Time-Dependent Autoregulation of Renal Blood Flow in Conscious Rats

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Abstract. Response of renal vasculature to changes in renal perfusion pressure (RPP) involves mechanisms with different frequency characteristics. Autoregulation of renal blood flow is mediated by a rapid myogenic response and a slower tubuloglomerular feedback mechanism. In 25 male conscious rats, ramp-shaped changes in RPP were induced to quantify dynamic properties of autoregulation. Decremental RPP ramps immediately followed by incremental ramps were made for four different rates of change, ranging from 0.118 to 1.056 mmHg/s. Renal blood flow and cortical and medullary fluxes were assessed, and the corresponding relative conductance values were calculated continuously. During RPP decrements, conductance increased. With increasing rate of change of RPP decrements, maximum conductance increased from 10% to 80%, as compared with control. This response, which indicates the magnitude of autoregulation, was more pronounced in cortical versus medullary vasculature. Pressure at maximum conductance decreased with increasing rate of change of RPP decrements from 88 to 72 mmHg. During RPP increments, dependence of maximum conductance changes on the rate of change was enhanced (−20 to 110% of control). Thus, a hysteresis-like asymmetry between RPP decrements and increments, a resetting of autoregulation, was observed, which in direction and magnitude depended on the rate of change and duration of RPP changes. In conclusion, renal vascular responses to changes in RPP are highly dependent on the dynamics of the error signal. Furthermore, the method presented allows differentiated stimulation of various static and dynamic components of pressure-flow relationship and, thus, a direct assessment of the magnitudes and operating pressure range of active mechanisms of pressure-flow relationships.

Two mechanisms participate in renal blood flow (RBF) autoregulation: a rapid myogenic response and a slower tubuloglomerular feedback mechanism (TGF). The myogenic response is to some degree an open-loop mechanism, i.e., the local vasoaction will not directly feedback onto the input signal. Conversely, TGF operates in a closed-loop mode. Both elements of autoregulation can be discerned according to their frequency response; the TGF responds with a frequency of 0.02 to 0.06 Hz and has a half-life of roughly 10 to 30 s. The myogenic response is quicker, acting in a range between 0.1 and 0.3 Hz and having a half-life of 1 to 4 s (1–9).

Conventional studies of kidney autoregulation use stepwise changes in renal perfusion pressure (RPP) to construct pressure-flow relationships (PFR) (10). Artificial sine wave and square wave functions in RPP have been used to obtain the frequency response of autoregulation (7). The frequencies of these input signals are discrete, which has the disadvantage that the frequency, amplitude, and mean values of RPP must be varied to obtain a valid characterization of the autoregulatory process. This methodologic shortcoming can be partly circumvented by using an RPP input signal with a broad frequency spectrum. For instance, naturally occurring RPP variations can be studied, or the input signal can be superimposed by stochastic frequencies with different amplitudes (i.e., broad-band forcings) (5,7,9–11). In both cases, the resulting transfer function is valid only for the given mean RPP. Repeated experiments at other mean RPP are necessary for extending the observed pressure range (2,10), or the pressure steps must be maintained constant until a sufficiently large data set is obtained (1).

In addition to the aforementioned approaches to study frequency responses (sine waves, step functions, and broad-band noise), additional established input patterns with specific advantages exist (12,13). Ramp, step, and pulse functions have been used for assessing a variety of physiologic processes, such as endothelial function (14–16), the myogenic response of smooth muscles (17), and isolated vessels (18). Moreover, much more complex systems have been described by these input patterns, e.g., the control of muscle tension (19), the respiratory and cardiovascular response to the baroreflex (20,21), and eye movements (22). The model for vergence eye movement resembles autoregulation of the kidney in that it includes a rapid open-loop control component as well as a slow closed-loop mechanism. In the eye, when the responses to different ramp changes were studied, it became apparent that the slow closed-loop component compensated slow ramp changes. With increasing ramp velocities, the rapid open-loop component gained importance (22). Therefore, we hypothe-

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sized that ramp-shaped RPP changes can differentially stimulate the two components of RBF autoregulation. The primary aim of this study was to analyze the renal pressure-flow relationship using ramp functions of appropriate frequency contents.

In a previous study of ours (23), ramp-like RPP changes of a single velocity were used to describe renal autoregulation. The blood flow response during decremental RPP ramps differed considerably from that during incremental RPP ramp. In other words, a pronounced hysteresis-like asymmetry was found, as also has been described by others (24–26). An additional aim of this study was to examine whether this hysteresis of renal autoregulation depends on the frequency component and length of the stimulus.

