Assessment of renal autoregulation

William A. Cupples¹ and Branko Braam²

¹Centre for Biomedical Research, Department of Biology, University of Victoria, Victoria, British Columbia; and ²Departments of Medicine and Physiology, University of Alberta, Edmonton, Alberta, Canada

Cupples WA, Braam B. Assessment of renal autoregulation. Am J Physiol Renal Physiol 292: F1105–F1123, 2007. First published January 16, 2007; doi:10.1152/ajprenal.00194.2006.—The kidney displays highly efficient autoregulation so that under steady-state conditions renal blood flow (RBF) is independent of blood pressure over a wide range of pressure. Autoregulation occurs in the preglomerular microcirculation and is mediated by two, perhaps three, mechanisms. The faster myogenic mechanism and the slower tubuloglomerular feedback contribute both directly and interactively to autoregulation of RBF and of glomerular capillary pressure. Multiple experiments have been used to study autoregulation and can be considered as variants of two basic designs. The first measures RBF after multiple stepwise changes in renal perfusion pressure to assess how a biological condition or experimental maneuver affects the overall pressure-flow relationship. The second uses time-series analysis to better understand the operation of multiple controllers operating in parallel on the same vascular smooth muscle. There are conceptual and experimental limitations to all current experimental designs so that no one design adequately describes autoregulation. In particular, it is clear that the efficiency of autoregulation varies with time and that most current techniques do not adequately address this issue. Also, the time-varying and nonadditive interaction between the myogenic mechanism and tubuloglomerular feedback underscores the difficulty of dissecting their contributions to autoregulation. We consider the modulation of autoregulation by nitric oxide and use it to illustrate the necessity for multiple experimental designs, often applied iteratively.

Why is Autoregulation Important?

RBF is extraordinarily large (2% of body mass receives ~25% of cardiac output) to sustain filtration at the glomerulus. The importance of autoregulation is that RBF is driven by blood pressure, which varies continuously and extensively in rats and other mammals (e.g., 2, 60, 62, 79, 114, 205), including humans (e.g., 54, 126). The fact that RBF is substantially stabilized, as illustrated by the blood pressure and RBF traces shown in Fig. 1, indicates pressure-dependent adjustment of renal vascular conductance. We are all familiar with Ohm’s law: \( \Delta P = Q \times R \) (the pressure drop equals the product of flow and resistance). However, in autoregulation studies, pressure is both the input variable and the one that is manipulated; we feel it is more suitable to express the relationship as \( \dot{Q} = \Delta P \times G \) (flow is the product of driving pressure and conductance) (108, 147).

The importance of autoregulation is variably assigned to a role in regulation of body salt content and fluid balance, or to preservation of glomerular structure. The first perception arises from the understanding that final urine production is the out-

Most if not all vascular beds display active stabilization of their blood flow when blood pressure fluctuates. This process can be demonstrated in an isolated preparation perfused with a defined medium, thus precluding any central neural or hormonal input. It is termed autoregulation because the entire process of sensation, transduction, and actuation occurs within the organ or tissue in question. The kidney displays highly efficient autoregulation so that under steady-state conditions, renal blood flow (RBF) is independent of pressure over a wide range of pressure. In many organs, flow is regulated and autoregulated to satisfy the metabolic needs of the parenchyma. In the kidney, however, the situation is reversed; parenchymal metabolic work is a function of RBF. More specifically, it depends on glomerular filtration rate (GFR) and thus the amount of sodium that is to be reabsorbed. This concordance of RBF and metabolic rate makes putative metabolic mechanisms of autoregulation unlikely. Two different rationales have been proposed for the importance of autoregulation: regulation of body salt content and fluid balance on the one hand and preservation of glomerular structure on the other.

The purpose of this review is to summarize the available knowledge of autoregulation. It is specifically written for the nonexpert because it has been our experience that the implications and limitations of data acquired from different experimental designs often are not fully appreciated even by the nephrology and renal physiology community. We provide a summary of the mechanisms of renal autoregulation and then discuss in some detail the experimental designs used to provide information about these mechanisms. Finally, we consider the role of nitric oxide (NO) in autoregulation and use its elucidation to illustrate the necessity for multiple experimental designs, often applied iteratively.
come of three flow-dependent processes, i.e., filtration, reabsorption, and secretion, all highly regulated (218), and from the recognition that regulation of sodium excretion is a, perhaps the, primary function of the kidney. This formulation recognizes that RBF is the input flow to glomerular filtration and that stabilization of RBF contributes strongly to stabilization of GFR and tubular function. If, as is normally the case, fractional excretion of sodium is <1%, then the organism is clearly conserving salt. If at the same time GFR were allowed to vary with blood pressure, then a blood-pressure spike, as occurs with physical activity, could cause disproportionate salt loss. To the extent that tubuloglomerular feedback (TGF) participates in autoregulation, one can make the argument that TGF matches tubular load to reabsorptive capacity of the distal nephron. As such, there is a good case for the involvement of autoregulation in maintaining sodium and volume balance.

Those who consider that the primary role of autoregulation is to preserve glomerular structure do so based on the recognition that the cardiovascular system is primarily mechanical, and that when a mechanical system consistently breaks in the same place one should suspect a mechanical cause (149). The glomerulus is a high-pressure capillary bed and thus prone to physical injury. Transmission of systemic hypertension to the glomerulus is widely recognized to cause structural damage to glomeruli (4, 11, 50, 60) and hypertension-induced renal disease begins with a characteristic glomerular injury that occurs in the first capillary loop (57, 72, 105). In addition, there is a strong association between progression of hypertensive renal disease and constitutive (105) or relative failure of renal autoregulation (11, 60, 85, 94, 183). Several recent studies of consomic and congenic strains based on the Fawn-hooded hypertensive rat have shown that failure of autoregulation is linked to the Rf-1 quantitative trait locus that was previously shown to predict development of proteinuria and renal failure in these rats (120, 199, 200, 201, 202). These findings make a very strong case for a renal-protective role of autoregulation.

It should be remembered, however, that the structure of autoregulation, with conductance changes occurring primarily in the afferent arteriole, ensures that stabilization of RBF must also stabilize glomerular capillary pressure and vice versa. Readers wishing to consider this issue in more depth are referred to the recent review by Loutzenhiser et al. (123).

How and Where Is RBF Regulated?

Conceptually, one can consider the regulators of RBF in three classes. The first class consists of systemic mechanisms, both neural and hormonal, that transfer information from the rest of the organism to the kidney. These include the endocrine renin-angiotensin system, efferent sympathetic nerve activity, vasopressin, and the family of natriuretic peptides (ANP, BNP, CNP). Although these systems can have profound effects on RBF, their actions are not directionally consistent with autoregulation; nor are they consistent with its autonomous operation. The second class consists of paracrine mechanisms including the intrarenal renin-angiotensin system, NO, endothelin, and eicosanoids that transfer information from one part of the kidney to another. These agonists can profoundly affect RBF (139) but are ruled out as mediators by the same directional argument or by the rapid kinetics of myogenic autoregulation that effectively preclude all but electromechanical coupling of pressure to contraction (121). Blood pressure alone (or more correctly renal perfusion pressure) forms the third class of RBF regulators.

