxyphenylalanine (DOPA) and dihydroxyphenylglycol (DHPG). All samples were analysed in a single-batch analysis.

An additional group of animals ($n = 8$) was used to determine the smallest dose of the angiotensin $AT_1$ receptor antagonist, valsartan, that still blocked the pressor response secondary to an angiotensin II bolus (1 μg/kg/ml, i.a.) at the end of the entire time period required for isoprenaline infusions.

Experiment 3 was designed to demonstrate the involvement of the renin–angiotensin system in the generation of blood pressure oscillations. Each animal ($n = 8$) was first infused with saline (20 μl/min) for 15 min to allow the recording of baseline blood pressure. A bolus injection of valsartan (2 mg/kg/ml, i.a.) was performed and the infusion was continued. The effect of valsartan was recorded 20 min after its administration. Then isoprenaline was infused according to the concentrations given for experiment 1.

### 2.5. Drugs

Sodium pentobarbital (6 g/100 ml) was purchased from Sanofi (Libourne, France), Penicillin G was from Diamant (Puteaux, France), angiotensin II was obtained from Sigma (St Louis, Missouri, USA) and isoprenaline was Isuprel® from Winthrop (Clichy, France). Valsartan was generously donated by Novartis Pharma (Basle, Switzerland).

### 2.6. Statistical analysis

Data are presented as means ± S.E.M. Statistical analyses of data from experiments 1 and 2 and for the group infused with vehicle only were done according to the following criteria. When the variance ratios were significantly different ($F > 0.05$), a logarithmic transformation was applied. Changes vs. control values (saline perfusion for experiment 1 and effect of valsartan for experiment 2)

were calculated and statistical analysis was performed using Student's paired *t*-test. Comparison of blood sample data included first the calculation of variance ratios with subsequent logarithmic transformation if necessary. A one-way analysis of variance (ANOVA) was then performed. Post-hoc testing was performed using the method of Dunnett. The null hypothesis was rejected at $P < 0.05$.

## 3. Results

### *3.1. Effects of isoprenaline on blood pressure and heart rate levels*

Table 1 shows the changes in blood pressure, heart rate and their corresponding S.D. when isoprenaline was infused locally in conscious rats. At the concentration of 10 ng/kg/min, the S.D. of the systolic and the diastolic blood pressure were increased ($+0.60 \pm 0.15$, $P < 0.01$ and $+0.42 \pm 0.12$, $P < 0.01$, respectively) with no change in the absolute blood pressure and heart rate levels. Blood pressure was further lowered by more concentrated isoprenaline, and the heart rate was increased concomitantly; the changes in these variables reached a peak at the highest concentation of isoprenaline, $-22 \pm 2$ mm Hg ($P < 0.001$) and $+170 \pm 8$ bpm ($P < 0.001$) respectively.

### *3.2. Plasma renin activity and catechols*

Plasma renin activity was determined from five separate groups of conscious animals that were infused with isoprenaline (Fig. 1). One group ($n = 7$) had no infusion at all to check the lack of effect of the rate of infusion (20 μl/min) on plasma renin activity (isoprenaline 0 group: $0.91 \pm 0.14$ ng angiotensin I/ml/h vs. $1.19 \pm 0.23$ ng angiotensin I/ml/h for no infusion group, ns). Isoprenaline increased plasma renin activity; the highest concentration of isoprenaline induced an eight and half-fold increase of plasma renin activity vs. saline infusion (group isuprel 0).

Table 1
Changes in average values and standard deviations (S.D.) of the systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR), provoked by increased infused concentrations of isoprenaline (ng/kg/min; 20 μl/min in conscious rats ($n = 8$). Values are means ± S.E.M.

