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P2 Receptors in Renal Autoregulation

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Abstract

Accomplishing autoregulation of renal blood flow and glomerular filtration rate is an essential function of the renal microcirculation. While the existence of this phenomenon has been known for many years, the exact mechanisms that underlie this unique regulatory capability remain poorly understood. The work of many investigators has provided insights into many aspects of the autoregulatory mechanism, but many critical components remain elusive. This review is intended to update the reader on the role of P2 purinoceptors as a postulated mechanism responsible for renal autoregulatory resistance adjustments. It will summarize recent advances in normal function and it will touch on more recent ideas regarding autoregulatory insufficiency in hypertension and inflammation. Current thoughts on the nature of the mechanosensor responsible for myogenic behavior will be discussed as well as current thoughts on the mechanisms involved in ATP release to the extracellular fluid space.

Keywords

ATP; adenosine; afferent arterioles; P2 receptors; connexins; myogenic behavior; tubuloglomerular feedback

Introduction

Autoregulatory control is an intrinsic property of blood vessels in most vascular beds and particularly in the brain, heart and kidney [1–3]. Renal autoregulatory control is largely manifested by the preglomerular arteries and arterioles and is largely responsible for maintaining a constant glomerular capillary pressure [1, 2]. Impaired autoregulatory behavior is linked to end organ injury in many pathological settings including hypertension, diabetes, renal failure, and heart failure [2, 4–10]. Protection of glomerular capillary pressure against increases in arterial pressure is accomplished by regulation of preglomerular vascular resistance (Figure 1). In healthy kidneys, automatic adjustments in afferent arteriolar resistance occur within seconds of changes in arterial perfusion pressure. The kidney accomplishes autoregulatory behavior through the combined influences of two distinct mechanisms. The preglomerular vasculature exhibits inherent myogenic control that is intrinsic to vascular smooth muscle. In addition, renal autoregulation includes a second component with input from the tubuloglomerular feedback mechanism [11]. Myogenic reactivity is clearly manifested by preglomerular arteries but is most prominent in the

afferent arterioles, which are the major renal vascular resistance vessels primarily responsible for autoregulatory efficiency [2]. Tubuloglomerular feedback is a mechanism by which the macula densa senses changes in NaCl delivery in the distal tubule and releases paracrine signals that modulate afferent arteriole resistance, thus conferring an additional level of fine control over glomerular hemodynamics. The combined influences of the myogenic and tubuloglomerular feedback mechanisms, working through extracellular ATP and/or adenosine, enable the kidney to maintain a stable renal blood flow, glomerular capillary pressure and glomerular filtration rate essential to the kidney's ability to maintain fluid and electrolyte homeostasis [11–14]. Impairment of autoregulatory capability can inappropriately elevate glomerular capillary pressure, and contribute to glomerular injury [3, 4]. This review will focus on current questions to be answered regarding the P2 receptor hypothesis and how it integrates with the tubuloglomerular feedback system.

Purinoceptor Signaling Mechanisms and Renal Autoregulation

P2 receptors are a large family of receptors that can be divided into two structurally distinct families [15, 16]. The P2Y receptor family is represented by eight distinct receptor subtypes (P2Y₁, P2Y₂, P2Y₄, P2Y₆, P2Y_{11–14}). More recent evaluation suggests the existence of two subfamilies with the first involving P2Y₁, P2Y₂, P2Y₄, P2Y₆, and P2Y₁₁ receptors. These receptors are coupled to G_q and stimulation leads to the activation of phospholipase C β (PLC β). A second subfamily has been proposed for the P2Y_{12–14} receptors which appear to be coupled to G_i resulting in inhibition of adenylyl cyclase [15]. The second P2 receptor family is termed P2X receptors which were first identified in 1994. The P2X receptor family is made up of seven distinct receptor subtypes (P2X_{1–7}) which function as ligand gated channels [16]. P2X receptors are known to play key roles in neural signaling of pain, regulation of renal blood flow and renal tubular function, vascular endothelial function and some inflammatory responses [16–18].

Autoregulatory resistance adjustments rely on calcium influx through the voltage-gated L-type calcium channels [1, 19, 20]. P2 receptor activation causes vasoconstriction by increasing the intracellular calcium concentration in vascular smooth muscle cells [21–23]. P2 purinoceptors signal specifically through activation by adenine and uridine nucleotides. Calcium responses evoked by activation of G-protein coupled P2Y receptors primarily rely on inositol trisphosphate-dependent calcium release from intracellular stores [15]. In contrast, P2X receptors are ligand-gated channels that directly influence intracellular calcium concentration through ligand-mediated sodium and calcium influx with subsequent opening of voltage-dependent calcium channels [21–23].

