MINIREVIEW

Purinoceptors in the Kidney

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The multiple roles of extracellular ATP and its metabolite adenosine include broad areas, such as regulating vascular tone and inducing inflammation. This review will discuss purinoceptor-induced effects on renal vascular resistance, highlighting the key experiments providing a significant contribution to our current understanding of autoregulatory mechanisms. Emphasis will be placed on the purinoceptor subtypes involved in autoregulatory control by ATP and adenosine. Additionally, the role of purinoceptors in hypertension-associated impairment of autoregulatory efficiency will be discussed.
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Key words: P1 receptors; P2 receptors; adenosine; ATP; renal microvasculature; renal autoregulation

Introduction

Since Burnstock's discovery that extracellular ATP functions as a nonadrenergic, noncholinergic neurotransmitter, purinoceptors have been identified in nearly every tissue and have been implicated in a broad range of physiologic processes (1). These processes include neurotransmission, modulation of vascular tone, contraction of muscular cells, aggregation of platelets, and signal transduction in cardiac cells (1–10). Evidence also implicates purinoceptors in ischemia, inflammation, cell growth, differentiation and proliferation in development, and regeneration (5, 11–13). Two major families of purinoceptors, P1 and P2, have been described.

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P1 and P2 receptors are both expressed throughout the kidney and are found especially in glomeruli and tubules (14–18). Over the past two decades, determination of the roles purinoceptors play in the kidney has become an area of intensive investigation. Physiologic studies of extracellular ATP and adenosine have revealed their importance in the control of renal function *via* regulation of the renal microcirculation, renin secretion, and tubular function (4, 19–25). This review will focus on ATP and adenosine as extracellular regulators of renal microcirculation, renal hemodynamics, and autoregulation by summarizing the recent findings. The relationship between P1 and P2 receptor function and the autoregulatory impairment observed in some forms of hypertension also will be considered.

Overview of Purinoceptors

Historically, the nucleotide ATP was mainly considered an energy source or enzyme cofactor involved in cellular biochemical processes. Since Burnstock's discovery that ATP functions as a principal nonadrenergic, noncholinergic neurotransmitter in the gut and bladder (26, 27), purinergic nucleotides are increasingly recognized as paracrine mediators contributing to the regulation of neuronal and nonneuronal tissue function (28). P1 receptors are metabotropic and have a higher affinity for adenosine and AMP than for ATP or ADP. Alternatively, P2 receptors have higher affinity for ATP and ADP than for AMP or adenosine.

Adenosine is formed by breakdown of ATP by ubiquitous intracellular or extracellular ectonucleotidases and exerts its effects by activating P1 receptors. The P1 receptor family comprises four receptor subtypes classified A_1, A_{2a}, A_{2b}, and A_3 (28–31). A_1 and A_3 receptors are coupled to a G_o/G_i protein and cause inhibition of adenylyl cyclase and, hence, decrease the production of cyclic AMP (Fig. 1; Refs. 32–36). In contrast, A_{2a} and A_{2b} receptors are