| | Saline | 0.003 | 10 | 100 | 300 |
|---|---|---|---|---|---|
| SBP (mm Hg) | | | | | |
| Average | 128 ± 3 | 128 ± 4 | 129 ± 4 | 115 ± 3[c] | 106 ± 3[c] |
| S.D. | 2.8 ± 0.2 | 3.0 ± 0.3 | 3.4 ± 0.3[b] | 4.2 ± 0.2[c] | 4.7 ± 0.2[c] |
| DBP (mm Hg) | | | | | |
| Average | 92 ± 3 | 93 ± 3 | 93 ± 3 | 83 ± 3[a] | 72 ± 3[c] |
| S.D. | 2.4 ± 0.2 | 2.5 ± 0.2 | 2.8 ± 0.2[b] | 3.5 ± 0.2[b] | 3.6 ± 0.1[b] |
| HR (bpm) | | | | | |
| Average | 351 ± 7 | 362 ± 9 | 364 ± 9 | 464 ± 15[c] | 521 ± 6[c] |
| S.D. | 8.9 ± 1.6 | 6.8 ± 1.0 | 6.6 ± 0.9 | 10.3 ± 0.5 | 10.0 ± 0.3 |

[a] $P < 0.05$ for change relative to the saline value.
[b] $P < 0.01$ for change relative to the saline value.
[c] $P < 0.001$ for change relative to the saline value.

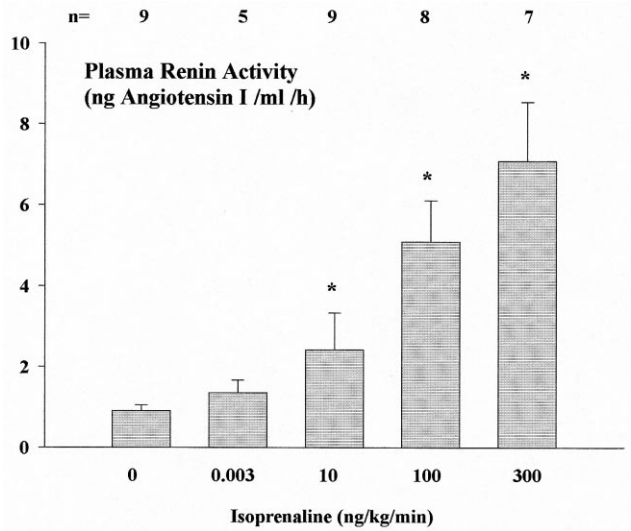


Fig. 1. Plasma renin activity (mean ± S.E.M.) in five groups of conscious rats infused with isoprenaline at a rate of 20 μl/min. *$P < 0.05$, for the change relative to the concentration of isoprenaline 0 (saline).

Table 2 shows the plasma catechol concentrations in five groups of animals given one concentration of isoprenaline. No significant changes were induced by isoprenaline except for DHPG which increased with the highest concentration infused ($7.69 \pm 0.81$ nmol/l vs. $4.40 \pm 0.37$ nmol/l, $P < 0.05$).

### *3.3. Changes in blood pressure and heart rate variability induced by isoprenaline*

Fig. 2 illustrates the modifications of systolic blood pressure variability induced by isoprenaline. Compared to saline infusion, isoprenaline infusion, at the rate of 10 ng/kg/min that did not modify either blood pressure or heart rate levels, changed the profile of the blood pressure tracings. The changes in the systolic blood pressure signals induced by isoprenaline were reflected by the modification of the corresponding spectrum especially in the low-frequency range where the power was enhanced.

The analysis of the systolic blood pressure variability in the different frequency ranges is shown in Fig. 3. In the control group, the systolic blood pressure variability was partitioned as follows: low-frequency: $2.90 \pm 0.44$ mm $Hg^2$, mid-frequency: $2.22 \pm 0.61$ mm $Hg^2$ and high-frequency oscillations: $0.21 \pm 0.05$ mm $Hg^2$. The changes in systolic blood pressure variability induced by isoprenaline showed modifications in the spectrum evidenced as enhancement of the power in the low-frequency domain. This effect was observed for the isoprenaline concentration of 10 ng/kg/min ($4.16 \pm 0.62$ mm $Hg^2$ vs. $2.90 \pm 0.44$ mm $Hg^2$, $P < 0.05$), whereas systemic blood pressure and

Table 2
Plasma catechol concentrations measured in conscious animals infused with isoprenaline (ng/kg/min; 20 μl/min). DOPA, dihydroxyphenylalanine; NE, norepinephrine; DHPG, dihydroxyphenylglycol; E, epinephrine. Values are means ± S.E.M.