Adenosine signals through the modulation of intracellular cAMP levels [24]. A₁ receptor activation leads to cAMP suppression and vasoconstriction, while A₂ receptor activation stimulates cAMP accumulation and generally evokes vasorelaxation. A₁ and A₂ receptors are not generally attributed to initiating calcium signaling responses, but the more recently identified A₃ receptor may be linked to calcium signaling events [24]. The clear association of voltage-dependent calcium signaling with typical autoregulatory resistance adjustments suggests that adenosine receptor activation is not a major contributor to the major resistance changes elicited by preglomerular vascular smooth muscle cells in response to increases in

renal perfusion pressure. Importantly however, adenosine is reported to modulate calcium sensitivity in mouse afferent arteriolar smooth muscle cells, so adenosine-dependent modulation of calcium could be involved in regulating calcium sensitivity in mouse vascular smooth muscle and thus could influence mouse afferent arteriolar autoregulatory behavior [25].

Activation of P2X₁ receptors is an essential mechanism for pressure-mediated afferent arteriolar vasoconstriction (Figure 1) [21, 22, 26]. Pharmacological blockade of P2X₁ receptors or deletion of P2X₁ receptors in knockout mice leads to blunted pressure-mediated afferent arteriolar vasoconstriction [26]. Furthermore, pressure-mediated afferent arteriolar vasoconstriction is lost during blockade of voltage-dependent L-type calcium channels [21]. 20-HETE has also been strongly implicated in the myogenic reactivity of many vascular beds and may be important in myogenic regulation of the afferent arteriolar autoregulatory response [27–29]. Blockade of 20-HETE with 20-HEDE, blocks P2X₁ receptor mediated afferent arteriole vasoconstriction and 20-HEDE suppresses P2X₁ receptor-mediated calcium signaling responses [30]. This suggests that endogenous 20-HETE is playing an important role in the calcium signaling events initiated by P2X₁ receptor activation. It is also reported that 20-HETE plays an integral role in the autoregulatory response of cerebral arteries to autoregulate cerebral blood flow [31]. These more recent data suggest that 20-HETE also plays an important role in the calcium-dependent signaling events involved in renal microvascular autoregulatory responses [30].

Rho-kinase is postulated to enhance calcium sensitivity in vascular smooth muscle cells [32, 33]. Inhibition of Rho-kinase with Y-27632 significantly blunts myogenic behavior in hydronephrotic kidneys and autoregulatory reactivity in normal kidneys [34, 35]. Inhibition of Rho-kinase blunts vascular reactivity to potent vasoconstrictors such as endothelin, angiotensin II (Ang II) and the P2X₁ agonist, α β -methylene ATP [32, 33, 35]. Reactive oxygen species are also known to be important modulators of vascular and microvascular reactivity [36, 37]. Rat afferent arterioles acutely exposed to TGF- β exhibited impaired pressure-mediated vasoconstriction, and the impairment was prevented during exposure of afferent arterioles to the antioxidants, apocynin or tempol [36]. In contrast, increasing luminal pressure of isolated perfused mouse afferent arterioles enhanced superoxide production and myogenic vasoconstriction [37]. Reduction of superoxide levels can reduce renal vascular resistance, whereas increasing renal superoxide levels can lead to increased renal vascular resistance. Thus, the redox status plays an integral role in regulating renal vascular function and could be an important signaling mechanism influencing renal microvascular autoregulatory behavior.

Mechanisms of Autoregulatory Behavior

The concept that increases in transmural pressure cause vasoconstriction of preglomerular arterioles to maintain a constant renal blood flow and glomerular filtration rate is based on seminal experiments investigating the hemodynamic principles required to maintain stable glomerular capillary pressure and kidney function [38, 39]. The kidney utilizes at least two, and possibly as many as four, separate mechanisms to affect renal resistance changes in response to changes in perfusion pressure, and tubular fluid flow with the two best defined

mechanisms being the myogenic response and tubuloglomerular feedback (Figure 1) [13, 14, 40–42]. Evidence suggests that there is a fast acting (~5sec.) component of the autoregulatory response, ascribed to the myogenic mechanism, and then a slower (~25–60 sec.) response attributed to the tubuloglomerular feedback mechanism, with the possible involvement of other mechanisms including a slower tubuloglomerular feedback mechanism (~2 min.) and a connecting tubule glomerular feedback mechanism which are postulated but not clearly resolved [13, 14, 40–42]. Furthermore, the respective contributions of these different mechanisms to the overall autoregulatory response may depend on a combination of physiological and pathophysiological mediators.

