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 contransmitter 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 P2X6/7). P2X1 and P2X3 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, P2Y12, and P2Y14 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.5–8 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/collection 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.3–5,14–16 P2X1, P2X4, and P2X7 receptors are detected in proximal tubular microvessels.15–18 Western blot analysis and immunohistochemical staining reveal intense P2X1 receptor expression in vascular smooth muscle cells of proximal tubular microvessels, especially afferent arterioles, whereas efferent arterioles, glomeruli, and renal tubules show no detectable staining, suggesting that P2X1 receptors are predominantly expressed by proximal tubular microvessels.15–18 Recently, patch-clamp studies combined with mRNA analysis suggest that proximal tubular vascular smooth muscle cells also express heteromeric P2X1/4 receptors.19 In addition, low level P2X7 receptor expression was reported in glomeruli and proximal tubular vessels, but expression levels reportedly increase dramatically under pathological conditions, such as diabetes mellitus, inflammation, and hypertension,8 suggesting an...

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of the components for synthesis and metabolism of ATP exist in the kidney. Therefore, the extracellular ATP concentration appears tightly controlled by ectonucleotidases.

**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 arteriolar 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 \( \beta, \gamma \)-methylene ATP caused a rapid transient reduction in RBF followed by an increase of RBF in anesthetized rabbits.\(^{28}\) The increased RBF was significantly attenuated by the adenosine receptor antagonist, 8-(p-Sulfophenyl) theophylline, implicating A\(_2\) receptors in the ATP-induced increase in RBF. In a subsequent study, infusion of the P2X agonist \( \alpha, \beta \)-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 P2X\(_1\) receptor antagonist, \( \text{P1,P5-Di-inosine-5'-pentaphosphate pentasodium salt} \) to assess the role of renal P2X\(_1\) receptors in vivo.\(^{31}\) Bolus intravenous infusion of \( \alpha, \beta \)-methylene ATP dose-dependently decreased RBF. This response was prevented by P2X receptor blockade with pyridoxal-phosphate-6-azophenyl-2',4'-disulfonic acid (PPADS) or \( \text{P1,P5-Di-inosine-5'-pentaphosphate pentasodium salt} \), consistent with renal vascular expression of \( \alpha, \beta \)-methylene ATP-sensitive P2X\(_1\) and P2X\(_3\) receptors. Notably, P2X\(_1\) receptor expression has not been convincingly demonstrated for the preglomerular microvasculature,\(^{17,19}\) thereby implicating P2X\(_1\) receptors in the response to \( \alpha, \beta \)-methylene ATP and highlighting a specific role for P2X\(_1\) 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

**Figure 1.** Schematic diagram illustrating the catabolic pathways for degrading ATP to form ADP, AMP, and adenosine by a series of ectonucleotidases (NTPDases: nucleotide triphosphate diphosphohydrolases; NPPs: ectonucleotide pyrophosphatase; ecto-5'-NT: ecto-5'-nucleotidases; and ALP: ectoalkaline phosphatase). Figure shows ATP, its metabolites, and its associated intracellular signaling pathways. The signaling pathways depicted for P1 receptor activation reflect conventionally held mechanisms.\(^{23}\)
effects were eliminated by P2 receptor blockade with PPADS. 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). For example, under basal tone conditions, ATP and its agonists evoked marked vasoconstriction with the following rank order potency: \( \alpha, \beta\)-methylene ATP \( > \beta, \gamma\)-methylene ATP \( > \) ATP, \( \gamma\)-S \( > \) 2-methylthio ATP \( > \) ATP > ADP = UTP, 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 \( N^G\)-nitro-L-arginine methyl ester, removal of endothelium, or by nonspecific blockade of \( K^+ \) channels during exposure to high extracellular \( K^+ \) (25 mmol/L). Collectively, these observations indicate that P2X receptor stimulation causes vasodilation, whereas activation of P2Y receptors causes vasoconstriction or tone-dependent vasodilation. The ability of \( N^G\)-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 P2Y 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. Administration of ATP and \( \alpha, \beta\)-methylene ATP elicited concentration-dependent vasoconstriction of isolated-perfused rabbit afferent arterioles. The addition of an \( A_1 \) 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 \( A_1 \) receptors in more proximal arteriolar regions. Using blood-perfused rat juxtamedullary nephron afferent arterioles, superfusion of ATP; \( \alpha, \beta\)-methylene ATP; or \( \beta, \gamma\)-methylene ATP evokes biphasic afferent arteriolar vasoconstriction with a rapid initial vasoconstriction, followed by a stable plateau phase. 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 \(<100 \mu\text{mol/L.} \)