Since all the arterial segments of the preglomerular circulation and the postglomerular efferent arteriole are vasoactive, it was necessary to identify the sites at which regulators of RBF and autoregulation adjust renal vascular conductance. Early studies showed that RBF and GFR are autoregulated largely in parallel, indicating a preglomerular site (56, 171, 174). The distributing arteries of the rat kidney are optimized as conductance vessels to transmit the maximum amount of blood with the minimum energy loss (222), consistent with regulation of conductance in the downstream resistance vessels. Studies using a variety of approaches demonstrated that the afferent arteriole is the primary site at which RBF is regulated and autoregulated. Segmental diameters have been measured in intact kidneys by vascular casting (182), in the hydronephrotic kidney both in vivo (181) and in vitro (73), in the isolated perfused juxtamedullary nephron preparation (23, 24, 71, 83, 165, 184), and in isolated, perfused afferent arterioles (86). In addition, perfusion pressure in the various segments has been measured in vivo (74), and pressure gradients (24) and perfusate flow (184) have been measured in the isolated, perfused juxtamedullary nephron preparation. Collectively, these studies have provided irrefutable evidence that autoregulation occurs primarily in the afferent arteriole and to some extent in the interlobular artery, the next upstream segment (74, 182).

None of these studies has provided evidence consistent with pressure-dependent vasoactivity in the efferent arteriole (71, 73, 181).

At Least Two Mechanisms Mediate Renal Autoregulation

Thus far we have spoken of autoregulation as though it were a mechanism, but autoregulation is in fact a phenomenon that, in the kidney, is mediated by at least two mechanisms. Studies of RBF dynamics and kinetics consistently show a fast myo-
genic mechanism and the slower TGF mechanism. There is also increasing evidence that interactions between the two, combined perhaps with a third very slow mechanism, contribute to the overall effectiveness of autoregulation.

**Myogenic mechanism.** The hydronephrotic kidney, which lacks tubules and thus TGF, shows only one autoregulatory mechanism and its afferent arterioles display graded vasoconstriction when perfusion pressure is elevated in a stepwise fashion (73). The kinetics and dynamics of this system in vitro are consistent with those of the faster autoregulatory system in vivo (38, 121). Because it is independent of tubules and because its operating frequency is independent of perfusate flow, this mechanism is presumed to be myogenic, that is to reside entirely within the vascular smooth muscle. Studies of blood flow dynamics indicate that other vascular beds also show a fast, presumably myogenic, autoregulatory mechanism.

The myogenic mechanism senses a variable related to transmural pressure (presumably wall tension or hoop stress) rather than a variable related to blood flow as it can be demonstrated in isolated afferent arterioles perfused in the absence of flow (86). The signal appears to be transduced through the membrane potential of vascular smooth muscle cells which exhibit graded depolarization when perfusion pressure is increased (69, 70, 122, 176). This depolarization spans the voltage range in which L-type calcium channels are activated (122). Certainly calcium entry is necessary (143) but not sufficient (177) to initiate myogenic constriction, indicating the presence of intracellular regulatory elements.

The key enzymes that govern tone of smooth muscle are myosin light chain kinase and myosin light chain phosphatase for contraction and relaxation, respectively. The details of the function and regulation of these enzymes are beyond the scope of this article and the reader’s attention is drawn to several excellent recent reviews (177–179). What is relevant here is that constrictor and dilator pathways are independently regulated (Fig. 2). Myosin light chain kinase is activated by calcium-calmodulin and phosphorylates myosin light chain, leading to enhanced ATPase activity of the myosin which, in turn, increases actin-myosin cross-bridge cycling. Myosin light chain phosphatase appears to be constitutively active and is regulated by an inhibitory phosphorylation mediated by rho-kinase. Inhibition of rho-kinase paralyzes renal autoregulation (137, 172) and perhaps all vasoactivity (172), suggesting that regulation of myosin light chain phosphatase plays an important role in determination of renal vascular tone (e.g., Refs. 26, 137, 172). The important point is that contraction and relaxation of smooth muscle are both active processes that are mediated and regulated separately, whether activated by perfusion pressure or by agonists.

An immediate implication of the different routes to contraction and relaxation is that their kinetics need not be identical and, indeed, the kinetics of constriction and dilatation in the afferent arteriole differ considerably (121; reviewed in Ref. 123). The delay from the start of a pressure transient to the start of constriction is ~0.3 s, much shorter than had been considered possible (177), whereas the delay to dilatation is ~1 s. The rapid onset of constriction means that the system can respond to systolic pressure. Recently, Loutzenhiser et al. (121) have shown that the system responds to individual pressure spikes of 0.3 s, the shortest duration they were able to generate. Thus steady-state myogenic tone is likely to emerge from the difference in the delays with repeated constrictor responses occurring within the time of a single dilator delay. Figure 3 illustrates this, showing equivalent afferent arteriolar constriction whether peak pressure is delivered as a steady-state or as a 2-Hz pulse train.

**TGF.** There are several detailed reviews on the TGF system (19, 168, 198, 215), so a brief discussion of the system will suffice here. In each nephron the distal tubule loops back and makes intimate contact with the vascular pole of its own glomerulus, in particular with its afferent artery. In the evolutionary sense, this appears to be a robust design feature of glomerular nephrons since it is present in the pronephric kidneys of elasmobranchs (107), the mesonephric kidney of amphibia (150), and the metanephric kidneys of birds and mammals (6, 43). It provides an obvious and unique route for passing information about tubular fluid composition in the distal nephron to the afferent arteriole to regulate GFR. TGF has a sensor ([Cl]− at the macula densa), effector (altered preglomerular conductance), and direction (increased early distal delivery and transport reduces conductance) that are all consistent with autoregulation. It should be noted that there remains some uncertainty as to which specific component of tubular fluid is sensed at the macula densa. A recent study (101) and the accompanying commentary (13) highlight the potential for changes in luminal osmolality to contribute to signaling at the macula densa. Presumably changes in osmolality modulate Cl− signaling, although current understanding is limited. There is strong evidence that the downstream constrictor signal of TGF is adenosine (reviewed in Ref. 68), although ATP has also been implicated (141). Parenthetically, this provides metabolic stabilization to the kidney because reducing RBF inevitably reduces tubular work.

TGF is routinely studied by interrupting proximal tubular flow and perfusing the loop of Henle at varied flows. One measures either nephron GFR or a related variable such as stop-flow pressure (proximal tubular pressure once it has risen...
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proximal flow-dependent changes in TGF are consistent with a than of GFR (190). Both the blood pressure-dependent and the flow that adjusts to changes in volume status (186); it also adjusts to sustained alterations of proximal reabsorption (189, 191) by a mechanism that involves nitric oxide synthase (NOS) at the macula densa (45, 187), but apparently not ANG II (45). Model-based interpretation of such data suggests that TGF may be a less efficient regulator of glomerular capillary pressure than of GFR (190). Both the blood pressure-dependent and the proximal flow-dependent changes in TGF are consistent with a role in autoregulation.

The hydronephrotic kidney displays effective autoregulation both in vivo and in vitro (73, 181). In contrast, kidneys of Fawn-hooded hypertensive rats show strongly impaired myogenic responses (204) and autoregulation (203), despite normal or even enhanced TGF responses (206). These data suggest that TGF is neither necessary nor sufficient for effective autoregulation and that the myogenic mechanism is both necessary and sufficient. However, many reports from a number of laboratories employing a variety of experimental designs have made it abundantly clear that TGF contributes significantly to autoregulation of RBF and GFR. In amphibians (150) and mammals (24, 214, 221), TGF regulates RBF. Many laboratories have shown that TGF accounts for some part of autoregulation in mammals (21, 36, 41, 87, 92, 131, 138, 208, 219). Assessments of RBF dynamics routinely show a TGF signature although it has proven difficult, for technical reasons (see Limitations of Transfer Functions), to determine the contribution of TGF to autoregulation using time-series analysis. Experiments assessing the response of RBF to pressure steps have attempted to quantify the contribution of TGF to autoregulation under various experimental conditions (36, 88, 89, 134). Es-

alternative TGF can be assessed in closed loop mode with small perturbations of proximal tubular flow around control levels and early proximal flow assessed as a surrogate of single-nephron GFR (34, 186). These studies have shown that TGF provides substantial stabilization of early proximal flow that adjusts to changes in volume status (186); it also adjusts to sustained alterations of proximal reabsorption (189, 191) by a mechanism that involves nitric oxide synthase (NOS) at the macula densa (45, 187), but apparently not ANG II (45). Model-based interpretation of such data suggests that TGF may be a less efficient regulator of glomerular capillary pressure than of GFR (190). Both the blood pressure-dependent and the proximal flow-dependent changes in TGF are consistent with a role in autoregulation.

