Mysteries of Renal Autoregulation
Edward W. Inscho

Autoregulation is an important renal regulatory mechanism that provides an important protective role in glomerular hemodynamics.1 The phenomenon of autoregulatory behavior has been recognized for many decades, but a clear understanding of the underlying mechanisms involved in mediating resistance adjustments remains to be established. As illustrated in Figure 1, pressure-mediated autoregulatory behavior operates by recognizing a change in renal perfusion pressure, or renal vascular transmural pressure, and initiating induction of autoregulatory resistance changes intended to stabilize renal blood flow and/or glomerular filtration rate. The net result is that renal blood flow and glomerular filtration rate remain relatively stable over a wide range of renal perfusion pressures. Autoregulatory behavior is exquisitely sensitive in healthy normal kidneys but significantly less efficient in pathological settings, such as diabetes mellitus and some forms of hypertension. Complete loss of autoregulatory control results in renal blood flow that behaves more passively, thus increasing variability and rendering flow more directly determined by renal perfusion pressure.1,2

Whole kidney autoregulation reflects regulatory input from the myogenic and tubuloglomerular feedback mechanisms at the very least.3–7 Recently, contributions of a third and possibly a fourth mechanism have been suggested.6,7 Consequently, with continued study, the mechanisms underlying renal autoregulatory resistance adjustments are slowly becoming clearer. In addition to the P1 receptor hypothesis put forth to explain signal transduction for tubuloglomerular feedback-dependent resistance changes, we have explored the hypothesis that pressure-mediated autoregulatory responses are mediated by extracellular ATP and activation of P2X receptors.3,8,9 Conclusive identification of the signaling molecules that mediate autoregulation remains a point of debate, but it appears clear that the release of a purine-based moiety is required for autoregulatory adjustments in afferent arteriolar resistance.3–5,8–10 This review tries to focus on the new and current questions that need resolution regarding the P2 receptor hypothesis and how the tubuloglomerular feedback (TGF) system overlaps with those questions. For a more detailed discussion and review of the mechanisms of TGF and myogenic contributions to autoregulatory behavior, the reader is referred to several recent and excellent reviews that dwell more on the relative contributions of both systems to overall autoregulation.1–7,9,11

Signalizing Mechanisms

It is generally accepted that autoregulatory resistance adjustments are calcium dependent and rely, in part, on calcium influx through voltage-gated L-type calcium channels.4,12,13 P2 receptors produce vasoconstriction by increasing the intracellular calcium concentration in renal vascular smooth muscle cells.8,14 P2Y receptors modulate the intracellular calcium concentration mainly by mechanisms involving calcium mobilization from intracellular stores rather than by stimulating voltage-dependent calcium influx.8 In contrast, P2X receptor activation stimulates calcium influx directly through the receptor protein, which is a ligand-gated channel that passes a nonselective cation current.8,15 Influx of sodium and calcium through this channel increases intracellular calcium concentration directly and causes depolarization, which subsequently activates L-type calcium channels.

Adenosine traditionally signals through modulation of intracellular cAMP levels.16,17 Activation of A1 receptors suppresses cAMP formation, and A2 receptor activation increases cAMP production. Neither of these receptors is reported to stimulate calcium influx events in preglomerular smooth muscle cells, making adenosine poorly suited to the calcium influx–dependent autoregulatory events known to occur. However, adenosine receptors are reported to modulate calcium sensitivity in mouse afferent arteriolar vascular smooth muscles.18 Thus, adenosine-dependent modulation of calcium sensitivity could provide a means to elicit some calcium dependency to adenosine-dependent regulation of afferent arteriolar resistance. Regulation of calcium sensitivity is an important component of regulating vascular and microvascular function, and significantly more research needs to be done to understand its contributions to controlling renal microvascular function in health and disease.