Materials and Methods
Input Signals

An adequate stimulus is required for characterizing a system’s response. As mentioned above, the myogenic response and the TGF have different response times; their operating points are approximately 0.04 and 0.2 Hz. Thus, stimuli with appropriate frequency content are required. The spectral density of a pulse function is constant along the frequency, i.e., it is independent of the frequency. The spectral density of a step function declines by 1/angular frequency ω, whereas for a ramp function it diminishes according to 1/ω² (12). This was the rationale for choosing a ramp function to separate the response times of the TGF and myogenic responses.

Most studies of renal autoregulation use RPP staircases. A comparison of different ramp functions with various step and staircase functions is depicted in Figure 1. It is clear that the spectral power (Figure 1D) of the ramps (Figure 1A) reveal a rapid decrease. The decline depends on the ramp steepness. The slower the ramp is, the greater the decrease in spectral amplitude. The ramp velocities shown in Figure 1 correspond to those used in the experiments. The sharp decline in spectral power is of considerable advantage, as it allows a more selective stimulation of the autoregulatory components (i.e., the myogenic response versus the TGF).

The single-step function (Figure 1B) or staircase functions (Figure 1C) are less suited as a stimulus, as can be seen by their power spectra in Figure 1, E and F. Different steps do not lead to major differences in the frequency contents (Figure 1E). The step resembles, more or less, the most rapid ramp function used in this study (Figure 1D). The staircase functions in Figure 1C have similar power spectra as the single-step response (Figure 1, E versus F). However, periodic changes in power that depend on the shape of the steps used occur (Figure 1F). Taken together, the sharp decline in spectral amplitude as seen for ramp functions make this stimulus suited for discriminating the fast and slow component of kidney autoregulation.

Figure 1. Transformation of different artificial input signals (pressure changes; A, B, and C) into the frequency domain (D, E, and F). (A) Ramp-shaped pressure changes with different velocities. (B) Step-shaped pressure changes with different step height. (C) Staircase-shaped pressure changes with different step width and different step height.
The functions in Figure 1 were generated electronically. To avoid aliasing, the signals were low-pass filtered with a corner frequency of 1 Hz and then resampled at 0.1 s. Leakage was alleviated by tapering the signal with a Hanning window.

**Surgical Procedures**

All experiments were performed on 25 male, adult, 3- to 4-mo-old, Wistar rats (Charles River, Sulzfeld, Germany). Body weight ranged from 300 to 400 g. 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 the Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85 to 23, revised 1996).

Surgical procedures and measurements were reported previously in detail (23). Briefly, implantation surgery was performed during general anesthesia (chloralhydrate 4% in saline, 1 ml/100 g body wt intraperitoneally). A catheter connected to a telemetry transmitter (11 PA C40; DSI, St. Paul, MN) was implanted into the infrarenal aorta. An ultrasound transit time flow probe (Type 1RB; Transonic Systems, Ithaca, NY) was positioned around the left renal artery and was fixed with tissue glue and a suture on the iliopsoas muscle. Two 500-μm optical fibers (PF500; Fiberware, Berlin, Germany) were inserted into the renal tissue, one at 2-mm depth (cortical region, [LFC]) and another one at 4-mm depth (outer medullary region [LFM]). Finally, an inflatable cuff was placed around the suprarenal aorta, below the junction of the superior mesenteric artery. The leads and catheters were routed subcutaneously to exit at the nape. After surgery, which took [lt]40 min, the rats were housed under constant room temperature and received a single dose of an antibiotic and an analgesic (1 ml/kg body wt total). After sufficient training for several days, the rats freely positioned themselves into a restraining tube. PFR were determined immediately before the start of the protocols, did not differ among the protocols. Mean control values (n = 25) of local flux data resemble the one obtained in earlier studies in conscious as well as anesthetized rats (23,27). Mean control values of LFC ranged from 93 ± 15 AU to 51 ± 14 AU. Mean control values of LFM ranged from 93 ± 23 AU to 99 ± 37 AU with a biologic zero signal ranging from 38 ± 15 AU to 51 ± 14 AU. Both of these local flux data resemble the one obtained in earlier studies in conscious as well as anesthetized rats (23,27–29). Pressure changes with time were accurate and reproducible: the error bars (SEM) seldom exceeded symbol size (Figure 2D).

**Data Analysis**

The data were averaged by filtering using a finite impulse response low-pass filter with corner frequency of 0.2 Hz to obtain mean values in steps of 1 mmHg in RPP. For clarity of the figures, data at every fourth mmHg were depicted only.

