Role of Adenosine 5’-Triphosphate in Regulating Renal Microvascular Function and in Hypertension

Zhengrong Guan, Edward W. Inscho

Abstract—ATP is an essential energy substrate for cellular metabolism, but it can also influence many biological processes when released into the extracellular milieu. Research has established that extracellular ATP acts as an autocrine/paracrine factor that regulates many physiological functions. Alternatively, excessive extracellular ATP levels contribute to pathophysiological processes, such as inflammation, cell proliferation and apoptosis, and atherosclerosis.

Renal P2 receptors are widely distributed throughout glomeruli, vasculature, and tubular segments and participate in controlling renal vascular resistance, mediating renal autoregulation, and regulating tubular transport function. This review will focus on the role of ATP-P2 receptor signaling in regulating renal microvascular function and autoregulation, recent advances on the role of ATP-P2 signaling in hypertension-associated renal vascular injury, and emerging new directions. (Hypertension. 2011;58:00-00.)

Since ATP’s discovery as a coneurotransmitter nearly 40 years ago,1 ATP is no longer regarded as just an intracellular energy source. Extracellular ATP is an autocrine/paracrine factor that regulates many physiological functions, including neuronal signaling, control of vascular tone and reactivity, mechanosensation, and ion transport. Alternatively, excessive extracellular ATP levels contribute to pathophysiological processes, such as inflammation, cell proliferation and apoptosis, and atherosclerosis.7 Extracellular ATP exerts its effects by activating distinct P2 purinoceptors, P2X and P2Y (Figure 1). P2X receptors are ligand-gated ion channels and exist as homotrimers (P2X1 to P2X7), meaning that 3 of the same subunit type associate to form a functional channel. They can also associate as multimeric complexes involving different types of P2X receptor subunits to form functional channels (P2X1/2, P2X1/5, P2X2/3, P2X2/6, P2X4/6 and P2X1/4). P2X and P2Y receptors display unique responses to ATP. Both receptors and their heteromers desensitize rapidly, although the mechanism responsible for desensitization is not clear. In contrast, P2Y receptors are G protein–coupled receptors including 8 members (P2Y1, P2Y2, P2Y4, P2Y6, P2Y11, P2Y12, P2Y13, and P2Y14). UTP is a potent agonist for P2Y2, P2Y4, and P2Y6 receptors.

The importance of P2 receptors in regulating renal function has been clarified over the past 2 decades by the identification and characterization of P2 receptors throughout renal structures.3–6 Renal P2 receptors are widely distributed throughout glomeruli, vasculature and tubular segments, and participate in controlling renal vascular resistance, mediating renal autoregulation, and regulating tubular transport function. Recent studies indicate that alteration of ATP-P2 receptor signaling occurs in hypertension.7–11 In this review, we will integrate recent findings on ATP-P2 receptor signaling in renal microvascular function, autoregulation, and hypertension-associated renal vascular injury with existing information, and emerging new directions will be discussed.

Expression of P2 Receptors in the Kidney

Renal P2 receptor expression has been explored extensively. The vasculature, glomerulus, and nearly every nephron segment express ≥1 P2 receptor subtype. Each tubular segment expresses different P2X and P2Y receptor subtypes at apical or basolateral membranes or both. P2Y2 receptors expressed in the distal tubule/collecting duct are important for sodium and fluid reabsorption. Deletion of P2Y2 receptors reportedly leads to salt-resistant hypertension.12 and the P2Y2 receptor gene is linked to essential hypertension.13 More specific information on how P2 receptor subtypes influence tubular solute transport can be found in several excellent reviews.1–3,14 P2X1, P2X3, and P2X7 receptors are detected in glomerular microvessels.15–18 Western blot analysis and immunohistochemical staining reveal intense P2X7 receptor expression in vascular smooth muscle cells of glomerular microvessels, especially afferent arterioles, whereas efferent arterioles, glomeruli, and renal tubules show no detectable staining, suggesting that P2X7 receptors are predominantly expressed by glomerular microvessels.16–18 Recently, patch-clamp studies combined with mRNA analysis suggest that glomerular vascular smooth muscle cells also express heteromeric P2X1/4 receptors.19 In addition, low level P2X7 receptor expression was reported in glomeruli and glomerular vessels, but expression levels reportedly increase dramatically under pathological conditions, such as diabetes mellitus, inflammation, and hypertension,8 suggesting an

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From the Department of Physiology, Georgia Health Sciences University, Augusta, GA.