| | Isoprenaline | | | | |
|---|---|---|---|---|---|
| | 0 ($n = 5$) | 0.003 ($n = 5$) | 10 ($n = 6$) | 100 ($n = 5$) | 300 ($n = 5$) |
| DOPA (nmol/l) | 3.21 ± 0.46 | 2.46 ± 0.15 | 2.21 ± 0.18 | 2.64 ± 0.27 | 2.38 ± 0.15 |
| NE (nmol/l) | 2.00 ± 0.89 | 1.98 ± 0.57 | 0.76 ± 0.11 | 1.70 ± 0.17 | 2.65 ± 0.47 |
| DHPG (nmol/l) | 4.40 ± 0.37 | 5.69 ± 0.62 | 4.41 ± 0.12 | 5.17 ± 0.29 | 7.69 ± 0.81[a] |
| E (nmol/l) | 2.13 ± 0.67 | 1.83 ± 0.60 | 1.76 ± 0.40 | 1.51 ± 0.42 | 1.20 ± 0.23 |

[a] $P < 0.001$, for change relative to the control value.

heart rate levels were not yet modified. Blood pressure variability was not affected by isoprenaline in other frequency bands except the high-frequency oscillations, which increased with the highest concentration used.

### 3.4. Effects of valsartan on the blood pressure and heart rate changes induced by isoprenaline

Blockade of the renin–angiotensin system by the angiotensin $AT_1$ receptor antagonist, valsartan, was efficient at the dose of 2 mg/kg/ml (Table 3). Blood pressure was slightly lowered (not significant) by valsartan and heart rate increased ($+16 \pm 13$ bpm, $P < 0.01$). Three hours after the administration of valsartan, the blockade of the renin–angiotensin system was of the order of 88%.

In the group which received valsartan before infusion of isoprenaline, the levels of blood pressure and the corresponding S.D. were not modified. Valsartan's effect was to elicit tachycardia ($+25 \pm 7$ bpm, $P < 0.01$) (Table 4). Subsequent values during infusions of isoprenaline were

