Published in final edited form as:
Hypertension. 2011 September ; 58(3): 333–340. doi:10.1161/HYPERTENSIONAHA.110.155952.

ROLE OF ATP IN REGULATING RENAL MICROVASCULAR FUNCTION AND IN HYPERTENSION

Zhengrong Guan and **Edward W. Inscho***
Department of Physiology, Georgia Health Sciences University, Augusta, Georgia

Abstract

Adenosine triphosphate (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.

Introduction

Since ATP's discovery as a co-neurotransmitter 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.[2] Extracellular ATP exerts its effects by activating distinct P2 purinoceptors, P2X and P2Y (Fig 1). P2X receptors are ligand-gated ion channels and exist as homotrimers ($P2X_1$–$P2X_7$), meaning that three of the same subunit-type associate to form a functional channel. They can also associate as multi-meric complexes involving different types of P2X receptor subunits to form functional channels ($P2X_{1/2}$, $P2X_{1/5}$, $P2X_{2/3}$, $P2X_{2/6}$, $P2X_{4/6}$, and $P2X_{1/4}$). $P2X_1$ and $P2X_3$ 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 eight members ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$). UTP is a potent agonist for $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors.

***Correspondence and Reprint Requests to:** Edward W. Inscho, PhD, Department of Physiology CA3137, Georgia Health Sciences University, 1120, 15th Street, Augusta, Georgia 30912, U.S.A. Phone: 706-721-5615, Fax: 706-721-7299, einscho@georgiahealth.edu.

Disclosures
None.

The importance of P2 receptors in regulating renal function has clarified over the past two 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. 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 at least one P2 receptor subtype. Each tubular segment expresses different P2X and P2Y receptor subtypes at apical or basolateral membranes or both. $P2Y_2$ receptors expressed in distal tubule/collecting duct are important for sodium and fluid reabsorption. Deletion of $P2Y_2$ receptors reportedly leads to salt-resistant hypertension[12] and the $P2Y_2$ 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]

$P2X_1$, $P2X_2$ and $P2X_4$ receptors are detected in preglomerular microvessels.[7, 15–18] Western blot analysis and immunohistochemical staining reveal intense $P2X_1$ receptor expression in vascular smooth muscle cells of preglomerular microvessels, especially afferent arterioles whereas efferent arterioles, glomeruli and renal tubules show no detectable staining, suggesting that $P2X_1$ receptors are predominantly expressed by preglomerular microvessels.[7, 16–18] Recently, patch-clamp studies combined with mRNA analysis suggest that preglomerular vascular smooth muscle cells also express heteromeric $P2X_{1/4}$ receptors.[19] Additionally, low level $P2X_7$ receptor expression was reported in glomeruli and preglomerular vessels, but expression levels reportedly increase dramatically under pathological conditions such as diabetes, inflammation, and hypertension,[8] suggesting an important role of $P2X_7$ receptors in inflammatory processes. Afferent arterioles also express $P2Y_1$ and $P2Y_2$ receptors, whereas efferent arterioles express only $P2Y_1$ 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 upon stimulation but it can also be released from non-excitatory cells.[20] Evidence suggests that ATP is released from glomerular cells,[21] juxtaglomerular cells,[22] macula densa,[5] and proximal tubular epithelium.[23] In the normal kidney, the extracellular ATP concentration is estimated to be 1 nmol/L in glomeruli,[21] 6~9 nmol/L in interstitial fluid,[9, 24] 100–300 nmol/L in proximal and 33 nmol/L in distal tubular fluid,[23] but may increase markedly under pathological conditions.[9, 11, 20] Extracellular ATP concentrations between 10 and 100 nmol/L vasoconstrict juxtamedullary 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 catabolized *in vivo* by ecto-nucleotidases (Fig 1), classified as ectonucleotide pyrophosphatase phosphodiesterases (NPP), ectonucleoside triphosphate diphosphohydrolases (NTPD), ecto-5'-nucleotidase (Ecto-5'-NT), and alkaline phosphatase. Among them, NTPDase 1, NTPDase2, NTPDase3, NPP1, NPP3, and Ecto-5'-NT are

expressed in rat or mouse kidneys with varying degrees of expression along the nephron, vasculature and peritubular spaces.[27, 28] Although the functional significance of intrarenal ecto-nucleotidases is poorly understood, all components for synthesis and metabolism of ATP exist in the kidney. Therefore, the extracellular ATP concentration appears tightly controlled by ecto-nucleotidases.

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 nitric oxide (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 A_2 receptors in the ATP-induced increase in RBF. In a subsequent study, infusion of the P2X agonist α, β-methylene ATP reduced RBF, 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 $P2X_1$ and $P2X_3$ receptor antagonist, P_1, P_5-Di-inosine-5'-pentaphosphate pentasodium salt (IP5I) to assess the role of renal $P2X_1$ 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 IP5I, consistent with renal vascular expression of α, β-methylene ATP-sensitive $P2X_1$ and $P2X_3$ receptors. Notably, $P2X_3$ receptor expression has not been convincingly demonstrated for the preglomerular microvasculature,[17, 19] thereby implicating $P2X_1$ receptors in the response to α, β-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 low-salt (0.15%). 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 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 vs. non-preconstricted).[33, 34] For example, under basal tone conditions, ATP and its agonists evoked marked vasoconstriction with the 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 pre-constricted, 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 L-NAME, removal of endothelium or by nonspecific blockade of K^+ channels during exposure to high extracellular K^+ (25 mmole/L).[33, 34] Collectively, these observations indicate that P2X receptor stimulation causes vasoconstriction while activation of P2Y receptors causes vasoconstriction or tone-dependent vasodilation. The ability of L-NAME 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 nitric oxide 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 clarified.