The top panel of Figure 2 illustrates a conceptual depiction of the autoregulatory response under normal conditions with two mechanisms contributing to the overall response and with all systems intact and operational. An increase in perfusion pressure should evoke an autoregulatory vasoconstriction and reduce vessel diameter to a caliber below the starting diameter (solid line). If both systems are inhibited, then an increase in perfusion pressure should yield a passive pressure-diameter relationship as illustrated by the dashed line in Figure 2. If two autoregulatory systems are contributing to increased wall tension through separate signaling mechanisms, then loss of one system should result in an intermediate pressure-diameter response depicted by the gray shaded area reflecting the contribution of a single mechanism, which provides some adjustment but the adjustment is incomplete.

Experimental data plotted in the lower panel of Figure 2 contrasts the pressure-mediated afferent arteriole autoregulatory vasoconstriction obtained in normal kidneys (black symbols), with the response obtained during P2X₁ receptor blockade with NF-279 (white symbols) [26]. P2X₁ receptor blockade consistently yields a flat pressure-diameter relationship, rather than the hypothetical passive relationship (gray symbols) suggesting that partial tension-generating capacity remains even though the complete autoregulatory response is not manifested. A similar flat pressure-diameter relationship has been observed in other experimental settings such as the during non-selective P2 receptor blockade, Ang II infused hypertension model, the two-kidney, one clip Goldblatt model of hypertension and during acute exposure to transforming growth factor β 1 (TGF- β 1) [36, 43–46].

Additionally, Figure 3 shows that P2X₁ receptor deletion in global knockout mice yields the same flat pressure-diameter profile that is observed during pharmacological P2 receptor or P2X₁ receptor blockade [21, 26]. This is in marked contrast to the normal pressure-induced decline in afferent arteriole diameter observed in wild type littermate mice. Conversely, removal of the papilla, which severs the loops of Henle and thus interrupts the tubular fluid component of the tubuloglomerular feedback mechanism, or treatment with furosemide which inhibits the Na-K-2Cl co-transporter and thus inhibits the tubuloglomerular feedback mechanism, changed the normal autoregulatory response in wild type mice to a flatter pressure-diameter relationship but had no detectable effect on the responses obtained from kidneys of P2X₁ knockout mice [47]. These data suggest that P2X₁ receptor blockade or deletion has only intervened in one of the two recognized mechanisms that participate in the overall autoregulatory response and supports the hypothesis that P2X receptors play an important role in the overall autoregulatory response.

Adenosine is another signaling molecule proposed to play a role in autoregulation of glomerular blood flow and filtration rate through tubuloglomerular feedback by activating A₁ receptors [42, 48, 49]. Adenosine A₁ receptor deficient mice have blunted renal blood flow autoregulation and attenuated tubuloglomerular feedback responses [49–51]. Studies have also shown that gene deletions or mutation to the ectonucleotidases that catalyze the production of adenosine from ATP accentuate tubuloglomerular feedback [52]. However, other studies have shown modest or no blunting of autoregulatory function in response to pharmacological A₁ receptor blockade so the role of adenosine in overall renal autoregulation remains unclear [53, 54].

As suggested by the data above, other influences also contribute to setting afferent arteriole resistance to achieve autoregulatory control. The myogenic component of the autoregulatory response accounts for the fast response to alterations in renal perfusion pressure and is a major contributor to the overall autoregulatory response. Myogenic reactivity is an intrinsic property of vascular smooth muscle whereby increases in transmural pressure induce a reactive increase in active tension. This increase in active tension is calcium-dependent and results in a decrease in lumen diameter to provide the increase in resistance needed to offset the increase in transmural pressure and maintain a relatively constant blood flow [1]. The nature of the mechanosensor(s) that transduce(s) vascular wall stretch into vasoconstriction remain unknown.