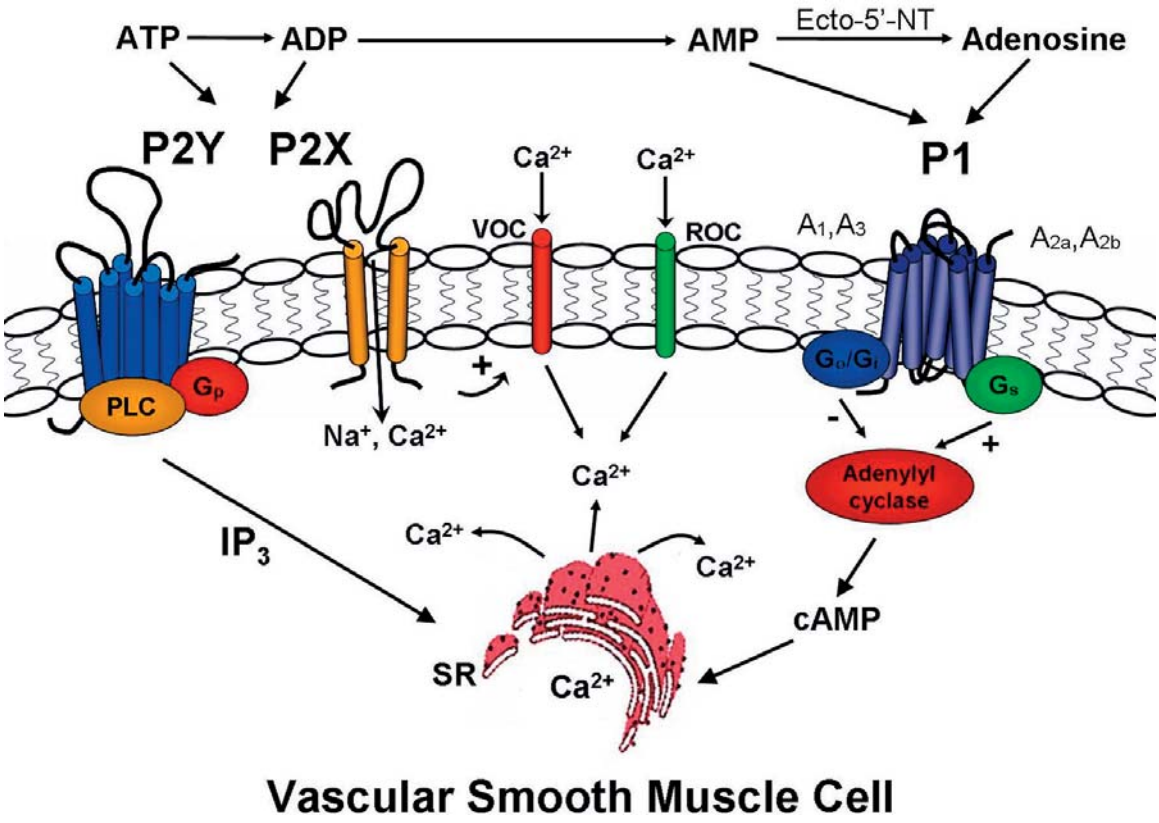

Figure 1. Purinoceptors (P1 and P2 receptors) and their postulated intracellular cascades in vascular smooth muscle cells. This diagram shows the postulated mechanisms for activation of P1 and P2 purinoceptors by AMP, adenosine, ATP, and ADP, respectively, as shown in arrows. Gp, GTP-binding proteins; Gi, inhibitory G protein; Gs, stimulatory G protein; IP3, inositol-1,4,5-triphosphate; SR, sarcoplasmic reticulum; PLC, phospholipase C; VOC, voltage-operated calcium channel; ROC, receptor-operated calcium channel; ECTO-5′-NT, ecto-5′-nucleotidase. Plus sign indicates stimulation, and minus sign indicates inhibition.

coupled to G_s protein, leading to stimulation of adenylyl cyclase (Fig. 1; Refs. 37, 38).

ATP achieves its effects by activating P2 receptors (Fig. 1). P2 receptors are membrane bound and widely expressed in neural, vascular, connective, and immune tissues (1, 2). The P2 receptor family is divided into two distinct subsets (P2X and P2Y) based on important structural differences and mechanistic differences in the intracellular signal transduction pathways used. The ionotropic P2X receptor family is composed of seven unique subtypes classified as $P2X_{1–7}$. P2Y receptors are metabotropic and include approximately eight subtypes ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$; Refs. 10, 39, 40). Although the physiologic roles and intracellular signaling mechanisms of these receptor subtypes are still under investigation, considerable attention has been directed toward determining the vascular response to P2X and P2Y receptor activation. Vascular P2X receptor activation generally produces a rapid vasoconstriction, whereas P2Y receptor activation can produce either vasodilation or vasoconstriction. Vasodilation occurs by stimulating the release of endothelial vasodilators, such as nitric oxide or prostaglandin I_2 (12, 41–43). P2Y receptors expressed by vascular smooth muscle can stimulate vasoconstriction by increasing intracellular calcium concentration (7, 44) and/or by activating the Rho kinase pathway (45, 46). In addition to functional differences, the cellular mechanisms underlying activation of P2X and P2Y receptors also are different. P2X receptors are ligand-gated ion channels composed of two transmembrane domains coupled to a large extracellular loop and short intracellular tails (Fig. 1; Ref. 47). When ATP binds to the extracellular loop, the receptor is activated and the ligand-gated channel opens, allowing nonselective passage of Ca^{2+} and Na^+ into cells and K^+ out of cells (8, 48, 49). Activation of P2Y receptors may initiate numerous events, including inhibition of adenylyl cyclase, activation of phospholipase C (PLC), and elevation of cytosolic Ca^{2+} (Fig. 1; Refs. 50–53).