Correspondence to Edward W. Inscho, Department of Physiology CA3137, Georgia Health Sciences University, 1120 15th St, Augusta, GA 30912.

E-mail einscho@georgiahealth.edu

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The varied distribution of P2 receptors in the kidney indicates their important role for P2X7 receptors in inflammatory processes. Afferent arterioles also express P2Y1 and P2Y2 receptors, whereas efferent arterioles express only P2Y1 receptors.16,18 The varied distribution of P2 receptors in the kidney indicates that ATP-P2 signaling pathways play important roles in regulating renal vascular, glomerular, and tubular function.

**ATP Release and Metabolism**

ATP is released from sympathetic nerve fibers on stimulation, but it can also be released from nonexcitatory cells.20 Evidence suggests that ATP is released from glomerular cells,21 juxtaglomerular cells,22 macula densa,23 and proximal tubular epithelium.24 In the normal kidney, the extracellular ATP concentration is estimated to be 1 nmol/L in glomeruli,25 6 to 9 nmol/L in interstitial fluid,26,27 and 100 to 300 nmol/L in proximal and 33 nmol/L in distal tubular fluid28 but may increase markedly under pathological conditions.9,11,20 Extracellular ATP concentrations between 10 and 100 nmol/L vasoconstrict juxtedudillary afferent arterioles under in vitro conditions.7,10,25,26 It is important to note, however, that these concentrations reflect superfused ATP solutions that encounter competing currents, diffusion barriers, and unstirred water layers. Given that the ATP concentration in microdomains is probably important, it is difficult to directly translate interstitial fluid measurements with in vitro data. Extracellular ATP is rapidly catalyzed in vivo by ectonucleotidases (Figure 1), classified as ectonucleotide triphosphatase phosphodiesterases, ectonucleoside triphosphate diphosphohydrolases (NTPDases; nucleotide triphosphate phosphohydrolases; ecto-5′-NT: ecto-5′-nucleotidases; and ALP: ectoalcaline phos-phatases). Figure shows ATP, its metabolites, and its associated intracellular signaling pathways. The signaling pathways depicted for P1 receptor activation reflect conventionally held mechanisms.73

**ATP-P2 Receptor Signaling in Renal Hemodynamics**

Normal kidney function is critical for maintenance of physiological blood volume, blood pressure, and normal cellular metabolism. Efficient renal function relies on a relatively constant renal blood flow (RBF), glomerular capillary pressure, and glomerular filtration rate. The kidney achieves these stable hemodynamic conditions primarily through precise adjustment of afferent arteriole resistance, which is influenced by numerous extrinsic and intrinsic factors, including NO, endothelium-derived hyperpolarizing factor(s), angiotensin II (Ang II), endothelin, and prostaglandins. These autocrine, paracrine, and endocrine factors directly or indirectly modulate afferent arteriolar resistance to achieve the stable hemodynamic conditions needed for efficient renal function. Loss, or reduction, of afferent arteriolar reactivity to vasoactive agents may contribute to renal injury in hypertension.