Jernigan and Drummond have pioneered the hypothesis that epithelial sodium channels (ENaC) may serve as mechanosensors for myogenic vasoconstriction of upstream renal arteries [55]. Using siRNA targeted to the β , or γ subunits of ENaC, Jernigan *et.al.* showed that reducing expression of functional ENaC channels reduced the myogenic vasoconstriction induced by pressure increases in isolated renal interlobular arteries [55]. While compelling, this idea still has not been universally accepted [56]. Data reported by Wang *et. al.* failed to show ENaC expression in isolated afferent arterioles, calling into question the role of ENaC in regulating myogenic control of the preglomerular vasculature [56]. Furthermore, the ENaC inhibitors, amiloride and benzamil had no detectable effect on the pressure-mediated myogenic response of afferent arterioles in hydronephrotic kidneys [56]. More recent work is consistent with ENaC participating in the myogenic response. Guan *et al* demonstrated that the autoregulatory response of afferent arterioles was markedly blunted in kidneys where the tubuloglomerular feedback response was eliminated by papillectomy, implying that ENaC blockade indeed inhibited myogenic signals which are retained in papillectomized kidneys [57]. Additionally there is more recent work from the Drummond laboratory in mouse models that have genetic reductions in fully functional β -ENaC expression in lung, kidney and vascular smooth muscle cells. Using these animals, Ge *et.al.* and Grifoni *et. al.* showed that whole kidney autoregulatory responses were smaller in magnitude and slower to develop than in wild type litter-mates with intact β -ENaC [58, 59]. These controversies and other open questions show that more work is needed to elucidate the specifics of this purported component of the autoregulatory mechanism.

A number of other systems may contribute significantly to autoregulatory control in ways that may be dependent, or independent, of the myogenic and tubuloglomerular feedback mechanisms. Modulation of cytoskeletal elements such as integrins, gap junctions,

connexins, or interactions among many of these systems could be involved [60, 61]. For example, pannexin-1 is a connexin-like protein which forms mechano-sensitive channels in the plasma membrane that facilitate the release of ATP into the extracellular fluid space where it participates in autocrine or paracrine signaling or where it can simply be broken down [62, 63]. There is also evidence that the Rho-kinase pathway contributes to renal microvascular responses to autoregulatory and vasoconstrictor signals, purportedly by increasing calcium sensitivity of the contractile proteins [64–67]. Inhibition of Rho-kinase with Y-27632 yields a concentration-dependent decline in autoregulatory behavior as well as inhibition of Ang II-dependent vasoconstriction [35]. These studies also reveal that Rho-kinase inhibition blocks P2X₁ but not P2Y₂ receptor-mediated vasoconstriction. Accordingly, the loss of autoregulatory control coincides with the loss of P2X₁ receptor-dependent vasoconstriction providing indirect support for the postulate that P2X₁ receptors are essential elements in the autoregulatory resistance cascade. The data also strongly implicate the Rho-kinase system in modulating P2X₁ receptor signaling and thus may play a role in regulating the sensitivity of autoregulatory mechanisms [34, 68].

While the phenomenon of autoregulation has been accepted for many decades, work continues on understanding the mechanisms involved. Those continued efforts have led to identification of a novel new mechanism called connecting tubule glomerular feedback, or CTGF which is thought to modulate autoregulation [41, 69, 70]. CTGF is postulated to influence autoregulatory behavior by modulating tubuloglomerular feedback responses by dilating the afferent arteriole in response to increased connecting tubule NaCl delivery. The dilatory influence is thought to involve local release of epoxyeicosatrienoic acids and prostaglandins but the true nature of the signal(s) has (have) not been determined [69, 70]. Consequently, CTGF could function by resetting tubuloglomerular feedback signaling to reduce tubuloglomerular feedback-dependent vasoconstriction under conditions of increased NaCl delivery to the connecting tubule.

Mechanisms of ATP Release

Many of the aforementioned mechanisms rely on signaling via the second messenger ATP in the extracellular space. Identifying a source and mechanism for extracellular ATP release is required to establish ATP, or ATP hydrolysis products as critical extracellular messenger molecules mediating pressure-dependent autoregulatory resistance adjustments. ATP is commonly released from many different cell types in response to stretch, osmotic stimuli, oxygen/nutrient status or membrane perturbation or deformation [71–75]. For the P2 receptor hypothesis in particular, the sources and mechanism(s) for increased extracellular ATP, via local autocrine or paracrine release remain to be clearly established.

