The step response: a method to characterize mechanisms of renal blood flow autoregulation

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Wronski, T., E. Seeliger, P. B. Persson, C. Forner, C. Fichtner, J. Scheller, and B. Flemming. The step response: a method to characterize mechanisms of renal blood flow autoregulation. Am J Physiol Renal Physiol 285:F758–F764, 2003. First published July 8, 2003; 10.1152/ajprenal.00420.2002.—Response of renal vasculature to changes in renal perfusion pressure (RPP) involves mechanisms with different frequency characteristics. Autoregulation of renal blood flow (RBF) is mediated by the rapid myogenic response, by the slower tubuloglomerular feedback (TGF) mechanism, and, possibly, by an even slower third mechanism. To evaluate the individual contribution of these mechanisms to RBF autoregulation, we analyzed the response of RBF to a step increase in RPP. In anesthetized rats, the suprarenal aorta was occluded for 30 s, and then the occlusion was released to induce a step increase in RPP. Three dampened oscillations were observed; their oscillation periods ranged from 9.5 to 13 s, from 34.2 to 38.6 s, and from 100.5 to 132.2 s, respectively. The two faster oscillations correspond with previously reported data on the myogenic mechanism and the TGF. In accordance, after furosemide, the amplitude of the intermediate oscillation was significantly reduced. Inhibition of nitric oxide synthesis by N\textsuperscript{-}nitro-L-arginine methyl ester significantly increased the amplitude of the intermediate oscillation was significantly reduced. Inhibition of nitric oxide synthesis by N\textsuperscript{-}nitro-L-arginine methyl ester

Various studies are aimed at determining the pressure range and the efficiency of renal blood flow (RBF) autoregulation under various conditions (3, 7, 25). Typically, in these studies, renal perfusion pressure (RPP) is changed according to a staircase-shaped or a ramp-shaped function to obtain pressure-flow relationships (7, 8, 22). Although this experimental approach allows us to determine the autoregulatory pressure range and to compare the autoregulatory efficiency under different conditions, it does not allow us to determine the individual contribution of the underlying mechanisms to the overall response.

Our understanding of the relative importance of the myogenic response and the tubuloglomerular feedback (TGF) mechanism stems mainly from studies in which vascular diameters of isolated vessels, isolated nephrons, or hydronephrotic kidneys were measured (1, 26). In these studies, step changes in perfusion pressure were used as a stimulus, and the time constant of the vascular response was used to assess the contribution of the mechanisms. This was also done in preparations, which lack the TGF [hydronephrotic kidney, isolated nephron without distal tubule (4)].

Other experimental approaches, which were applied in whole animal preparations, employed simultaneous recordings of spontaneous variations in arterial pressure and RBF for several hours (2, 5, 6, 15) or broadband forcings (9), followed by the calculation of transfer functions. In these studies, extreme values of the gain of transfer function were observed at distinct frequencies, i.e., the myogenic response was found to operate at frequencies of 0.1 to 0.2 Hz and the TGF at frequencies of 0.03 to 0.05 Hz. This approach, however, is very time consuming, and, in addition, it requires the long-term stability of the biological system, a condition that is hard to fulfill in vivo.

Here, we present a new approach to study the mechanisms contributing to RBF autoregulation in whole animal preparations. The frequency response of RBF to a step increase of RPP, as induced by release of a 30-s occlusion of the suprarenal aorta, is analyzed. This allows to calculate the contribution of oscillations of different frequencies and thus of different autoregulatory mechanisms. As with the transfer-function method, this procedure does not allow determination of the autoregulatory pressure range and the autoregulatory efficiency.

The mathematical analysis of the step response is based on procedures widely used in technical control theory. The application of control theory to physiological systems has recently been described by Rosengarten et al. (24). If mathematical models of second order are sufficient to represent the experimentally obtained time course, the calculation yields two time constants.

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(frequency and dampening), which allow an unequivocal interpretation (24). If models of higher order are required to represent the time course (i.e., the time course is more complicated), then the number of time constants corresponds with the order of the model. Again, these time constants unequivocally characterize the control system (21), but a direct physiological interpretation of these time constants is impossible.

