Spontaneous renal blood flow autoregulation curves in conscious sinoaortic baroreceptor-denervated rats

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Pires, Silene L. S., Claude Julien, Bruno Chapuis, Jean Sassard, and Christian Barrès. Spontaneous renal blood flow autoregulation curves in conscious sinoaortic baroreceptor-denervated rats. Am J Physiol Renal Physiol 282: F51–F58, 2002. First published August 8, 2001; 10.1152/ajprenal.00186.2001.—These experiments examined whether the conscious sinoaortic baroreceptor-denervated (SAD) rat, owing to its high spontaneous arterial pressure (AP) variability, might represent a model for renal blood flow (RBF) autoregulation studies. In eight SAD and six baroreceptor-intact rats, AP and RBF were recorded (1-h periods) before and after furosemide (10 mg/kg followed by 10 mg·kg⁻¹·h⁻¹ iv) administration. In control conditions, AP variability was markedly enhanced in SAD rats (coefficient of variation: 16.0 ± 1.2 vs. 5.4 ± 0.5% in intact rats), whereas RBF variability was only slightly increased (8.7 ± 0.6 vs. 6.1 ± 0.5% in intact rats), suggesting buffering by autoregulatory mechanisms. In SAD rats, but not in intact rats, the AP-RBF relationships could be modeled with a four-parameter sigmoid Weibull equation (r² = 0.24 ± 0.07, 3,600 data pairs/rat), allowing for estimation of an autoregulatory plateau (10.1 ± 0.7 ml/min) and a lower limit of RBF autoregulation (P_{LL} = 93 ± 6 mmHg, defined as AP at RBF 5% below the plateau). After furosemide treatment, autoregulation curves (r² = 0.49 ± 0.07) in SAD rats were shifted downward (plateau = 8.6 ± 0.8 ml/min) and rightward (P_{LL} = 102 ± 5 mmHg). In five of six intact rats, P_{LL} became measurable (104 ± 1 mmHg), albeit with limited accuracy (r² = 0.09 ± 0.03). In conclusion, the conscious SAD rat offers the possibility of describing RBF autoregulation curves under dynamic, unforced conditions. The tubuloglomerular feedback and myogenic mechanisms cooperate in setting P_{LL} and thus in stabilizing RBF during spontaneous depressor episodes.

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that the TGF mechanism was not required for RBF autoregulation in response to spontaneously occurring AP changes (16). Therefore, to evaluate the participation of the TGF mechanism in RBF responses to spontaneous variations of AP in the conscious SAD rat, hemodynamic recordings were performed before and after administration of furosemide, a potent inhibitor of TGF activity (27).

METHODS

Animal preparation. All experiments were conducted in accordance with the guidelines of the French Ministry of Agriculture for animal experimentation. Fourteen male Sprague-Dawley rats, weighing 250–300 g (Iffa-Credo, L’Arbresle, France), were used and prepared as previously described (20). Briefly, 14 days before the beginning of the study a bilateral sinoaortic baroreceptor denervation was performed in eight rats. One week later, SAD rats and six baroreceptor-intact rats were submitted to left renal denervation and instrumented with an ultrasonic transit-time flow probe (1R8, Transonic Systems, Ithaca, NY) around the left renal artery for RBF measurement. Five days later, i.e., 2 days before the beginning of the study, femoral arterial and venous catheters were inserted for AP measurement and drug administration, respectively. Both catheters and the probe cable were exteriorized between the scapulae and protected in a small plastic cap sewn to the skin. Antibiotic (neomycin sulfate) was applied topically. After each intervention, rats received a single injection of penicillin G (40,000 IU ip).

Measurements. AP was measured by connecting the arterial catheter to a precalibrated pressure transducer (TNP-R, Ohmeda, Bilthoven, The Netherlands) through a two-way stopcock, which allowed the continuous infusion of a 5% glucose solution (0.5 ml/h). The pressure transducer was coupled to an amplifier (model 13–4615–52, Gould, Cleveland, OH) chart recorder (model 8802, Gould). Absolute RBF was measured with a flowmeter (T106, Transonic Systems) without filtering. After analog-digital conversion (AT-MIO-16E converter board, National Instruments, Austin, TX), AP and RBF signals were simultaneously and continuously recorded on a computer (500-Hz sampling rate) using LabVIEW 5.0 software (National Instruments).