Purinoceptors in Kidney

Purinoceptors and Their Expression in the Kidney. By using a variety of immunohistochemical and molecular biologic techniques, both P1 and P2 receptor protein or mRNA have been detected in different regions of the nephron, the glomerulus, and the renal vascular system (4, 17, 20, 54–59). Table 1 summarizes the recent findings

Table 1. Expression of Purinoceptors in the Kidney[a]

Subtype	Preglomerular vessels	Afferent arterioles	Efferent arterioles	Glomerulus	Proximal tubules	Loop of Henle	Distal tubule	Collecting duct
A_1	WB, mRNA (63)	IM (61)	—	IM (61, 62), mRNA (139)	IM (61)	mRNA (18)	—	IM (61), mRNA (18, 60)
A_{2a}	—	mRNA (18)	—	mRNA (18, 60)	—	—	—	mRNA (60)
A_{2b}	WB, mRNA (63)	—[b]	—	—	—	mRNA (18)	mRNA (18)	—
A_3			—	—	—	—	—	—
$P2X_1$	IM (15, 65), WB (137)	IM (15, 65, 137)	—	—	—	—	—	—
$P2X_2$	IM (15, 140)	—	—	—	—	—	—	—
$P2X_4$	—	—	—	—	IM (15)	—	IM (15)	—
$P2X_5$	—	—	—	—	IM (15)	—	—	IM (15)
$P2X_6$	—	—	—	—	IM (15)	—	IM (15)	
$P2X_7$	—	—	—	IM, WB, mRNA (135)	—	—	—	IM (141)
$P2Y_1$	IM (15)	IM (15, 17), mRNA (17)	IM (15)	IM (15)	IM (15), mRNA (66)	mRNA (66)	—	mRNA (66)
$P2Y_2$	—	—	IM, mRNA (17)	IM (15)	mRNA (66)	IM (15), mRNA (66)	—	IM (15, 142), WB (142), mRNA (66, 142)
$P2Y_4$	—	—	IM, mRNA (17)	—	IM (15), mRNA (66)	mRNA (66)	—	—
$P2Y_6$	—	—	IM, mRNA (17)	—	mRNA (143)	mRNA (143)	—	mRNA (143)

[a] The expressions of purinoceptor protein and mRNA are restricted to native kidneys. IM, immunostaining; WB, Western blotting; mRNA, messenger ribonucleic acid. Numbers in parentheses refer to the reference citation number.
[b] For A_3, WB (62, 64) and mRNA (62) in cortical and medullary tissue.

of purinoceptors expressed in native kidneys rather than cultured kidney cell lines. Using Northern blot, *in situ* hybridization, and reverse transcription–polymerase chain reaction (RT-PCR) techniques, Vitzthum and colleagues (18) demonstrated the presence of A_1 receptor mRNA in the thin loops of Henle and collecting ducts in both rat and mouse kidneys. A_{2b} receptor mRNA was present in the cortical thick ascending loops of Henle and in the distal convoluted tubule, whereas A_{2a} receptor mRNA was only present in glomeruli (18, 60). By employing immunohistochemical techniques, A_1 receptors were found to be expressed mainly in the afferent arteriole, glomerulus, mesangial cells, proximal tubule, and collecting ducts (61, 62). Finally, by combining RT-PCR and Western and Northern blotting techniques, abundant expression of A_1 and A_{2b} but not A_{2a} and A_3 receptor protein and mRNA was detected in renal preglomerular microvascular tissue (60, 63). However, other studies reported that both A_3 receptor protein expression and mRNA were found in rat cortical and medullary tissue (62, 64).

Numerous studies also have focused on determining the expression of P2 receptors in renal vascular, glomerular, mesangial, and tubular epithelial cells (17, 20, 54, 55, 57–59, 65). Using immunohistochemical techniques, Chan and colleagues demonstrated positive immunostaining for $P2X_1$ receptors on the intrarenal vascular smooth muscle cells of arcuate and interlobular arteries and afferent arterioles, but not glomeruli, efferent arterioles, or renal tubules (65). Recently, the same group reported $P2Y_1$ receptor expression by both afferent and efferent arterioles and $P2X_2$ receptor expression in larger intrarenal arteries and veins (15). $P2Y_1$, $P2Y_2$, and $P2X_7$ receptors were expressed in glomeruli, whereas $P2Y_1$ receptors were mainly in mesangial cells, and $P2Y_2$ receptors were detected in podocytes (15, 17). Several subtypes of P2X and P2Y receptors are expressed by renal tubular epithelial cells. For example, $P2Y_4$ receptors are expressed along the basolateral membrane of the proximal convoluted tubule, whereas $P2Y_1$ and $P2X_5$ receptors are expressed along the apical membrane of the proximal convoluted and straight tubule cells (15, 66). Moreover, $P2X_4$ and $P2X_6$ receptors are present throughout the tubular epithelium, from the proximal tubule to the collecting duct (15). Recently, using Western blot we demonstrated protein expression for $P2X_2$, $P2X_4$, $P2X_7$, $P2Y_2$, and $P2Y_4$ receptors in cultured mouse mesangial cells. No evidence of $P2X_1$ and $P2X_3$ receptor protein expression was detected (67). RT-PCR analysis in the same mouse mesangial cell line revealed mRNA expression for $P2X_1$, $P2X_2$, $P2X_3$, $P2X_4$, $P2X_7$, $P2Y_2$, and $P2Y_4$ receptors (67). Although the functional roles of intrarenal P2 receptor subtypes have not been fully clarified, these studies provide compelling evidence indicating that purinoceptors play diverse roles in regulating renal hemodynamics and electrolyte homeostasis.