P2 Receptor Action on Pre- and Post-glomerular 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.[6, 24, 25, 35, 36] Administration of ATP and α, β-methylene ATP elicited concentration-dependent vasoconstriction of isolated-perfused rabbit afferent arterioles.[35] 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, 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 transit vasoconstriction at ATP concentrations below 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.[25] These findings suggest that ATP-mediated vasoconstriction involves P2 receptor activation rather than stimulation of A_1 receptors by ATP metabolites. Interestingly, α, β-methylene ATP-mediated vasoconstriction was blocked by $P2X_1$ receptor desensitization induced by pulsatile exposure to α, β-methylene ATP (5.0 μmol/L 60 sec intervals).[26] Importantly, ATP evoked no detectable response from efferent arterioles with ATP concentrations as high as 100 μmol/L.[6, 24, 36] Since afferent arterioles are the primary resistance vessels regulating renal vascular resistance and autoregulatory efficiency, the potent vasoconstrictor influence of ATP-$P2X_1$ receptor signaling in afferent arterioles implicates $P2X_1$ receptors in regulating glomerular hemodynamics.

Afferent arterioles also exhibit significant vasoconstrictor responses to P2Y receptor stimulation.[24, 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 $P2Y_2$ receptors, elicited greater vasoconstrictions than 2-methylthio-ATP, providing functional evidence for $P2Y_2$ 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 Utilized by P2X and P2Y Receptors

Generally, P2X and P2Y receptors signal through modulation of intracellular Ca^{2+}, albeit by different mechanisms.[37–41] Studies using freshly isolated preglomerular microvascular smooth muscle cells (MVSMC)[38, 40, 42] showed that ATP and UTP both produce biphasic increases in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) typified by a rapid peak response followed by a more stable plateau phase.[40] Removal of Ca^{2+} from the bath, blockade of L-type voltage-dependent calcium channels (L-VDCC) with diltiazem, or depletion of intracellular Ca^{2+} stores markedly attenuates the ATP-induced increase of $[Ca^{2+}]_i$ while UTP-mediated increases in $[Ca^{2+}]_i$ are essentially unchanged by L-VDCC blockade or Ca^{2+}-free medium.[40] In contrast, P2X receptor stimulation with α, β-methylene ATP elicited a more monophasic increase in $[Ca^{2+}]_i$ which was eliminated by Ca^{2+}-free medium, diltiazem or a specific $P2X_1$ 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 Ca^{2+} via activation of L-VDCC, whereas P2Y receptor activation largely accesses Ca^{2+} 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 $[Ca^{2+}]_i$ and afferent arteriolar vasoconstriction is coupled with several intracellular signaling pathways. ATP- or α, β-methylene ATP-mediated afferent arteriolar vasoconstriction, or elevation of $[Ca^{2+}]_i$ was significantly attenuated during cytochrome P450 hydroxylase inhibition or application of a 20-hydroxyeicosatetraenoic acid (20-HETE) antagonist,[43, 44] suggesting that 20-HETE modulates L-VDCC 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 MVSMC suggest that P2X receptor-induced elevation of $[Ca^{2+}]_i$ is mainly mediated by inositol trisphosphate receptors and less by ryanodine receptors.[41]

Additionally, the Rho-RhoA pathway is known to stimulate vasoconstriction by increasing Ca^{2+} sensitivity. Inhibition of Rho-kinase activation not only attenuated ATP and α, β-methylene ATP-mediated afferent arteriolar vasoconstriction but also blunted pressure-mediated vasoconstriction while UTP-mediated vasoconstriction remained intact.[45] 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 two distinct mechanisms, the intrinsic myogenic response and the tubuloglomerular feedback (TGF) mechanism.[6, 46] Recent studies propose a third component contributing to autoregulation but the mechanism remains unclear.[47] 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.[48]

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 behaviour 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.[49] Afferent arteriolar autoregulatory behavior was inhibited by deliberate $P2X_1$-receptor desensitization,[26] non-selective P2 receptor blockade using suramin or PPADS,[36] and selective $P2X_1$ receptor blockade with NF-279.[50] RBF autoregulation was inhibited *in vivo* by P2 receptor blockade using either PPADS or the highly selective $P2X_1$ antagonist, IP5I, yielding a passive pressure-flow relationship.[31] In contrast, the *in vivo* autoregulatory response was not significantly altered during A_1 receptor blockade with 8-cyclopentyl-1, 3-dipropylxanthine (DPCPX). Thus an intact $P2X_1$ receptor system appears required for manifestation of normal autoregulatory behaviour.

Studies in $P2X_1$ knockout mice provide valuable information supporting a role of ATP-$P2X_1$ receptor signaling in renal autoregulation. Afferent arterioles from $P2X_1$-deficient mice displayed impaired pressure-mediated autoregulatory behavior.[50] 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. While 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 due 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.[24, 51] Microdialysis studies in anesthetized dogs showed that renal interstitial ATP concentrations correlated directly with renal arterial pressure between 130 to 75 mmHg. The interstitial fluid ATP concentration averaged 6.5 nmol/L with renal perfusion at 130 mmHg and decreased to 4.5 and 2.8 nmol/L during a stepwise perfusion pressure reduction to 105 and 80 mmHg, 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 L-VDCC blockade,[51] indicating that ATP release occurs prior to vascular smooth muscle cell depolarization. The interstitial adenosine concentration remained unchanged despite changes in renal perfusion pressure.[24] 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 and co-workers provide compelling evidence demonstrating ATP release from macula densa cells via a maxi anion channel in response to the TGF

stimulation.[5, 52] By monitoring $[Ca^{2+}]_i$ in biosensor cells over-expressing P2X receptors, they found that increasing the luminal NaCl concentration at the macula densa significantly increased $[Ca^{2+}]_i$ 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.[5] This is the first direct evidence that ATP is released from the basolateral surface of macula densa in response to a TGF stimulus (Fig. 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 ecto-nucleotidases to adenosine leading to A_1 receptor-dependent TGF adjustments remains to be determined.[53]