The likelihood that P2 receptors regulate renal hemodynamics and vascular reactivity is supported by in vivo and in vitro studies. Intravenous infusion of ATP or the P2X agonist β, γ-methylene ATP caused a rapid transient reduction in RBF followed by an increase of RBF in anesthetized rabbits.29 The increased RBF was significantly attenuated by the adenosine receptor antagonist, 8-(p-Sulfophenyl) theophylline, implicating A2 receptors in the ATP-induced increase in RBF. In a subsequent study, infusion of the P2X agonist α, β-methylene ATP reduced RBF and cortical and medullary blood flow by 63%, 58%, and 49%, respectively, without changes in mean arterial pressure,30 suggesting that the renal vasculature is more sensitive to P2 receptor stimulation than other vascular beds. We recently applied a more selective P2X1 receptor antagonist, P1,P5-Di-inosine-5′-pentaphosphate pentasodium salt to assess the role of renal P2X1 receptors in vivo.31 Bolus intravenous infusion of α, β-methylene ATP dose-dependently decreased RBF. This response was prevented by P2 receptor blockade with pyridoxal-phosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS) or P1,P5-Di-inosine-5′-pentaphosphate pentasodium salt, consistent with renal vascular expression of α, β-methylene ATP-sensitive P2X1 and P2X3 receptors. Notably, P2X1 receptor expression has not been convincingly demonstrated for the pregglomerular microvasculature,17,19 thereby implicating P2X1 receptors in the response to α, β-methylene ATP and highlighting a specific role for P2X1 receptors in regulating RBF. Others report that ATP evoked either vasoconstriction or vasodilation under different dietary salt conditions. For example, low-dose ATP infusion did not change total or cortical RBF but increased inner medullary blood flow in anesthetized rats fed a low-salt (0.15%) diet. The same dose decreased both inner and outer medullary blood flow in rats fed 4% salt.32 The increase in inner medullary blood flow in low-salt rats was prevented by blockade of NO production, whereas the ATP-mediated medullary vasoconstriction with high-salt intake was prevented by inhibition of cytochrome P450 activity.32 Both
effects were eliminated by P2 receptor blockade with PPADS.32 Therefore, renal vascular responses to ATP can vary under different “environmental” conditions, and these differences could reflect direct receptor-specific actions or vasoactive responses mediated, or modulated, by vasoactive substances, such as NO or cytochrome P450 products.

The influence of ATP on renal hemodynamics has also been demonstrated using isolated perfused rat kidney preparations under different basal tone conditions (preconstricted versus nonpreconstricted).33,34 For example, under basal tone conditions, ATP and its agonists evoked marked vasoconstriction with the following rank order potency: α, β-methylene ATP > β, γ-methylene ATP > ATP-γ-S > 2-methylthio ATP > ATP > ADP=UTP,33 indicating greater sensitivity of the renal vasculature to P2X receptor stimulation than P2Y. In contrast, in preconstricted, isolated perfused rat kidneys, ATP, 2-methylthio ATP, and UTP caused vasodilation at low concentrations and vasoconstriction at higher concentrations. The vasodilation was blunted by N5-nitro-L-arginine methyl ester, removal of endothelium, or by nonspecific blockade of K+ channels during exposure to high extracellular K+ (25 mmol/L).33,34 Collectively, these observations indicate that P2X receptor stimulation causes vasoconstriction, whereas activation of P2Y receptors causes vasoconstriction or tone-dependent vasodilation. The ability of N5-nitro-L-arginine methyl ester and removal of the endothelium to blunt ATP-mediated renal vasodilation argues that P2 receptors are expressed by endothelial cells and that these receptors couple through NO synthase. The observation that inhibition of K+ channel function also blunted P2X agonist-mediated renal vasodilation loosely suggests that endothelium-derived hyperpolarizing factor(s) may also be involved, although this remains to be clarified.