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Putative third mechanism. Two other vascular mechanisms have been observed that are intrinsic to the renal circulation. One is an autonomous oscillator with a natural frequency of ~0.7 Hz. This oscillator is seen occasionally in normotensive rats, more commonly after nonselective inhibition of NOS (e.g., Ref. 210), and routinely in stroke-prone spontaneously hypertensive rats (2) and after intrarenal ETB blockade (173). It does not appear to contribute significantly to autoregulation, and its origin and role, if any, are unclear at the present time.

In several reports Just and colleagues (87–89) have suggested the presence of a third autoregulatory system that is considerably slower than TGF in both dogs and rats. The dynamics of such a mechanism appear to be consistent with the reversed-phase oscillation of RBF induced by very slow sinusoidal forcing of blood pressure (135). To date, little is understood about it mechanistically and one must consider that it...
appears to operate in a frequency range (~0.01 Hz) in which the systemic renin-angiotensin system can be expected to contribute to vasomotor fluctuation (58). In addition, there are several known contributors to renal autoregulation that could contribute to the putative third mechanism. Hypertension-induced reduction of proximal reabsorption operates on the appropriate time scale (33, 34) and is partly ANG II dependent (115). Resetting of autoregulation to operate around a new mean arterial pressure also operates over a similar time scale and is ANG II dependent (36, 75, 180). At this time, it is not clear whether these are separate mechanisms or different manifestations of a common mechanism. The physiological function of such a slow mechanism is at this moment unclear.

Involvement of the renin-angiotensin system. Another important consideration is that the TGF response and renin secretion share the same sensor. When loop flow and salt delivery are low, afferent arteriolar conductance is increased and renin secretion is stimulated (118, 175). ANG II is formed, acts at multiple sites, and is degraded within the kidney (160). This suggests that the relevant actions of ANG II are largely paracrine in nature although its endocrine role must also be considered. Due to the high convective fluxes within the kidney and to the very high metabolic clearance of ANG II by the kidney (160), paracrine effects of ANG II are likely to display rapid on- and off-kinetics. On the other hand, endocrine actions of ANG II can be expected to have a slower off-response than on-response because of the asymmetry in renin kinetics, rapid secretion and accumulation in the extracellular fluid coupled with a half-life on the order of 15–30 min (96). Recent findings indicate that proximal tubular reabsorption and local ANG II concentration can under some conditions operate in an opposite fashion to the systemic renin-angiotensin system: salt loading is accompanied by enhanced proximal tubular reabsorption and is still under strong influence of ANG II (188).

ANG II acts on at least four sites within the TGF circuit. It acts directly as a vasoconstrictor on both afferent and efferent arterioles. Although mesangial cells in vitro are contractile (e.g., Ref. 129), it is unlikely that they play a significant role in autoregulation because they express α-smooth muscle actin only in vitro (49, 51, 97); this isoform is a marker of the contractile phenotype in smooth muscles (67). ANG II is a major regulator of reabsorption from the proximal tubule, thus altering the signal that reaches the macula densa (119). Importantly, it is also a strong modulator of the magnitude of the TGF response; this effect occurs at the afferent arteriole and is apparent in the absence of ANG II-dependent vasoconstriction (17, 155, 156). Thus modulation by ANG II of both myogenic (99) and TGF-mediated (130, 140) autoregulation is at least conceptually independent of its vasoconstrictor effect. The separation of GFR and RBF at low perfusion pressures (GFR is autoregulated to lower pressure than is RBF) results from ANG II-mediated efferent constriction combined with autoregulatory afferent vasodilatation (66, 161).

Interactions in Autoregulation

TGF-mediated vascular interactions between nephrons. In a modeling study, we showed that TGF, acting in individual nephrons, could account for autoregulation of glomerular blood flow, but only over a narrow pressure range of ~30 mmHg (41). This result could be achieved only if a faster, presumably myogenic, mechanism had removed all compliance from the renal arterial circulation, allowing TGF to operate on a constant basal preglomerular conductance. Thus it became clear that TGF, operating in a single nephron, could account for only a portion of the autoregulatory plateau. At that time, it was not appreciated that there is a TGF-mediated, vascular interaction (i.e., cross talk) among nephrons whose afferent arterioles arise from the same interlobular artery (93). This interaction is constructive and therefore has the potential to increase the contribution of TGF to autoregulation (27, 76, 93). The signal propagates rapidly between afferent arterioles and is thus presumed to involve depolarization and electrotonic transmission, as does propagation of KCl-induced constriction in the afferent arteriole (207). For this reason, and because vascular smooth muscle cells, which are electrically continuous with the endothelium (104, 185), exhibit graded, pressure-dependent depolarization, one might expect the radius of interaction to increase with perfusion pressure.

One implication of this interaction is immediately apparent. Even today, it is often assumed that TGF in each nephron operates independently of all other nephrons. Clearly, this assumption is untenable, and we should be thinking of a unit of autoregulation that is larger than an individual nephron and its arterioles (the “nephrovascular unit”). We should consider that the size of this unit probably varies with blood pressure and with the ambient influences of modulators such as ANG II, which can significantly enhance conducted (remote) responses, at least in the mesenteric circulation (64).

In a technical tour de force, Casellas et al. (25) demonstrated the anatomic foundation for such interactions, showing that average interglomerular spacing is similar to length constants measured in the renal microcirculation (207). Although the difficulty of working with the renal microcirculation in intact kidneys has limited exploration of the implications of the TGF-mediated, vascular interaction (164), the study of conduct responses and their implications is an active area of research in more accessible vascular beds (e.g., Refs. 46, 48, 64, 65, 106). Recently, there has been significant progress in an understanding of both the mechanisms of and the consequences for vascular regulation of conduct responses. In particular, there is strong potential for smoothing in both spatial and temporal dimensions (47).

Interaction between myogenic and TGF mechanisms. Understanding the interactions between TGF and the myogenic mechanism is of major importance. Both operate on the afferent arteriole, and the TGF sensor is downstream of all other components of both control mechanisms. Both have been demonstrated to induce vasoconstriction in response to a blood pressure increase and vasodilatation in response to a blood pressure decrease. Schnermann and Briggs (167) showed that the dynamic range of TGF increases with renal perfusion pressure and that autoregulation of glomerular capillary pressure is strongly dependent on flow through the loop of Henle. This finding is strong evidence for an interaction between TGF and the myogenic mechanism. It is transparently obvious from these and other data (e.g., Refs. 131, 184, 208) that the two mechanisms interact and that the interaction is constructive. What is much less obvious is how the constructive interaction occurs. If the two mechanisms operated independently, then one would expect their outputs to collide and thus impair
autoregulation (i.e., a destructive interaction). Feldberg et al. (52) concluded that it is difficult to construct a model in which both negative feedback controllers operate on the same actuator and generate a constructive interaction.

Perhaps the first question is what is the benefit of having two separate autoregulatory mechanisms in the kidney? As shown in a variety of systems, including various motor control tasks, two or more systems operating in parallel with appropriate parameters can provide both high gain and stability (e.g., Refs. 133, 144). There is strong evidence from the motor control literature that multiple control mechanisms with different kinetics and operating together can provide optimal regulation in conditions where the input signal can display a wide range of amplitudes and frequencies (53, 133, 144). In such cases, the faster mechanism may be ballistic, with negative feedback being provided by the slow mechanism, or both may be negative feedback mechanisms. The relative contribution of the two mechanisms to any given input perturbation is variable and depends on the input amplitude and its rate of change.