To validate the new method, we tested whether it is able to reproduce well-known physiological results originally obtained by use of different methodical approaches. To this end, we used established experimental interventions to alter autoregulatory mechanisms. The TGF was abolished by furosemide (4, 12, 20). The myogenic response was enhanced by inhibition of nitric oxide synthesis (10, 13, 16, 25, 27, 28).

MATERIALS AND METHODS

The experiments were performed on 18 male, adult, 3- to 4-mo-old Wistar rats and 10 male Sprague-Dawley rats of the same age (Charles River). Body weight ranged from 300 to 400 g. The experimental procedures used to verify the method [administration of furosemide and Nω-nitro-l-arginine methyl ester (L-NAME), respectively] were part of more extensive protocols performed in separate series of experiments in Wistar and Sprague-Dawley rats, respectively. The rats received a standard rat diet. One day before the preparatory surgery, the animals were deprived of food but allowed free access to tap water. The investigation conformed with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH) (NIH Publication No. 85–23, revised 1996).

Surgical procedures and measurements. The rats were anesthetized by an intraperitoneal injection of urethane solution (2% in water; 6 ml/kg body wt; Sigma, Steinheim, Germany). During the surgery and experiment, the rats were positioned on a heated table (39°C). A tracheal cannula was inserted, and the rats breathed spontaneously. A catheter was advanced into a jugular vein, which was later on used to administer furosemide or L-NAME. Then, the abdominal cavity was opened by a midventral incision. An inflatable cuff was placed around the suprarenal aorta just below the junction of the superior mesenteric artery. A catheter was inserted into the infrarenal aorta and then connected to a pressure transducer and an amplifier (Gould, Valley View, OH). Finally, an ultrasound transit time flow probe (Type 1RB, Transonic Systems, Ithaca, NY) was positioned around the left renal artery by use of a micromanipulator. During the surgery and experiment, the abdominal cavity was continuously flushed with isotonic saline thermostatted at 37°C. RPP (infrarenal aortic pressure) and RBF (left kidney) were recorded continuously. Following analog-to-digital conversion, the data were stored on-line with a sampling rate of 100 Hz.

Experimental protocols. Each experiment started with a stabilization period of 10 min, after which baseline values of RPP and RBF were obtained.

The first set of experiments was performed in Wistar rats (n = 18). After baseline recordings, the aortic occluder cuff was rapidly filled such that RPP and RBF took on values of approximately zero. The occlusion was maintained for 30 s. Then, the occlusion was rapidly released, i.e., a step increase of RPP was induced to obtain the time course of RBF restoration (step response). Then, L-NAME was intravenously administered (Sigma; bolus injection of 1 mg followed by continuous infusion of a 1 mM solution), and, after a period of 15 min, the procedure was repeated.

The second set of experiments was performed in Sprague-Dawley rats (n = 10). After baseline recordings, the step-response procedure was performed as described above. Then, furosemide was intravenously administered (Lasix, Hoechst, Germany; bolus injection of 20 mg in 2 ml), and, after a period of 15 min, the step-response procedure was repeated.

Data analysis. The original data were averaged by sliding average with a window size of 1 s. Relative values of RPP and RBF were obtained by relating the absolute data to the absolute baseline values measured immediately before the start of the protocol. Relative conductance values were calculated by dividing the relative RBF values by the respective relative RPP values.

Analysis of the step response and theoretical background. The rapid release of the aortic occluder results in an immediate increase in RPP and, in turn, in an increase in RBF. Thus, in terms of control theory, the step increase in RPP constitutes the input signal, and RBF the output signal, of the physiological system. According to the literature (12), RBF autoregulation is mediated by the rapid myogenic response, by the slower TGF, and, possibly, by an even slower third mechanism. Thus we presume that RBF autoregulation consists of three parallel subsystems. Each of the subsystems is exposed to the same input signal, and the individual output signals of the subsystems overlap one another, thus a lumped overall output signal is formed.