The studies from Bell's group were extended by combining confocal imaging techniques and highly sensitive calcium fluorophores, to directly link TGF activity, ATP release and afferent arteriolar responses.[52] Peti-Peterdi et al. reported that activation of TGF signals triggered rapid propagation of a Ca^{2+} wave from the macula densa towards the afferent arteriole and glomerulus, leading to significant arteriolar constriction within 10 seconds.[52] Propagation of Ca^{2+} signals and reduction of arteriolar diameter was eliminated by inhibiting P2 receptor activation with suramin or suffusing with ATP hydrolyzing enzymes (apyrase and hexokinase), but not by A_1 receptor blockade with DPCPX.[52] These studies suggest that ATP released from the macula densa cells, rather than adenosine metabolized from ATP, plays an essential role in transmitting TGF signals to the afferent arterioles. Overall, these observations support the hypothesis that extracellular ATP is an important paracrine signaling molecule participating in TGF responses and regulating afferent arteriolar resistance, but they also underscore the need for more direct micropuncture studies.

Adenosine-P1 receptor signaling in TGF

ATP released from the macula densa could be rapidly degraded to adenosine by ecto-nucleotidases expressed in renal interstitial tissue and microvessels prior to P2 receptor activation.[27, 28] Adenosine is postulated to mediate TGF-dependent vasoconstriction by activating A_1 receptors on afferent arterioles.[46, 53] In contrast to the Peti-Peterdi study described in the previous paragraph,[52] micropuncture studies showed that TGF responses were blunted by A_1 receptor blockade or by inhibition of 5'-nucleotidase.[54] In *in-vitro* studies, TGF-mediated afferent arteriolar vasoconstriction was enhanced by application of apyrase or hexokinase but abolished by inhibiting ecto-5'-NT or by blockade of A_1.[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 A_1 receptors, ecto-5'-NT 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 employing *in vivo* micropuncture indicates that P2 receptor blockade using either PPADS or suramin did not significantly alter TGF responses in two 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 paper, TGF responses also tended to be smaller in $P2X_1$ receptor knockout mice.[63] Small decrements in TGF magnitude could be attributed to many technical, experimental or nonphysiologic causes, but it 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 the past decades, numerous studies have established that ATP contributes to many pathophysiolgical 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 ($P2X_1$, $P2X_2$, $P2X_3$, $P2X_4$, $P2X_7$) and some P2Y receptor subtypes.[2] Both $P2X_1$ and $P2X_4$ receptor deficient mice exhibit a small but significant increase in systolic blood pressure as measured by tail cuff plethysmography (116±2 in $P2X_1$ knockout vs. 108±2 mmHg in wild-type control mice on a 129Ola-MF-1 genetic background).[64, 65] $P2X_4$ receptor-deficient mice exhibit blunted ATP-mediated vasodilation in cremaster muscle arterioles and mesenteric arteries, and reduced urinary excretion of nitrate and nitrite.[65] 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.[8]

Studies also showed a close link between blood pressure and a genetic variation in the region of the human $P2X_7$ 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 interstitial levels of ATP and gene expression of ecto-nucleotidases are reportedly increased in Ang II and L-NAME-induced hypertensive rats, respectively.[9, 11] Chronic Ang II infusion for 2 weeks increased renal interstitial ATP concentration from 5.6 to 11.8 nmol/L.[9] Simultaneous treatment with the P2 receptor antagonist, PPADS, or the $P2Y_{12}$ receptor antagonist, clopidogrel, attenuated afferent arteriolar hypertrophy and glomerular injury despite persistent hypertension. Our group has begun investigating the mechanisms involved in impaired renal microvascular function 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 $P2X_1$ receptor reactivity. Afferent arterioles from Ang II-infused rats exhibit markedly attenuated vasoconstriction to ATP and β, γ-methylene ATP when compared to vessels from normotensive rats.[7, 10] Interestingly, responses to $P2Y_2$ receptor activation and P1 receptor activation were unaffected.[7] Parallel loss of pressure-mediated autoregulatory vasoconstriction and $P2X_1$ receptor signaling is consistent with the ATP-$P2X_1$ receptor system being important for autoregulatory reactivity. Taken together, these studies support the hypothesis that impaired ATP-P2 receptor signaling in hypertension could contribute to hypertensive 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 non-specific anti-inflammatory agent, pentosan polysulphate (PPS) preserved normal afferent arteriolar autoregulatory behavior in Ang II-infused hypertensive rats despite sustained hypertension.[10] Simultaneously, afferent arteriolar responses to ATP and β, γ-methylene ATP were also preserved. Treatment with PPS also corrected the elevated plasma transforming growth factor-beta 1 (TGF-β1)

concentration and ameliorated renal microvascular injury in Ang II-infused rats, suggesting that increased TGF-β may contribute to renal microvascular dysfunction in hypertension. This possibility is supported by previous observations that acute exposure to TGF-β diminished autoregulatory capability of afferent arterioles.[69] Normalization of autoregulatory behavior and microvascular reactivity to $P2X_1$ receptor activation by treatment with PPS 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 to this phenomenon. It is unlikely that impairment of $P2X_1$ receptor activation in Ang II-induced hypertensive rats reflects down-regulation of $P2X_1$ receptors as $P2X_1$ receptor protein expression is similar in preglomerular microvessels from control and hypertensive rats; however where the protein is localized remains to be determined.[7] Chronic increases in interstitial ATP concentration could desensitize or internalize $P2X_1$ receptors in Ang II hypertensive rats.[9] 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 MVSMC. Indeed a recent study in MVSMC isolated from rat preglomerular microvessels suggests the presence of heteromeric $P2X_{1/4}$ receptors that are only sensitive to very high concentrations of α, β-methylene ATP and NF279 compared to homomeric $P2X_1$ receptors.[19] 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 remains to be clarified.