Along these lines, it has been suggested that the myogenic mechanism is a predicting or ballistic system with negative feedback being provided by the slower TGF mechanism (53). The TGF mechanism is a negative feedback control system that regulates distal delivery, although it is less obvious that the myogenic mechanism acts as a negative feedback system to regulate RBF.

Interacting control systems commonly generate nonadditive behavior. Technically, such processes are considered to be nonlinear and/or nonstationary. A process is linear if the input and output remain proportional within the range of input perturbations that is used. Similarly, a process is stationary if it is stable over time; for a control mechanism, this means that the same input perturbation always induces the same output response. In a series of studies, Chon, Marsh, and Holstein-Rathlou separately and jointly have addressed the interaction using newly developed analytic techniques to extract information from time-series data. They have shown the presence of a high-order, nonlinear interaction between the two systems (28–30, 32, 78) that is particularly apparent in hypertensive rats (220, 227); the implications of this finding with respect to hypertension are not yet clear. While these methods demonstrated the presence of a nonlinear interaction, they have been less successful in providing physical insight into its origin and consequences. In addition, there is a caveat that needs to be considered. To date, all studies of interactions between the two mechanisms have used rats, and it is not clear how far these findings can be generalized. In rats, one routinely sees low coherence between blood pressure and RBF at frequencies <0.03 Hz, consistent with a nonlinear (or nonstationary) interaction between the myogenic and TGF mechanisms. Interestingly, in dogs the reduction of coherence at low frequencies is less pronounced (89, 90, 92), suggesting that the interaction may be different in this species. The Laytons and their colleagues (110–113, 145, 154) have pointed out that the internal dynamics of the TGF response itself can contribute significantly to macroscopically observable complexity.

Recently, Chon and colleagues (157, 224, 225) have taken a different approach. Recognizing that stochastic events or other nonstationary behavior would previously have been characterized as nonlinearities, his laboratory has concentrated on developing techniques to extract information about the time-varying behavior of autoregulation. The resulting time-varying coherence and transfer functions have shown that TGF often contributes intermittently to autoregulation. Not only do these methods identify a dynamic complexity, they also provide physical insight into its origins.

Several proposals have been put forward to explain the function of the TGF-myogenic interaction. One early proposal was that the myogenic mechanism dithered TGF (221; to dither a control system is to add jitter and thus minimize error due to internal stickiness). This can be a useful strategy, although in the present case tubular compliance and transport kinetics act as a low-pass filter to prevent the relatively high-frequency oscillations generated by the myogenic mechanism from reaching the macula densa (112, 162). A second proposal was that TGF, acting in the most distal segment of the afferent arteriole, altered upstream resistance and thus upstream pressure, provoking an ascending myogenic response (132). A test of this idea using a dynamic model suggested that the system requirements were unlikely to be encountered under physiological conditions (52). Current thinking is that TGF modulates the myogenic mechanism so that, for instance, myogenic vasoconstriction is enhanced when TGF is activated (208). Such modulation has been identified in a variety of experimental designs (88, 172, 208). Figure 4 illustrates one such experiment, in which the response of afferent arteriolar diameter to a step increase in perfusion pressure was measured with TGF intact and interrupted. Interrupting TGF not only removed the

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**Fig. 4.** Results obtained in the isolated, blood-perfused juxtamedullary nephron preparation. A pressure step from 100 to 140 mmHg was imposed, and the response of afferent arteriolar diameter (AAD) was tracked until the resulting constriction was complete. TGF-intact (●) and -independent (○) kinetic responses are shown. A: exponential stripping analysis revealed that the TGF-independent (striped horizontal bar) initial response starts at >6 s and ends at ≈13 s. The same analysis revealed a modulation of the initial response (A) because in the TGF-intact (checkered bar) system the early response starts at >6 s and ends at >24 s. B: late response of the TGF-intact system starts at >24 s and plateaus after >68 s when TGF was intact and was completely abrogated when TGF was interrupted. *P < 0.05 vs. TGF intact. From Ref. 208.
late constrictor response but also truncated the early response, consistent with modulation by TGF of the myogenic mechanism. Evidence of modulation has also been extracted from pressure-flow data using sophisticated time-series analysis (32, 157). Interestingly, the modulation appears to involve NO generated at the macula densa (88, 172). Aspects of these interactions are discussed in some detail elsewhere (37).

General Considerations When Renal Autoregulation Is Assessed

To this point, we have seen that mechanistically renal autoregulation is remarkably complex. Consequently, methodological considerations play an important part in the choice and interpretation of experimental designs. For any analysis, be it static or dynamic, considerable processing or reduction of the data occurs to extract important features. In all cases, one should be able to visually examine the original data and predict what the finished analysis will look like.

When assessing autoregulation, one can measure the response to altered perfusion pressure of RBF, GFR, diameters of arteriolar segments, or glomerular capillary pressure. Because pressure-dependent conductance changes occur in the preglomerular circulation, it is generally held that measurement of any one of the four variables produces similar information. That being so, technical issues play a major role in experimen
tal design. The diameters of relevant vascular segments are accessible only in reduced preparations such as the hydronephrotic kidney or the isolated, perfused juxtamedullary nephron preparation and are of course nonlinearly related to conductance and RBF. Similarly, glomerular capillary pressure can be assessed only by micropuncture and only by a limited number of laboratories. The advent of blood flowmeters that were reliable, accurate, and gave essentially instantaneous measurement led to routine choice of RBF as the most robust and rapid measurement. Nevertheless, one should always be aware that there are situations in which GFR or glomerular capillary pressure is the more appropriate variable to measure.

To assess autoregulation, one needs an adequate range of renal perfusion pressure, both within and below the “autoregulatory range” in which RBF is independent of blood pressure. Renal perfusion pressure can be increased above control levels by one or more of several means. Use of an extracorporeal reservoir and direct pump perfusion of the kidney has been productive (e.g., Ref. 174) but has fallen out of favor due to the extensive manipulation required and the recognition that autoregulation is often susceptible to such manipulation. The problem was recognized 50 years ago (55), has been described in a number of important vascular beds including hindlimb, liver, and intestine (55, 109), and may involve activation of a blood component that is cleared by the lungs (55, 172). This susceptibility in no way precludes protocols involving extracorporeal circuits or intra-arterial hardware. It does mean that such studies must be internally controlled and should not be compared quantitatively with studies that use less invasive protocols. This constraint applies to both in vivo studies and to blood-perfused in vitro preparations.

Commonly, bilateral carotid occlusion is used to unload the baroreflexes. Concern has been expressed about the potential effects on autoregulation of changing sympathetic activity within an experiment. In rats, pressor amounts of phenylephrine have little or no effect on RBF (100) or on RBF dynamics (172) while acute renal denervation has little effect on steady-state autoregulation (35) or on RBF dynamics (1, 92, 125). In dogs, infusion of norepinephrine has little effect on steady-state autoregulation (128, 136), although the lower limit of autoregulation may be shifted right during bilateral carotid occlusion (151). Another approach is to mechanically increase total peripheral resistance by partial occlusion of major vascular beds, usually the distal aorta and splanchnic beds. This approach can be used alone or combined with infusion throughout the experiment of a cocktail containing pressor concentrations of phenylephrine, vasopressin, and ANG II (159). While these approaches avoid the problems associated with carotid occlusion, they have their own problems. For example, it is possible to induce hypoxia in the gut with deleterious effects on the organism. Equally, ANG II is known to increase the gain of both autoregulatory mechanisms (99, 156) and to affect the range of blood pressure in which they are effective (36, 75, 180). In Brown Norway rats, although not other strains, pressor amounts of vasopressin impair myogenic autoregulation (211). This is noteworthy because the Brown Norway rat is the source of the rat genome and is often used to construct consomic and congenic strains (e.g., Ref. 120).