**P2 Receptor Action on Preglomerular and Postglomerular Microvessels**

ATP’s ability to regulate renal microvascular function has been established by determining segment-specific vascular responses to exogenously applied ATP or ATP analogues.5,24,25,35,36 Administration of ATP and α, β-methylene ATP elicited concentration-dependent vasoconstriction of isolated-perfused rabbit afferent arterioles.35 The addition of an A1 receptor blocker abolished ATP-induced vasoconstriction in the proximal region of the arteriole, but a significant vasoconstriction still existed in the distal region of the arteriole, suggesting that ATP-induced vasoconstriction reflects activation of P2 receptors in distal arteriolar segments but partially via activation of A1 receptors in more proximal arteriolar regions. Using blood-perfused rat juxtamedullary nephron afferent arterioles, superfusion of ATP; α, β-methylene ATP; or β, γ-methylene ATP evokes biphasic afferent arteriolar vasoconstriction with a rapid initial vasoconstriction, followed by a stable plateau phase.6,24,36 Sustained P2 receptor–mediated vasoconstriction was clearly manifested in afferent arterioles at low ATP concentrations, but upstream arcuate and interlobular arteries exhibited only transient vasoconstriction at ATP concentrations <100 μmol/L. By contrast, ADP, AMP, or adenosine did not mimic the afferent arteriole vasoconstrictor profiles of ATP or α, β-methylene ATP. The magnitude of ATP-mediated vasoconstriction was enhanced during adenosine receptor blockade.35 These findings suggest that ATP-mediated vasoconstriction involves P2 receptor activation rather than stimulation of A1 receptors by ATP metabolites. Interestingly, α, β-methylene ATP-mediated vasoconstriction was blocked by P2X1 receptor desensitization induced by pulsatile exposure to α, β-methylene ATP (5.0 μmol/L for 60-second intervals).36 Importantly, ATP evoked no detectable response from efferent arterioles with ATP concentrations as high as 100 μmol/L.24-36 Because afferent arterioles are the primary resistance vessels regulating renal vascular resistance and autoregulatory efficiency, the potent vasoconstrictor influence of ATP-P2X1 receptor signaling in afferent arterioles implicates P2X1 receptors in regulating glomerular hemodynamics.

Afferent arterioles also exhibit significant vasoconstrictor responses to P2Y receptor stimulation.34,36 Application of the P2Y receptor agonist, 2-methylthio-ATP, evoked concentration-dependent vasoconstriction of afferent arterioles. UTP or ATP-γ-S, more selective agonists for P2Y2 receptors, elicited greater vasoconstrictions than 2-methylthio-ATP, providing functional evidence for P2Y2 receptor expression by afferent arterioles. These studies establish ATP as a potent vasoconstrictor of afferent arterioles and suggest that renal microvascular function is regulated by activation of P2X and P2Y receptors.

**Intracellular Signaling Pathway Used by P2X and P2Y Receptors**

Generally, P2X and P2Y receptors signal through modulation of intracellular Ca2+, albeit by different mechanisms.37-41 Studies using freshly isolated preglomerular microvascular smooth muscle cells showed that ATP and UTP both produce biphasic increases in intracellular Ca2+ concentration ([Ca2+]i), typified by a rapid peak response, followed by a more stable plateau phase.40 Removal of Ca2+ from the bath, blockade of L-type voltage-dependent calcium channels (L-VDCC) with diltiazem, or depletion of intracellular Ca2+ stores markedly attenuates the ATP-induced increase of [Ca2+]i, whereas UTP-mediated increases in [Ca2+]i are essentially unchanged by L-VDCC blockade or Ca2+-free medium.40 In contrast, P2X receptor stimulation with α, β-methylene ATP elicited a more monophasic increase in [Ca2+]i, which was eliminated by Ca2+-free medium, diltiazem, or a specific P2X1 receptor antagonist, NF-279.42 These observations are consistent with responses to ATP, UTP, or α, β-methylene ATP in afferent arterioles treated with diltiazem.37,38 Collectively, in preglomerular microvessels, vasoconstriction by P2X receptor activation is largely achieved by influx of extracellular Ca2+ via activation of L-VDCC, whereas P2Y receptor activation largely accesses Ca2+ from intracellular stores. L-VDCC activation is a prerequisite for afferent arteriolar autoregulatory responses. Elimination of P2X receptor signaling by blocking L-VDCC provided a circumstantial association between P2X receptors and renal autoregulation.