Experimental protocol. All recordings were performed while the rats were conscious and unrestrained. AP and RBF were recorded continuously during two consecutive periods of 1-h duration each. The first 1-h recording period occurred without any pharmacological intervention and was initiated after stabilization of cardiovascular variables. The second 1-h recording period started 10 min after an intravenous bolus injection of furosemide (10 mg/kg, Lasilix, Hoechst Houdé), followed by a continuous intravenous infusion of 10 mg·kg⁻¹·h⁻¹ maintained until completion of the study. The infusion flow rate was 1 ml·kg⁻¹·h⁻¹. Rats had free access to water throughout the study. At the end of the experiment, rats received an intravenous overdose of pentobarbital sodium, and both kidneys were removed, weighed, and frozen for subsequent determination of norepinephrine concentration (9).

Data analysis. The individual 500-Hz data files were first replayed and carefully examined to eliminate occasionally occurring artifacts. The 500-Hz data files were resampled at 1 Hz by averaging over consecutive 1-s periods for subsequent analysis. Therefore, each 1-h experimental period consisted of 3,600 AP-RBF data pairs.

From each 1-h period, the 3,600 AP-RBF data pairs were sorted according to an ascending-order AP, and AP and corresponding RBF values were averaged within 2.5-mmHg intervals. After the AP intervals that were common to all SAD or intact rats were considered, average RBF values were plotted as a function of AP in each group of rats.

Finally, the equation of a sigmoidal model was fitted to the different sets of experimental data (3,600 individual data pairs, individual data organized in AP intervals, and group-average data organized in AP intervals), using an iterative least-squares procedure (SigmaPlot 5.0, SPSS, Chicago, IL).

In each rat and for each period, the overall spontaneous variability of AP and RBF was estimated by calculating the coefficient of variation of 1-Hz data.

Statistics. All data are presented as means ± SE. Comparisons between intact and SAD rats were performed with the use of the nonparametric Mann-Whitney U-test. Within each group of rats, comparisons between periods were performed by using the Wilcoxon signed-rank test.

RESULTS

Body weights were similar in intact (331 ± 13 g) and SAD (322 ± 9 g) rats. Norepinephrine concentrations in the left kidney (12.2 ± 2.7 and 15.5 ± 7.5 ng/g kidney wt in intact and SAD rats, respectively) were reduced by >90% compared with the norepinephrine concentrations measured in the right kidney (141 ± 12 and 240 ± 25 ng/g kidney wt in intact and SAD rats, respectively).

As indicated in Table 1, in control conditions chronic sinoaortic denervation did not modify the 1-h mean levels of AP and RBF but induced a threefold increase in AP variability and a 40% increase in RBF variability. Furosemide administration increased AP and decreased RBF in intact rats, whereas it decreased both AP and RBF in SAD rats. In addition, after furosemide, the RBF variability was increased in both groups of rats without a significant change in AP variability.

Group-average RBF autoregulation curves. Figure 1 summarizes the spontaneous RBF-AP relationships observed in six intact (A) and eight SAD (B) rats after data reduction in 2.5-mmHg AP intervals. In intact rats with low AP variability, a zone of nonefficient autoregulation (RBF decreases associated with AP decreases) was not clearly apparent in control conditions.

Table 1. One-hour average values of mean level and variability for AP and RBF in conscious intact and SAD rats before and after furosemide administration

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<th>Intact (n = 6)</th>
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<td>Control</td>
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<td>Control</td>
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<td>AP</td>
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<tr>
<td>Mean, mmHg</td>
<td>106 ± 1</td>
<td>116 ± 2</td>
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<tr>
<td>CV, %</td>
<td>5.4 ± 0.5</td>
<td>5.4 ± 0.3</td>
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<td>113 ± 3</td>
<td>16.0 ± 1.2†</td>
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<td></td>
<td>98 ± 5†</td>
<td>16.6 ± 1.3†</td>
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<tr>
<td>RBF</td>
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<tr>
<td>Mean, ml/min</td>
<td>11.1 ± 1.2</td>
<td>9.1 ± 0.8*</td>
</tr>
<tr>
<td>CV, %</td>
<td>6.1 ± 0.5</td>
<td>7.6 ± 0.6*</td>
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<td>9.9 ± 0.7</td>
<td>8.7 ± 0.6†</td>
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<td>7.8 ± 0.8*</td>
<td>14.0 ± 2.3†</td>
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Values are means ± SE, n, No. of rats; AP, arterial pressure; RBF, renal blood flow; SAD, sinoaortic baroreceptor-denervated; CV, coefficient of variation; control, before furosemide. *P < 0.05 vs. control. †P < 0.05 vs. intact rats under the same experimental conditions.
By contrast, in SAD rats with high AP variability, a plateau and a zone of nonefficient autoregulation could be clearly identified in control conditions. Such a profile with a plateau and a zone of nonefficient autoregulation was also observed in both intact and SAD rats after furosemide.