Activation of P2X1 receptors is an essential step in pressure-mediated afferent arteriolar vasoconstriction.8,19 Blockade of P2X1 receptors or deletion of P2X1 receptors in knockout mice eliminates pressure-mediated reductions in the afferent arteriolar diameter.19 Sustained P2X1 receptor–mediated vasoconstriction is blocked during blockade of L-type calcium channels.8 The participation of other intracellular signaling pathways remains a distinct possibility. For example, 20-HETE has been strongly implicated in myogenic responses in many vascular beds and may well be involved in...
myogenic responses of afferent arterioles.\textsuperscript{20–23} Similarly, blockade of 20-HETE inhibits P2X\textsubscript{1} receptor–mediated afferent arteriolar vasoconstriction to approximately the same degree.\textsuperscript{24} Inhibition of 20-HETE also suppresses P2X\textsubscript{1} receptor–mediated calcium signaling responses, suggesting that endogenous 20-HETE plays an integral role in calcium-dependent events initiated by P2X\textsubscript{1} receptor activation.\textsuperscript{25} Indeed, 20-HETE is known to participate in autoregulatory responses and P2X\textsubscript{1} receptor activation and to enhance calcium currents in cerebral vascular smooth muscles, and the data in renal afferent arterioles support the postulate that 20-HETE facilitates calcium-dependent events.\textsuperscript{25,26} Therefore, the specific mechanisms involved in the linkage among pressure-dependent autoregulatory vasoconstriction, P2X\textsubscript{1} receptor activation, and calcium-dependent events is intriguing and needs further clarification.

Rho-kinase is an important candidate enzyme postulated to enhance calcium sensitivity in vascular smooth muscles.\textsuperscript{27,28} Nakamura et al\textsuperscript{29} have shown that inhibition of Rho-kinase activity with Y-27632 significantly blunts myogenic behavior in hydropnephrotic kidneys. Similarly, Rho-kinase inhibition also blunts renal vascular reactivity to important renal vasoconstrictors, such as endothelin or angiotensin.\textsuperscript{29,30} Rho-kinase may also be involved in mechanisms that influence ATP release from cells given an osmotic stimulus.\textsuperscript{31} Reactive oxygen species are also known modulators of vascular and microvascular functions.\textsuperscript{32–34} Acute exposure of afferent arterioles to TGF-\beta reversibly impairs pressure-mediated vasoconstriction, and this impairment was prevented during exposure to Tempol or apocynin.\textsuperscript{33} Degradation of superoxide can reduce renal vascular resistance, whereas increased superoxide levels can lead to renal vasoconstriction.\textsuperscript{33} Thus, the endogenous redox status can have important implications for renal vascular function and autoregulatory capability.\textsuperscript{32,34} Many physiological and pathophysiological conditions involve oxidative signaling mechanisms, and their roles in specific circumstances, especially in conditions leading to autoregulatory impairment, remain poorly understood.

**Mechanisms of Renal Microvascular Impairment of Autoregulatory Behavior**

As shown in the left panel of Figure 2, the general concept that increases in transmural pressure result in autoregulatory reductions in vascular diameter is based on the hemodynamic concept that vessel diameter must become smaller than it was before the increase in transmural pressure to maintain stable downstream pressures and flows. As presented in Figure 1, the kidney uses at least 2 distinct mechanisms to effect autoregulatory resistance adjustments, myogenic and tubuloglomerular feedback, with the possibility that a third or more mechanisms may also contribute. In simple terms, with 2 functionally intact systems for generating autoregulatory vascular wall tension, increased perfusion pressure would result in reductions in the resting diameter to counter the transmural pressure increase (Figure 2). When both systems are deleted, the expected outcome would be a passive pressure-diameter relationship, such that increases in perfusion pressure result in pressure-mediated increases in diameter. Loss of 1 of the 2 systems would likely result in a pressure-induced diameter response somewhere in between. As we have studied autoregulatory behavior in the kidney, it has become apparent that, under conditions of autoregulatory impairment, afferent arteriolar diameter tends to remain static in the face of perfusion pressure changes rather than behaving passively. The right panel of Figure 2 depicts actual data for normal (black symbols) and impaired autoregulation (white symbols), as well as hypothetical data (gray symbols) expected if all of the tension-generating capacity were lost. Pharmacological blockade of P2X\textsubscript{1} receptors changes the autoregulatory response from a pressure-mediated reduction in afferent arteriolar diameter (black symbols) to a flat pressure-diameter relationship (white symbols) instead of the
hypothetical passive relationship. This flat pressure-diameter relationship occurs despite the marked increase in perfusion pressure (100 to 160 mm Hg). Similarly, as shown in the left panel of Figure 3, deletion of P2X1 receptors in knockout mice yields a flat pressure-diameter relationship compared with wild-type littermates (black circles). Removal of the papilla (Figure 3) or treatment with furosemide (data not shown) to eliminate tubuloglomerular feedback influences (ie, removing 1 of the 2 known autoregulatory elements) changed the normal autoregulatory response in wild-type mice to a flat pressure-diameter relationship, whereas it had no effect in mice lacking functional P2X1 receptors. However, as shown in the right panel of Figure 3, removal of all of the tension-generating capacity, by removal of extracellular calcium, resulted in a passive pressure-diameter relationship. Similar flat pressure-diameter relationships have been observed in other experimental settings, such as the general P2 receptor blockade, acute exposure to TGF-β, 2K1C Goldblatt hypertension, and angiotensin II–infused hypertension. Whether retention of a static diameter in the face of increasing arterial pressure in this setting reflects enhanced myogenic influences or uncoupling of mechanosensation remains unclear.