Multiple mechanisms have been proposed to account for cellular release of ATP. These include vesicular ATP release and release through anion channels such as maxi-anion channels [76]. The cystic fibrosis transmembrane conductance regulator (CFTR) has been postulated to play a role in ATP transport but experiments have failed to support this hypothesis [77]. Maxi-anion channels can be activated by stretch and reportedly allow passage of ATP to the extracellular fluid space [76, 78]. The prevailing hypothesis is that transmission of autoregulatory signals utilizing ATP as a second messenger are being

facilitated by both intercellular and extracellular movement of ATP via gap junctions and hemichannels formed by connexins and pannexins [60, 75, 79].

For the ATP hypothesis to be validated, it is important to demonstrate that appropriate changes in extracellular ATP occur in response to autoregulatory stimuli. Nishiyama et. al. approached this issue by determining whether or not ATP could be detected in renal cortical interstitial fluid and whether or not ATP levels fluctuate in a manner consistent with pressure-mediated autoregulatory responses [80]. Using a small surgically implanted microdialysis catheter they were able to demonstrate that ATP was present in the interstitial fluid of the renal cortex and that interstitial ATP concentrations decreased in direct proportion to decreases in renal arterial pressure, though the levels they detected in these studies were 30- to 40-fold lower than shown effective for ATP-mediated vasoconstriction of afferent arterioles [81]. This discrepancy could reflect the technical limitations of the dialysis technique, ATP stability in the dialysis fluid or in the effective ATP concentration reaching the receptor in *in vitro* superfusion experiments [82]. Nishiyama's findings do support the involvement of interstitial ATP as the signaling molecule effecting autoregulation and importantly that ATP concentrations tracked appropriately with changes in arterial pressure. Of additional note, measured interstitial adenosine concentrations remain unchanged as a function of renal arterial pressure in these same studies [81].

With similar implications, Bell and colleagues show ATP release was detected adjacent to rabbit macula densa cells in response to stimuli imposed to elicit a tubuloglomerular feedback response [83]. Biosensor cells expressing P2X receptors were placed in close proximity to the basolateral surface of macula densa cells. These biosensor cells responded to tubular autoregulatory stimuli in a manner consistent with P2X receptor activation. Activation of the biosensor cell was dependent on proximity to the basolateral surface of the macula densa and was ATP dependent. Bell et. al. postulated that changes in tubular Na⁺ and Cl⁻ delivery to the macula densa cells activates a depolarizing current, which is believed to activate a signaling cascade involving voltage-dependent calcium influx through nifedipine-sensitive calcium channels in the basolateral membrane which culminates in ATP release through maxi-anion channels [84, 85]. The ATP that is released stimulates P2 receptors expressed by mesangial cells and preglomerular smooth muscle cells, as well as perhaps in an autocrine fashion on the basolateral surface of macula densa cells themselves [84].

Similar work by Peti-Peterdi also examined the role of ATP signaling by the juxtaglomerular apparatus by assessing afferent arteriolar calcium wave propagation induced by tubuloglomerular feedback signals [60]. They noted that in glomeruli with attached afferent arterioles isolated from rabbits, tubuloglomerular feedback stimuli increased intracellular calcium concentration in afferent arterioles, and this response was propagated retrograde along the length of the arteriole. The response could be blocked with furosemide applied to the apical membrane of macula densa cells, which inhibits tubuloglomerular feedback. The response was also inhibited by the P2 receptor blocker, suramin, but they were unaffected by DPCPX, a selective adenosine A₁ receptor antagonist. These data suggest that ATP is playing a direct role in stimulating afferent arteriole smooth muscle

contraction in response to tubuloglomerular feedback signals, however they do not reveal the mechanisms by which macula densa cells may be releasing ATP.

The gap junction hypothesis posits that transmission of the ATP autoregulatory signal between the juxtaglomerular apparatus and afferent arterioles occurs via gap junctions and/or hemichannels formed from connexins. ATP has been shown to mediate calcium waves between adjacent cells by release from gap junctions and hemichannels and subsequent purinergic receptor activation [86]. Connexin 40 is highly expressed in preglomerular endothelial cells, glomerular mesangial cells and renin-containing cells of rats and mice [87]. To specifically examine the involvement of gap junctions in this signaling cascade, Peti-Peterdi utilized agents that uncouple, or block, gap junctions and examined tubuloglomerular feedback responses in afferent arterioles [60]. The gap junction blocker, heptanol, inhibited calcium wave propagation along the arteriole length. The nonspecific gap junction inhibitor, 18 α -glycyrrhetic acid, markedly blunted both the propagation of a calcium wave along the vessel length but also the initial calcium response at the afferent arteriole. 18 α -glycyrrhetic acid is also known to inhibit the function of hemi-channels and the difference between these observations implies that hemi-channels may also be a functional mechanism at the juxtaglomerular apparatus.