Because this profile matched a portion of a sigmoidal curve, the equations of various sigmoidal models were tested for fitting to experimental data. The four-parameter Weibull equation was found to satisfactorily fit to experimental data ($r^2 = 0.984$, $n = 28$ AP intervals, $P < 0.0001$ in SAD rats in control conditions; $r^2 = 0.997$, $n = 27$ AP intervals, $P < 0.0001$ in SAD rats after furosemide; $r^2 = 0.935$, $n = 17$ AP intervals, $P < 0.0001$ in intact rats after furosemide). The equation of the model is

$$RBF = a \left[ 1 - e^{-\left(\frac{AP - x_0 + b \ln(2)^c}{b}\right)^c} \right]$$

where $a$ is the upper plateau of the sigmoid, $x_0$ is the AP at the RBF value corresponding to half the plateau, and $b$ and $c$ are scale and shape parameters of the Weibull distribution, respectively. From the fitted curves (Fig. 1), $P_{LL}$ was computed as the AP corresponding to the RBF value 5% lower than the estimated plateau. In SAD rats, plateau and $P_{LL}$ were found at 9.9 ml/min and 89 mmHg and at 8.5 ml/min and 101 mmHg in control conditions and after furosemide, respectively. In intact rats after furosemide, the plateau and $P_{LL}$ were 9.2 ml/min and 105 mmHg.

Analysis of individual autoregulation curves. In a second step, the model was fitted to individual data organized in AP intervals of 2.5 mmHg (Figs. 2C and 3C). If the estimated plateau was not reached by the experimental data or if no data point was present below the estimated $P_{LL}$, the fitting was not considered. According to these criteria, in control conditions plateau and $P_{LL}$ could not be determined in intact rats and were estimated in five of eight SAD rats. After furosemide, plateau and $P_{LL}$ were estimated in three of six intact rats and in six of eight SAD rats.

When the model was applied to the individual sets of 3,600 data pairs obtained initially in each intact and SAD rat (Figs. 2B and 3B), it was found to satisfactorily fit to data in the eight SAD rats in both experimental conditions (Table 2). In intact rats, the model was able to fit to experimental data in one of six rats in control conditions and in five of six rats after furosemide (Table 2). In SAD rats after furosemide, the plateau was significantly decreased and $P_{LL}$ was increased (Table 2). In addition, the percentage of data pairs below $P_{LL}$ was markedly increased after furosemide (Table 2). $P_{LL}$ values were comparable in both intact and SAD rats after furosemide. Figure 4 summarizes the individual fitted curves for SAD rats and shows the average model for each condition. The $P_{LL}$ values calculated from the average models were similar to the mean values presented in Table 2.

DISCUSSION

The major finding of this study is that the conscious SAD rat, having high spontaneous AP variability, may represent a convenient model for studying the characteristics of RBF autoregulation. The frequent occurrence of large pressor and depressor episodes permitted the study of RBF responses over a wide range of AP fluctuations. After AP-RBF relationships were modeled with a sigmoidal equation, an autoregulatory plateau and $P_{LL}$ were identifiable. Under control condi-
tions, \( P_{LL} \) was found to be \( \sim 90 \) mmHg. After inhibition of the TGF mechanism with furosemide, \( P_{LL} \) was shifted to \( \sim 100 \) mmHg.