The original RBF recordings of the step response revealed that the time course of the RBF increase roughly followed an exponential function (y = 1 – e⁻ᵗ), which gradually approaches a steady state at RBF values comparable to preocclusion values. In most cases, visible oscillations were superimposed on this exponential time course. In accordance with technical systems, we thus presumed that each autoregulatory mechanism is best described by a lag element of second order [low-pass element (PT2)] (21). The step response of a single PT2 element is described by

\[ u_a = u_i k_1 [1 - e^{-t \delta} \cos (\omega t + \varphi)] \]  

where \( u_a \) is output signal, \( u_i \) is input signal, \( k_1 \) is amplitude, \( t \) is time, \( \omega \) is angular frequency, \( \delta \) is dampening, and \( \varphi \) is phase angle.

At time 0, this function yields a \( u_i \) value of zero, and, with increasing time, \( u_a \) increases exponentially to approach a steady state. The values of \( \delta \) and \( \omega \) respectively, determine the time course of the transient. If \( \omega > \delta \), then oscillations overlay the exponential time course; if \( \omega < \delta \), then the transient is aperiodic.

In case of three parallel PT2 elements, the overall output signal is the sum of the three individual output signals

\[ u_a = u_i \sum [1 - e^{-t \delta_i} \cos (\omega_0 t + \varphi_i)] \]

where \( i = 1 \ldots 3 \)

\( u_i \) and \( u_a \) are measured variables (in our case, RBF and RPP). The aim of the mathematical procedure is to determine the unknown variables, i.e., phase angles \( \varphi_i \), angular frequencies \( \omega_0 i \), dampenings \( \delta_i \), and amplitudes \( k \), by means of a least-square fitting (Levenberg-Marquardt algorithm).

As three oscillations were presumed to be involved, 12 unknown parameters had to be determined according to equation 2. These 12 parameters were not determined simultaneously but by three subsequent fitting procedures, each yielding the four unknown parameters of one of the involved oscillations, as described by Mautz (19). Figure 1 depicts the three subsequent fitting procedures as applied to a typical
individual step response following the administration of L-NAME.

It is well known that the adequate choice of starting values is pivotal for the success of any iterative fitting procedure. Because the step response presumably contains oscillations of different frequencies, the fitting procedure can yield different results dependent on the starting values chosen for the angular frequency $\omega$ and the dampening $\delta$. Therefore, within each of the three subsequent fitting procedures, the fitting was done 18 times using six different starting values for $\omega$ (0.96, 0.48, 0.24, 0.12, 0.06, and 0.03 s) and, for each $\omega$, three different values of the dampening, i.e., $\delta = \omega/10$ (mild dampening), $\delta = \omega/2$ (strong dampening), and $\delta = 2\omega$ (aperiodic). These ranges of starting values of the frequency were chosen in accordance with the literature on the individual mechanisms of RBF autoregulation, which are reported to have oscillation periods of 10, 30, and 100 s, respectively. Of course, the values for $\omega$ and $\delta$ listed above were only used as starting points to run the respective fitting, yet the starting values, as all other parameters, were free to change during the following iterations. From the 18 runs, the mathematical function that fit best to the original RBF step response was taken as the result of the respective fitting procedure.

In Fig. 1A, the results of the first fitting procedure are depicted alongside the original step response of flow (thin line). In this individual step response, an oscillation with a high frequency dominates. By the first fitting procedure, this dampened oscillation (see inset) was extracted according to equation 1. The four parameters $\varphi_1$, $\omega_1$, $\delta_1$, and $k_1$ were used to reconstruct the time course (bold line). This first approximation yielded $r^2 = 0.830$.

In the second fitting procedure, the remaining difference between the original step response of flow (thin line in Fig. 1A) and the first approximation (bold line in Fig. 1A), i.e., the residuum, was used to extract a second oscillation according to equation 1. This dampened oscillation is depicted in Fig. 1B, inset. The eight parameters $\varphi_2$, $\omega_2$, $\delta_2$, $k_2$, $\varphi_3$, $\omega_3$, $\delta_3$, and $k_3$ (from the second fitting procedure) were used to reconstruct a new time course (bold line in Fig. 1B). The inclusion of the second oscillation improved the approximation considerably ($r^2 = 0.953$). As shown in Fig. 1C, the third fitting procedure, which was done in analogy to the second, yielded a third dampened oscillation (see inset). The time course was reconstructed from the 12 parameters calculated in all three fitting procedures, i.e., the third approximation (bold line) fits very well with the original step response ($r^2 = 0.978$).