P2 receptor-induced elevation of [Ca2+]i and afferent arteriolar vasoconstriction is coupled with several intracellular signaling pathways. ATP- or α, β-methylene ATP-
mediated afferent arteriolar vasoconstriction or elevation of \([\text{Ca}^{2+}]_i\) was significantly attenuated during cytochrome P450 hydroxylase inhibition or application of a 20-hydroxyecosatetraenoic acid antagonist, suggesting that 20-hydroxyecosatetraenoic acid modulates \(\alpha\)-VDCC activity elicited by P2X receptor activation. In contrast to the dependency of P2X receptors on \([\text{Ca}^{2+}]_i\) influx in pregglomerular arterioles, recent patch clamp and \([\text{Ca}^{2+}]_i\) imaging studies in microvascular smooth muscle cells suggest that P2X receptor–induced elevation of \([\text{Ca}^{2+}]_i\) is mainly mediated by inositol trisphosphate receptors and less by ryanodine receptors.

In addition, the Rho-RhoA pathway is known to stimulate vasodilation by increasing \([\text{Ca}^{2+}]_i\) sensitivity. Inhibition of Rho-kinase activation not only attenuated ATP and \(\alpha\)-methylene ATP–mediated afferent arteriolar vasodilation but also blunted pressure-mediated vasoconstriction, whereas UTP-mediated vasoconstriction remained intact. These data implicate Rho-kinase activation in the ATP-P2X receptor signaling pathway associated with renal autoregulation.

### Renal Autoregulation

Efficient renal function depends on stable RBF and glomerular filtration rate. Kidneys achieve these stable hemodynamic conditions in part through autoregulation of afferent arteriolar resistance. Accurate adjustment of afferent arteriolar resistance provides an essential buffer preventing transmission of high arterial pressure to the glomerulus. This protective property of afferent arterioles is achieved primarily by 2 distinct mechanisms, the intrinsic myogenic response and the tubuloglomerular feedback (TGF) mechanism. Recent studies propose a third component contributing to autoregulation, but the mechanism remains unclear. The myogenic response is inherent to the preglomerular arteries and arterioles. The TGF response regulates vascular resistance in the terminal juxtaglomerular segment of the afferent arteriole in response to changes in NaCl concentration sensed by macula densa cells. Therefore, afferent arterioles are the principal resistance vessels determining renal autoregulatory efficiency. Lack of accurate resistance adjustments renders glomeruli susceptible to elevated glomerular capillary pressure in hypertension, reflected in glomerular injury and progression to renal failure.

### ATP-P2 Receptor Signaling in Renal Autoregulation

An important challenge facing renal physiologists is to understand the signaling mechanisms linking the distal tubular NaCl delivery and macula densa cells to autoregulatory function. Both ATP and its breakdown product, adenosine, are indicated as extracellular messenger molecules mediating aggregate autoregulatory behavior.

Evidence supporting extracellular ATP as a messenger molecule mediating autoregulatory behavior is derived from in vitro and in vivo work. An initial study in dogs showed that RBF autoregulation was significantly blunted during saturation of P2 receptors with continuous intra-arterial administration of ATP. Afferent arteriolar autoregulatory behavior was inhibited by deliberate P2X\(_1\) receptor desensitization, nonselective P2 receptor blockade using suramin or PPADS, and selective P2X\(_1\) receptor blockade with NF-279. RBF autoregulation was inhibited in vivo by P2 receptor blockade using either PPADS or the highly selective P2X\(_1\) antagonist, P\(1\).P\(5\)-Di-inosine-5’-pentaphosphate penta-sodium salt, yielding a passive pressure-flow relationship. In contrast, the in vivo autoregulatory response was not significantly altered during A\(_1\) receptor blockade with 8-cyclopentyl-1, 3-dipropylxanthine. Thus, an intact P2X\(_1\) receptor system appears required for manifestation of normal autoregulatory behavior.

Studies in P2X\(_1\) knockout mice provide valuable information supporting a role for ATP-P2X receptor signaling in renal autoregulation. Afferent arterioles from P2X\(_1\)-deficient mice displayed impaired pressure-mediated autoregulatory behavior. Inhibition of TGF responses by papillectomy or furosemide failed to further modify the pressure-diameter relationship in P2X\(_1\) knockout mice but not in wild-type control mice. Although TGF responses were not directly measured in these studies, the data suggested that the TGF response was blunted or absent in P2X\(_1\)-deficient mice. Meanwhile, A\(_1\) receptor–mediated vasoconstriction in P2X\(_1\)-deficient mice was similar to their wild-type littermates. These data provide compelling evidence that impaired pressure-mediated arteriolar vasoconstriction is attributed to a lack of P2X\(_1\) receptor activation in afferent arterioles rather than loss of A\(_1\) signaling.