In baroreceptor-intact rats, AP fluctuations occurred mainly in the RBF autoregulatory range, which precluded the determination of pressure limits of RBF autoregulation. After SAD, large spontaneous dips in AP were frequently associated with decreases in RBF, suggesting that autoregulatory mechanisms were unable to maintain RBF during these depressor episodes. Classically, in studies using artificially induced AP reductions, \( P_{LL} \) is determined from autoregulatory curves exhibiting a plateau and a subautoregulatory zone (2, 4, 17, 18, 21, 22, 24). From SAD rat raw data, even after being resampled at 1 Hz, the drawing of autoregulation curves was not obvious, because of the large spontaneous variability of RBF. Averaging RBF data over 1-s periods eliminated most of the respiratory fluctuations and compliance effects, which predominate at frequencies above 1 Hz (20). At lower frequencies, sources of RBF variability include a prominent oscillation of \( \sim 0.25 \)-Hz frequency related either to vasomotion or to the resonance of myogenic responses (7) and a slower oscillation, centered around 0.05 Hz, that probably derives from instability in the TGF loop (12). It is likely that the averaging procedure within 2.5-mmHg intervals effectively limited the influence of such RBF fluctuations without affecting the global trends of the AP-RBF relationships, as similar autoregulatory curves were obtained before and after data reduction in AP intervals (Fig. 3, B and C). The 2.5-mmHg range for AP intervals was chosen to favor the presence of several data pairs in each AP interval, especially in the lowest and highest AP ranges. In addition, this procedure of data reduction offered the possibility of easily obtaining mean RBF autoregulatory curves in a given group of rats (Fig. 1). From RBF autoregulatory curves, several methods have been proposed for the determination of \( P_{LL} \). It is frequently calculated as the intersection of the two straight lines corresponding to the autoregulatory plateau and to the subautoregulatory zone after the data points were fitted either by eye (21) or by progressive linear regression (17, 18). Recently, a new method of determination has been proposed (22). After the AP-RBF data pairs are subjected to nonlinear regression analysis using a sigmoid equation, \( P_{LL} \) is defined as the AP where the third derivative of the fitted curve is null, which mathematically corresponds to the shoulder in a sigmoidal curve. When the averaged AP-RBF data pairs corresponding to the SAD group in control conditions were submitted to nonlinear regression analysis using the three-parameter sigmoid equation, an excellent coefficient of determination was obtained (0.971 with 28 AP intervals). When the same data were submitted to nonlinear regression analysis using the four-parameter Weibull equation, although the coefficient of determination was only slightly improved, the fitted curve was found to more accurately describe the region at the break-off point of the autoregulatory curve (see Fig. 1). Because similar observations were made from fittings of RBF autoregulation curves obtained in SAD or intact rats after furosemide, the Weibull equation was preferred to the three-parameter sigmoid equation. After modeling, \( P_{LL} \) was calculated as the AP where the fitted RBF was equal to 95% of the plateau. This value calculated from the autoregulatory curve obtained in the group of SAD rats under control conditions (89 mmHg) was intermediate to \( P_{LL} \) calculated by the third derivative method (86 mmHg) or estimated by eye (92 mmHg). Similar observations were made in both SAD and intact rats after furosemide treatment. Because the four-parameter Weibull equation was not able to fit all individual experimental data obtained in SAD rats under control conditions, we applied the model to the 1-Hz data sets. A plateau and \( P_{LL} \) were measurable in all SAD rats, whatever the experimental condition, and in five of six intact rats after furosemide.

The estimated \( P_{LL} \) value in conscious SAD rats under control conditions (93 ± 6 mmHg) agrees well with previous observations made in conscious intact normotensive rats using artificially induced stepwise reductions of AP (90 mmHg in Long-Evans rats (4), 88 ± 2 mmHg in Wistar rats (24), and 98 ± 3 mmHg in Sprague-Dawley rats (2)). Such a similarity strongly suggests that classic methods, based on the observation of steady-state effects, and the present one, based on the observation of dynamic responses, explore the same phenomena. This would imply that the largest depressor episodes in SAD rats induced a maximal activation (saturation) of autoregulatory mechanisms. We have previously shown that spontaneous AP fluctuations in SAD rats are able to induce powerful renal autoregulatory responses. Furthermore, as could be assessed from transfer-function analysis between AP and RBF, the upper frequency limit of AP lability (0.15 Hz) coincides with that of renal autoregulatory responses (20).

As previously reported in this model of the conscious SAD rat with renal denervation, the RBF was found to be well maintained during marked pressor episodes (20). Therefore, a higher pressure limit of RBF autoregulation was never detected. However, the pressor episodes allowed us to extend the range of AP describing the autoregulatory plateau and thus to improve its
determination and that of \( P_{LL} \). Whether a higher pressure limit of RBF would be detectable in hypertensive SAD rats remains to be determined.

Acute administration of furosemide decreased both RBF and AP in SAD rats, whereas it decreased RBF and increased AP in intact rats. These observations confirm that sinoaortic baroreceptors are essential for maintaining AP after furosemide administration (15), probably through a mechanism involving sympathetic activation (19). The downward shift of the RBF autoregulatory plateau observed in SAD rats after furosemide points to tonic renal vasoconstriction. In vitro experiments in renal artery segments ruled out a possible direct vasoconstrictor effect of furosemide (14). Because in our experiments the kidney was denervated, a contribution of sympathetic nerves to renal vasoconstriction can be excluded. Moreover, as there was no indication of sympathoexcitation in SAD rats after furosemide (no significant change in heart rate, data not shown), the participation of increased levels of plasma catecholamines is unlikely, although it cannot be completely ruled out, especially because of denervation supersensitivity to \( \alpha \)-adrenoceptor stimulation. Furosemide is known to increase renin secretion (5), and the renal vasoconstrictor action of furosemide in conscious rats has been shown to depend on the stimulation of angiotensin II receptors (14). Therefore, activation of the renin-angiotensin system seems the most likely explanation for the renal vasoconstriction observed in SAD rats after furosemide.