Common experimental designs: implications and limitations. Assessment of autoregulation. Pressure ladders. In the classic experiment illustrated in Fig. 5, blood pressure is first raised and RBF allowed to stabilize at a new steady state. Blood pressure is then reduced in steps by progressive tightening of a clamp on the renal artery (in large mammals) or on the suprarenal aorta (in rodents). At each step, RBF is allowed to stabilize at a new steady state and then recorded. The resulting pressure-flow curve defines steady-state autoregulation. This procedure is commonly called a “pressure ramp,” but is referred to here as a “pressure ladder” to differentiate it from a ramp in which pressure is varied continuously. The pressure-flow relationship is interpreted in terms of the efficiency of autoregulation and the lower and upper limits of autoregulation that define the operating range. The range is the pressure region in which RBF is largely independent of renal perfusion pressure and typically spans from ~75 (dogs) or 85 mmHg (rats, but somewhat dependent upon the strain of rat and on experimental conditions) to >160 mmHg. Efficiency is simply the slope (%ΔRBF/%Δpressure), or gain, within the autoregulatory range. This range is only valid when baseline and experimental RBF levels are similar or, more commonly, when the data are normalized to the RBF at some reference pressure (typically 100 mmHg). To the extent that the autoregulatory range is discrete, the lower and upper limits are those pressures beyond which autoregulation fails because maximum/minimum achievable conductance has been reached. In practice, the upper limit is rarely approached unless pump perfusion is used. The derived parameters define 1) the effectiveness of autoregulation within the autoregulatory range and 2) the actual autoregulatory range itself.

Large mammals demonstrate a fairly discrete autoregulatory range, and the lower limit is often assessed as the intersection of two lines describing the data within and below the autoregulatory range. This procedure is not reliable when small mammals are used because the autoregulatory range is less discrete. In addition, rat studies typically have fewer and more widely spaced data points, resulting in considerably less certainty.
about the location of the lower limit. Turkstra et al. (196) derived a formal procedure to determine the lower limit of autoregulation. They fitted their pressure-flow data to a logistic equation. The shoulder of this curve is determined as the point at which the third derivative equals zero and is defined to be the lower limit. Although the data are fitted to one of several equations that could have been chosen, the determination of the lower limit is robust. Pires et al. (153) assessed the pressure-flow relationship in conscious rats that had been subjected to chronic sinoaortic denervation to increase fluctuation of blood pressure and compared the ability of several sigmoidal equations to fit their data. However, they then defined the lower limit as the pressure at which RBF had declined 5% below its value at the plateau, a procedure that lacks theoretical underpinning.

In a series of studies, Bidani, Griffin, and colleagues (2, 10, 11, 12, 59, 60, 61, 62, 65) have demonstrated beyond question that the efficiency of autoregulation assessed using this pressure ladder approach predicts the rate of progression of hypertensive renal disease. Recently, autoregulatory efficiency has been genetically linked to the Rf-1 quantitative trait locus in Fawn-hooded rats that predicts proteinuria and renal failure (120, 199–202). Thus the experiment possesses important predictive power. Nevertheless, three significant limitations are immediately apparent.

First, because it assesses the steady-state response, it neglects the information about mechanism that is provided in the time course of a control system. Some information concerning the mechanisms of autoregulation can be inferred by use of agents known to specifically affect one component of autoregulation, e.g., furosemide, which effectively blocks the TGF mechanism (168, 217). Recently, a note of caution has been introduced by the demonstration that loop diuretics can also inhibit myogenic responsiveness directly (212) and, in general, the selectivity of inhibitors tends to decline with time. Second, it assesses only pressure-induced dilatation. Some laboratories that use in vitro preparations such as the hydronephrotic kidney employ an ascending pressure sequence which assesses only constrictor responses so that the same limitation applies. Third, to be useful the experiment must include pressures that are below the autoregulatory range and thereby within the range in which renin secretion is extremely sensitive to blood pressure (98). This results in ANG II-dependent hysteresis between the pressure-flow curve obtained during pressure reduction and the reciprocal curve obtained during pressure restoration (36, 180).

One variant of the basic design is to hold renal perfusion pressure at a control level of ~100 mmHg; periodically, blood pressure is altered up or down by a variable amount and for a short time, then restored to the holding pressure (shown in Fig. 5). This experiment assesses autoregulatory dilatation for pressures below the holding pressure and autoregulatory constriction for pressures above the holding pressure. Furthermore, it does not truly mitigate the problem of hysteresis (53).

**ASSESSMENT OF AUTOREGULATION: PRESSURE RAMPS.** A somewhat different approach was taken by Flemming et al. (53), who began with the observation that, in frequency terms, a step is a more complex signal than a ramp and has substantially more high-frequency content. Consequently, a ramp, in which blood pressure is changed continuously and smoothly, allows much better isolation of slow and fast inputs. This is relevant because TGF responds preferentially to inputs slower than 0.1 Hz (112, 162). By using ramps that varied over an eightfold range of velocity, this study demonstrated rate-dependent differences in the efficiency of autoregulation. The faster ramps provided rates of pressure change that would be expected to stimulate both TGF and myogenic mechanisms; these ramps provoked stronger autoregulatory responses than did the slower ramps, whose frequencies more closely approximated that of TGF. It is thus a reasonable inference that the myogenic mechanism responds preferentially to faster inputs. Furthermore, it is reasonable to conclude that the relative contribution of the two autoregulatory mechanisms varies with the shape of the input signal; this, of course, contains implications for attempts to partition the contributions of the two mechanisms. Finally, these results show that the down-up hysteresis is present in any experiment, whether steady state or dynamic, in which renal perfusion pressure has been below the critical level for long enough to engage renin secretion (53). Recall that changing ambient ANG II concentration can affect autoregulation.
luation in a number of ways, some of which are predictable (36, 99) and others that are less well understood (18, 188, 197).

**ASSESSMENT OF AUTOREGULATION: KINETICS.** This experiment is designed to extract the kinetics of autoregulation and to partition the contribution of each mechanism to the whole and has been used both in vivo and in vitro. One analyzes the response of RBF to either a single pressure step or to repetitions of the same step. There are several technical and conceptual issues that always need to be considered in this experiment. First, to reliably assess kinetics, one needs a pressure step that is effectively instantaneous. This requirement can be met using properly designed in vitro systems (e.g., Ref. 121), but this is difficult to achieve under in vivo conditions. When a suprarenal aortic clamp is used to adjust renal perfusion pressure, as is usually the case, a large pressure drop across the clamp is required to generate a rapid change in renal perfusion pressure. It is rare that this can be achieved while maintaining renal perfusion pressure within the autoregulatory range. In addition, compliance in the systemic circulation limits the rate of pressure change. Another consideration is that to reliably step between two perfusion pressures, one needs a feedback control circuit to drive the aortic occluder. The requirement for stability limits the gain that can be used and demands a low-pass filter in the control circuit at a frequency below that of heart rate (36). Therefore, most in vivo experiments are probably not adequate to properly characterize rapid events that occur within the first 2–3 s after a pressure change. Second, a high signal-to-noise ratio is required to extract delays and rates of constriction and relaxation from the fitted data. This can be achieved by using large individual steps (e.g., 80 mmHg) (121) or smaller, repeated steps with coherent averaging. The former gives confidence in the signal-to-noise ratio but obscures known and suspected pressure-dependent changes in dynamics (38). We are not aware of in vitro studies that have employed coherent averaging of small steps at multiple pressures.