### ATP-P2 Receptor Signaling in TGF

Renal interstitial ATP concentration correlates directly with manipulation of TGF activity, consistent with extracellular ATP as an autoregulatory signaling molecule. Microdialysis studies in anesthetized dogs showed that renal interstitial ATP concentrations correlated directly with renal arterial pressure between 130 and 75 mm Hg. The interstitial fluid ATP concentration averaged 6.5 mmol/L with renal perfusion at 130 mm Hg and decreased to 4.5 and 2.8 mmol/L during a stepwise perfusion pressure reduction to 105 and 80 mm Hg, respectively. Stimulation of TGF by distal tubular NaCl loading with acetazolamide increased the interstitial ATP concentration, which was accompanied by an increase in renal vascular resistance. The change in interstitial ATP concentration was not blocked by \(\alpha\)-VDCC blockade, indicating that ATP release occurs before vascular smooth muscle cell depolarization. The interstitial adenosine concentration remained unchanged despite changes in renal perfusion pressure. These studies suggest that ATP is released in response to autoregulatory stimuli, and renal vascular resistance correlates directly with interstitial ATP concentrations but not adenosine concentrations.

Elegant in vitro studies by Bell et al provide compelling evidence demonstrating ATP release from macula densa cells via a maxi anion channel in response to the TGF stimulation. By monitoring \([\text{Ca}^{2+}]_i\) in biosensor cells overexpressing P2X receptors, they found that increasing the luminal NaCl concentration at the macula densa significantly increased \([\text{Ca}^{2+}]_i\) in the biosensor cell when it was placed adjacent to the macula densa’s basolateral surface but not if placed adjacent to thick ascending limb cells. This is the first direct evidence that ATP is released from the basolateral...
dependent vasoconstriction by activating A1 receptors on afferent arterioles.46,53 In contrast to the study by Peti-Peterdi et al52 reported that TGF responses were blunted by A1 receptor blockade or by inhibition of 5'-nucleotidase.54 In vitro studies, TGF-mediated afferent arteriolar vasoconstriction was enhanced by application of apyrase or hexokinase but abolished by inhibiting 5'-nucleotidase or by blockade of A155,56 The TGF response remained intact in this in vitro preparation during inhibition of P2 receptors with suramin.56 Recent studies using mice deficient in A1 receptors ecto-5'-nucleotidase gene or NTPDase1 support adenosine as a TGF signaling molecule.47,57–61 TGF responses were either completely abolished or significantly attenuated. However, a recent report using in vivo micropuncture indicates that P2 receptor blockade using either PPADS or suramin did not significantly alter TGF responses in 2 different strains of mice.62 Interestingly, as noted by the author, TGF responses tended to be numerically lower during P2 receptor blockade, although not significantly so. In a set of preliminary data published in a review article, TGF responses also tended to be smaller in P2X1 receptor knockout mice.63 Small decrements in TGF magnitude could be attributed to many technical, experimental, or nonphysiologic causes; but they could also represent some "low-level interaction" between significant influences of P1 receptor activation and smaller influences from P2 receptor activation in TGF-dependent resistance adjustments. Interested readers are referred to excellent reviews on this topic.46,53,63 These results support adenosine as a mediator of TGF.