Recent studies also indicate that localization of P2X receptors in lipid-rafts is important for P2X receptor signaling.[70] Lipid-rafts are plasma membrane platforms supporting a variety of receptor-mediated signaling cascades. Disruption of lipid-rafts with β-cyclodextrin which moves $P2X_1$ receptors from a lipid-raft component to a non-lipid-raft component, leads to attenuated α, β-methylene ATP-induced contractility of the rat tail artery, while the P2Y receptor response was retained,[70] highlighting that lipid-raft integrity is necessary for efficient $P2X_1$ receptor signaling. Interestingly, β-cyclodextrin also attenuated myogenic responses 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 $P2X_1$ receptors in lipid-rafts may be altered by immune-factors leading to receptor dysfunction and impaired vascular smooth muscle cell contractility.

Conclusion

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-$P2X_1$ receptor signaling is important in renal autoregulation. Renal autoregulation and afferent arteriolar $P2X_1$ 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 $P2X_1$ receptor activation and thus autoregulatory impairment, it is unclear how hypertension impairs $P2X_1$ receptor activation. It is therefore important to understand the ontogeny of

renal autoregulation and its control. It would be also very interesting to examine if impaired $P2X_1$ receptor signaling in $P2X_1$ 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 prevention of renal injury.

Acknowledgments

Sources of Funding

Some of the work in this review was supported by National Institute of Health grants (DK44628, HL074167 and HL098135) for E.W.I. and grants from the American Heart Association (0825465E and 10SDG3770010) for Z.G.

References

1. Burnstock G. Purinergic nerves. Pharmacol Rev. 1972; 24:509–581. [PubMed: 4404211]
2. Burnstock G, Verkhratsky A. Long-term (trophic) purinergic signalling: purinoceptors control cell proliferation, differentiation and death. Cell Death Dis. 2010; 1:e9. [PubMed: 21364628]
3. Schwiebert EM, Kishore BK. Extracellular nucleotide signaling along the renal epithelium. Am J Physiol Renal Physiol. 2001; 280:F945–F963. [PubMed: 11352834]
4. Bailey MA, Shirley DG. Effects of extracellular nucleotides on renal tubular solute transport. Purinergic Signal. 2009; 5:473–480. [PubMed: 19308675]
5. Bell PD, Komlosi P, Zhang ZR. ATP as a mediator of macula densa cell signalling. Purinergic Signal. 2009; 5:461–471. [PubMed: 19330465]
6. Inscho EW. ATP, P2 receptors and the renal microcirculation. Purinergic Signal. 2009; 5:447–460. [PubMed: 19294530]
7. Zhao X, Cook AK, Field M, Edwards B, Zhang S, Zhang Z, Pollock JS, Imig JD, Inscho EW. Impaired Ca^{2+} signaling attenuates P2X receptor-mediated vasoconstriction of afferent arterioles in angiotensin II hypertension. Hypertension. 2005; 46:562–568. [PubMed: 16116048]
8. Vonend O, Turner CM, Chan CM, Loesch A, Dell'Anna GC, Srai KS, Burnstock G, Unwin RJ. Glomerular expression of the ATP-sensitive P2X receptor in diabetic and hypertensive rat models. Kidney Int. 2004; 66:157–166. [PubMed: 15200422]
9. Graciano ML, Nishiyama A, Jackson K, Seth DM, Ortiz RM, Prieto-Carrasquero MC, Kobori H, Navar LG. Purinergic receptors contribute to early mesangial cell transformation and renal vessel hypertrophy during angiotensin II-induced hypertension. Am J Physiol Renal Physiol. 2008; 294:F161–F169. [PubMed: 17989111]
10. Guan Z, Fuller BS, Yamamoto T, Cook AK, Pollock JS, Inscho EW. Pentosan polysulfate treatment preserves renal autoregulation in Ang II-infused hypertensive rats via normalization of $P2X_1$ receptor activation. Am J Physiol Renal Physiol. 2010; 298:F1276–F1284. [PubMed: 20200092]
11. Furstenau CR, Ramos DB, Vuaden FC, Casali EA, Monteiro Pde S, Trentin Dda S, Gossenheimer AN, Bogo MR, Bonan CD, Barreto-Chaves ML, Sarkis JJ, Wofchuk ST. L-NAME-treatment alters ectonucleotidase activities in kidney membranes of rats. Life Sci. 2010; 87:325–332. [PubMed: 20655932]
12. Rieg T, Vallon V. ATP and adenosine in the local regulation of water transport and homeostasis by the kidney. Am J Physiol Regul Integr Comp Physiol. 2009; 296:R419–R427. [PubMed: 19020292]
13. Wang Z, Nakayama T, Sato N, Izumi Y, Kasamaki Y, Ohta M, Soma M, Aoi N, Ozawa Y, Ma Y. The purinergic receptor P2Y, G-protein coupled, 2 (P2RY2) gene associated with essential hypertension in Japanese men. J Hum Hypertens. 2010; 24:327–335. [PubMed: 19710694]
14. Kishore BK, Nelson RD, Miller RL, Carlson NG, Kohan DE. $P2Y_2$ receptors and water transport in the kidney. Purinergic Signal. 2009; 5:491–499. [PubMed: 19319665]

15. Bailey MA, Turner CM, Hus-Citharel A, Marchetti J, Imbert-Teboul M, Milner P, Burnstock G, Unwin RJ. P2Y receptors present in the native and isolated rat glomerulus. Nephron Physiol. 2004; 96:79–90.

16. Chan CM, Unwin RJ, Bardini M, Oglesby IB, Ford AP, Townsend-Nicholson A, Burnstock G. Localization of $P2X_1$ purinoceptors by autoradiography and immunohistochemistry in rat kidneys. Am J Physiol Renal Physiol. 1998; 274:F799–F804.