Physiologic Role of Purinoceptors in the Kidney. *Purinoceptors and Renal Microcirculation.* The functional effect of nucleosides in the kidney was first noticed in the 1920s (68). The effect of P1 receptors in regulating renal vascular reactivity has been intensively studied (21, 36, 69–80). Unlike the vasodilatory response of adenosine in other organs or tissues, whole-kidney intraarterial infusion of adenosine evokes a biphasic response, with an initial transient decrease of renal blood flow (RBF) followed by a progressive and sustained increase of flow (70). The initial vasoconstriction is completely abolished by infusion of the selective A_1 receptor antagonist, KW-3902 (81), and a monophasic renal vasodilation is elicited by infusion of the A_{2a} receptor agonist, CGS-21860 (82, 83). These data suggest that adenosine-induced vasoconstriction and vasodilation are mediated via activation of A_1 and A_{2a} receptors, respectively.

The effect of adenosine on the renal microvasculature also has been examined using several different experimental preparations. For example, in the hydronephrotic rat kidney preparation, administration of an adenosine A_1 receptor agonist produced vasoconstriction of the afferent arteriole without changing efferent arteriolar diameter, whereas administration of an A_{2a} receptor agonist caused vasorelaxation in both afferent and efferent arterioles (71). Studies using isolated afferent arterioles from rabbit superficial cortex demonstrated a similar vasoconstriction in response to selective A_1 receptor activation and a vasodilation following selective A_2 receptor activation (74, 79). These observations support the finding that the renal effects of adenosine are mediated *via* activation of A_1 and A_{2a} receptors. However, controversy exists regarding the presence and function of A_1 receptors in efferent arterioles. Despite the fact that A_1 receptor expression has not been localized to efferent arterioles, functional studies reveal that efferent arterioles are responsive to P1 receptor stimulation (84). Using *in vitro* blood-perfused rat juxtamedullary nephrons, Nishiyama and colleagues (84) established that superfusion of 10 μM adenosine caused reduction of both afferent and efferent arteriolar diameters. The response of the afferent and efferent arterioles to adenosine was attenuated in the presence of the A_1 receptor blocker, KW-3902, and was enhanced in the presence of the A_{2a} receptor antagonist, KF-17837, suggesting that adenosine may exert a direct effect on the efferent arteriole. Moreover, an *in vitro* study using isolated microperfused rabbit efferent arterioles with adherent tubular segments and with the macula densa attached (85) found that increases of NaCl delivery to the macula densa caused vasodilation of efferent arterioles preconstricted with norepinephrine. This vasodilatory response was abolished in the presence of the A_1/A_2 receptor antagonist, PSPX, and the specific A_2 receptor antagonist, 3,7-dimethyl-1-propargylxanthine (DMPX), but not in the presence of the A_1 receptor antagonist, FK838. These observations indicate that adenosine-mediated vasodilation of efferent arterioles occurs through stimulation of A_2 receptors.