As a result of the three subsequent fitting procedures, three separate eigenoscillations are detected, each of which is characterized by certain values of angular frequency, amplitude, dampening, and phase angle. By use of the parameter angular frequency (or oscillation period, respectively), the observed oscillation can be ascribed to one of the known autoregulatory mechanisms, as long as the observed frequency closely corresponds with the frequency or oscillation period values reported for the individual mechanism in the literature. By use of the parameter “square of the individual amplitudes,” the distribution of energy among the autoregulatory mechanisms is characterized.

L-NAME administration resulted in a marked increase of arterial blood pressure. Higher preocclusion RPP values result in a greater height of the RPP step, which would increase the absolute amplitude of RBF oscillations. Therefore, the parameter square of amplitudes was normalized, i.e., it was divided by the individual preocclusion RPP ($p_0$). In addition, the relative contributions of the individual mechanisms were determined. The parameter dampening characterizes the stability of regulation, i.e., the larger the absolute amount of the negative dampening constant, the faster the system approaches a steady state. The parameter phase angle was left unconsidered in this study.

Statistical analysis. Statistical comparisons were made by Kruskall-Wallis test for unpaired data. The probability level was set at $P < 0.05$ to indicate significance. All data are depicted as means ± SE.

RESULTS

Resting values averaged 106 ± 1 mmHg for arterial pressure and 5.9 ± 0.1 ml/min for RBF in Wistar rats and 91 ± 3 mmHg and 6.2 ± 0.8 ml/min, respectively, in Sprague-Dawley rats. To verify the new methodical approach, established procedures to alter autoregulatory mechanisms were used, i.e., the step response was studied with and without L-NAME and with and without furosemide. Administration of L-NAME, as expected, decreased the resting value (before starting the RPP reduction) of RBF from 5.9 ± 0.1 to 2.4 ± 0.3 ml/min and increased arterial pressure from 106 ± 1 to 154 ± 1 mmHg.

In Fig. 2A, the relative changes in RBF as induced by the step increase in RPP following a 30-s occlusion of the suprarenal aorta are depicted. L-NAME amplified the initial RBF oscillation markedly. For quantitative
comparison between control conditions and L-NAME, the square values of the calculated oscillatory amplitudes (normalized by preoclusion RPP, \(p_0\)) are depicted as a function of their respective oscillation periods (in classes with a class width of 4 s). Under control conditions (Fig. 2B), oscillation periods between 5 and 20 s are frequently found. There is a second accumulation at oscillation periods between 20 and 60 s, whereas several small oscillations are widely scattered over the range beyond 60 s. After L-NAME (Fig. 2C), the square amplitudes of fastest oscillations increased by about one order of magnitude.

The three ranges of oscillation periods observed, i.e., 5–20, 20–60, and 60–200 s, were each summarized to three classes of oscillations, named OP1, OP2, and OP3. As shown by Fig. 3A, the average period durations of these three classes of oscillations were unaffected by L-NAME. Figure 3B depicts the respective dampening coefficients. L-NAME did not alter the dampening significantly. In Fig. 3C, the square amplitudes (normalized by \(p_0\)) of the classes are depicted on a logarithmic scale. L-NAME dramatically increased the amplitude of the fastest oscillation (OP1), whereas it did not significantly alter the amplitudes of the slower oscillations (OP2 and OP3). The square value of an oscillatory amplitude is a proportional measurement of the oscillations’ energy content. To evaluate the relative contribution of the individual oscillations to the overall response, the portion of the individual oscillations’ energy content to the overall energy content was calculated. To this end, the square amplitudes of the individual classes of oscillation were related to the sum of square amplitudes of all classes (Fig. 3D).