Hemi-channels, which are uncoupled subunits that can form gap junctions, reportedly serve as membrane pathways for ATP release into the interstitial fluid when activated by a variety of stimuli in different cell types [88]. Toma et al examined connexin expression by glomerular endothelial cells and the involvement of specific connexins, functioning as hemi-channels, in transmitting mechanical signals [61]. They reported that connexin 40 was highly expressed by cells in the juxtaglomerular region which is consistent with the findings of Wagner and coworkers, who implicate connexin 40 in the pressure-dependent control of renin secretion by juxtaglomerular cells [89]. Focusing on connexin 40, Toma et al reported that propagation of mechanical signals could be markedly inhibited when glomerular endothelial cells were pretreated with small-interfering RNA directed against connexin 40 [61]. Similar degrees of inhibition were obtained in those studies by gap junction uncoupling agents, ATP scavengers, and P2 receptor blockers.

In vitro experiments reveal that homomeric connexin 40 hemi-channels can be induced to make a calcium-dependent conformational change from an open state in low calcium media to a closed state in high calcium media [90]. This mechanism could be akin to a negative feedback loop that would induce ATP release only at a rate commensurate with maintaining a basal cell tension dependent on P2X₁ receptor density and/or hemi-channel expression. In a similar mechanism, evidence shows connexin 30 forms hemi-channels in murine renal tubules which facilitates ATP release in response to changes in tubular fluid flow rate [91].

The previously mentioned pannexin-1 could also play a key role in mediating ATP release as a mechanism for autoregulation. As a connexin-like protein which forms mechano-sensitive hemi-channel like structures in the plasma membrane, this could constitute a role for ATP release by smooth muscle cells as an autocrine vascular smooth muscle component of the myogenic response [63, 65, 75].

These data are supportive of the hypothesis that hemi-channels could play an important role in transmitting myogenic and/or tubuloglomerular feedback responses to effect autoregulatory resistance adjustments. It is exciting to postulate that mechanical stimuli could induce ATP release for paracrine/autocrine signaling of vasoconstriction as an initial response to perturbations of the endothelial cell membranes caused by pressure changes along the afferent arteriole. Given that hemi-channels are present in the plasma membrane, that they are capable of forming substrate-passing pores, and the constituent protein expression has been demonstrated in these cell types, hemichannels are hypothesized to act as an autocrine/paracrine signaling component of the myogenic mechanism.

An additional mechanism that could participate in autoregulatory ATP signaling could be modulation of the rate of conversion of released ATP into adenosine by ecto-5'-nucleotidases. Either through reductions of extracellular ATP or increases in adenosine acting as a signaling molecule itself, expression of enzymes capable of catabolizing extracellular ATP could constitute another control mechanism for fine tuning overall autoregulation [92, 93]. For example, released ATP could also be hydrolyzed to adenosine, which could act on A₁ receptors expressed by preglomerular smooth muscle cells. As previously mentioned, adenosine has been hypothesized to affect autoregulation through modulation of tubuloglomerular feedback. Some studies have shown that gene deletions or mutation to the ectonucleotidases that catalyze the production of adenosine result in stimulation of tubuloglomerular feedback [52, 94, 95]. These effects could be due to either a decrease in adenosine or an increase in ATP.

