Dynamic autoregulation in the in vitro perfused hydronephrotic rat kidney

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Cupple, William A., and Rodger D. Loutzenhiser. Dynamic autoregulation in the in vitro perfused hydronephrotic rat kidney. Am. J. Physiol. 275 (Renal Physiol. 44): F126–F130, 1998.—Renal autoregulation is mediated by tubuloglomerular feedback, operating at 0.03–0.05 Hz, and a faster system, operating at 0.1–0.2 Hz, that has been attributed to exclusion of myogenic vasoconstriction. In this study, we examined dynamic autoregulation in the hydronephrotic rat kidney, which lacks tubuloglomerular feedback but exhibits pressure-induced afferent arteriolar vasoconstriction. Kidneys were harvested under anesthesia from Sprague-Dawley rats and perfused in vitro using defined, colloid-free medium. Renal perfuse flow was assessed during forced pressure fluctuations at mean pressures of 60–140 mmHg. Transfer function analysis revealed passive behavior at 60 mmHg and active, pressure-dependent responses at higher pressures. In all cases, coherence was high (0.89 ± 0.03 between 0.01 and 0.9 Hz). There was a resonance peak in admittance gain at ~0.3 Hz and an associated broad peak in phase angle. Below this frequency, gain declined progressively. The minimum gain achieved at 0.01–0.05 Hz was pressure sensitive, being 1.08 ± 0.02 at 60 mmHg and 0.71 ± 0.04 at 140 mmHg. These findings are consistent with in vivo results and with model-based predictions of the dynamics of myogenic autoregulation, supporting the postulate that the rapid component of autoregulation reflects operation of a myogenic mechanism.

Dynamic autoregulation; myogenic; transfer function; pressure; flow

Autoregulation of renal blood flow is achieved by two dynamic mechanisms. Tubuloglomerular feedback involves a distal sensor coupled to early distal tubular flow rate or fluid composition and a proximal effector limb regulating afferent arteriolar tone. This system exhibits a characteristic signature at 0.03–0.05 Hz and is blocked by loop diuretics (see Ref. 12 for review). The coexistence of a much faster mechanism, operating at 0.1–0.3 Hz, is also revealed by frequency domain analysis. The underlying process responsible for this second dynamic signature of renal autoregulation has not been firmly established. The rapid kinetics of the process and the observation that it is not blocked by loop diuretics have prompted the suggestion that the faster system may reflect the operation of an intrinsic preglomerular myogenic mechanism (12). The evidence to date supporting this postulate is based primarily on exclusion, and direct support of the hypothesis is currently lacking.

The isolated perfused hydronephrotic kidney has been used to study myogenic vasoconstriction at the level of the arteriole (9, 10). In the model, chronic ureteral ligation and tubular atrophy, induced to facilitate direct visualization of the renal microcirculation (24), eliminate the possibility of vasoconstriction mediated by tubuloglomerular feedback. Nevertheless, graded, pressure-induced afferent arteriolar vasoconstriction is elicited in response to stepped increases in renal perfusion pressure (9). The kinetics of this pressure-induced vasoconstriction correspond to that ascribed to the rapid component of renal autoregulation (i.e., <10 s for full activation). The sensitivity of myogenic vasoconstriction in this model to pharmacological interventions corresponds to the effects of such manipulations on renal autoregulation in vivo (reviewed in Ref. 17). In addition, significant alterations in autoregulation seen in vivo in spontaneously hypertensive and Dahl salt-sensitive rats and in diabetic rat models are also apparent in studies of afferent arteriolar myogenic reactivity with this model (9, 10, 26).

In the present study, we applied frequency domain analysis to examine the dynamics of autoregulation in this in vitro model. Our findings are consistent with experimental data acquired during blockade of tubuloglomerular feedback (1, 8, 9) and with model-based predictions of the dynamics of myogenic autoregulation (13) and provide direct evidence supporting the postulate that myogenic vasoconstriction contributes to the rapid component of the dynamic signature of renal autoregulation.