Together, these data suggest that P2X1 receptors are important for autoregulatory vasoconstriction, but they also suggest that other influences may contribute to the generation of autoregulatory tension. The mechanisms responsible for the maintenance of arteriolar diameter in the face of increases in perfusion pressure remain unclear, but some possibilities can be postulated. If it is assumed that arteriolar diameter depends on active tension to counter the hydrostatic influence of arterial pressure, then one must postulate that the increase in perfusion pressure depicted in Figure 2 was indeed countered by an increase in active tension that was not sufficient to reduce the diameter below control. Active tension-generating capacity could be enhanced by increased calcium signaling or calcium sensitivity or by the generation of other intracellular regulators of active tension that still remain to be identified. Arteriolar wall tension may be regulated by a mechanosensor that is uncoupled, thus separating the increase in transmural pressure from compensatory events, such as cytoskeletal rearrangement or the increase in other second messenger accumulation. Indeed, Jernigan and Drummond have shown that epithelial sodium channels may serve as mechanosensors for myogenic vasoconstriction of upstream renal arteries, but this has been contested recently by Wang et al. Certainly, cytoskeletal components, integrins, gap junctions, connexins, or the interaction among many of these elements could be involved. It could indicate that P2X1 receptor blockade has only intervened in 1 of the autoregulatory mechanisms that participate in the overall autoregulatory response. Continued influence of TGF or other as-yet-undefined autoregulatory messenger systems, could combine to retain arteriolar diameter and, thus, confer some degree of autoregulatory buffering of increased arterial pressure, making the renal vascular autoregulatory system more complicated than previously believed. Indeed, a third mechanism has been postulated to be involved in autoregulatory control, and Rho-kinase, purported to increase calcium...
sensitivity, contributes significantly to renal vascular responses to vasoconstrictor signals.6,7,27,29 In preliminary studies, we have found that inhibition of Rho-kinase with Y-27632 results in concentration-dependent inhibition of autoregulatory behavior, and, interestingly, it blocks P2X1, but not P2Y2 receptor–mediated vasoconstriction (unpublished observations).18,44 Given that A1 receptor activation reportedly involves enhanced vascular sensitivity to calcium and the unique effect of Rho-kinase inhibition on P2X1 receptor signaling, it is reasonable to postulate that calcium sensitization plays an important role in autoregulatory control of renal vascular resistance. Certainly, the mechanisms of pressure-induced active tension development by afferent arterioles subjected to autoregulatory stimuli need further investigation.