By contrast, ADP, AMP, or adenosine did not mimic the afferent arteriole vasoconstrictor profiles of ATP or \( \alpha, \beta\)-methylene ATP. The magnitude of ATP-mediated vasoconstriction was enhanced during adenosine receptor blockade. These findings suggest that ATP-mediated vasoconstriction involves P2 receptor activation rather than stimulation of \( A_1 \) receptors by ATP metabolites. Interestingly, \( \alpha, \beta\)-methylene ATP-mediated vasoconstriction was blocked by P2X1 receptor desensitization induced by pulsatile exposure to \( \alpha, \beta\)-methylene ATP (5.0 \( \mu\text{mol/L for 60-second intervals.} \)) Importantly, ATP evoked no detectable response from efferent arterioles with ATP concentrations as high as 100 \( \mu\text{mol/L.} \) 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. Application of the P2Y receptor agonist, 2-methylthio-ATP, evoked concentration-dependent vasoconstriction of afferent arterioles. UTP or ATP, \( \gamma\)-S, more selective agonists for P2Y receptors, elicited greater vasoconstrictions than 2-methylthio-ATP, providing functional evidence for P2Y 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 \( \text{Ca}^{2+} \), albeit by different mechanisms. Studies using freshly isolated preglomerular microvascular smooth muscle cells showed that ATP and UTP both produce biphasic increases in intracellular \( \text{Ca}^{2+} \) concentration (\([\text{Ca}^{2+}]_i\)), typified by a rapid peak response, followed by a more stable plateau phase. Removal of \( \text{Ca}^{2+} \) from the bath, blockade of \( \alpha \)-type voltage-dependent calcium channels (\( \alpha \)-VDCC) with diltiazem, or depletion of intracellular \( \text{Ca}^{2+} \) stores markedly attenuates the ATP-induced increase of \([\text{Ca}^{2+}]_i \), whereas UTP-mediated increases in \([\text{Ca}^{2+}]_i \) are essentially unchanged by \( \alpha \)-VDCC blockade or \( \text{Ca}^{2+} \)-free medium. In contrast, P2X receptor stimulation with \( \alpha, \beta\)-methylene ATP elicited a more monophasic increase in \([\text{Ca}^{2+}]_i \), which was eliminated by \( \text{Ca}^{2+} \)-free medium, diltiazem, or a specific P2X1 receptor antagonist, NF-279.

These observations are consistent with responses to ATP, UTP, or \( \alpha, \beta\)-methylene ATP in afferent arterioles treated with diltiazem. Collectively, in preglomerular microvessels, vasoconstriction by P2X receptor activation is largely achieved by influx of extracellular \( \text{Ca}^{2+} \) via activation of \( \alpha \)-VDCC, whereas P2Y receptor activation largely accesses \( \text{Ca}^{2+} \) from intracellular stores. \( \alpha \)-VDCC activation is a prerequisite for afferent arteriolar autoregulatory responses. Elimination of P2X receptor signaling by blocking \( \alpha \)-VDCC provided a circumstantial association between P2X receptors and renal autoregulation.

P2 receptor-induced elevation of \([\text{Ca}^{2+}]_i \) and afferent arteriolar vasoconstriction is coupled with several intracellular signaling pathways. ATP- or \( \alpha, \beta\)-methylene ATP-based signaling pathways include calcium-dependent mechanisms, such as calcium/calmodulin-dependent protein kinase II (CaMKII) and protein kinase C (PKC). ATP-induced calcium release can activate CaMKII, which then phosphorylates various downstream targets to mediate vasoconstriction. Furthermore, ATP has been shown to activate PKC, which can modulate various signaling pathways, including those involved in vasoconstriction. These pathways are crucial for mediating the complex effects of ATP on renal microvascular function.
mediated afferent arteriolar vasoconstriction or elevation of 
\([Ca^{2+}]_i\), was significantly attenuated during cytchrome P450 
hydroxylase inhibition or application of a 20-hydroxysteroid 
tetraenoic acid antagonist, suggesting that 20-hydroxysteroid 
tetraenoic acid modulates \(\alpha\)-VDC activity elicited by 
P2X receptor activation. In contrast to the dependency of P2X 
receptors on \(Ca^{2+}\) influx in preglomerular arterioles, recent patch clamp and \(Ca^{2+}\) imaging studies in microvascular 
smooth muscle cells suggest that P2X receptor–induced 
elevation of \([Ca^{2+}]_i\), is mainly mediated by inositol trisphos- 
phate receptors and less by ryanodine receptors.