The relative contribution (or power) of the individual oscillations is markedly changed by L-NAME: the relative power of the fastest oscillation (OP1) increased to reach almost 100%.

Fig. 2. A: step response of relative RBF values during control conditions and during \(N^\omega\)-nitro-L-arginine methyl ester (L-NAME) administration. \(B\) and \(C\): average (means ± SE) of normalized (division by preoclusion pressure; \(p_0\)) square amplitudes of eigenoscillations vs. oscillation period.

Fig. 3. Average values (means ± SE) of oscillation period (OP; \(A\)), absolute amount of dampening (\(B\)), normalized square amplitudes (\(C\)), and relative contribution of the individual eigenoscillation’s energy content to the sum of the energy content (\(D\)) during control conditions and during L-NAME. OP refers to classes of eigenoscillations according to the oscillation periods: OP1: 5–20 s, OP2: 20–60 s, OP3: >60 s. *Significant difference (\(P < 0.05\)).
Figure 4 summarizes the effects of furosemide on the step response. Furosemide did not significantly change the absolute resting values of RBF and arterial pressure. With regard to the RBF step response (Fig. 4A), differences between control conditions and furosemide appear rather small. However, depicting the square amplitudes (normalized by $p_0$) as a function of oscillation period (Fig. 4, B and C) reveals a marked difference. After furosemide, oscillations in the range between 20 and 60 s are diminished or almost completely abolished.

In accordance, the square amplitude (Fig. 5C) and the relative contribution or power (Fig. 5D) of the medium frequency oscillations (OP2) are significantly reduced after furosemide compared with control conditions. The average period durations (Fig. 5A) and the dampening (Fig. 5B) were not significantly altered.

**DISCUSSION**

In this study, we present a new experimental approach to elucidate the individual contribution of autoregulatory mechanisms to the overall response in whole animal preparations. With the use of established interventions to alter autoregulatory mechanisms to verify the new method, we could corroborate earlier results obtained by different methodical approaches.

In Fig. 1A, the step increase in RPP that was used as input signal is depicted alongside the step response of RBF, i.e., the output signal. Although the output signal RBF contains marked oscillation, the input signal RPP does not. Thus possible oscillations in RPP are excluded as a reason behind the observed RBF oscillations. Rather, these RBF oscillations reflect eigenoscillations of intrarenal systems capable of oscillating, which are triggered by the step change of the input signal RPP.
The vasculature plays a significant role in the regulation of blood pressure. It is known that passive elastic systems, particularly, if the mass in the vasculature plays a significant role. However, the finding that the observed oscillations are significantly altered, when autoregulatory mechanisms are altered, strongly supports the assumption that these oscillations are brought about by active regulation. Administration of L-NAME and furosemide, respectively, i.e., experimental interventions that alter individual autoregulatory mechanisms, resulted in significant changes of the amplitude of distinct oscillations, i.e., oscillations within a certain range of period duration.

The average oscillation periods in the classes OP1 and OP2 (see Table 1) correspond quantitatively with the time constants of the myogenic mechanism and the TGF, respectively, as reported in the literature (2, 5, 6, 9, 18). Furthermore, Just et al. (11–14) recently observed that renal vascular resistance increases very slowly following a 60-s incomplete aortic occlusion. The authors postulated that a third mechanism contributes to RBF autoregulation. This mechanism’s time constant was ~100 s. The time constant of the slow oscillations (OP3) observed in our study come close to this time constant. One may speculate that these slow oscillations represent an intrarenal mechanism related to metabolic changes, i.e., the accumulation of metabolites brought about by the ischemia associated with aortic occlusion could impinge on the vasculature.

In this context, it should be noted that the duration and degree of the prior occlusion could influence the RBF step response. Our experimental protocol was based on a study by Pfueger et al. (23), who used a 30-s complete aortic occlusion. Given the usual duration of (warm) kidney ischemia during transplantation surgery, it seems unlikely that a 30-s ischemia would result in cellular damage, which would compromise autoregulatory mechanisms. However, even within this short period, some metabolites would be accumulated that may influence the vascular response to the pressure step. In line with this notion, Just et al. (12) reported that the step response following a 60-s complete occlusion differed from that observed after reducing RPP to 50 mmHg only.