Mechanisms of ATP Release
The requirement for identifying a source and mechanism for extracellular messenger release is incumbent on any hypothesis relying on extracellular second messengers as mediators of pressure-dependent autoregulatory resistance adjustments. Indeed, ATP is released from many different cell types in response to stretch, osmotic stimuli, or almost any form of membrane perturbation or deformation.10,45–48 For the P2 receptor hypothesis, the sources and mechanism(s) remain to be clearly established. ATP release has been studied in several different cell types and is thought to include vesicular release and release through anion channels such as CFTR, maxi-anion channels, and voltage-dependent anion channels.47,49

Nishiyama et al50,51 approached this issue by determining whether ATP could be detected in the renal cortical interstitial fluid and whether detected levels would fluctuate in a manner consistent with autoregulatory behavior. They implanted a microdialysis catheter in the renal cortex and measured ATP collected in the dialysate. They reported that, indeed, ATP was collected in the dialysate after it equilibrated with renal cortical interstitial fluid. In addition, as one would expect if extracellular ATP is the autoregulatory messenger molecule, interstitial ATP concentrations increased in direct proportion to arterial pressure. Thus, as the P2 hypothesis would predict, increases in renal perfusion pressure would stimulate increases in ATP release, increased extracellular ATP concentration, and, presumably, increased P2X1 receptor activation. From the standpoint of measured ATP concentration, extracellular ATP levels vary as a function of arterial pressure, but the levels of ATP measured in the dialysate tended to be 30- to 40-fold lower than has been shown effective for ATP-mediated vasoconstriction of afferent arterioles.51 This discrepancy could reflect technical limitations of the dialysis technique, ATP measurements/stability, or limitations of topical ATP delivery in in vitro experiments.52 Nevertheless, the measured values for interstitial ATP concentration track appropriately with changes in arterial pressure. Interestingly, interstitial adenosine concentrations were found to remain unchanged as a function of renal arterial pressure.51

In pioneering work by Bell and colleagues,10,46 ATP release was detected adjacent to the basolateral membrane of rabbit macula densa cells induced by stimuli imposed to elicit a tubuloglomerular feedback response. A biosensor PC12 cell expressing P2 receptors was placed in close proximity to the macula densa. Increasing NaCl delivery to the apical membrane of the macula densa resulted in the generation of whole-cell patch clamp currents and increases in intracellular calcium concentration in the biosensor cell. This biosensor cell response could be eliminated by moving the biosensor away from the basolateral surface of the macula densa, by pretreating the bath with a P2 receptor antagonist, or by enhancing ATP breakdown by adding apyrase and hexoki-
nase (ATP hydrolysis enzymes) to the bathing medium. Subsequent studies suggested that released ATP may have exited macula densa cells via a large conductance chloride channel. As a result of these studies, Bell et al.\(^5\) postulated that tubular sodium and chloride enter the apical membrane of macula densa cells via the Na\(^+\)/K\(^+\)/2Cl\(^-\) transporter and increase intracellular chloride concentration. The increase in cytosolic chloride concentration activates chloride efflux through chloride channels in the basolateral membrane, imposing a depolarizing current.\(^5\) The depolarization is believed to activate voltage-dependent calcium influx through nifedipine-sensitive calcium channels in the basolateral membrane, leading to an increase in macula densa cell calcium concentration.\(^5,53\) These events culminate in ATP release through maxi-anion channels, and this released ATP exerts its effects on P2 receptors expressed by mesangial cells and preglomerular smooth muscle cells, as well as in an autocrine fashion on the basolateral surface of macula densa cells themselves.\(^5,54\) Released ATP could also be hydrolyzed to adenosine, which could also act on A\(_1\) receptors expressed by preglomerular smooth muscle cells. In addition, they postulated that transmission of ATP-dependent signals on mesangial cells could be transmitted to preglomerular vascular smooth muscles via gap junctions.