**ATP-P2 Receptor Signaling in Hypertension**

Hypertensive renal injury is a major risk factor associated with progressive cardiovascular disease. Although in past decades numerous studies have established that ATP contributes to many pathophysiological processes, including cell proliferation, necrosis, inflammation, and vascular remodeling, studies of ATP-P2 receptor signaling in hypertension are few. Knockout mice have been developed for several P2 receptor subtypes (P2X1, P2X2, P2X3, P2X4, and P2X7) and some P2Y receptor subtypes.2 Both P2X1 and P2X7 receptor-deficient mice exhibit a small but significant increase in
systolic blood pressure as measured by tail-cuff plethysmogra-
phy (116±2 mm Hg in P2X1 knockout versus 108±2 mm Hg in
wild-type control mice on a 129Ola-MF-1 genetic back-
ground).64,65 P2X4 receptor-deficient mice exhibit blunted
ATP-mediated vasodilation in cremaster muscle arterioles
and mesenteric arteries and reduced urinary excretion of
nitrate and nitrite.65 Although the role of P2X4 receptors in
regulating the renal microvasculature is unknown, this study
implies that altered ATP-P2 signaling can lead to vascular
dysfunction. Furthermore, P2X7 receptor expression is mark-
edly increased in Ren-2 hypertensive rat kidneys.8 Studies
also showed a close link between blood pressure and a
genetic variation in the region of the human P2X7 gene.66
These studies indicate that altered ATP-P2 receptor signaling
might lead to vascular dysfunction and contribute to renal
injury under hypertensive conditions.

Although the role of ATP-P2 receptor signaling in the pathophysiology of hypertension remains unclear, the in-
trstitial levels of ATP and gene expression of ectonucleotidases
are reportedly increased in Ang II and Nω-nitro-L-arginine
methyl ester–induced hypertensive rats, respectively.7,9,11
Chronic Ang II infusion for 2 weeks increased renal intersti-
tial ATP concentration from 5.6 to 11.8 nmol/L.9 Simulta-
neous treatment with the P2 receptor antagonist PPADS or
the P2Y12 receptor antagonist clopidogrel attenuated affer-
ent arteriolar hypertrophy and glomerular injury despite persist-
ent hypertension. Our group has begun investigating the
mechanisms involved in impaired renal microvascular func-
tion and autoregulation under hypertensive conditions.7,10
Studies using Ang II–infused hypertensive rats revealed that,
apart from impaired afferent arteriolar autoregulation, these
rats also exhibit impaired P2X1 receptor reactivity. Afferent
arterioles from Ang II–infused rats exhibit markedly attenu-
ated vasoconstriction to ATP and β, γ-methylene ATP when
compared with vessels from normotensive rats.7,10 Interest-
ingly, responses to P2Y1 receptor activation and P1 receptor
activation were unaffected. Parallel loss of pressure-
mediated autoregulatory vasoconstriction and P2X1 receptor
signaling is consistent with the ATP-P2X1 receptor inter-
stitial concentration being important for autoregulatory reactivity. Taken together,
these studies support the hypothesis that impaired ATP-P2
receptor signaling in hypertension could contribute to hy-
tensive renal injury through impaired autoregulatory control
of glomerular perfusion pressure.

Growing evidence suggests that inflammatory factors play
crucial roles in the development of cardiovascular disease and
hypertensive renal injury.67 Inflammatory factors, such as
nuclear factor κB and monocyte chemotactic protein 1, are
increased in hypertensive animal models, and anti-
inflammatory treatment prevents progressive renal injury.67,68
Thus, inflammatory factors may contribute to hypertension-
induced renal injury by impairing afferent arteriolar reactivity
and reducing autoregulatory capability in Ang II–infused
hypertensive rats. Interestingly, treatment with the nonspe-
cific anti-inflamatory agent pentosan polysulphate pre-
served normal afferent arteriolar autoregulatory behavior in
Ang II–infused hypertensive rats despite sustained hyperten-
sion.10 Simultaneously, afferent arteriolar responses to ATP
and β, γ-methylene ATP were also preserved. Treatment with
pentosan polysulphate also corrected the elevated plasma
transforming growth factor-β1 concentration and ameliorated
renal microvascular injury in Ang II–infused rats, suggesting
that increased transforming growth factor-β may contribute
to renal microvascular dysfunction in hypertension. This
possibility is supported by previous observations that acute
exposure to transforming growth factor-β diminished auto-
regulatory capability of afferent arterioles.69 Normalization of
autoregulatory behavior and microvascular reactivity to P2X1
receptor activation by treatment with pentosan polysulphate
supports an active role for ATP-P2X1 signaling in autoregu-
lation and suggests that inflammatory processes contribute
to the decline in autoregulatory efficiency in hypertension.
Collectively, these data suggest a potentially important mech-
anism whereby reduced P2X1-mediated vasoconstriction of
afferent arterioles accounts for impairment of autoregulation
and promotes progression to renal injury.