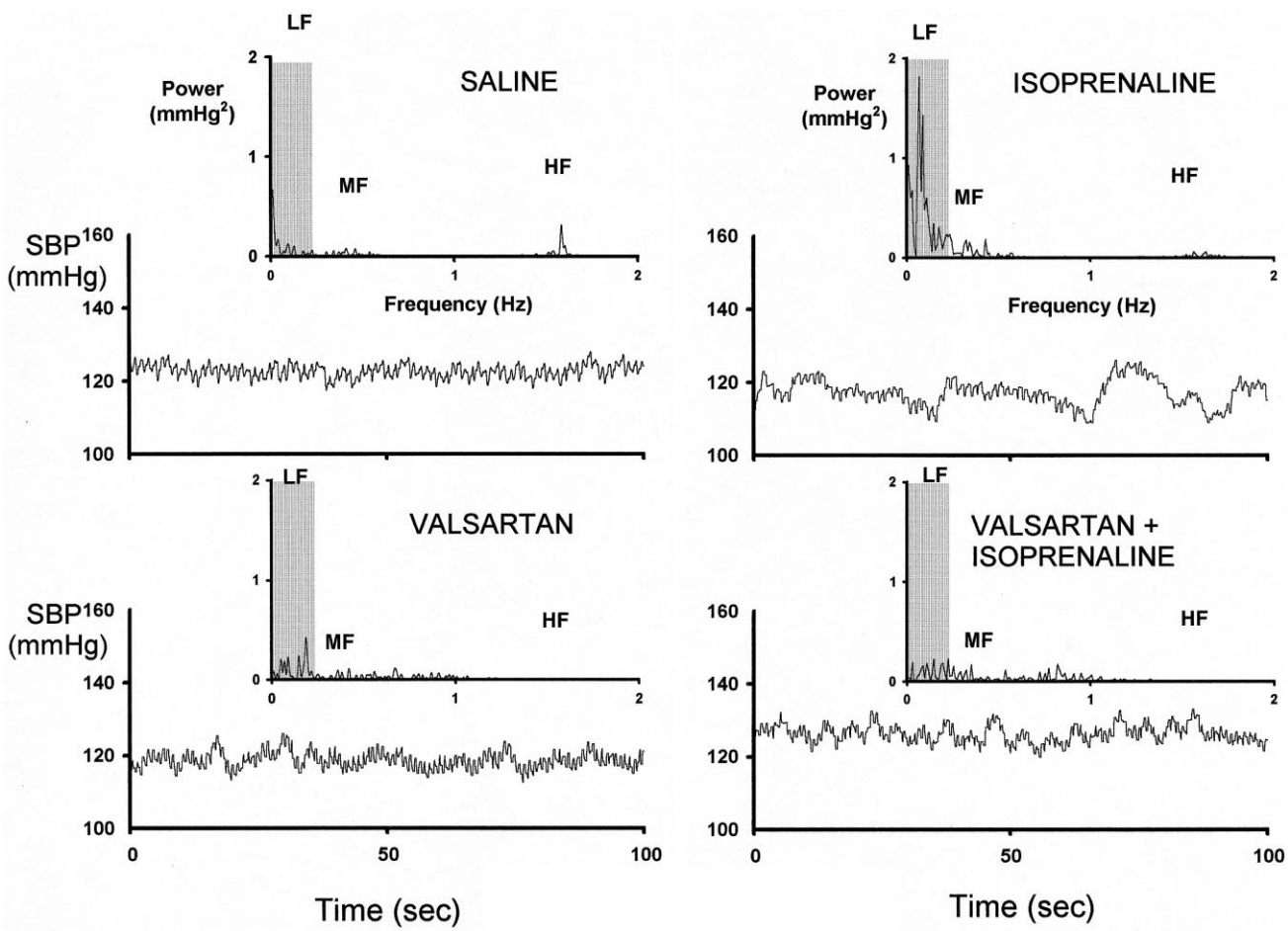


Fig. 2. Examples of systolic blood pressure digitized recordings for 100 s and their corresponding spectra from one rat infused with saline and then with isoprenaline (10 ng/kg/min at a rate of 20 μl/min) and one rat given a bolus of valsartan (2 mg/kg/ml) and then infused with isoprenaline (10 ng/kg/min at a rate of 20 μl/min).

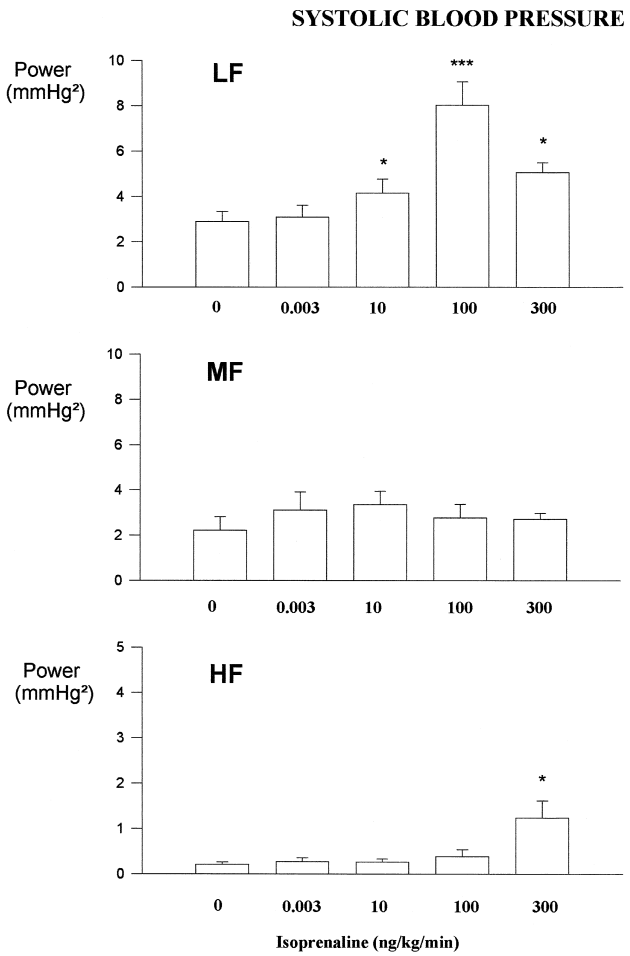


Fig. 3. Areas of the low-frequency (LF: 0.02–0.2 Hz), mid-frequency (MF: 0.2–0.6 Hz) and high-frequency (HF: within 1–2 Hz) components of the systolic blood pressure spectra in vehicle or isoprenaline treated rats ($n = 8$). Isoprenaline was infused at a rate of 20 μl/min. Values are means ± S.E.M. $^{*}P < 0.05$; $^{***}P < 0.001$ for the comparisons vs. isoprenaline 0.