More recent work by Peti-Peterdi\(^11\) examined the gap junction hypothesis by assessing afferent arteriolar calcium wave propagation induced by tubuloglomerular feedback signals. They noted that tubuloglomerular feedback stimuli produced an increase in intracellular calcium concentration in afferent arterioles, and this response was propagated retrograde along the arteriole length. These tubuloglomerular feedback–induced calcium waves were blocked by furosemide that inhibits tubuloglomerular feedback at the apical membrane of macula densa cells. They were also prevented by P2 receptor blockade with suramin or by an ATP hydrolysis mixture, but they were unaffected by DPCPX, a selective adenosine A\(_1\) receptor antagonist. Together these data suggest that activation of tubuloglomerular feedback signals involves release of ATP, which influences calcium signaling in afferent arteriolar vascular smooth muscle. These data do not reveal whether the actions of ATP are directly on afferent arteriolar receptors or through an indirect effect involving other cells or mechanisms. To examine the involvement of gap junctions in this signaling cascade, they tested responses in the presence of agents that uncouple gap junctions (heptanol; \(\alpha\)-glycyrrhetinic acid).\(^11\) In the presence of heptanol, tubuloglomerular feedback stimuli still elicited a calcium response at the terminal end of the afferent arteriole near its fusion with the glomerulus, but this response was not propagated along the arteriole length. \(\alpha\)-Glycyrrhetinic acid markedly blunted both the calcium response and its propagation. Thus, gap junctions appear to play an important role in transmitting ATP-mediated tubuloglomerular feedback responses to afferent arterioles and propagating it along the arteriole length. These studies were later extended by Toma et al.\(^43\) who examined connexin expression by glomerular endothelial cells and the involvement of specific connexins, functioning as hemi-channels, in transmitting mechanical signals. The underlying idea behind this work is that hemi-channels, which are uncoupled gap junctional units, may serve as membrane pathways for ATP release to the interstitial fluid environment. They reported that many connexins were found to be expressed by renal tissue, but connexin 40 was highly expressed by these glomerular cells. Their observation for connexin 40 expression in this region of the renal microcirculation agrees with the findings of Wagner and coworkers,\(^55–57\) who implicate connexin 40 in the pressure-dependent control of renin secretion by juxtaglomerular cells. Focusing on connexin 40, Toma et al.\(^43\) reported that propagation of mechanical signals could be markedly inhibited when the glomerular endothelial cells were pretreated with small-interfering RNA directed against connexin 40. In those same studies, similar degrees of inhibition were obtained by gap junction uncoupling agents, ATP scavengers, and P2 receptor blockers. Notably, the inhibitory effects of the blockers were reversible. These data argue that connexins such as connexin 40 play an important role in the transmission of mechanical and/or tubuloglomerular feedback responses. It is exciting to postulate that connexin 40 is functioning as a hemi-channel capable of releasing ATP on stimulation. Given that these hemi-channels are invested in the plasma membrane and that they are capable of forming substrate-passing pores, it is interesting to postulate that hemi-channels may respond to membrane stretch or perturbation with the local release of ATP, which acts in an autocrine/paracrine manner. Although such things are interesting to think about, much more work is needed to clarify the mechanisms involved in ATP release or the release of other autoregulatory messenger molecules.

Impact of Hypertension on Autoregulatory Behavior and Afferent Arteriolar Reactivity

Hypertension is frequently associated with progressive renal injury that arises through a number of poorly understood factors. Loss of autoregulatory efficiency could be one contributing factor that leads to glomerular stress, glomerular compensation, and perhaps glomerular injury and failure. Autoregulatory behavior does not need to be completely eliminated to cause glomerular stress. Rather, a small reduction in efficiency could facilitate the transmission of elevated arterial pressure to the glomerulus, causing a chronic elevation of glomerular capillary pressure and contributing to subsequent glomerular injury. Evidence indicates that renal autoregulation is impaired in some experimental animal models of hypertension.\(^35,36,58–61\) In contrast, young spontaneously hypertensive rats are a model of significant hypertension that retains efficient autoregulatory control.\(^62,63\) Considering the role of purinoceptors in the regulation of renal microvascular function, autoregulation, modulation of hemodynamic function, and tubular transport, it is reasonable to examine the role of purinoceptors in the pathophysiology of hypertension-related renal injury. Evidence suggests that purinoceptors participate in the functional adaptations that occur during the development of hypertension and that they contribute to the pathophysiology of hypertension.\(^36,64,65\) For example, the renal injury that occurs in angiotensin II–infused hypertensive rats is reduced by treatment with the P2Y\(_{12}\) receptor antagonist clopidogrel, while having no effect on the...
hypertensive arterial pressure.\textsuperscript{66} ATP-stimulated elevation of intracellular calcium concentration is enhanced in glomerular mesangial cells from spontaneously hypertensive rats, but the response is desensitized on repeated stimulation.\textsuperscript{67} NO was found to re sensitize P2Y receptors in mesangial cells from Wistar-Kyoto rats but not in spontaneously hypertensive rats. Resensitization by NO restored the magnitude of the calcium response to P2Y receptor stimulation. In addition, mesangial cells increase P2X\textsubscript{7} receptor expression in Ren-2 transgenic hypertensive rat kidneys.\textsuperscript{65} Thus, potentially important changes occur in P2 receptor expression and perhaps function, but the ramifications of these changes remain unclear.