The mechanisms underlying impairment of ATP-P2X1
signaling in hypertension remain unclear. From studies of P2
receptor signaling in other cell types and vascular beds, we
can speculate on possible explanations for this phenomenon.
It is unlikely that impairment of P2X1 receptor activation in
Ang II–induced hypertensive rats reflects downregulation
of P2X1 receptors, because P2X1 receptor protein expression
is similar in pregglomerular microvessels from control and
hypertensive rats; however, where the protein is localized
remains to be determined.7 Chronic increases in interstitial
ATP concentration could desensitize or internalize P2X1
receptors in Ang II hypertensive rats.9 Inflammatory media-
tors invoked by hypertension could uncouple important P2X1
receptor–dependent signaling pathways, thereby separating
receptor activation from vasoconstriction. Hypertension
could alter the multimeric receptor complement expressed by
pregglomerular microvascular smooth muscle cells. Indeed,
a recent study in microvascular smooth muscle cells isolated
from rat pregglomerular microvessels suggests the presence
of heteromeric P2X1A receptors that are only sensitive to very
high concentrations of α, β-methylene ATP and NF279
compared with homomeric P2X1 receptors.19 Alteration of
the multimeric receptor profile could reduce P2X1 receptor
reactivity by shifting to a less sensitive multimeric receptor
isoform. Thus, the potential role and expression of hetero-
meric receptors of P2X1 in pregglomerular microvessels re-
main to be clarified.

Recent studies also indicate that localization of P2X
receptors in lipid rafts is important for P2X receptor signal-
ing.70 Lipid rafts are plasma membrane platforms supporting
a variety of receptor-mediated signaling cascades. Disruption
of lipid rafts with β-cyclodextrin, which moves P2X1
receptors from a lipid raft component to a non-lipid raft
component, leads to attenuated α, β-methylene ATP–
duced contractility of the rat tail artery, whereas the P2Y
receptor response was retained,70 highlighting that lipid raft
integrity is necessary for efficient P2X1 receptor signaling.
Interestingly, β-cyclodextrin also attenuated myogenic re-
ponses in de-endothelialized skeletal muscle arterioles.71
Loss of lipid raft integrity might occur in hypertension or in
inflammatory states.70,72 Although not studied in the renal
vasculature, it is possible that localization of P2X1 receptors
in lipid rafts may be altered by immune factors leading to receptor dysfunction and impaired vascular smooth muscle cell contractility.

Conclusions
Evidence strongly supports the notion that extracellular ATP is an autocrine/paracrine factor that plays important roles in regulating renal hemodynamics, microvascular reactivity, autoregulation, and tubular transport. ATP-P2X1 receptor signaling is important in renal autoregulation. Renal autoregulation and afferent arteriolar P2X1 receptor reactivity are compromised in Ang II–induced hypertension. Reduction or loss of autoregulatory efficiency in hypertension can promote hypertensive renal injury. Although inflammatory processes make important contributions to renal injury by blunting microvascular reactivity to P2X1 receptor activation and, thus, autoregulatory impairment, it is unclear how hypertension impairs P2X1 receptor activation. It is, therefore, important to understand the ontogeny of renal autoregulation and its control. It would also be very interesting to examine whether impaired P2X1 receptor signaling in P2X1 receptor–deficient mice could cause severe renal damage under hypertension. Better understanding of the mechanisms responsible for the deterioration of afferent arteriolar function in hypertension might reveal efficient therapeutic interventions for the prevention of renal injury.

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Zhengrong Guan and Edward W. Inscho

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