compared with the values of valsartan. Isoprenaline began to have a tachycardiac effect ($+21 \pm 6$ bpm, $P < 0.01$) from the concentration of 10 ng/kg/min. At the end of the experiment the heart rate values were similar to those of the group that did not receive valsartan before isopre-

Table 3
Valsartan blockade (2 mg/kg/ml) was confirmed 3 h after its administration by the absence of a pressive response to a bolus of angiotensin II (AII, 1μg/kg/ml).
SBP: Systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate

| | Control | Delta AII | 3 h after Valsartan | Delta AII 3 h after Valsartan |
|---|---|---|---|---|
| SBP (mm Hg) | 127 ± 5 | 49 ± 4[a] | 117 ± 5 | 6 ± 3 |
| DBP (mm Hg) | 90 ± 2 | 35 ± 3[a] | 81 ± 4 | 5 ± 2 |
| HR (bpm) | 365 ± 5 | −52 ± 8[a] | 381 ± 18[b] | −10 ± 4 |

[a] $P < 0.001$ vs. control; $n = 8$.
[b] $P < 0.01$.

Table 4
Changes in average values and standard deviations (S.D.) of systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) in conscious rats ($n = 8$) 30 min after a bolus of valsartan (2 mg/kg/ml, i.a.) followed by infusion of increased isoprenaline concentrations (ng/kg/min; 20 μl/min). Values are means ± S.E.M.

| | Saline | Valsartan 0.003 | 10 | 100 | 300 |
|---|---|---|---|---|---|
| SBP (mm Hg) | | | | | |
| Average | 130 ± 4 | 125 ± 6 | 123 ± 5 | 127 ± 6 | 123 ± 5 | 112 ± 4[b] |
| S.D. | 2.8 ± 0.2 | 3.1 ± 0.2 | 3.0 ± 0.2 | 3.1 ± 0.2 | 3.4 ± 0.5 | 4.7 ± 0.6[b] |
| DBP (mm Hg) | | | | | | |
| Average | 89 ± 2 | 87 ± 4 | 88 ± 4 | 89 ± 4 | 81 ± 2[b] | 67 ± 2[d] |
| S.D. | 2.6 ± 0.3 | 2.9 ± 0.3 | 2.8 ± 0.3 | 2.7 ± 0.2 | 2.9 ± 0.4 | 3.5 ± 0.5 |
| HR (bpm) | | | | | | |
| Average | 349 ± 10 | 374 ± 9[a] | 374 ± 10 | 396 ± 13[c] | 432 ± 20[c] | 521 ± 15[d] |
| S.D. | 7.5 ± 0.7 | 9.1 ± 0.6 | 8.1 ± 0.4 | 9.7 ± 0.8 | 11.0 ± 1.4 | 11.0 ± 0.7 |

[a] $P < 0.01$, for the change relative to the saline value.
[b] $P < 0.05$ for the change relative to the valsartan value.
[c] $P < 0.01$, for the change relative to the valsartan value.
[d] $P < 0.001$, for the change relative to the valsartan value.