Beginning in the early 1980s, increasing attention has

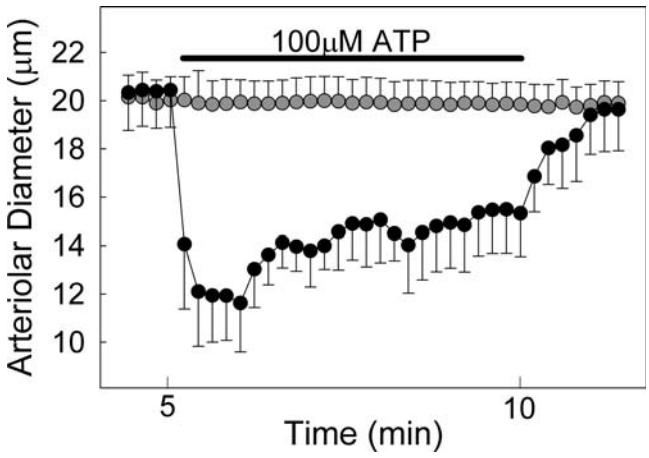

Figure 2. The effect of ATP on blood-perfused rat juxtamedullary afferent and efferent arterioles *in vitro*. The diameter of the afferent arterioles ($n = 7$) decreased rapidly within the initial 60 secs in response to superfusion of ATP and then increased slightly but remained significantly smaller than control (black circles). The diameter of the efferent arterioles ($n = 4$), however, did not change to this high concentration of ATP (gray circles). Data taken from Inscho *et al.* (86).

focused on determining the effects of ATP and P2 receptor activation on renal function. There is compelling functional evidence supporting extracellular ATP as a major local autocrine and paracrine regulator of preglomerular microvascular reactivity involving activation of P2X and P2Y receptors (4, 21, 74, 86, 87). A more recent *in vivo* study showed that renal arterial infusion of the $P2X_1/P2X_3$ agonist, α,β-methylene ATP, at a dose that does not modify systemic arterial blood pressure, caused a significant decrease in both cortical and medullary blood flow in rabbit kidneys (88). These studies indicate that P2 receptors are expressed by the renal vasculature. The direct effect of P2 receptor activation on renal microvessels has been demonstrated using *in vitro* microperfused rabbit afferent arterioles with their glomeruli attached. Afferent arteriolar diameter declined significantly in response to superfusion of ATP or $P2X_1$ agonist β,γ-methylene ATP (74). Moreover, lower concentrations of ATP caused rapid and sustained afferent arteriolar vasoconstriction, even during adenosine receptor blockade (86). Sustained ATP-mediated vasoconstriction is uniquely an afferent arteriolar response, since only this preglomerular segment exhibits a sustained reduction in vascular diameter at low ATP concentrations. In contrast, essentially only transit vasoconstrictor responses are observed for arcuate and interlobular arteries at ATP concentrations below 100 μ*M*. Importantly, efferent arterioles are completely unresponsive to ATP concentrations as high as 100 μ*M* (Fig. 2; Ref. 86). These observations strongly support the argument that extracellular ATP represents an important paracrine regulator of the renal microvasculature, mainly *via* contraction of afferent arteriolar smooth muscle cells.

There is substantial evidence that activation of P2Y receptors may result in the release of endothelium-derived relaxing factors (89–91). Using isolated, perfused rat kidneys preconstricted with phenylephrine, Wangensteen and colleagues (92) found that inhibition of nitric oxide production by L-NAME markedly reduced the vasodilation by ATP, 2-methylthio ATP, and UTP. Similar results were obtained by increasing extracellular K^+ to inhibit K^+ channel function. Collectively, these data indicate that both nitric oxide and endothelium-derived hyperpolarizing factor might contribute to the vasodilation evoked by P2Y receptor activation. ATP was found to be a more potent vasoconstrictor of arcuate arteries in kidneys treated with L-NAME (91). These results suggest nitric oxide involvement in P2 receptor–mediated vasodilation. Regulation of renal vascular tone by ATP may reflect the balance of cellular signals arising from activation of P2 receptors on vascular smooth muscle cells and endothelial cells (10).

The mechanisms underlying the intracellular second messenger systems used by ATP also have been investigated (44, 93–96). Using freshly isolated preglomerular vascular smooth muscle cells, Inscho *et al*. determined that ATP evoked a biphasic increase in intracellular Ca^{2+} concentration (94). ATP increases intracellular Ca^{2+} in preglomerular smooth muscle cells by stimulating Ca^{2+} release from intracellular stores and the influx of Ca^{2+} from the extracellular fluid space. Calcium influx in isolated smooth muscle cells was significantly attenuated by blockade of L-type Ca^{2+} channels (95), and sustained ATP-mediated afferent arteriolar vasoconstriction was eliminated by superfusion with Ca^{2+} free medium or blockade of L-type Ca^{2+} channels (44). These studies indicate that activation of voltage-dependent Ca^{2+} channels is an essential signaling element for ATP-mediated vasoconstriction of afferent arterioles. Voltage-dependent Ca^{2+} influx is also an essential component of pressure-mediated afferent arteriolar vasoconstriction (autoregulation).