METHODS

All experiments received approval of the University of Calgary Animal Care Committee and were conducted under the guidelines promulgated by the Canadian Council on Animal Care (20). Unilateral hydronephrosis was induced by ligation of the left ureter in male Sprague-Dawley rats (150 g) under halothane-induced anesthesia. Within 6–8 wk, the tubules of the hydronephrotic kidney undergo complete atrophy, eliminating the possibility of tubuloglomerular feedback and allowing direct visualization of the renal microcirculation. At this stage, the rats were anesthetized (methoxyflurane), and the renal artery of the hydronephrotic kidney was cannulated in situ. The kidney was then excised and perfused in vitro. During the in vivo cannulation and throughout the excision process, kidneys were continuously perfused to avoid any disruption of nutritive flow.

The perfusion apparatus employed a single-pass presentation of perfusate to the kidney. Medium was pumped on demand through a heat exchanger to a pressurized reservoir.
supplying the renal artery. Perfusion pressure was monitored at the level of the renal artery and controlled by adjusting the pressure within the perfusion reservoir. Kidneys were perfused with modified Dulbecco's medium containing 1.6 mM Ca\(^{2+}\), 30 mM bicarbonate, 5 mM glucose, 1 mM pyruvate, and 5 mM HEPES. The perfusate was equilibrated with 5% CO\(_2\) and 95% air (P\(_{O2}\) = 150 torr). Temperature and pH were maintained at 37°C and 7.40, respectively. A model T106 Transonic flow meter with extracorporeal flow probe was used to monitor renal perfusate flow. Diameter of an afferent arteriole was measured continuously as previously described (17).

Kidneys were allowed to equilibrate at a perfusion pressure of 80 mmHg for 1 h. Perfusion pressure was then reduced to 60 mmHg. Perfusion pressure and flow were digitized continuously at 3 Hz. Random fluctuations in perfusion pressure were introduced using a solenoid valve in the reservoir exhaust line operated by a foot switch and by manual pressure applied to the outlet of the back-pressure regulator. Average perfusion pressure was increased to 80, 100, 120, and 140 mmHg at 400-s intervals. Original data illustrating the time-course of these experiments is depicted in Fig. 1.

Data segments of 1,024 points (341 s) were subjected to linear trend removal and low-pass filtered with a 1.35 Hz cutoff using the Hann window. Fast Fourier transforms were computed on 256 point segments shaped by the Blackmann window with 50% overlap (1, 19). Coherences and transfer functions (magnitude and phase angle) employed 256 point segments, the Hann window, and 69% overlap (1). Fractional admittance gain was calculated as magnitude divided by conductance. Thus, gain > 1 means that pressure fluctuations are actually amplified into perfusate flow; gain = 1 means that the vasculature behaved as a stiff tube; and gain < 1 means that pressure fluctuations were being attenuated and flow was being stabilized. Under passive conditions, or with no kidney in the circuit, phase angle declined linearly toward zero as frequency declined. This reflects a constant delay that originated in the fine-bore cannula used to measure pressure in the root of the renal artery. To correct for this delay, in each experiment, phase angle at 60 mmHg was fitted by least squares to frequency from 0.01 to 0.75 Hz. This procedure reliably removed the delay, r\(^2\) = 0.86 ± 0.06, but also limits interpretation of phase angle, particularly at frequencies >0.5 Hz. Coherence, which can vary from 0 to 1, is an index of the degree to which two signals correspond. High coherence indicates that the pressure and flow signals are closely and linearly related (12).

Results are presented as means ± SE of original data. Two-tailed t-tests were used to test for differences between two groups. One-way analysis of variance with completion by the Tukey-Kramer method was used to test for differences among multiple groups, and P < 0.05 was considered to indicate a significant difference.

**RESULTS**

In six of seven kidneys, the vasculature behaved passively when perfused at 60 mmHg; in the remaining preparation, it was necessary to reduce perfusion pressure to 40 mmHg to obtain passive dynamics. Below 100 mmHg, vascular responses were variable, whereas between 100 and 140 mmHg, autoregulation of both afferent arteriolar diameter and renal perfusate flow was evident. Arteriolar diameter declined from 14.6 ± 1.7 µm at 100 mmHg to 12.0 ± 1.4 µm at 140 mmHg (P < 0.01). Similarly, conductance declined from 0.124 ± 0.011 ml·min\(^{-1}\)·mmHg\(^{-1}\) at 100 mmHg to 0.119 ± 0.010 ml·min\(^{-1}\)·mmHg\(^{-1}\) at 140 mmHg (P < 0.05).