17. Lewis CJ, Evans RJ. P2X receptor immunoreactivity in different arteries from the femoral, pulmonary, cerebral, coronary and renal circulations. J Vasc Res. 2001; 38:332–340. [PubMed: 11455204]

18. Turner CM, Vonend O, Chan C, Burnstock G, Unwin RJ. The pattern of distribution of selected ATP-sensitive P2 receptor subtypes in normal rat kidney: an immunohistological study. Cells Tissues Organs. 2003; 175:105–117. [PubMed: 14605489]

19. Harhun MI, Povstyan OV, Gordienko DV. Purinoreceptor-mediated current in myocytes from renal resistance arteries. Br J Pharmacol. 2010; 160:987–997. [PubMed: 20590593]

20. Erlinge D, Burnstock G. P2 receptors in cardiovascular regulation and disease. Purinergic Signal. 2008; 4:1–20. [PubMed: 18368530]

21. Karczewska J, Martyniec L, Dzierzko G, Stepinski J, Angielski S. The relationship between constitutive ATP release and its extracellular metabolism in isolated rat kidney glomeruli. J Physiol Pharmacol. 2007; 58:321–333. [PubMed: 17622700]

22. Yao J, Suwa M, Li B, Kawamura K, Morioka T, Oite T. ATP-dependent mechanism for coordination of intercellular Ca^{2+} signaling and renin secretion in rat juxtaglomerular cells. Circ Res. 2003; 93:338–345. [PubMed: 12869388]

23. Vekaria RM, Unwin RJ, Shirley DG. Intraluminal ATP concentrations in rat renal tubules. J Am Soc Nephrol. 2006; 17:1841–1847. [PubMed: 16790512]

24. Nishiyama A, Rahman M, Inscho EW. Role of interstitial ATP and adenosine in the regulation of renal hemodynamics and microvascular function. Hypertens Res. 2004; 27:791–804. [PubMed: 15824461]

25. Inscho EW, Mitchell KD, Navar LG. Extracellular ATP in the regulation of renal microvascular function. Faseb J. 1994; 8:319–328. [PubMed: 8143938]

26. Inscho EW, Cook AK, Navar LG. Pressure-mediated vasoconstriction of juxtamedullary afferent arterioles involves P2-purinoceptor activation. Am J Physiol Renal Physiol. 1996; 271:F1077–F1085.

27. Kishore BK, Isaac J, Fausther M, Tripp SR, Shi H, Gill PS, Braun N, Zimmermann H, Sevigny J, Robson SC. Expression of NTPDase1 and NTPDase2 in murine kidney: relevance to regulation of P2 receptor signaling. Am J Physiol Renal Physiol. 2005; 288:F1032–F1043. [PubMed: 15632415]

28. Shirley DG, Vekaria RM, Sevigny J. Ectonucleotidases in the kidney. Purinergic Signal. 2009; 5:501–511. [PubMed: 19333785]

29. Eppel GA, Ventura S, Evans RG. Regional vascular responses to ATP and ATP analogues in the rabbit kidney *in vivo*: roles for adenosine receptors and prostanoids. Br J Pharmacol. 2006; 149:523–531. [PubMed: 16981003]

30. Eppel GA, Ventura S, Denton KM, Evans RG. Lack of contribution of P2X receptors to neurally mediated vasoconstriction in the rabbit kidney *in vivo*. Acta Physiol. 2006; 186:197–207.

31. Osmond DA, Inscho EW. $P2X_1$ receptor blockade inhibits whole kidney autoregulation of renal blood flow in vivo. Am J Physiol Renal Physiol. 2010; 298:F1360–F1368. [PubMed: 20335318]

32. Dobrowolski L, Walkowska A, Kompanowska-Jezierska E, Kuczeriszka M, Sadowski J. Effects of ATP on rat renal haemodynamics and excretion: role of sodium intake, nitric oxide and cytochrome P450. Acta Physiol. 2007; 189:77–85.

33. Eltze M, Ullrich B. Characterization of vascular P2 purinoceptors in the rat isolated perfused kidney. Eur J Pharmacol. 1996; 306:139–152. [PubMed: 8813626]

34. Wangensteen R, Fernandez O, Sainz J, Quesada A, Vargas F, Osuna A. Contribution of endothelium-derived relaxing factors to P2Y-purinoceptor-induced vasodilation in the isolated rat kidney. Gen Pharmacol. 2000; 35:129–133. [PubMed: 11744234]