Renal Autoregulation in Hypertension

As we discussed in the aforementioned sections of this review, the role of renal autoregulatory capacity is to protect kidneys from transmission of high systolic blood pressure to glomeruli and therefore, prevent development of intra-glomerular hypertension and subsequent glomerular injury. Hypertension with renal injury is recognized as a major risk factor for developing cardiovascular disease. The fundamental mechanisms leading to renal injury in hypertensive subjects are complex and certainly involve numerous pathophysiological processes that are occurring simultaneously. Studies from both animal and human subjects indicate that reduction or loss of autoregulatory efficiency may initiate glomerular hyperfiltration, lead to the development of intra-glomerular hypertension, and ultimately lead to progression of renal injury in hypertension [4, 5, 7–10, 96]. For example, African Americans afflicted with essential hypertension have a higher incidence of end-stage renal disease compared to Caucasians while they also exhibit impaired autoregulation of glomerular filtration rate [5, 8]. Hypertension with impaired autoregulatory capability may put these patients at a higher risk for development of renal injury [5]. Studies in animal models also provide compelling evidence that impaired renal autoregulation in hypertension is correlated with a high incidence of compromised renal vascular and tubular function [9, 44, 45, 96–101]. Reduced, or lack of, autoregulatory efficiency are observed in several hypertensive animal models including chronic Ang II-infusion, L-NAME-induced, Dahl salt-sensitive, the Fawn Hooded rat model and two-kidney, one-clip Goldblatt hypertensive rats [9, 44–46, 96–101], but does not seem to be as clear in the spontaneously hypertensive

rat model [102, 103]. Interestingly, although spontaneously hypertensive rats exhibit comparable hypertension, renal injury is less severe [104].

Accumulating evidence indicates that ATP-P2 receptor signaling not only plays a critical role in many physiological processes, it is also involved in many pathophysiological processes including inflammation, vascular remodeling, and cell proliferation and necrosis [105]. Given the ubiquitous expression of P2 receptors in kidneys, and the diverse roles of extracellular ATP signaling in regulating renal hemodynamics, microvascular function, renal autoregulation, tubular function, and sodium excretion, it is reasonable to speculate that alteration of ATP-P2 receptor signaling pathways are associated with development of hypertension and hypertensive kidney injury [18, 54, 84, 106, 106–113]. For example, P2X₁, P2Y₂, and P2X₄ receptor deficient mice display hypertension or a significant increase in systolic blood pressure [114–116]. Besides the genetic evidence, studies in hypertensive animal models also show significant impairment of ATP-P2 receptor signaling in kidneys [100, 101, 117–122]. Chronic Ang II-infusion for 2 weeks leads to increased interstitial ATP concentrations in the renal cortex coincident with hypertension [117]. Antagonism of P2Y₁₂ receptor activation with clopidogrel provides a renoprotective effect in Ang II-infused hypertensive rats without changing systolic blood pressure [117]. Inhibition of P2X₇ receptor activation prevents hypertension and renal injury in Dahl salt-sensitive rats [119]. Genetic variation in P2X₇ and P2Y₂ genes has also been linked to hypertension in human subjects [123, 124]. Although the fundamental mechanisms by which ATP-P2 receptor signaling contribute to the pathophysiology of hypertension are not well understood, there may be significant differences from one hypertensive setting to another. Nevertheless, the existing data clearly suggest that there is a close link between ATP-P2 receptor signaling and hypertension.

We have focused on investigating mechanisms and signaling pathways involved in renal microvascular function and autoregulation, and the mechanisms that contribute to impaired renal autoregulatory behavior in hypertensive rat models. As shown in Figure 4, chronic Ang II-infusion for 2 weeks exhibited little to no pressure-mediated vasoconstriction of afferent arterioles compared to normotensive control Sprague-Dawley rats. These observations indicate that the ability of afferent arterioles to make appropriate adjustments in resistance in response to acute changes in renal perfusion pressure is significantly impaired. However, the attenuated autoregulatory responsiveness observed in Ang II hypertensive rats was preserved when the hypertensive response to chronic Ang II was blocked by “triple therapy” treatment (Figure 4A). The rats receiving triple therapy were normotensive despite chronic Ang II infusion and the autoregulatory response was essentially identical to control rats, suggesting that hypertension per se triggers the decline in renal autoregulatory capability [46]. Consistent with the autoregulatory impairment observed in Ang II-infused hypertension, Zhou et al. reported that chronic Ang II infusion also markedly attenuated the afferent arteriolar response to ATP and to a selective P2X₁ receptor agonist, β γ -methylene ATP [121]. In contrast, Ang II hypertensive rats maintained normal afferent arteriolar vasoconstrictor responses to the P2Y receptor agonist, UTP and adenosine [121]. The coincidence of the reduced vasoconstriction to P2X₁ receptor activation and impaired pressure-mediated autoregulatory behavior in this hypertensive model highlights the possibility that impairment of autoregulatory behavior caused by Ang