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

Renal Autoregulation

Efficient renal function depends on stable RBF and glomerular 
filtration rate. Kidneys achieve these stable hemodynamic con-
ditions in part through autoregulation of afferent arteriolar 
resistance. Accurate adjustment of afferent arteriolar resis-
tance 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 autoregu-
lation, but the mechanism remains unclear. The myogenic 
response is inherent to the preglomerular arteries and arteri-
oles. The TGF response regulates vascular resistance in the 
terminal juxtapglomerular 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 effi-
ciency. Lack of accurate resistance adjustments renders 
glomeruli susceptible to elevated glomerular capillary pres-
sure 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 tubu-
lar \(NaCl\) 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 satura-
tion of P2 receptors with continuous intra-arterial adminis-
tration of ATP. Afferent arteriolar autoregulatory behavior 
was inhibited by deliberate P2X receptor desensitization, 
nonselective P2 receptor blockade using suramin or 
PPADS, and selective P2X receptor blockade with NF-
279. RBF autoregulation was inhibited in vivo by P2 
receptor blockade using either PPADS or the highly selective 
P2X antagonist, P4-P5-Di-inoosine-5’-pentaphosphate penta-
sodium salt, yielding a passive pressure-flow relationship. In 
contrast, the in vivo autoregulatory response was not 
significantly altered during A1 receptor blockade with 
8-cyclopentyl-1, 3-dipropylxanthine. Thus, an intact P2X1 
receptor system appears required for manifestation of normal 
autoregulatory behavior.

Studies in P2X1 knockout mice provide valuable informa-
tion supporting a role for ATP-P2X1 receptor signaling in 
renal autoregulation. Afferent arterioles from P2X1-deficient 
mouse displayed impaired pressure-mediated autoregulatory 
behavior. Inhibition of TGF responses by papillectomy or 
furosemide failed to further modify the pressure-diameter 
relationship in P2X1 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 P2X1-deficient mice. 
Meanwhile, A1 receptor–mediated vasoconstriction in P2X1-
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 P2X1 receptor activation in afferent arterioles rather 
than loss of A1 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. Microdi-
alysis studies in anesthetized dogs showed that renal intersti-
tial ATP concentrations correlated directly with renal arterial 
pressure between 130 and 75 mm Hg. The interstitial fluid 
ATP concentration averaged 6.5 nmol/L with renal perfusion 
at 130 mm Hg and decreased to 4.5 and 2.8 nmol/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 \(\alpha\)-VDC blockade, indic-
ating that ATP release occurs before vascular smooth 
muscle cell depolarization. The interstitial adenosine concen-
tration remained unchanged despite changes in renal perfu-
sion pressure. These studies suggest that ATP is released in 
response to autoregulatory stimuli, and renal vascular resis-
tance 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 stimula-
tion. By monitoring \([Ca^{2+}]_i\), in biosensor cells overexpress-
ping P2X receptors, they found that increasing the luminal 
NaCl concentration at the macula densa significantly in-
creased \([Ca^{2+}]_i\), 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
surface of macula densa in response to a TGF stimulus (Figure 2). These data support the idea that TGF signals begin with ATP release from macula densa cells; however, whether ATP released from the macula densa cells acts directly on P2 receptors of afferent arterioles or whether ATP is degraded by ectonucleotidases to adenosine leading to A₁ receptor–dependent TGF adjustments remains to be determined.53

The studies from Bell et al.5 were extended by combining confocal imaging techniques and highly sensitive calcium fluorophores to directly link TGF activity, ATP release, and afferent arteriolar responses.53 Peti-Peterdi described that activation of TGF signals triggered rapid propagation of a Ca²⁺ wave from the macula densa toward the afferent arteriole and glomerulus, leading to significant arteriolar constriction within 10 seconds. Propagation of Ca²⁺ signals and reduction of arteriolar diameter were eliminated by inhibiting P2 receptor activation with suramin or by inhibition of ecto-5'-nucleotidase or by blockade of P2_Y receptors of afferent arterioles or whether ATP is degraded by ectonucleotidases to adenosine leading to A₁ receptor–dependent TGF adjustments remains to be determined.53

In vitro studies, TGF-mediated afferent arteriolar vasoconstriction was enhanced by application of apyrase or hexokinase but abolished by inhibiting ecto-5'-nucleotidase or by blockade of A₁ receptor.55,56 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, P2X₇, and P2Y₁) and some P2Y receptor subtypes. Both P2X₁ and P2X₇ receptor-deficient mice exhibit a small but significant increase in