Comparable input fluctuations were achieved at all pressures. This is illustrated in Fig. 2, which depicts the power spectra of perfusate pressure and flow at mean pressures of 60, 100, and 140 mmHg. Induced fluctuations occurred over a wide range of frequencies and were sufficient in the band from 0.5 to 0.1 Hz to drive autoregulation. Although pressure spectral power declined above 0.4 Hz, it should be noted that this also occurs in conscious (6) and anesthetized rats (1, 7).

Average coherences and transfer functions acquired at pressures of 60 and 140 mmHg are presented in Fig. 3. Coherence was high (0.89 ± 0.03) over a frequency
range of 0.01–0.9 Hz at all perfusion pressures (Fig. 3, top). When perfused at 60 mmHg, the renal vasculature behaved passively, as illustrated by both a monotonic decline in gain to a value approaching unity and by the featureless phase angle. At 140 mmHg, the signature of an active autoregulatory mechanism is evident, shown by the resonance peak in gain at 0.3–0.4 Hz and subsequent decline to significantly less than unity, and by the associated broad peak in phase angle. Values are means ± SE of 7 kidneys.

To examine the relationship between autoregulatory efficiency and mean perfusion pressure, the mean gain observed between 0.01 and 0.05 Hz was plotted against mean perfusion pressure. As illustrated in Fig. 4, the minimal gain achieved declined significantly between 60 and 100 mmHg (P < 0.01). Minimal gain stabilized at higher pressures so that there were no differences among gains at 100, 120, and 140 mmHg. At 60 mmHg, pressure fluctuations were transmitted unattenuated, or even enhanced, into perfusate flow. In contrast, at 80 mmHg and above, there was significant autoregulation of perfusate flow when perfusion pressure was forced.

**DISCUSSION**

The major finding in this study is that the hydronephrotic kidney perfused in vitro displays dynamic autoregulation of perfusate flow. The pressure-flow transfer function of this preparation is largely consistent with transfer functions acquired from intact kidneys in vivo in which tubuloglomerular feedback had been blocked either by acute ureteral occlusion (8) or by combined treatment with furosemide plus losartan (1, 15). Furthermore, the transfer function reported here is consistent with model-based prediction for renal autoregulation mediated only by a myogenic mechanism (13).

Autoregulation by normal kidneys in vivo is highly efficient (16) and involves active constriction and dilatation of the preglomerular microcirculation, in particular, the afferent arteriole (2, 3, 4, 25). It is well known that afferent arterioles of reduced preparations such as the blood-perfused juxtamedullary nephron preparation (2, 3, 4, 18) or the hydronephrotic kidney either in vivo (22, 23, 24) or in vitro (9, 10) exhibit pressure-induced constriction. Significant pressure-induced afferent arteriolar constriction in the present study confirms the presence of autoregulation in these hydronephrotic kidneys. Coherence was uniformly high in these experiments, indicating a close linear relationship between pressure and flow. Above 0.4 Hz, gain was >1 indicating that vascular compliance permitted amplification of pressure fluctuations into flow. Gain became <1 below 0.1 Hz, indicating the presence of an active mechanism that stabilized flow. Since alterations in input other than pressure (e.g., sympathetic activation) are eliminated by use of an isolated kidney, this system clearly reflects the operation of an autoregulatory mechanism. In addition, as shown in Fig. 4, the minimum gain achieved was pressure sensitive. This

![Fig. 3. Renal vascular dynamics at 60 and 140 mmHg mean perfusion pressure. Top: coherence was high across the entire range of frequencies examined. Middle: fractional admittance gain. Bottom: phase angle. At 60 mmHg, the renal vasculature behaved passively, shown by the monotonic decline in gain toward unity and by the featureless phase angle. At 140 mmHg, the signature of an active autoregulatory mechanism is evident, shown by the resonance peak in gain at 0.3–0.4 Hz and subsequent decline to significantly less than unity, and by the associated broad peak in phase angle. Values are means ± SE of 7 kidneys.](http://ajprenal.physiology.org/)