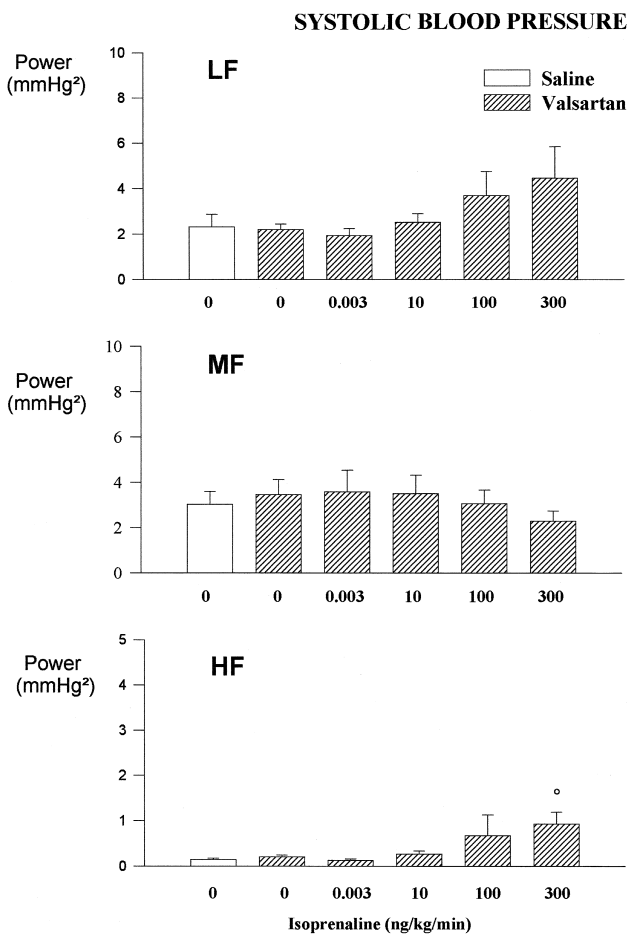


Fig. 4. Areas of the low-frequency (LF: 0.02–0.2 Hz), mid-frequency (MF: 0.2–0.6 Hz) and high-frequency (HF: within 1–2 Hz) components of the systolic blood pressure spectra in vehicle (open columns), and valsartan (bolus of 2 mg/kg/ml, i.a.; hatched columns)-treated rats ($n = 8$). Isoprenaline was infused at a rate of 20 μl/min. Values are means ± S.E.M. $^{\circ}P < 0.05$ for the change relative to the valsartan-isoprenaline 0 (control) value.

naline (521 ± 6 bpm in control rats (Table 1), 521 ± 15 bpm in valsartan treated rats, (Table 4)). Blood pressure was lowered at the end of the experiment (−18 ± 5 mm Hg, $P < 0.05$ for systolic blood pressure and −22 ± 1 mm Hg, $P < 0.001$ for diastolic blood pressure) compared to saline levels. The S.D. of the systolic blood pressure was increased by the greatest concentration of isoprenaline (4.66 ± 0.63 mm Hg vs. 2.76 ± 0.23 mm Hg, $P < 0.05$).

### *3.5. Effects of valsartan on blood pressure variability changes induced by isoprenaline*

Valsartan prevented the changes in the profile of the blood pressure tracings induced by isoprenaline (Fig. 2). This effect was reflected in the systolic blood pressure spectrum where the low-frequency oscillations were not enhanced by isoprenaline. The quantitative changes of systolic blood pressure variability are shown in Fig. 4. The control values for power in the different frequency bands were, respectively, 2.32 ± 0.56 mm $Hg^2$, 3.03 ± 0.57 mm $Hg^2$, 0.15 ± 0.03 mm $Hg^2$ for the low-, mid- and high-frequency oscillations. Valsartan did not modify blood pressure variability. Isoprenaline given after valsartan had no further increasing effect on low-frequency oscillations. High-frequency oscillations were enhanced with the highest concentration of isoprenaline (0.93 ± 0.26 mm $Hg^2$ vs. 0.21 ± 0.03 mm $Hg^2$, $P < 0.05$).

## 4. Discussion

The aim of this study was to investigate the influence of the activity of the renin–angiotensin system on the variability of blood pressure in conscious animals. This was achieved by infusing isoprenaline in order to stimulate plasma renin activity. The major finding of this work was that stimulation of renin, in a range where blood pressure and heart rate levels were not modified, induced amplification of blood pressure fluctuations in the low-frequency range. This effect was independent of sympathetic nervous system activation and was mediated by angiotensin II $AT_1$ receptors, given that blockade of the renin–angiotensin system activity by valsartan prevented the modifications of blood pressure variability induced by isoprenaline.