Because the calculated oscillation periods correspond closely to the time constants of the autoregulatory mechanisms as reported in the literature, the assumption seems justified that these oscillations reflect the eigenoscillations of the autoregulatory mechanisms. Comparison of the step response between control conditions and L-NAME reveals that inhibition of nitric oxide (NO) synthesis increased the amplitude of the fastest oscillation (OP1) markedly (Fig. 2). The average oscillation period (9.5 ± 1.2 s during control conditions; 11.5 ± 1.1 s during L-NAME) and the dampening were unchanged.

It is generally accepted that the rapid (stepwise) increase in RPP as exerted by release of the aortic occluder induces a myogenic response, i.e., the vessels contract. Under control conditions, this is counteracted by the dilating effect of NO. Accordingly, the myogenic response becomes more pronounced following inhibition of NO synthesis, and the relative contribution of the myogenic mechanism to the overall reaction increases. This in line with several recent reports on the effect of NO on the myogenic mechanism (10, 13, 16, 17, 25, 27, 28).

Furosemide is well known to abolish TGF, beside its other renal effects. This has been observed, among others, in isolated nephron preparations (4, 20) and recently confirmed by Just et al. (12), who analyzed the time course of renovascular resistance changes following an incomplete 60-s aortic occlusion. Comparison of the step response between control conditions and furosemide (Fig. 2) reveals a marked reduction of oscillatory amplitudes within the range of oscillation periods from 20 to 60 s following furosemide. The relative contribution of these oscillations to the step response is strikingly diminished. We conclude that the mechanism responsible for the eigenoscillations in OP2, i.e., the TGF, is almost completely abolished. This result clearly demonstrates that this new method reliably describes the alteration of autoregulatory mechanisms.

Taken together, the oscillation period allows us to attribute the individual eigenoscillation to one of the autoregulatory mechanisms, i.e., myogenic response, TGF, and a third mechanism possibly related to metabolisms. Furthermore, the distribution of the percent of energy contents among the individual eigenoscillations, as measured by the square values of amplitudes, allows the estimation of the relative contribution of these individual mechanisms to the overall autoregulatory response. The dampening, which would characterize the stability of regulation, was not significantly changed by the experimental interventions used in this study.

The present study did not aim at new physiological insights but at a methodological progress that seems warranted with regard to further physiological analysis of RBF autoregulatory mechanisms, as mentioned previously. Moreover, the simple manipulation to occlude a vessel and to analyze the step response induced by release of the occlusion in terms of eigenoscillations should easily be applicable to other vascular beds as well.

Table 1. OPs of eigenoscillations during control conditions, during L-NAME administration, and during furosemide administration

<table>
<thead>
<tr>
<th></th>
<th>OP1, s</th>
<th>OP2, s</th>
<th>OP3, s</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.5 ± 1.2</td>
<td>35.1 ± 1.3</td>
<td>116.6 ± 6.3</td>
<td>0.978 ± 0.004</td>
</tr>
<tr>
<td>L-NAME</td>
<td>11.5 ± 1.1</td>
<td>38.6 ± 1.9</td>
<td>122.2 ± 5.6</td>
<td>0.946 ± 0.017</td>
</tr>
<tr>
<td>Control</td>
<td>12.5 ± 1.0</td>
<td>35.6 ± 1.8</td>
<td>117.2 ± 5.5</td>
<td>0.990 ± 0.002</td>
</tr>
<tr>
<td>Furosemide</td>
<td>13.0 ± 1.2</td>
<td>34.2 ± 2.1</td>
<td>100.5 ± 8.0</td>
<td>0.977 ± 0.008</td>
</tr>
</tbody>
</table>

Values are means ± SE. OP refers to classes of eigenoscillations according to the oscillation periods: OP1: 5–20 s; OP2: 20–60 s; OP3: >60 s. R² values are after the third fitting procedure to characterize the goodness of the approximation of the step response (for details, please refer to MATERIALS AND METHODS). L-NAME, Nω-nitro-L-arginine methyl ester.
REFERENCES