![Fig. 4. Pressure dependency of dynamic autoregulation. Fractional admittance gain achieved between 0.01 and 0.05 Hz is plotted as a function of mean perfusion pressure. In this interval, gain at 60 mmHg was different from gain at all other pressures (P < 0.01), whereas gain at 80 mmHg was different from that 140 mmHg (P < 0.01). Gain did not differ significantly among 100, 120, and 140 mmHg. Values are means ± SE of 7 kidneys.](http://ajprenal.physiology.org/)

also is expected from prior studies showing pressure-sensitive vasoconstriction in steady-state experiments, both in vivo and in vitro (5, 9).

Over the past decade several laboratories have used frequency domain analysis to address issues related to renal autoregulation (e.g., 1, 8, 11, 14, 15, 21, 27). Collectively, these studies have shown that autoregulation is mediated by two active systems that in the rat operate at 0.03–0.05 Hz and at 0.1–0.2 Hz (14). The slower system is undoubtedly tubuloglomerular feedback (1, 8, 11, 15), whereas the faster one has been attributed, largely by exclusion, to a myogenic mechanism (14). Three groups have reported transfer functions acquired in vivo under conditions in which tubuloglomerular feedback was inhibited. Daniels et al. (8) used acute ureteral occlusion and observed that the low frequency rise of phase angle below 0.05 Hz and the local maximum in gain at 0.03–0.05 Hz were abrogated. Ajikobi et al. (1) employed furosemide plus ANG II blockade to inhibit tubuloglomerular feedback in rats and observed the same pattern of response, whereas Just et al. (15) achieved a very similar result in conscious dogs. In all cases, autoregulation was preserved after inhibition of tubuloglomerular feedback and was characterized by a single reduction of gain from ≈1.5 above 0.2 Hz to ≈0.4 below 0.1 Hz. A broad peak in phase angle was present in the same frequency band. In the present study, we observed a similar reduction in gain from ≈1.5 above 0.4 Hz to ≈0.7 below 0.1 Hz (Fig. 4). This change in gain was also associated with a broad peak in phase angle (Fig. 3). Except for the apparently faster operating frequency, the present results are thus consistent with the in vivo data obtained from intact kidneys in which tubuloglomerular feedback was inhibited.

Recently, Holstein-Rathlou and Marsh (13) derived and exercised a model specifically to predict the transfer functions generated by a myogenic mechanism and tubuloglomerular feedback, alone and in combination. The present results are consistent with the output of this model when it was run with only the myogenic mechanism active. In particular, the model predicts the resonance peak in gain and subsequent modest attenuation of input fluctuations and the small, broad peak in phase angle that are present in the present results (Fig. 3). There are two quantitative differences between the model output and our results. First, the model predicts ≈40% attenuation at 0.01–0.05 Hz, whereas the hydropnephrotic kidneys achieved only 31 ± 4% attenuation. Most likely, this small difference arises from the use of colloid-free perfusate with consequently low viscosity. The second difference is that the model predicts slower autoregulation (0.1–0.2 Hz; Ref. 13) than was observed in these experiments (0.3–0.35 Hz). The time constants used in the model were selected to best replicate the authors’ extensive data set, which was acquired under halothane anesthesia. In our hands, there are significant differences in the operating frequency of this mechanism, depending upon the presence or absence (and choice) of anesthetic (=0.25 Hz in conscious rats vs. ≈0.2 Hz under isoflurane anesthesia and ≈0.15 Hz under halothane anesthesia; Refs. 6 and 7). Thus the faster responses seen in the present in vitro model more closely reflect the velocity of the system as observed in conscious animals.

In conclusion, we have demonstrated that the fast component of renal autoregulation mediates autoregulation in the in vitro perfused hydronephrotic rat kidney. Since this model exhibits intact myogenic vasoconstriction and lacks tubuloglomerular feedback, these findings strongly support the postulate that the rapid component of renal autoregulation revealed by frequency domain analysis reflects the operation of the renal myogenic mechanism.

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