The mixed β-adrenoceptor agonist, isoprenaline, has been much used to induce renin secretion, especially to investigate the dipsogenic action of angiotensin II (Leenen and Mcdonald, 1974; Evered and Robinson, 1981; Kirby et al., 1994). Selective blockade of either β-adrenoceptor subtype significantly reduced isoprenaline-induced drinking (Kirby et al., 1994). It is well established that $\beta_1$-adrenoceptors located in the kidney mediate renin release from the juxtaglomerular cells into the blood, and $\beta_2$-adrenoceptors, located in the peripheral vasculature, stimulate vasodilation of skeletal muscle, inducing hypotension. In our study, in order to limit both the hypotensive effect and the tachycardia resulting from cardiac $\beta_1$-adrenoceptor stimulation, we developed a supra-renal infusion technique in the conscious rat. Resembling the direct intra-renal infusion technique, which is applied solely in anesthetized rats (Parekh, 1995), supra-renal infusion allowed the local stimulation of $\beta_1$-adrenoceptors of the juxtaglomerular cells of the kidney. Thus, a renin response was elicited in our study by concentrations of isoprenaline that did not change the blood pressure level. Intra-renal infusion of isoproterenol in the anesthetized rat was demonstrated to increase plasma renin activity while renal blood flow and mean arterial blood pressure remained unchanged (Pfeifer et al., 1995). These authors showed a moderate activation of the renin–angiotensin system of a magnitude similar to that we saw in the conscious rat. Exposure to β-adrenergic stimulation resulted in dose-dependent upregulation of renin gene expression and renin secretion, albeit with a different time course (Holmer et al., 1997). Several studies have provided evidence that important increases in plasma renin activity are induced by subcutaneous (Leenen and Mcdonald, 1974; Rowland and Fregly, 1993; Kirby et al., 1994) or intravenous (Vargas et al., 1989) administration of isoprenaline. In these latter studies, marked decreases in blood pressure were present. As pressure-dependent renin release is not activated spontaneously, and is therefore unlikely to play a role in mean arterial pressure maintenance, a threshold pressure, with mean arterial pressure below 86 mm Hg, inducing a large increase in plasma renin concentration, was described (Bertolino et al., 1994, 1996). The difference between our maximal eight and half-fold stimulation of plasma renin activity by isoprenaline and the 16-fold (Julien et al., 1997) described in the literature may be attributable to pressure-dependent renin release.

From previous studies using the angiotensin II $AT_1$ receptor antagonist, valsartan (Criscione et al., 1993, 1995), we adapted our experimental conditions to use a dose which maintained blockade of the renin–angiotensin system throughout our experimental period. Valsartan at a dose of 2 mg/kg did not decrease mean arterial pressure, supporting the thought that the vasoconstrictor action of angiotensin II is not part of the main pressor system under physiological conditions (Hasser and Bishop, 1988). The slight tachycardiac effect of valsartan could result from a reflex sympathetic activation subsequent to the vasodilation induced by blockade of the angiotensin $AT_1$ receptors and therefore compensate for an undetected slight decrease in blood pressure.

When valsartan was given prior to isoprenaline infusions, despite a general blood pressure lowering and a tachycardiac response similar to that seen in the non-valsartan-treated group, the time course of the action of isoprenaline changed. When the renin–angiotensin system was blocked, we found the depressor effect of subsequent infusion of isoprenaline only with the highest dose; on the other hand, the tachycardiac effect of isoprenaline was

present with the first active dose (the second one for the other group). Although the heart rate increase could result from the combined effects of direct stimulation of the cardiac $\beta_1$-adrenoceptors by isoprenaline and the reflex response to a possible $\beta_2$-mediated vasodilator effect of isoprenaline, we could hypothesize that the early tachycardia concomitant with the delayed hypotension observed in the valsartan-treated group could reflect an improvement of the cardiac baroreflex induced by the withdrawal of the action of the angiotensin II. Thus, it has been reported that blockade of the renin–angiotensin system activity either chronically (Kawano et al., 1994) or acutely (Kumagai et al., 1993) improves the sensitivity of the baroreflex control of heart rate (Reid, 1992). Interestingly, the maximal depressor and tachycardiac effects of isoprenaline were both similar in the two groups. However, at this stage of the experiment, plasma renin activity was eight and half-fold increased by isoprenaline. It was paradoxical that elimination of the circulating angiotensin II vasoconstrictor action as a counter-regulatory mechanism to attenuate the reduction of blood pressure did not lead to greater depressor response.