35. Weihprecht H, Lorenz JN, Briggs JP, Schnermann J. Vasomotor effects of purinergic agonists in isolated rabbit afferent arterioles. Am J Physiol Renal Physiol. 1992; 263:F1026–F1033.
36. Inscho EW. P2 receptors in regulation of renal microvascular function. Am J Physiol Renal Physiol. 2001; 280:F927–F944. [PubMed: 11352833]
37. Inscho EW, Ohishi K, Cook AK, Belott TP, Navar LG. Calcium activation mechanisms in the renal microvascular response to extracellular ATP. Am J Physiol Renal Physiol. 1995; 268:F876–F884.
38. Inscho EW, Cook AK. P2 receptor-mediated afferent arteriolar vasoconstriction during calcium blockade. Am J Physiol Renal Physiol. 2002; 282:F245–F255. [PubMed: 11788438]
39. Gutierrez AM, Kornfeld M, Persson AE. Calcium response to adenosine and ATP in rabbit afferent arterioles. Acta Physiol Scand. 1999; 166:175–181. [PubMed: 10468653]
40. Inscho EW, LeBlanc EA, Pham BT, White SM, Imig JD. Purinoceptor-mediated calcium signaling in preglomerular smooth muscle cells. Hypertension. 1999; 33:195–200. [PubMed: 9931104]
41. Povstyan OV, Harhun MI, Gordienko DV. Ca^{2+} entry following P2X receptor activation induces IP3 receptor-mediated Ca^{2+} release in myocytes from small renal arteries. Br J Pharmacol. 2010; 162:1618–1638. [PubMed: 21175582]
42. White SM, Imig JD, Kim TT, Hauschild BC, Inscho EW. Calcium signaling pathways utilized by P2X receptors in freshly isolated preglomerular MVSMC. Am J Physiol Renal Physiol. 2001; 280:F1054–F1061. [PubMed: 11352845]
43. Zhao X, Inscho EW, Bondlela M, Falck JR, Imig JD. The CYP450 hydroxylase pathway contributes to P2X receptor-mediated afferent arteriolar vasoconstriction. Am J Physiol Heart Circ Physiol. 2001; 281:H2089–H2096. [PubMed: 11668070]
44. Zhao X, Falck JR, Gopal VR, Inscho EW, Imig JD. P2X receptor-stimulated calcium responses in preglomerular vascular smooth muscle cells involves 20-hydroxyeicosatetraenoic acid. J Pharmacol Exp Ther. 2004; 311:1211–1217. [PubMed: 15316085]
45. Inscho EW, Cook AK, Webb RC, Jin LM. Rho-kinase inhibition reduces pressure-mediated autoregulatory adjustments in afferent arteriolar diameter. Am J Physiol Renal Physiol. 2009; 296:F590–F597. [PubMed: 19129253]
46. Schnermann J, Levine DZ. Paracrine factors in tubuloglomerular feedback: adenosine, ATP, and nitric oxide. Annu Rev Physiol. 2003; 65:501–529. [PubMed: 12208992]
47. Just A, Arendshorst WJ. A novel mechanism of renal blood flow autoregulation and the autoregulatory role of A_1 adenosine receptors in mice. Am J Physiol Renal Physiol. 2007; 293:F1489–F1500. [PubMed: 17728380]
48. Bidani AK, Griffin KA, Williamson G, Wang X, Loutzenhiser R. Protective importance of the myogenic response in the renal circulation. Hypertension. 2009; 54:393–398. [PubMed: 19546375]
49. Majid DS, Inscho EW, Navar LG. P2 purinoceptor saturation by adenosine triphosphate impairs renal autoregulation in dogs. J Am Soc Nephrol. 1999; 10:492–498. [PubMed: 10073599]
50. Inscho EW, Cook AK, Imig JD, Vial C, Evans RJ. Physiological role for $P2X_1$ receptors in renal microvascular autoregulatory behavior. J Clin Invest. 2003; 112:1895–1905. [PubMed: 14679185]
51. Nishiyama A, Jackson KE, Majid DS, Rahman M, Navar LG. Renal interstitial fluid ATP responses to arterial pressure and tubuloglomerular feedback activation during calcium channel blockade. Am J Physiol Heart Circ Physiol. 2006; 290:H772–H777. [PubMed: 16214849]
52. Peti-Peterdi J. Calcium wave of tubuloglomerular feedback. Am J Physiol Renal Physiol. 2006; 291:F473–F480. [PubMed: 16495210]
53. Castrop H. Mediators of tubuloglomerular feedback regulation of glomerular filtration: ATP and adenosine. Acta Physiol. 2007; 189:3–14.
54. Thomson S, Bao D, Deng A, Vallon V. Adenosine formed by 5'-nucleotidase mediates tubuloglomerular feedback. J Clin Invest. 2000; 106:289–298. [PubMed: 10903345]
55. Ren Y, Arima S, Carretero OA, Ito S. Possible role of adenosine in macula densa control of glomerular hemodynamics. Kidney Int. 2002; 61:169–176. [PubMed: 11786098]
56. Ren Y, Garvin JL, Liu R, Carretero OA. Role of macula densa adenosine triphosphate (ATP) in tubuloglomerular feedback. Kidney Int. 2004; 66:1479–1485. [PubMed: 15458441]