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**Figure 2.** Schematic diagram representing the postulated tubuloglomerular feedback (TGF) mechanism in the juxtaglomerular region. Increased luminal [NaCl] delivery stimulates Na-K-2Cl, which leads to ATP release from the basolateral membrane of macula densa cells via maxi anion channels. ATP vasoconstricts afferent arterioles via activating P2X₁ receptors, and/or adenosine (ado) converted from ATP by ectonucleotidase pyrophosphatase (NPPs), ectonucleoside triphosphate diphosphohydrolases (NTPD), and ecto-5'-nucleotidases (ecto-5'-NT), respectively, vasoconstrict afferent arterioles via activating A₁ receptors.
systolic blood pressure as measured by tail-cuff plethysmography (116±2 mm Hg in P2X$_4$ knockout versus 108±2 mm Hg in wild-type control mice on a 129Ola-MF-1 genetic background). P2X$_4$ receptor-deficient mice exhibit blunted ATP-mediated vasodilation in cremaster muscle arterioles and mesenteric arteries and reduced urinary excretion of nitrate and nitrite. Although the role of P2X$_4$ receptors in regulating the renal microvasculature is unknown, this study implies that altered ATP-P2 signaling can lead to vascular dysfunction. Furthermore, P2X$_7$ receptor expression is markedly increased in Ren-2 hypertensive rat kidneys. Studies also showed a close link between blood pressure and a genetic variation in the region of the human P2X7 gene. 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 P2X1 receptor signaling in the pathophysiology of hypertension remains unclear, the interstitial levels of ATP and gene expression of ectonucleotidases are reportedly increased in Ang II and N$\text{^6}$-nitro-l-arginine methyl ester–induced hypertensive rats, respectively. Chronic Ang II infusion for 2 weeks increased renal interstitial ATP concentration from 5.6 to 11.8 nmol/L. Simultaneous treatment with the P2 receptor antagonist PPADS or N$\text{^6}$-methylene ATP were also preserved. Treatment with pentosan polysulphate also corrected the elevated plasma transforming growth factor-$\beta$ concentration and ameliorated renal microvascular injury in Ang II–infused rats, suggesting that increased transforming growth factor-$\beta$ may contribute to renal microvascular dysfunction in hypertension. This possibility is supported by previous observations that acute exposure to transforming growth factor-$\beta$ diminished autoregulatory capability of afferent arterioles. Normalization of autoregulatory behavior and microvascular reactivity to P2X$_1$ receptor activation by treatment with pentosan polysulphate supports an active role for ATP-P2X$_1$ signaling in autoregulation and suggests that inflammatory processes contribute to the decline in autoregulatory efficiency in hypertension. Collectively, these data suggest a potentially important mechanism whereby reduced P2X$_1$-mediated vasoconstriction of afferent arterioles accounts for impairment of autoregulation and promotes progression to renal injury.

The mechanisms underlying impairment of ATP-P2X$_1$ 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 P2X$_1$ receptor activation in Ang II–induced hypertensive rats reflects downregulation of P2X$_1$ receptors, because P2X$_1$ receptor protein expression is similar in preglomerular microvessels from control and hypertensive rats; however, where the protein is localized remains to be determined. Chronic increases in interstitial ATP concentration could desensitize or internalize P2X$_1$ receptors in Ang II hypertensive rats. Inflammatory mediators invoked by hypertension could uncouple important P2X$_1$ receptor–dependent signaling pathways, thereby separating receptor activation from vasoconstriction. Hypertension could alter the multimeric receptor complement expressed by preglomerular microvascular smooth muscle cells. Indeed, a recent study in microvascular smooth muscle cells isolated from rat preglomerular microvessels suggests the presence of heteromeric P2X$_1$/P2Y$_1$ receptors that are only sensitive to very high concentrations of $\alpha$, $\gamma$-methylene ATP and NF279 compared with homomeric P2X$_1$ receptors. Alteration of the multimeric receptor profile could reduce P2X$_1$ receptor reactivity by shifting to a less sensitive multimeric receptor isoform. Thus, the potential role and expression of heteromeric receptors of P2X$_1$ in preglomerular microvessels remain to be clarified.

Recent studies also indicate that localization of P2X receptors in lipid rafts is important for P2X receptor signaling. Lipid rafts are plasma membrane platforms supporting a variety of receptor-mediated signaling cascades. Disruption of lipid rafts with $\beta$-cyclodextrin, which moves P2X$_1$ receptors from a lipid raft component to a non-lipid raft component, leads to attenuated $\alpha$, $\beta$-methylene ATP–induced contractility of the rat tail artery, whereas the P2Y receptor response was retained, highlighting that lipid raft integrity is necessary for efficient P2X$_1$ receptor signaling. Interestingly, $\beta$-cyclodextrin also attenuated myogenic responses in de-endothelialized skeletal muscle arterioles. Loss of lipid raft integrity might occur in hypertension or in inflammatory states. Although not studied in the renal vasculature, it is possible that localization of P2X$_1$ 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 impacts 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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References


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