Thus, both adenosine and ATP play important roles in modulating renal microvascular function by acting on P1 and P2 receptors expressed by afferent and/or efferent arterioles.

Purinoceptors and Renal Autoregulation. For many decades, investigators have recognized the phenomenon of renal autoregulation as a means for maintaining a constant blood flow, glomerular filtration rate (GFR), and oxygen delivery to the kidney (97). Renal autoregulation is a process by which the kidney alters renal vascular resistance in response to alterations in transmural pressure in order to stabilize renal hemodynamics. Although the detailed mechanisms involved in renal autoregulation are incompletely understood, it is generally recognized that whole-kidney autoregulation is the manifestation of two distinct regulatory systems that include a local myogenic mechanism operating along the preglomerular vascular tree and the tubuloglomerular feedback (TGF) mechanism, which regulates preglomerular resistance in the juxtaglomerular segment of the afferent arteriole (4, 23, 98). Both the myogenic

response and TGF enable the kidney to maintain constant renal blood flow and GFR during fluctuations of systemic arterial pressure, and they facilitate the kidney's ability to maintain fluid and electrolyte homeostasis.

Considerable effort has been devoted to determining whether the renal autoregulatory mechanism can be linked to a specific intrarenal paracrine signaling system activated in response to changes in arterial pressure and/or TGF signals from macula densa cells. These signals influence afferent arteriolar tone, and modulate of glomerular capillary pressure and thus GFR (4, 7, 23). Several studies provide evidence that ATP or adenosine serves as a major signaling molecule responsible for juxtaglomerular transmission of the TGF response (4, 23, 99, 100). The evidence for adenosine in modulating TGF is supported by micropuncture studies in rat kidneys. The change in stop-flow pressure in response to an increase in loop of Henle flow rate, an index of the TGF response, was significantly attenuated by A_1 receptor blockade (101, 102). Moreover, by using a microperfused rabbit tubule with attached macula densa, Ren and colleagues (103) observed that the afferent arteriolar vasoconstriction in response to increased macula densa NaCl delivery was completely abolished by addition of the A_1 receptor antagonist, FK838, but was enhanced by infusion of adenosine. Several studies report attenuated TGF responses (104–106) and impaired renal autoregulatory efficiency despite a normal GFR under control conditions in A_1 receptor–deficient mice (107), further supporting adenosine mediation of TGF. Most studies have focused on the effect of TGF activation on the afferent arteriolar response. In another *in vitro* study, Ren and colleagues reported that increasing NaCl delivery past the macula densa to initiate a TGF response also caused dilation of efferent arterioles *via* activation of A_2 receptor (85). They found that activation of TGF responses led to vasodilation of the norepinephrine-preconstricted efferent arterioles, which was completely inhibited by a selective adenosine A_2 receptor antagonist but not by an adenosine A_1 receptor antagonist. Although this phenomenon has not been demonstrated *in vivo*, this study suggests that dilation of efferent arterioles might contribute to TGF-mediated regulation of GFR and glomerular capillary pressure. Taken together, these studies are supportive of a role for A_1 receptor activation in TGF responses.

However, several other *in vivo* and *in vitro* studies exploring the role of adenosine in mediating renal autoregulation have shown controversial results. For example, autoregulation of renal blood flow and GFR remain fairly constant during adenosine receptor blockade in the dog (73, 108). A micropuncture study in anesthetized Sprague Dawley rats showed that blockade of adenosine A_1 receptors with KW-3902 dilated afferent arterioles and their upstream arterial segments, but the TGF response remained intact (77). Pressure-mediated vasoconstriction of juxtamedullary afferent arterioles was not changed during blockade of A_1 receptors with 1,3-dipropyl-8-cyclopentylxanthine (DPCPX; Ref. 100). Contrary to previously mentioned findings, these studies suggest that adenosine is not essential.