Analysis of the response of afferent arteriolar diameter (121) or of RBF (36, 87–89, 134) to such a step change in renal perfusion pressure is greatly facilitated by monotonic responses. However, under some conditions the myogenic system in particular shows more complex kinetics that are difficult to interpret. This is much more of an issue when isoflurane (134) rather than either halothane (36) or pentobarbital sodium is used (87, 88, 89). Blood pressure and RBF dynamics assessed under isoflurane anesthesia more closely reflect those of the conscious state, suggesting that the older anesthetics interfere with autoregulation (116). Information about other anesthetic regimes such as ketamine-xylazine mixtures is lacking.

A conceptual issue with this experiment also needs to be considered. Partitioning the contribution of the different autoregulatory mechanisms is valid only if they are additive and independent. That is, if the two mechanisms interact in such a way that either one affects the contribution of the other, then assessing the contribution of either may well be misleading. We have seen above that the myogenic and TGF mechanisms interact in a nonlinear fashion, and that they are not additive. Moreover, as shown initially by Walker et al. (208) and subsequently explored by others (88, 172), TGF modulates the contribution of the myogenic mechanism. Thus interpretation of these experiments, and of equivalent experiments using frequency domain analysis, is by no means straightforward.

**ASSESSMENT OF AUTOREGULATORY DYNAMICS.** Visual inspection of blood pressure and RBF traces reveals both similarities and differences (e.g., Fig. 1). Both blood pressure and RBF show characteristic periodic (oscillating) components at heart rate, at respiratory rate, and at longer time scales that arise largely from sympathetic vasomotor activity. While these and other components are visible in raw data, they can be more concisely and informatively represented in power spectra (or periodograms) which display the variance in a signal as a function of frequency. If a signal varies (oscillates) at a particular rate, then it will have a large variance component (spectral power) at that frequency. Thus prominent features in one representation are also notable in the other. Technically, one can map from one representation to the other so that oscillations in a time series appear as discrete peaks in the corresponding power spectrum. Figure 6 shows the power spectrum of the blood pressure record illustrated in Fig. 1. Periodic events, notably heart rate and respiration, and its harmonics in this mechanically ventilated rat, appear as individual peaks in a power spectrum. In rats most blood pressure spectral power between 0.01 and 1 Hz arises from sympathetic vasomotor traffic (166, 213). The neural contribution to blood pressure variance occurs at somewhat higher frequencies in mice (91), and at lower frequencies in larger mammals. Lower frequency spectral power is dominated by aperiodic fluctuation that often results from individual or intermittent events and from circadian rhythms.

Our interest is in the relationship between pressure and flow, not simply in their individual representations. In particular, we wish to understand the processes that are initiated by fluctuations in blood pressure and alter renal vascular conductance to stabilize RBF and glomerular capillary pressure. Conceptually, these processes can be exposed and characterized as the transfer function that operates on input to generate output. While blood pressure is always the input variable, either RBF or vascular conductance (or resistance) can be considered as the system’s output. In our discipline, pressure-flow transfer func-

![Fig. 6. Power spectrum of the blood pressure (BP) record shown in Fig. 1. The power spectrum shown is the average of spectra of 7 overlapping segments (50% overlap) encompassing the entire pressure record. In this animal, which was treated with atropine and mechanically ventilated, periodic events (respiration and heartbeat) are evident as discreet peaks. Respiration produced the strong peak at 1 Hz and its harmonics at 2 and 3 Hz. Heart rate produced the strong peak at ~3 Hz. In addition, there is considerable power at frequencies <0.6 Hz that is not clearly periodic (see the low-frequency fluctuation of blood pressure in Fig. 1) and typically is generated by sympathetic vasomotor activity.](image-url)
tions are used more commonly because pressure and flow are independent measurements. However, pressure-conductance transfer functions are useful in some circumstances (e.g., Refs. 1, 80, 117). Interpretation of pressure-conductance transfer functions is discussed in some detail in those papers.

Figure 7 illustrates the information needed to assess RBF dynamics. Results from two groups of isoflurane anesthetized rats are plotted; the first (Wistar rats) display effective autoregulation mediated by both mechanisms; the second (Fawn-hooded hypertensive rats) display gravely impaired autoregulation. It is helpful to read these graphs from right to left, that is, from high-frequency (fast) events to low-frequency (slow) events. Figure 7A depicts the blood pressure power spectra resulting from spontaneous fluctuation of blood pressure. The
amount and pattern of pressure fluctuation can vary significantly depending on the experimental model employed, whether the animal is anesthetized, and the type of anesthetic used (1, 40). Spontaneous blood pressure fluctuation is sufficient to drive autoregulation in the kidney and in other vascular beds (e.g., Refs. 1, 3, 40, 116, 152, 169, 223). However, for technical reasons blood pressure is usually forced; that is, one adds fluctuation in a range of frequencies that is wide enough to span the region of interest (78, 95). Such “broadband” forcing ensures that all relevant input frequencies are equally represented and that the relevant control loops are opened. Comparison of the pressure spectra in Figs. 7A and 8A shows the effect of forcing on both the scale and the distribution in frequency of pressure fluctuation. Blood pressure has been successfully forced using a variety of techniques including atrial fibrillation (42, 163), paced breathing (142), and computer-driven devices such as a bellows connected to the distal aorta (80) and a suprarenal aortic clamp (213).

Fig. 8. Effect of the calcium channel blocker nifedipine on RBF dynamics. In this experiment, blood pressure was forced by a computer-driven suprarenal aortic clamp. In 5 Wistar rats, a control record was acquired (RPP = 104 ± 2 mmHg, RBF = 5.6 ± 1.4 ml/min), following which nifedipine was infused intravenously (10 μg/kg plus 15 μg·kg⁻¹·min⁻¹) and caused modest changes in renal hemodynamics (RPP = 98 ± 3 mmHg, RBF = 6.3 ± 1.4 ml/min). Blood pressure power spectra (A) and squared coherences (B) were similar in the 2 periods. Gain reduction by the myogenic mechanism (C) was substantially impaired during nifedipine infusion (P < 0.01) as was the associated phase peak (P < 0.01; D). These data indicate substantial impairment of myogenic autoregulation by a dose of nifedipine that caused only modest blood pressure reduction and renal vasodilatation.
Coherence (technically, squared coherence) is shown in Fig. 7B and is analogous to correlation. Coherence can vary from 0 to 1; a coherence of 1 indicates that fluctuations of RBF can be completely predicted from fluctuations of blood pressure, while a coherence of 0 indicates that blood pressure fluctuations do not predict RBF fluctuations. When coherence between two variables is low, three possible explanations must be considered. First, the two variables may be unrelated: obviously in this case there is no further analysis to be done. Second, low coherence may result when a linear and stationary input-output relationship is contaminated by measurement noise. This does not originate in the analysis but is instead a measurement problem that limits the ability of a transfer function, or any other analysis, to capture the underlying processes. Third, the two variables may be related, but with significant nonlinearities or nonstationarities in the relationship. In this case, caution is required in interpreting the transfer function and the interpretation must involve careful inspection of the original data to ensure that the transfer function has correctly captured the dynamics of the system being tested.

Under conditions of spontaneous pressure fluctuation as in Fig. 7, coherence declines below 0.2 Hz and again below 0.04 Hz; when pressure is forced, as shown in Fig. 8, only the decline below 0.04 Hz remains. Note that in both cases coherence is ≤0.5 at frequencies below ~0.01 Hz. It is generally considered that coherence <0.5 precludes drawing any but the most basic conclusions from the transfer function at the relevant frequencies.