Before starting the main study, we infused a group of animals with saline at a rate of 20 μl/min and checked that there was no influence of the infusion per se on the blood pressure level and its variability (results not shown). We then examined the relationship between activation of the renin–angiotensin system and sympathetic nervous activity, as estimated from measurements of plasma renin activity and plasma catecholamines, respectively, and blood pressure variability. A common estimate of blood pressure variability is given by the S.D. of the frequency distribution of the pressure values. Stimulation by isoprenaline increased the S.D. of the systolic blood pressure. This effect on variability was independent of the blood pressure level and was prevented by prior blockade of the angiotensin II $AT_1$ receptors. We did not focus on heart rate variability since the corresponding S.D. were not modified by the different treatments. Breakdown of the spectrum of the systolic blood pressure variability showed that the amplification of variability was restricted to within the low-frequency range (0.02–0.2 Hz). The fluctuations in blood pressure were not accompanied by simultaneous fluctuations in heart rate. This excluded the involvement of a direct chronotropic effect in the generation of these oscillations. Several studies provide evidence that overexpression of low-frequency fluctuations of blood pressure occur in situations where the activity of the renin–angiotensin system is amplified. Thus, the amplitude of the low-frequency fluctuations of the systolic blood pressure is reduced by angiotensin $AT_1$ receptor antagonism in renovascular hypertension (Ponchon and Elghozi, 1996) and by angiotensin converting enzyme inhibition in animals with combined blockade of the autonomic nervous system and circulating vasopressin (Elghozi et al., 1995). In both cases, blockade of the action of angiotensin II lowered blood pressure levels by about 20 mm Hg. In the present study, small quantities of isoprenaline allowed local stimulation of β-adrenoceptors in the kidney as evidenced by increasing plasma renin activity without systemic changes of blood pressure and heart rate. We showed that endogenous renin added low-frequency fluctuations to the combination of mid-frequency and high-frequency oscillations found with saline infusion. This effect was present independently of modifications of either blood pressure or heart rate levels. It is tempting to relate our observation to the non neurally mediated mechanism responsible for the generation of a very low-frequency oscillation identified in the dog (Wagner et al., 1997) where a circulating factor dependent on intact pathways from the cardiac receptors or baroreceptors to the central nervous system was suggested to account for the very low oscillation (Wagner et al., 1997). The lack of a significant difference in arterial norepinephrine levels in any of our groups would seem to preclude any involvement of the sympathetic nervous system in the generation of the low-frequency fluctuations of systolic blood pressure we now observed. However, when blood pressure was decreased by the highest concentration of isoprenaline, there was a trend to an increase in arterial norepinephrine levels and disproportionate elevations in arterial levels of DHPG. DHPG is the intraneuronally produced metabolite of norepinephrine. While we have no reason to believe that the intraneuronal disposition of norepinephrine is altered in this group of animals, such observations may be consistent with reflex activation of the sympathetic nervous system in response to hypovolemia. Thus, isoprenaline administration may mimic the hypovolemic situation. Hypovolemia increases sympathetic drive to the kidney, increasing renin release and as volume loss increases, hypotension contributes a second stimulus to increase the renin–angiotensin system activity. Moreover, the high-frequency peak of the blood pressure spectrum was increased at this stage of the experiment in both groups. It has been shown that graded normotensive hemorrhage in conscious rats induces a volume dependent increase in the high-frequency component of systolic blood pressure variability and that the high-frequency index is a sensitive measure of hypovolemia that could reflect a low preload state (Baujard et al., 1996). As the respiratory peak frequency was not modified during the experiment in any groups, the increased amplitude of systolic blood pressure oscillations synchronized with respiration could not be attributed to changes in breathing frequency (Laude et al., 1995). The increased high-frequency peak observed with the high dose of isoprenaline persisted with blockade of the renin–angiotensin system, confirming that this humoral system did not impinge on these too rapid oscillations of systolic blood pressure.

The present results demonstrated that a moderate activation of the renin–angiotensin system, which does not change either blood pressure or heart rate levels, increases the variability of blood pressure in the low-frequency

range in the conscious rat. It has been suggested that the vasculature has an inherent ability to oscillate or resonate and that the hormone level reveals or dampens this ability to oscillate (Malpas et al., 1999). While the mechanisms involved in the effect we describe require further clarification, our results indicate the involvement of angiotensin II $AT_1$ receptors. The sympathetic nervous system is not involved in the effects observed.

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