There is increasing evidence that supports the hypothesis that extracellular ATP functions as an important mediator of renal autoregulation (100, 109–117). By using a microdialysis technique, Nishiyama and colleagues found that increases in interstitial ATP concentration but not adenosine concentration were correlated with increases in mean arterial pressure and autoregulatory changes in renal vascular resistance in canine kidneys (118). The interstitial ATP concentration was increased by stimulating TGF responses by increasing distal volume delivery with acetazolamide. The interstitial ATP concentration also decreased when the TGF response was inhibited with furosemide (112). More recently, Nishiyama *et al*. demonstrated that the pressure- and acetazolamide-mediated increases in renal cortical interstitial ATP concentration were still maintained in the kidneys treated with L-type calcium channel inhibitor, nifedipine, which completely prevented the autoregulatory changes of renal vascular resistance (113). These results indicate that interstitial ATP concentrations are directly correlated with conditions that evoke TGF responses and increased renal vascular resistance, and they support a role for extracellular ATP as an effector of autoregulatory adjustments in renal vascular resistance. The development of the *in vitro* blood-perfused juxtamedullary nephron preparation has provided a useful tool for observing both the myogenic and TGF components of autoregulation (119, 120). In this preparation, the myogenic response is readily observed with and without inhibition of the TGF response by transection of the papilla or infusion of loop diuretic furosemide. Using this preparation, pressure-mediated afferent arteriolar vasoconstriction was markedly attenuated by P2 purinoceptor desensitization and pharmacologic blockade of P2 receptors, specifically $P2X_1$ blockade by NF279 (Fig. 3; Refs. 100, 110). Furthermore, mice lacking $P2X_1$ receptors exhibited impaired pressure-mediated afferent arteriolar vasoconstriction, and this impairment was unaffected by blockade of the TGF response through papillectomy or furosemide (100). Afferent arterioles from $P2X_1$ knockout mouse kidney still exhibited vasoconstriction to cyclopentyl adenosine during exposure to NF 279, as shown in Figure 4 (100). These data support a role for $P2X_1$ receptors in the TGF response and support an important role for $P2X_1$ receptors in the overall autoregulatory response. Thus, these studies provide strong evidence that extracellular ATP and P2X receptors are essential signaling components of the autoregulatory response.

ATP as a possible TGF mediator is also supported by other studies performed to determine whether macula densa cells release ATP in response to factors that should evoke a TGF response (114–117). Investigators found that intracellular Ca^{2+} concentration in macula densa cells increased significantly in response to bath administration of the P2

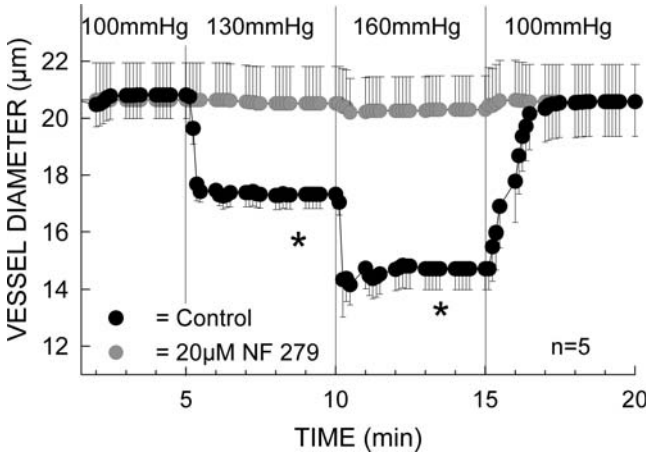

Figure 3. Effect of the $P2X_1$ receptor blocker, NF279, on blood-perfused juxtamedullary rat afferent arteriolar responses to increasing renal perfusion pressure (RPP) *in vitro*. The diameter of the afferent arterioles was significantly decreased in response to increasing RPP during the control period (black circles). NF279 completely inhibited the pressure-mediated afferent arteriolar response (gray circles). The responses during NF279 administration are overlaid on the control response for comparison. Each point represents mean vessel diameter measured at 12-sec intervals throughout each pressure period. $*P < 0.05$ versus diameter at 100 mm Hg. Data are taken from Inscho *et al.* (100).