In addition to the downward shift of the RBF autoregulatory plateau, \( P_{LL} \) was shifted rightward by \(-10\) mmHg. A similar value for \( P_{LL} \) was revealed in intact rats after furosemide, suggesting that the SAD procedure did not alter the effect of furosemide on RBF autoregulation. An impairment of RBF autoregulation after furosemide has been previously reported in conscious dogs by using stepwise AP reductions from spontaneous AP (16, 25, 26). However, in these experiments, a new value for \( P_{LL} \) could not be estimated, because an autoregulatory plateau could not be determined. In the SAD rats in the present study, the rightward shift of \( P_{LL} \) after furosemide did not parallel the mean change in AP and therefore cannot be regarded as a classic pressure-resetting phenomenon. Rather, this observation suggests that the TGF mech-

### Table 2. Characteristics of RBF autoregulation curves obtained from nonlinear modeling of individual sets of 3,600 data pairs

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<td></td>
<td>Control</td>
<td>Furosemide</td>
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<td></td>
<td>( r^2 )</td>
<td></td>
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<tr>
<td>Plateau, ml/min</td>
<td>11.5(1)</td>
<td>8.9 ± 1.0(5)</td>
</tr>
<tr>
<td>( P_{LL} ), mmHg</td>
<td>95(1)</td>
<td>104 ± 1(5)</td>
</tr>
<tr>
<td>Percentage of AP</td>
<td>11(1)</td>
<td>5 ± 2(5)</td>
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<td>values below ( P_{LL} )</td>
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Values are means ± SE, where nos. in parentheses indicate the no. of intact rats in which the plateau and \( P_{LL} \) could be estimated. \( n \), No. of rats; \( r^2 \), coefficient of determination (observed vs. predicted values); \( P_{LL} \), lower pressure limit of RBF autoregulation. *P < 0.05 vs. control. †P < 0.05 vs. intact rats.

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Fig. 3. Original 1-Hz time series of AP and RBF (A) and corresponding AP-RBF relationships obtained before (B) and after (C) data reduction in 2.5-mmHg AP intervals in 1 conscious SAD rat before (left) and after (right) furosemide administration. Only AP intervals containing at least 10 data pairs were considered. Solid lines in the scatterplots show the fitted model, and horizontal and vertical dotted lines indicate estimates of the plateau and \( P_{LL} \), respectively. Note the exaggerated AP variability and the occurrence of simultaneous AP and RBF decreases, especially after furosemide.

Fig. 4. Individual fitted curves (thin lines) obtained by applying the 4-parameter Weibull model to 3,600 AP-RBF data pairs in SAD rats before (A) and after (B) furosemide administration. Individual estimates of parameters were averaged to plot the average model (thick lines).
anism contributes to autoregulatory efficiency and co-operates with the myogenic mechanism in setting $P_{LL}$. Such an interaction between the TGF and myogenic mechanisms has been demonstrated in the in vitro blood-perfused juxtamedullary nephron preparation in response to step increases in renal perfusion pressure (23). One possible confounding factor in the present study is the activation of the renin-angiotensin system, which most probably occurred after furosemide administration, as discussed above. It has been reported in anesthetized rats that, after prolonged AP reduction, $P_{LL}$ was shifted toward lower AP values, an effect no longer observed after blockade of the renin-angiotensin system (6). Furthermore, clamping angiotensin II at a low level also induced a leftward shift of $P_{LL}$ (21). The authors suggested that potentiation of the TGF mechanism by angiotensin II might be responsible for the resetting of $P_{LL}$. Our observation of a rightward shift of $P_{LL}$ in furosemide-treated SAD rats supports the view that the integrity of the TGF mechanism is required for angiotensin II to shift $P_{LL}$ leftward. A quantitative evaluation of the role played by angiotensin II in $P_{LL}$ resetting after furosemide would require blockade of the renin-angiotensin system and restoration of RBF by the infusion of angiotensin II. Whatever its mechanism, the furosemide-induced rightward shift of $P_{LL}$ together with the mean decrease in AP, contributed to increase the percentage of AP values below $P_{LL}$, which in turn contributed to increase the overall RBF variability in SAD rats.

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