Relative conductances were calculated by dividing the relative flow values (for laser-Doppler signals after subtracting biologic zero values) by the respective relative RPP values. These relative values were obtained by relating the absolute data to the absolute control values measured immediately before the start of the protocols. From each pressure-conductance relationship curve, the maximum of relative conductance and the corresponding pressure value at this maximum were determined. For obtaining a quantitative measurement of hysteresis of blood flow autoregulation, the RBF value measured at a given RPP value during IR was related to the RBF value measured at the same RPP during DR in each animal.

**Statistical Analyses**

Statistical comparisons were made by Kruskal-Wallis test for unpaired data and by Friedman’s test for paired data. The probability level was set at P < 0.05 to indicate significance. All data are depicted as means ± SEM.

**Results**

Absolute control values of RBF, LFC, and LFM, as measured immediately before the start of the protocols, did not differ among the protocols. Mean control values (n = 25) of left kidney RBF ranged from 4.4 ± 0.96 ml/min to 5.5 ± 1.23 ml/min. Mean control values of LFC ranged from 287 ± 55 AU to 322 ± 52 AU, with a biologic zero signal ranging from 95 ± 12 AU to 106 ± 14 AU. Mean control values of LFM ranged from 93 ± 23 AU to 99 ± 37 AU with a biologic zero signal ranging from 38 ± 15 AU to 51 ± 14 AU. Both of these local flux data resemble the one obtained in earlier studies in conscious as well as anesthetized rats (23,27–29). Pressure changes with time were accurate and reproducible: the error bars (SEM) seldom exceeded symbol size (Figure 2D).

For quantitative comparison of the response of RBF with those of local fluxes, relative changes of total renal as well as local conductances are depicted in Figure 2. A through C. Changes of conductances of total renal and local fluxes are

1. DR and IR: RPP was reduced from 120 mmHg (or the control RPP, in case spontaneous RPP was lower than 120 mmHg) to 90 mmHg in 400 s. For the pressure range between 90 and 30 mmHg, another 400 s was required; hence, the rate of change was 0.118 mmHg/s. RPP was restored according to the same time regimen.
2. DR and IR: RPP was reduced and restored with a constant rate of change of 0.238 mmHg/s.
3. DR and IR: RPP was reduced and restored with a constant rate of change of 0.528 mmHg/s.
4. DR and IR: RPP was reduced and restored with a constant rate of change of 1.056 mmHg/s.

Before the first protocol was started, the rats were allowed 30 to 40 min to adjust to their environment. After a first control measurement, the first RPP reduction was performed, then RPP was immediately restored (protocol 1). A 5- to 10-min recovery period was allowed, then a new control measurement was made and the next protocol was started and so forth. In five rats, the order of protocols was inverted. This maneuver did not result in any qualitative differences of the measured parameters.
markedly influenced by the velocity of pressure changes. Increasing velocity of pressure changes is associated with larger increases of conductances. In general, this effect is more pronounced during IR as compared with DR.

In detail, the higher the velocity of DR was set, the higher the maxima of cortical conductance became (see Figures 3A and 4C). Maxima of medullary conductance (Figures 3B and 4C), however, only increased with the second and third veloc-
ity of pressure decrease as compared with the slowest velocity. With the highest velocity, maxima of medullary conductance returned to the level observed during the slowest velocity. Changes of maxima of total renal conductance (Figures 3C and 4C) seem to mirror a combination of the different responses of local conductances.

In contrast to different conductance responses during DR, a uniform response of total renal as well as local conductances

![Graph showing pressure-conductance relationship](https://example.com/graph.png)

**Figure 3.** Pressure-conductance relationship of cortical (A), medullary (B), and total renal (C) vasculature. Relative changes of conductances (means ± SEM; n = 25 rats) during ramp-shaped reductions and restorations of RPP with different velocities (symbols as in Figure 2). Bars indicate that conductances at the respective pressure differ significantly among the symbolized velocities of pressure changes (P < 0.05).
was observed during IR (Figures 3, A through C, and 4D). The faster the pressure was restored, the more the maxima increased.

Notably, the magnitude as well as the direction of these differences depends on velocity. With the lowest velocity, the maximum of total renal and local conductances is by far lower during IR as compared with DR. With a velocity of 0.238 mmHg/s, this difference is almost abolished. With higher velocities, the difference becomes inverse: here, maximum conductances are higher during IR as compared with the preceding DR.

Pressure at maximum conductances ranged between 87 and 63 mmHg during DR (Figure 4A). It is interesting that cortical conductance reached maxima at lower pressures than total renal conductance. Both pressures decreased with increasing velocity of DR (Figure 4C). The pressure at maximum medullary conductance (Figure 4A) did not change significantly with increasing velocity. With IR, pressures at maximum conductances (Figure 4B) ranged between 72 and 62 mmHg, i.e., lower than during DR.