We have examined the impact of hypertension on afferent arteriolar reactivity to P1 and P2 receptor stimulation.\textsuperscript{68} As shown in Figure 4, pressure-induced autoregulatory responses are markedly attenuated in the angiotensin II–infused model of hypertension.\textsuperscript{36,37,69} Elevation of arterial pressure plays an important role in the autoregulatory impairment, because pressure reduction with “triple therapy” normalizes autoregulatory behavior despite continued elevation of angiotensin II.\textsuperscript{37,70} Zhao et al\textsuperscript{68} found that ATP-mediated afferent arteriolar vasoconstriction was markedly blunted, whereas P2X\textsubscript{7} receptor–mediated vasoconstriction (\(\beta\) and \(\gamma\)-methylene ATP) was nearly completely eliminated (Figure 5). Interestingly, afferent arteriolar responses to adenosine and to the P2Y agonist UTP were unchanged.\textsuperscript{68} P2X\textsubscript{1} receptor–mediated impairment of afferent arteriolar vasoconstriction is associated with a reduction of agonist-induced elevation of intracellular calcium concentration in pregglomerular smooth muscle cells. In this regard, the reduced ATP-mediated vasoconstriction of rat afferent arterioles may contribute to hypertension-induced renal injury by impairment of renal autoregulatory efficiency, resulting in increased glomerular capillary pressure. The role of P1 receptors in hypertension-related renal injury, as well as the relationship between reduced P2 receptor–mediated vasoconstriction to autoregulatory impairment and hypertension-induced renal injury, remains to be clarified. As mentioned previously and as is consistent with genetic or pharmacological P2X\textsubscript{1} receptor inactivation, the pressure-diameter relationship in hypertension is flat over the entire pressure range (66 to 170 mm Hg) examined, indicating that the ability to increase or reduce the diameter appropriately for the change in perfusion pressure is compromised, yet the diameter remains static, suggesting that it is not behaving passively. Events must be taking place to hold the diameter constant while reactivity to the purported autoregulatory mediator is impaired. Interestingly, afferent arterioles from hypertensive kidneys are not unresponsive to vasoconstrictors. Indeed, responses to angiotensin II are augmented; responses to norepinephrine, UTP, and adenosine are normal; and responses to KCl appear to be normal or augmented.\textsuperscript{59,68} Thus, at present, abnormal afferent arteriolar function in hypertension seems to be related to P2 receptor function. Exactly what this means for the renal vascular adaptations to hypertension or other pathophysiologic settings, such as diabetes mellitus, where autoregulation is known to be impaired, remains unclear.

**Perspectives**

The kidney is an elegant and amazingly complex organ that subserves many tasks in preserving normal homeostatic balance. Renal autoregulatory capability is fascinating yet mysterious. Although it has been studied extensively, the kidney does not readily release the secrets that it uses for regulating renal vascular resistance and autoregulatory control and for effecting responses to vasoactive substances. Consequently, our appreciation for how the kidney regulates renal blood flow in health and why this ability may be compromised in some pathological conditions remain unclear. Also unclear are the ramifications of reduced autoregulatory efficiency and whether this is a sign of renal decline or whether it is a mechanism used by the kidney to respond to a physiological imbalance. For example, is reduced autoregulatory efficiency an attempt by the kidney to increase sodium excretion and reduce blood pressure in hypertension? Only through further study and new insights will we be able to understand the inner workings this unique and remarkably efficient system. Maybe then we will be able to understand what is physiological and what is pathological.

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None.

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