57. Sun D, Samuelson LC, Yang T, Huang Y, Paliege A, Saunders T, Briggs J, Schnermann J. Mediation of tubuloglomerular feedback by adenosine: evidence from mice lacking adenosine 1 receptors. Proc Natl Acad Sci U S A. 2001; 98:9983–9988. [PubMed: 11504952]
58. Brown R, Ollerstam A, Johansson B, Skott O, Gebre-Medhin S, Fredholm B, Persson AE. Abolished tubuloglomerular feedback and increased plasma renin in adenosine A_1 receptor-deficient mice. Am J Physiol Regul Integr Comp Physiol. 2001; 281:R1362–R1367. [PubMed: 11641103]
59. Castrop H, Huang Y, Hashimoto S, Mizel D, Hansen P, Theilig F, Bachmann S, Deng C, Briggs J, Schnermann J. Impairment of tubuloglomerular feedback regulation of GFR in ecto-5'-nucleotidase/CD73-deficient mice. J Clin Invest. 2004; 114:634–642. [PubMed: 15343381]
60. Huang DY, Vallon V, Zimmermann H, Koszalka P, Schrader J, Osswald H. Ecto-5'-nucleotidase (cd73)-dependent and -independent generation of adenosine participates in the mediation of tubuloglomerular feedback in vivo. Am J Physiol Renal Physiol. 2006; 291:F282–F288. [PubMed: 16525161]
61. Oppermann M, Friedman DJ, Faulhaber-Walter R, Mizel D, Castrop H, Enjyoji K, Robson SC, Schnermann J. Tubuloglomerular feedback and renin secretion in NTPDase1/CD39-deficient mice. Am J Physiol Renal Physiol. 2008; 294:F965–F970. [PubMed: 18256308]
62. Schnermann J. Maintained tubuloglomerular feedback responses during acute inhibition of P2 purinergic receptors in mice. Am J Physiol Renal Physiol. 2011; 300:F339–F344. [PubMed: 21147842]
63. Schnermann J, Briggs JP. Tubuloglomerular feedback: mechanistic insights from gene-manipulated mice. Kidney Int. 2008; 74:418–426. [PubMed: 18418352]
64. Mulryan K, Gitterman DP, Lewis CJ, Vial C, Leckie BJ, Cobb AL, Brown JE, Conley EC, Buell G, Pritchard CA, Evans RJ. Reduced vas deferens contraction and male infertility in mice lacking $P2X_1$ receptors. Nature. 2000; 403:86–89. [PubMed: 10638758]
65. Yamamoto K, Sokabe T, Matsumoto T, Yoshimura K, Shibata M, Ohura N, Fukuda T, Sato T, Sekine K, Kato S, Isshiki M, Fujita T, Kobayashi M, Kawamura K, Masuda H, Kamiya A, Ando J. Impaired flow-dependent control of vascular tone and remodeling in $P2X_4$-deficient mice. Nat Med. 2006; 12:133–137. [PubMed: 16327800]
66. Palomino-Doza J, Rahman TJ, Avery PJ, Mayosi BM, Farrall M, Watkins H, Edwards CR, Keavney B. Ambulatory blood pressure is associated with polymorphic variation in P2X receptor genes. Hypertension. 2008; 52:980–985. [PubMed: 18852390]
67. Harrison DG, Guzik TJ, Goronzy J, Weyand C. Is hypertension an immunologic disease? Curr Cardiol Rep. 2008; 10:464–469. [PubMed: 18950555]
68. Elmarakby AA, Quigley JE, Olearczyk JJ, Sridhar A, Cook AK, Inscho EW, Pollock DM, Imig JD. Chemokine receptor 2b inhibition provides renal protection in angiotensin II-salt hypertension. Hypertension. 2007; 50:1069–1076. [PubMed: 17938380]
69. Sharma K, Cook A, Smith M, Valancius C, Inscho EW. TGF-beta impairs renal autoregulation via generation of ROS. Am J Physiol Renal Physiol. 2005; 288:F1069–F1077. [PubMed: 15644487]
70. Vial C, Evans RJ. Disruption of lipid rafts inhibits $P2X_1$ receptor-mediated currents and arterial vasoconstriction. J Biol Chem. 2005; 280:30705–30711. [PubMed: 16006561]
71. Potocnik SJ, Jenkins N, Murphy TV, Hill MA. Membrane cholesterol depletion with beta-cyclodextrin impairs pressure-induced contraction and calcium signalling in isolated skeletal muscle arterioles. J Vasc Res. 2007; 44:292–302. [PubMed: 17406121]
72. Callera GE, Montezano AC, Yogi A, Tostes RC, Touyz RM. Vascular signaling through cholesterol-rich domains: implications in hypertension. Curr Opin Nephrol Hypertens. 2007; 16:90–104. [PubMed: 17293683]
73. Fredholm BB, Arslan G, Halldner L, Kull B, Schulte G, Wasserman W. Structure and function of adenosine receptors and their genes. Naunyn Schmiedebergs Arch Pharmacol. 2000; 362:364–374. [PubMed: 11111830]