As described above, RBF and conductance responses differed considerably between DR and IR. Furthermore, hysteresis of RBF response reveals a strong dependence on the velocity of pressure changes as depicted in Figure 5. It should be noted, however, that varying the velocity automatically affects the time during which pressure is below a certain value. Therefore, the lower the velocity is set, the longer pressure is below, e.g., 60 mmHg (Figure 2). With different ramp velocities and times of pressure reduction, the quality of hysteresis of RBF response changed (Figure 5).

Discussion

The are several ways to describe autoregulation of RBF (30–33). However, various methods for analyzing autoregulation are prone to substantial interobserver error, as described by Turkstra et al. (34). These authors suggested using the point

![Figure 4](https://example.com/figure4.png)

**Figure 4.** Influence of different velocities of pressure changes (rate of RPP change) during ramp-shaped pressure reduction (pressure decrease, left) and pressure restoration (pressure increase, right) on maximum values of conductances (C and D) and on pressures at time of maximum conductance (A and B). Values are means ± SEM (n = 25 rats) for cortical (LFC), medullary (LFM), and total renal (RBF) vasculature. Characters below indicate that significant changes (<>; P < 0.05) were found among different rates of pressure changes for the symbolized vasculature.
of RPP at which the third derivative of a fitted RPP-RBF curve equals 0 as a characteristic of autoregulation (34).

The autoregulatory behavior of a vascular bed is best characterized by its resistance or by the reciprocal value of resistance, vascular conductance. In rigid tubes, pressure changes leave the relative conductance unaffected at a value of 1. Elastic vessels, in contrast, exhibit a drop in conductance as perfusion pressure decreases. The elastic, i.e., passive, properties of the renal vasculature cannot be assessed satisfactorily under in vivo conditions. Thus, the active component, i.e., autoregulatory behavior, is only unequivocal when the relative conductance is greater than 1 in the face of decreasing RPP.

In the present study, a continuous assessment of RBF autoregulation was made possible by ramp changes in RPP. For characterizing RBF autoregulation, the greatest relative conductance increase was taken at its respective RPP (Figure 4C). For all cases, the peak relative conductance was greater than 1 during DR, thus indicating active vasodilatation for RBF, LFC, and LFM (Figure 4C).

The present study was performed in conscious rats. Remarkably, however, the autoregulatory parameters agree well with a previous study performed in anesthetized rats. Daniels et al. (6) found minimal renal vascular resistance at 87 mmHg, which is very close to maximum conductance of RBF in the present study (88 mmHg, Figure 4A).

**Decremental Pressure Ramp**

The passive response of vessels is determined by its viscoelastic properties and the surrounding tissue. Passive changes in vessel geometry will require a certain amount of time. This also is true for changes in the properties of surrounding tissue induced through tubular filling and renal volume during ramps. Thus, in a pressure range in which the vascular bed can only respond passively, e.g., below 40 mmHg, rapid changes in RPP should elicit a less pronounced decrease in conductance than slower pressure changes (18). As seen in Figure 3, this indeed was the case, in particular for the cortical vascular bed. It therefore seems likely that the passive viscoelastic properties of the vasculature participate in the alteration of PFR induced by rapid RPP changes.

Remarkably, a marked increase was found in myogenic vascular conductance for rapid ramps, as characterized by their maximum value, at lower RPP (Figures 3 and 4, A and C). This cannot be explained by vascular viscoelastic properties. The increased vascular conductance level may rely on shear stress-dependent release of vasoactive substances (14–16) or may depend on a rate-sensitive mechanism of myogenic response of the vascular smooth muscles (17,18,35–37). These stimuli are known to exhibit a greater response to the rate of changes in stimulus than to the absolute value of the stimulus itself (15–18,35–37). Taking these considerations into account, one may
conclude that the output signal depends on the time constants of the involved mechanisms and the ramp velocity. The larger the ratio of the last two is the greater the output signal will be. This relationship has a hyperbolic-like shape; the difference between input and output signals diminishes at lower ramp velocities (Figure 3) (19).

The medullary vascular bed did not reveal the same pronounced increase in conductance as seen in the cortical bed during the rapid decremental ramps (Figure 3). This may mirror the different vascular control mechanisms of the renal medulla and cortex. The latter may rely more on the myogenic response, whereas the medulla is mainly under control of the TGF. Hydronephrotic kidneys can autoregulate RBF only via the myogenic mechanism. In these kidneys, the intersection point of the fractional gain of RBF pressure relation with 1 is at 67 mmHg (1). This value corresponds mathematically with the parameter “pressure at maximum conductance” used in the present study. Despite considerable differences in the protocols, preparation, viscosity, and anesthesia, we remarkably obtained a similar value of LFC (66 mmHg) during DR of the highest velocity (Figure 4A).