Comparison of the two records in Fig. 7 aids in interpreting these patterns. In the Wistar rats, coherence is high at frequencies faster than 0.1 Hz and declines at slower frequencies. The Fawn-hooded rats, which show a different blood pressure power spectra, display high coherence at low frequencies and lower coherence at frequencies faster than 0.1 Hz. The low coherence above 0.1 Hz in Fawn-hooded rats results from the lack of blood pressure spectral power at these frequencies and consequent low signal-to-noise ratio. The high coherence shown by these rats at frequencies below 0.1 Hz reflects the close relationship between blood pressure and RBF fluctuations that results from the absence of autoregulation. In contrast, the Wistar rats have higher pressure spectral power above 0.1 Hz and consequently higher signal-to-noise ratio, and thus coherence, at these frequencies. The two reductions of coherence in the Wistar rats coincide with the operating frequencies of the myogenic and TGF mechanisms and are interpreted to reflect increased complexity of RBF dynamics that results from the operation of these mechanisms.

Figure 7, C and D, shows the transfer function, admittance gain and phase, respectively. Admittance is the dynamic equivalent to the steady-state variable conductance. As a dynamic response goes to steady state, admittance goes to conductance. As in steady-state experiments, interpretation is complicated by differences in the level of RBF, so admittance gain is typically normalized to conductance calculated over the duration of the data examined. Conductance is used for this purpose rather than the zero frequency point of the admittance spectrum because the latter is the point in the gain spectrum which typically has the lowest coherence, and of which one is least confident. Gain may be presented on a linear scale or on a log scale in decibels to highlight the operation of both mechanisms. A gain of 1 (0 dB) indicates that a pressure fluctuation is transmitted directly into flow; gain >0 dB means that pressure fluctuations are actually amplified into flow fluctuations; gain <0 dB means that flow is stabilized with respect to pressure. In both groups of rats shown in Fig. 7, the slightly positive gain in the band from 1 Hz down to ~0.3 Hz arises from arterial compliance. The Wistar rats show a strong reduction of gain between 0.2 and ~0.1 Hz; they also show a local gain maximum at 0.04–0.05 Hz, both consistent with autoregulation. In contrast, the Fawn-hooded rats show a monotonic decline of gain toward 0 dB, consistent with pressure-passive behavior in which gain is determined only by arterial compliance (38).

The phase spectrum shows the temporal relationship between the pressure and flow signals; a zero phase angle means that pressure and flow fluctuations are simultaneous. Consequently, pressure-passive behavior of the circulation results in a phase angle that is slightly positive and close to zero radians, as seen above 0.3 Hz in the Wistar rats and at all frequencies from 1 to 0.01 Hz in Fawn-hooded rats. (Note, however, that the term pressure-passive says nothing about the underlying vascular tone and implies only that the autoregulatory mechanisms are not actively adjusting admittance in response to transient pressure changes.) The Wistar rats show positive phase peaks at frequencies where gain reduction is maximal (0.1–0.2 and ~0.03 Hz). This pattern of gain reduction associated with a positive phase peak is diagnostic of an autoregulatory mechanism and arises because operation of the mechanism results in truncation of pressure-induced RBF fluctuations in the frequency band within which it operates. In contrast, the Fawn-hooded rats show gain, phase, and coherence spectra that are consistent with pressure-passive RBF dynamics (i.e., no autoregulation).

The mechanism that generates the autoregulatory signature at 0.1–0.2 Hz has been identified in the hydronephrotic kidney in vitro (38), indicating that it is confined to the vasculature and is therefore myogenic. A second phase peak is seen at ~0.03 Hz, associated with the local gain maximum at ~0.04 Hz. This signature is consistent with the dynamics of TGF (77), it can be blocked by furosemide (3, 92), and it is not present in the hydronephrotic kidney (38). Thus it is generally held that this is the autoregulatory signature of TGF.

LIMITATIONS OF TRANSFER FUNCTIONS. Ideally, one could fully characterize the transfer function and thus obtain a quantitative description of autoregulatory mechanisms. However, a transfer function has only one defined input (in this case blood pressure), while in reality there may be multiple inputs affecting organ blood flow. Examples include a baroreflex operating in the organ in question (this is defined because it is pressure related) and vasomotor neural traffic that is unrelated to pressure and is therefore undefined. The signature of a baroreflex is a strongly negative phase at the operating frequencies of the baroreflex and is routinely seen in the splanchnic circulation and rarely in the renal circulation, even though most efferent renal nerve traffic is baroreflex coded. The net effect of neural vasomotor traffic unrelated to blood pressure is to reduce gain at frequencies above 0.3 Hz to ~1 (0 dB) (1, 92, 125); effectively, it reduces compliance (92).

Even under the constraints imposed by methodological limitations, experimental noise, and biological complexity, these techniques based on the fast Fourier transform (FFT) provide insight into how autoregulation is achieved, subject to several
important assumptions and limitations. First, there is an inevitable trade-off between temporal and frequency resolution. Longer arrays give greater bandwidth (they reach lower frequencies) and better frequency resolution. The latter arises from segmenting the original record and performing transfer functions on each segment then averaging these. Of course, averaging reduces the temporal resolution. On the other hand, short arrays, sampled at the same frequency, do not reach low frequencies, and to improve temporal resolution the number of segments that are averaged is typically reduced. This provides increased temporal resolution at the cost of increased noise and decreased frequency resolution. Parametric methods such as autoregressive moving average (ARMA) techniques require considerably shorter data arrays (e.g., Refs. 28, 30, 31). However, these techniques are very sensitive to selection of the correct model order and to date it has proven difficult to generate reliable estimates of model order. Because of the large potential advantages of these techniques, significant effort is being expended to address this limitation (e.g., Ref. 146).

Another approach, pioneered by Chon and colleagues (209, 224, 225, 226, 227), has involved the development of time-varying coherence and transfer functions. These provide time-frequency maps of the variable in question and are particularly suited for the detection and characterization of intermittent events such as the intermittent contribution of TGF to the gain of autoregulation.

Second, the analysis is properly valid only if the input and output signals are linear and stationary. At or near its limits, any physiological process is nonlinear, but a linear approximation is often valid if the perturbation amplitude is limited to a small fraction (e.g., ±15%) of the mean of the input signal. A moment’s thought, or visual inspection of many data traces, indicates that the assumption of stationarity is not really fulfilled. Fortunately, FFT-based methods have proven to be robust and to provide reasonable descriptions of processes under less than perfect application of the starting assumptions (28, 95). Nevertheless, it remains important to calculate coherence between the two signals because coherence will determine the degree of confidence with which one can interpret the transfer function. For instance, there is reduced coherence at low frequencies and it has recently been recognized that TGF contributes intermittently to autoregulation. Because of these constraints it has proven difficult to determine quantitatively the contribution of TGF to autoregulatory efficiency.

Third, a transfer function is valid only for the range of pressure tested. Thus a study in which transfer functions have been acquired at the same or similar mean perfusion pressures does not test “autoregulation” as does the steady-state experiment, with its wide range of renal perfusion pressure. We are aware of only one study in which perfusion pressure was varied systematically and transfer functions acquired at mean pressures ranging from 80 to 140 mmHg (38). That study demonstrated substantial pressure dependency of achieved gain and of the operating frequency of the myogenic mechanism.