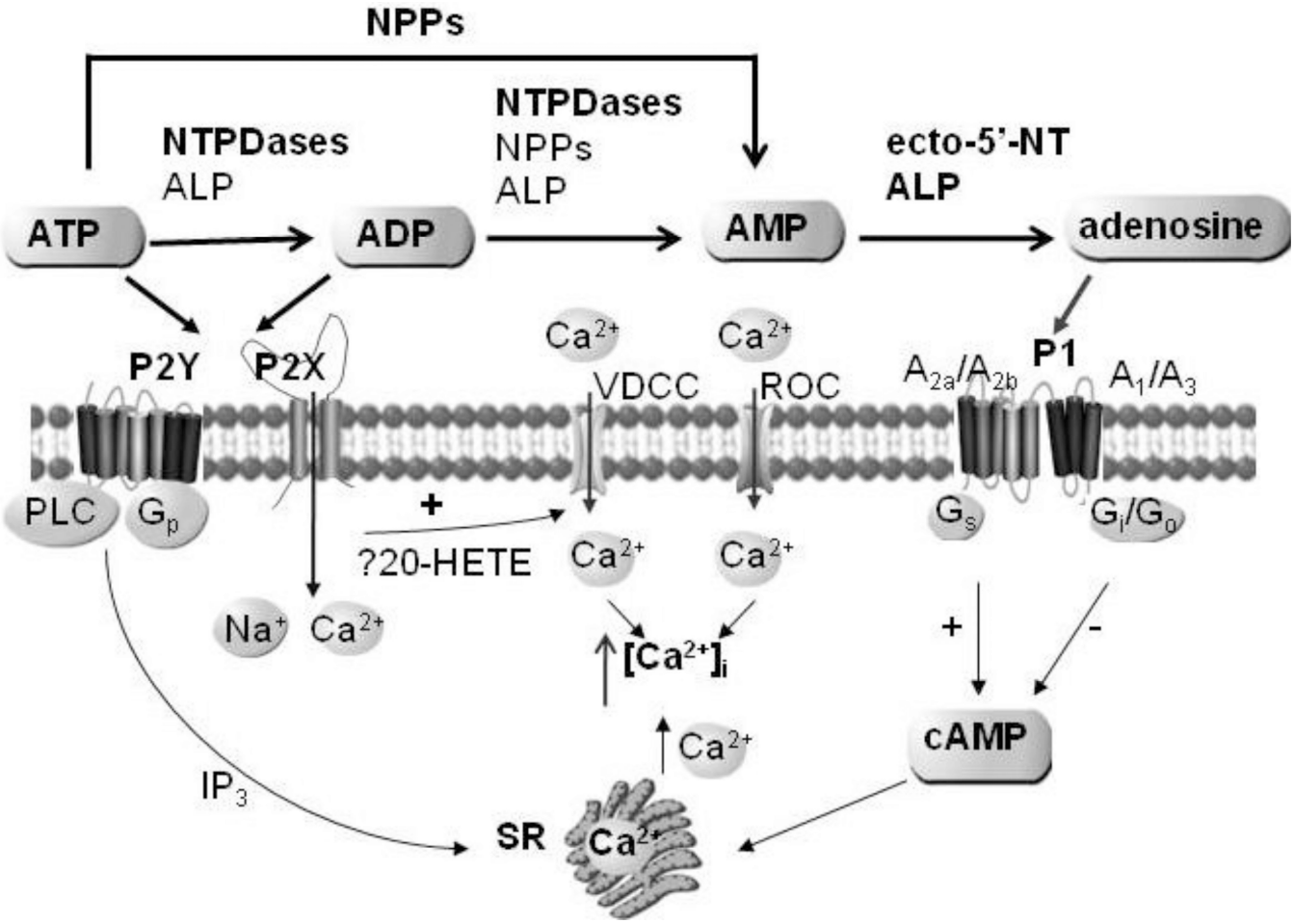

Figure 1.

Schematic diagram illustrating the catabolic pathways for degrading ATP to form ADP, AMP and adenosine by a series ecto-nucleotidases (NTPDases: nucleotide triphosphate diphosphohydrolases; NPPs: ecto-nucleotide pyrophosphatase; ecto-5'-NT: ecto-5'-nucleotidases, and ALP: ecto-alkaline phosphatases). ATP, its metabolites and its associated intracellular signaling pathways. The signaling pathways depicted for P1 receptor activation reflect conventionally held mechanisms.[73]

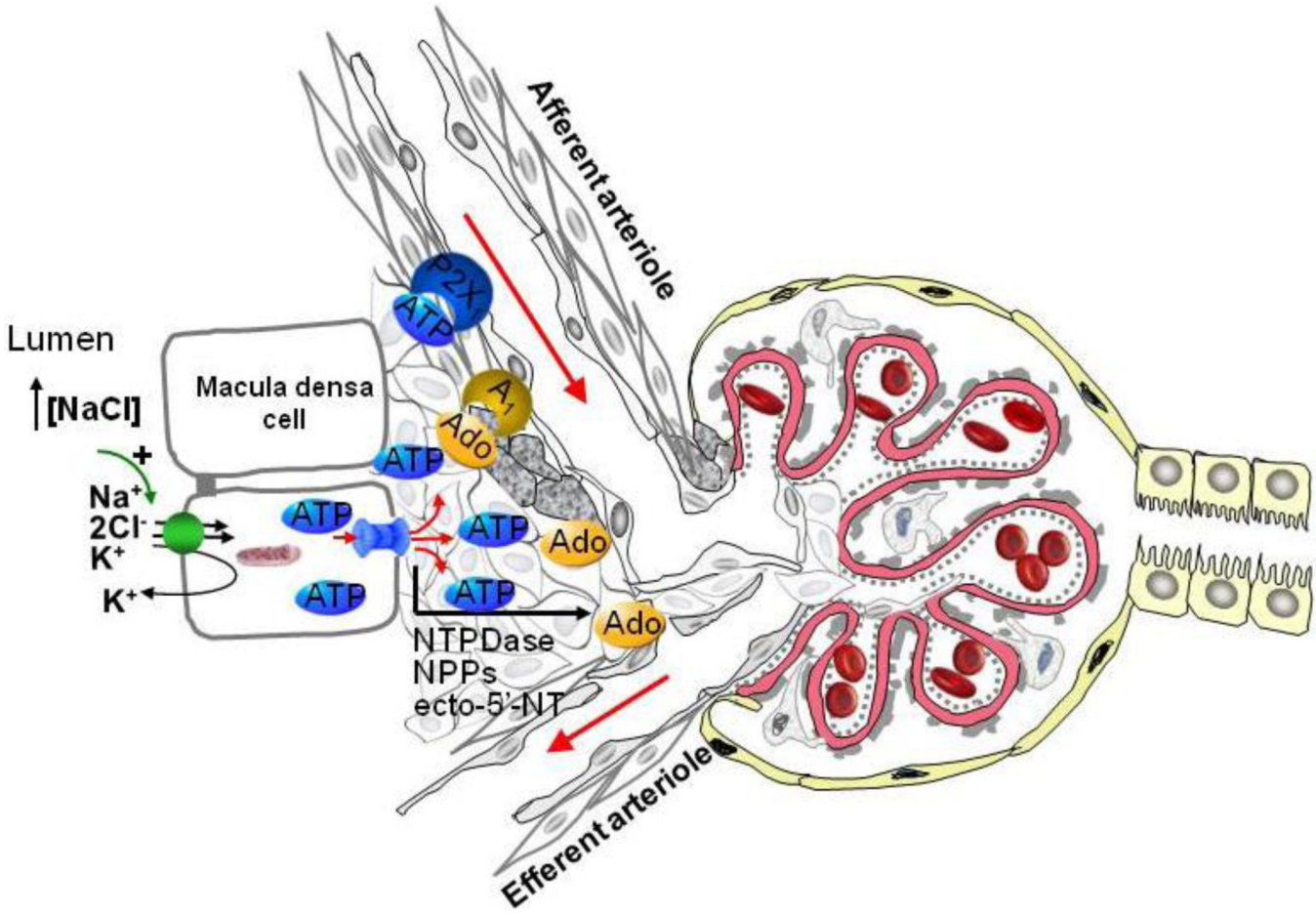

Figure 2.

Schematic diagram representing the postulated TGF mechanism in 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 $P2X_1$ receptors and/or adenosine (ado) converted from ATP by ecto-nucleotide pyrophosphatase (NPPs), ectonucleoside triphosphate diphosphohydrolases (NTPD), and ecto-5'-nucleotidases (ecto-5'-NT), respectively, vasoconstricts afferent arterioles via activating A_1 receptors.