The response time of TGF is in the range of the time required for the fastest RPP ramp (10 to 30 s) (3). Thus, TGF cannot fully participate in the response to the rapid ramp velocities. This can explain the behavior of medullary conductance seen in Figures 3B and 4C. The maximum conductance initially increases with the rate of change of stimulus as would be expected for the combined myogenic and TGF response. At higher rates of change (ramp velocities of 0.528 and 1.056 mmHg/s), maximum conductance decreases as a result of lacking TGF response (Figure 4A).

Total renal PFR mirrors the lumped cortical and medullary responses. LFC, however, accounts for the greater proportion of total RBF. Accordingly, a similar behavior in vascular conductance is seen for RBF and LFC (Figure 4, A and C). At the highest ramp velocity, however, the opposite behavior between medullary and cortical vascular conductance attenuates the overshooting increase of total renal vascular conductance.

Incremental Pressure Ramp

The PFR differed between the DR and the IR (Figures 3 through 5). This may be due to the different response mechanisms involved, which may have unlike time constants. Moreover, the response of any system involved may have a different off-time constant. Finally, humoral paracrine and autocrine factors change, thus producing an asymmetric time course.

The enhanced vascular resistance after restoration of RPP may rely on adenosine and angiotensin II (Ang II) formation. The concentration of these renal vasoconstrictors depends on the duration and intensity of the stimulus (38–40). Reduction of RPP elicits renin release (41), which, via Ang II, constricts the renal vasculature. Despite the assumed increased levels of renin during the incremental RPP ramp, autoregulation was maintained (Figures 3 and 4). This agrees with findings in isolated afferent arterioles. In this preparation, Ang II constricts the vessels without compromising their autoregulatory capacity (4,30).

Remarkably, the rapid ramps elicited a different response than the slower ramps. Rapid ramps caused transient overshooting of conductance during RPP increase as compared with RPP decrease. Conversely, during the slower ramps, conductance was not even fully restored (Figures 3 and 4). A diminished RBF after a reduction of RPP to 60 mmHg also was found by Holm et al. (25) in anesthetized rats. Converting enzyme inhibition fully restores posthypotensive RBF, underlining the potential importance of the renin-angiotensin system in this context (24–26).

The rapid ramps may enhance the renal vasoconstrictors adenosine and Ang II to a lesser degree, because the hypotensive episodes are shorter. This would explain the higher baseline values after occlusion. A dilating mechanism, however, is required for understanding the overshooting conductance response as seen for the more rapid ramps. A similar conductance response was observed by Cupples (24) using step functions. One reason for this behavior may be found in nitric oxide (NO). Endothelial NO release depends on two independent components: a steady-state release and a shear stress–dependent NO formation. Thus, the frequency content of the stimulus determines NO release in isolated endothelial cells and isolated arterioles (14,15). In this in vitro preparation, a step function increases NO threefold more than a ramp function of the same amplitude. Thus, the slowest RPP ramp in our study may not have a great effect on shear stress–dependent NO release, in contrast to the rapid RPP ramp. Prostacyclin formation also relies to a greater extent on shear stress changes than the absolute shear stress level (16). Another reason may be found in lower levels of vasoconstrictors and/or their combinations induced by shorter hypotensive episodes. For instance, low levels of Ang II influence myogenic vasoconstriction (42). In addition, adenosine and prostaglandin E2 elicit a biphasic response, eliciting vasodilation at low concentrations and reversing this effect at higher concentrations (43,44). These mechanisms could explain the overshooting conductance during rapid RPP restoration.

Conclusions

Renal autoregulation is usually considered as a stereotype response of renal vascular resistance to changes in RPP. The present findings indicate that the renal vascular responses to changes in RPP are highly dependent on the dynamics of the error signal. Rapid RPP changes can have the opposite effect to sluggish alterations in RPP. Furthermore, the direction of the RPP change can be of larger importance than the absolute RPP level. These results add to the understanding of the importance of BP variability for renal function, as recently reported (45).

The myogenic and TGF mechanisms, because of their different frequency properties, contribute differently to the response to slow and rapid pressure ramps according to their respective frequency contents. Slow pressure changes may elicit a predominant TGF response, whereas rapid pressure changes also involve the myogenic response. Moreover, conductance during incremental pressure ramps is different from
the decremental ramps. The reasons for this hysteresis is still not fully understood.

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References