II infusion arises from impaired afferent arteriolar vasoconstriction to P2X₁ receptor activation. The mechanisms that contribute to impaired renal autoregulatory behavior and P2X₁ receptor activation however, are largely unknown. Since growing evidence shows that inflammation plays a critical role in the development of hypertension and cardiovascular diseases characteristic of patients with chronic kidney diseases, we treated Ang II hypertensive rats simultaneously with a broad spectrum, anti-inflammatory agent, pentosan polysulfate (PPS) or a specific inhibitor of lymphocyte proliferation, mycophenolate mofetil (MMF) [100, 101]. We found that the decline in autoregulatory control in Ang II-infused hypertensive rats was preserved by PPS or MMF treatment (Figure 4B). Furthermore, PPS and MMF treatment preserved afferent arteriolar vasoconstrictor responses to ATP and β , γ -methylene ATP in addition to reduction of plasma TGF- β 1 levels [100, 101]. MMF treatment also significantly reduced albuminuria and proteinuria. These studies provide important mechanistic information linking inflammation, specially the activation of lymphocytes in the hypertensive kidneys, and impairment of renal microvascular function with progression to hypertensive renal injury. Overall, these studies imply that intact renal autoregulation is a critical determinant of progression of renal injury in hypertension.

Perspectives

The kidney constitutes less than 1% of body weight but it plays a crucial role in maintaining normal homeostasis of the body fluid and systemic blood pressure via its high filtration and reabsorption functions. One of the most remarkable features of kidney is the renal autoregulatory capacity which allows renal blood flow and glomerular filtration rate to be tightly regulated within a limited range. Although the phenomenon of renal autoregulation has been recognized for many decades and the mechanisms underlying renal autoregulatory control have been extensively investigated, there are still many questions to be answered. It is uncertain exactly how renal autoregulation is altered to allow greater flexibility in managing the variability in our normal daily fluid and electrolyte intake. It is also unclear whether reduced autoregulatory efficiency occurring before renal structural changes in hypertension is a mechanism employed by the kidney to facilitate excretion of excess salt and fluid to maintain normal homeostatic balance. This reduction in autoregulatory efficiency may come at the cost of glomerular hypertension which can lead to glomerular decline. In the current review, we try to provide an update of the existing evidence and recent advances in renal autoregulation and ATP-P2 receptor signaling in hypertension. Certainly, more work needs to be conducted to advance our understanding of the potential mechanisms contributing reduced autoregulatory efficiency and the impaired P2X₁ receptor-mediated renal microvascular function in hypertension.

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Figure 1.

A postulated pressure-induced signaling scheme for renal autoregulatory control of afferent arteriolar resistance. Ect-5'-NT: ecto-5'-nucleotidases; TGF: tubuloglomerular feedback; RBF: renal blood flow; GFR: glomerular filtration rate, CTGF: connecting tubule glomerular feedback; (+) indicates stimulation; (-) indicates inhibition.

Figure 2.

Conceptual view for assessment of autoregulatory responses. The upper panel depicts expected outcomes for a simple autoregulatory mechanism involving two contributing elements. The solid plot represents the expected response with both systems operating normally. The dashed plot represents the expected response with both systems inoperative. The gray shaded area represents predicted responses if just one system is inoperative. The lower panel combines actual data (black and white symbols) with hypothetical data

presented by the gray symbols. Actual data are modified from previously published work [26].

Figure 3.

Effect of renal perfusion pressure changes on afferent arteriolar diameter in wild type mice (left panel) and P2X₁ receptor knockout mice (right panel) before (dark symbols) and after removal of tubuloglomerular feedback by papillectomy (pap; gray symbols). Data are modified from previously published work [26].

Figure 4.

Autoregulatory behavior of afferent arterioles in kidneys from normotensive and hypertensive rats. Chronic Ang II-infusion (60 ng/min) for 10–14 days blunted pressure-mediated vasoconstriction of afferent arterioles (white symbols). Triple therapy treatment (A) or mycophenolate mofetil (MMF; B) preserved normal autoregulatory behavior of afferent arterioles in Ang II-infused hypertensive rats (gray symbols) in a pressure-dependent manner, which was indistinguishable from normal control rats (black symbols). Data are expressed as percent of control diameter at 100 mmHg during equilibration period.

Values are mean $\pm$ SEM. * P <0.05 indicates a significant difference from control diameter; $^{\dagger}P$ <0.05 indicates a significant difference between Ang II and Ang II treated with triple therapy or MMF. Data are adapted from previously published work [46, 101].