Comparison of Steady-State and Dynamic Analyses of Renal Autoregulation

A final point of discussion is whether different experimental designs have led to consistent or differing interpretations of the underlying events. Areas of difference are particularly important because they expose issues that are poorly understood. In general, there is substantial agreement between the conclusions drawn from time- and frequency-domain experiments. Both identify a similar autoregulatory range of blood pressure; both identify the myogenic and TGF mechanisms, and the kinetics and dynamics are internally consistent. However, both kinetics and dynamics have been assessed under a limited set of conditions, and both need to be characterized over a wider range of perfusion pressures. Both techniques identify strong and weak autoregulation in some cases, although in other cases there appear to be some discrepancies. For instance, the failure of autoregulation in the Fawn-hooded rat is readily detected using steady-state experiments (203, 204) and using transfer functions (Fig. 7). Both approaches have identified modulation by inhibition of NOS, indicating that both can detect impairment and enhancement of autoregulation. Recently, it has been claimed that transfer function analysis fails to detect the inhibition of myogenic autoregulation by calcium channel blockers (59). However, other laboratories have demonstrated clear inhibition of the myogenic mechanism, in both dogs (92) and rats (Wang and Cupples, unpublished data shown in Fig. 8), using intravenous infusions of nifedipine that caused minor reductions in blood pressure and modest increases in renal vascular conductance. Nevertheless, this discrepancy is important and requires resolution.

The most important discrepancy, however, lies in the gains achieved in the two experiments. Steady-state experiments often produce infinite gain (RBF is independent of blood pressure), whereas to date no study employing dynamic experiments has reported low-frequency gain approaching this. Instead, they typically report 50–70% attenuation of blood pressure fluctuations by autoregulation, even on a time scale of hours (127). This difference was observed very early in the use of transfer function analysis (127, 163) and has been explicitly investigated (10, 92). These studies have shown that the discrepancy is present in conscious dogs and rats. Whereas the steady-state experiment showed impaired autoregulation in rats with remnant kidneys, FFT-based transfer functions did not differentiate between remnant and normal kidneys (10). Furthermore, Julien’s group (152, 153) has shown that the difference is apparent even in the same data set, strongly suggesting methodological constraints. We speculate that these constraints may relate to nonstationary behavior of the renal vasculature and to undefined (at least in the pressure-flow transfer function) inputs governing RBF. It appears likely that current techniques do not reliably assess low-frequency gain in dynamic experiments, which further emphasizes the importance of developing techniques that can assess RBF dynamics below 0.01 Hz.

Role of NO, or the Need for Multiple Approaches

NO is an important modulator of renal autoregulation (15, 139). A brief description of the evolution of our understanding of this physiological role is given here to highlight the importance of multiple approaches.

Early studies exploring the role of NO in the kidney demonstrated that inhibition of NOS caused profound renal vasoconstriction. Initial reports indicated that NOS inhibition did not affect steady-state autoregulation of RBF (7, 8, 124), although it soon became apparent that the neuronal form of NOS is located in the macula densa and the efferent arteriole
(5). Either nonselective or neuronal-selective NOS inhibition clearly increased the dynamic range of TGF responses (17, 192, 195, 216), acting at the afferent arteriole (84). In addition, flow-induced NO generation was shown to modulate pressure-dependent vasoconstriction of isolated, perfused afferent arterioles (86). Thus the autoregulation results appeared anomalous and due perhaps to the difficulty in detecting positive modulation of the already highly effective autoregulation.

Subsequently, Turkstra et al. (196) demonstrated increased efficiency of autoregulation in the nonclipped kidney of two-kidney, one-clip hypertensive rats, a model in which autoregulatory efficiency tends to be reduced. Using their formal interpretation procedures, they demonstrated a significant shift of the lower limit of autoregulation to reduced arterial pressure (196), a finding that was also reported by Kramp et al. (102, 103). In addition, time-series analysis showed that the myogenic system is augmented during NOS inhibition (88, 213, 210). These effects were accompanied by acute hypertension and a marked rise in filtration fraction (210), suggesting that glomerular capillary pressure and afferent arteriolar pressure could be strongly elevated. Accordingly, several maneuvers have been employed in attempts to control for these changes. Arterial pressure has been forced at the same mean pressure, and with the same power spectrum, both before and after NOS inhibition (210). The responses to NOS inhibition have been compared with those of vasopressin, phenylephrine, and ANG II (88, 172, 210). Whether administered to mimic the pressor or the renal vasoconstrictor response to NOS inhibition, none of these agonists duplicates the effect of NOS inhibition on renal autoregulation. There is also some evidence for renal specificity since nonselective NOS inhibition affects neither blood flow dynamics in the splanchic circulation (39) nor myogenic autoregulation in the hindlimb (88). These results minimize, but do not exclude, the possibility that glomerular hypertension accounts for the augmentation of autoregulation by nonselective NOS inhibition.

For some years, studies of the behavior of the whole kidney were restricted to use of nonselective NOS inhibitors and thus were unable to differentiate consequences of inhibition of endothelial or neuronal NOS inhibition. In contrast, studies of TGF employed local delivery of NOS inhibitors either into the proximal tubular lumen or into the peritubular capillaries and suggested a substantial contribution of neuronal NOS at the macula densa (19, 17, 192, 193, 194). This suggestion has been amply confirmed by use of inhibitors that exhibit selectivity toward neuronal NOS (22, 81, 148). Recent studies employing combined inhibition of TGF and of neuronal NOS have shown augmented autoregulation in the presence of only minor vasoconstriction, making glomerular and afferent arteriolar hypertension unlikely as an explanation of the augmentation (172). These studies have demonstrated a role for TGF in the modulation of autoregulation by NOS inhibition and have shown also that it involves interaction between TGF and the myogenic mechanism (88, 172).

Thus it has required the use of most or all of the experimental designs discussed in this review, often in an iterative fashion, to understand how NO participates in renal autoregulation. Together, they have excluded a number of technical explanations for the results and have elucidated a plausible physiological role (i.e., participation in a negative feedback control circuit) for NO in the modulation of renal autoregulation.

Concluding Remarks

In summary, there are a number of different experimental designs that assess different aspects of renal autoregulation. No single experiment provides information sufficient to characterize autoregulation, and all current designs have significant strengths and limitations. The steady-state experiment remains important because it predicts progression of, and thus captures important elements of the susceptibility to, hypertensive renal disease. Dynamic analysis based on the FFT is informative about the mechanisms of autoregulation and how they are modulated. However, this analysis is not as successful in extracting information about gain at low frequencies (<0.01 Hz), perhaps because it cannot resolve the time-varying contribution of TGF. Careful consideration of the question being asked, of the design to be employed, and interpretation of the results obtained remain as important today as they ever were.

REFERENCES


RENALE AUTOREGULATION

Invited Review

Steinhausen M, Blum M, Fleming JT, Holz FG, Parekh N, Wiegman

Selen G, Persson AEG.

Schondorf R, Stein R, Roberts R, Benoit J, Cupples W.

Schnermann J, Traynor T, Yang T, Arend L, Huang YG, Smart A,

Schnermann J, Briggs JP.

Schenk D, Bishop VS.

Schenkman J, Forssmann WG.

Schmidt J, Craig DA, Wexler AS, Marsh DJ.

Schmidt J, Blum M, Fleming JT, Holz FG, Parekh N, Wiegman

Sanchez-Ferrer CF, Roman RJ, Harder DR.

Sanchez-Holgado P, Forssmann WG.

Salomonson M, Gustafsson F, Andreasen D, Jensen BL, Holstein-

Rathlou NH. Local electric stimulation causes conducted calcium re-

sponse in rat interlobular arteries. Am J Physiol Renal Physiol 283:


Sanchez-Perez JF, Blum M, Fleming JT, Holz FG, Parekh N, Wiegman

Rathlou NH.

Rathlou NH. Autoregulation of afferent arteriolar blood flow in juxtamedullary


Rathlou NH. Local electric stimulation causes conducted calcium re-

sponse in rat interlobular arteries. Am J Physiol Renal Physiol 283:


Rathlou NH. Autoregulation of afferent arteriolar blood flow in juxtamedullary


Rathlou NH. Local electric stimulation causes conducted calcium re-

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