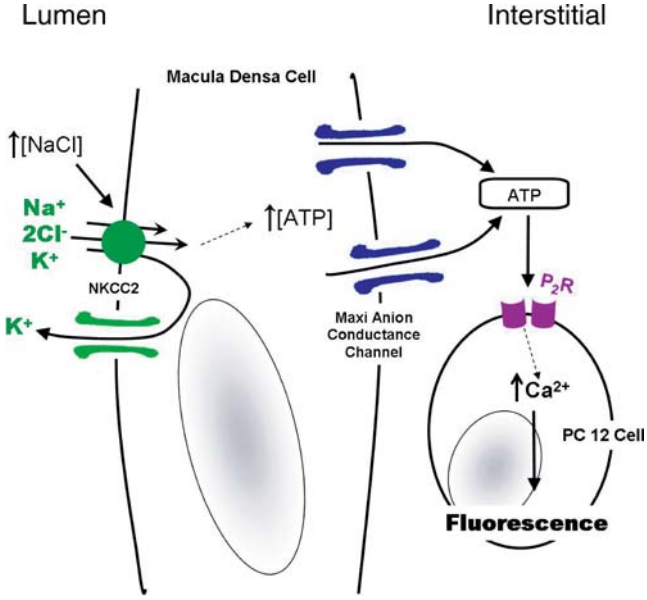

Figure 5. Illustration showing experimental methods used by Bell *et al.* (117). PC12 cells loaded with fura-2, an indicator of intracellular calcium concentration, were positioned in close proximity to the basolateral aspect of macula densa cells with attached thick ascending limb. In response to increasing luminal NaCl, ATP was released from the macula densa and induced fura-2 fluorescence, indicating activation of P2X receptors and an increase in $[Ca^{2+}]_i$.

receptor agonists ATP and UTP, but not to adenosine. The increased cytosolic Ca^{2+} concentration was completely inhibited by addition of a P2 receptor antagonist (114). Bell and colleagues used a microdissected glomerular preparation with an attached macula densa to determine whether ATP release could be detected using a biosensor (117). Using whole-cell patch clamp techniques, a single PC12 cell or cultured mouse mesangial cell overexpressing P2X receptors was placed in close proximity to the macula densa, adjacent to the basolateral membrane surface (Fig. 5). The Ca^{2+} concentration in the biosensor cells increased in response to either addition of ATP or an increase in tubular fluid NaCl concentration. A more recent *in vitro* study that employed isolated perfused rabbit juxtaglomerular apparatus combined with confocal fluorescence imaging to directly visualize propagation of calcium signals from macula densa to other cells of the juxtaglomerular apparatus provides an direct evidence supporting ATP as a mediator in TGF response (121). Peti-Peterdi (121) observed an upstream calcium signal from the macula densa toward the proximal afferent arteriole and the adjacent glomerulus and intraglomerular cells in response to increasing tubular flow past the macula densa. Propagation of the calcium signal and vasoconstriction of the afferent arteriole can be blocked by a P2 receptor antagonist but not an A_1 receptor antagonist. These studies demonstrate that ATP is released from the macula densa in response to a TGF stimulus and represents an important signaling molecule linking the macula densa with TGF-mediated afferent arteriolar vasoconstriction.

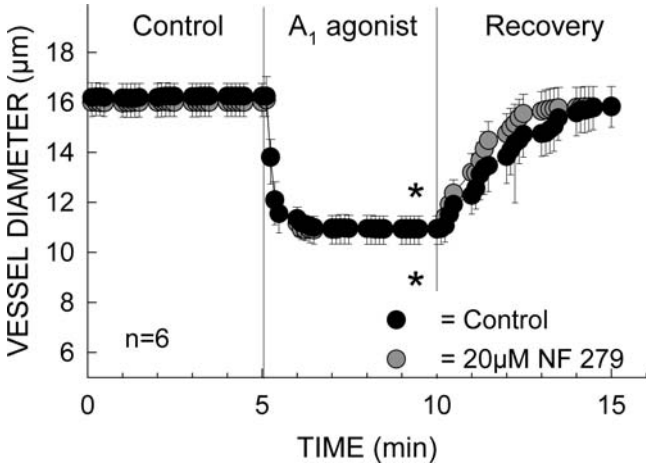

Figure 4. Effect of P2X blocker NF279 on the afferent arteriolar response to the A_1 agonist N^6-cyclopentyl adenosine (CPA) from $P2X_1$ knockout mouse kidney. The afferent arteriole constricted in response to administration of CPA in the absence (black circles) and presence (gray circles) of NF279. The response during NF279 administration is overlaid on the control response. $*P < 0.05$ versus diameter at 100 mm Hg. Data are taken from *Inscho et al.* (100).

ATP metabolism is accomplished by nucleotidases that are highly expressed in the kidney. Ecto-nucleotidases cleave ATP to AMP or adenosine and represent a possible mechanism by which the interstitial adenosine concentration can be regulated. It is possible that ecto-nucleotidases catabolize ATP released from the macula densa to generate adenosine which subsequently acts on P1 receptors. In order to distinguish the P2 receptor–mediated effect from that of

P1 receptors, various pharmacologic, biochemical, and molecular tools have been used, including metabolically stable P2 receptor agonists, P1 and P2 receptor antagonists, adenosine uptake inhibitors, and ecto-nucleotidase–deficient mice (122, 123). For example, the vascular response of ATP in isolated perfused rat kidneys remained unaffected by addition of the adenosine receptor antagonist, 8-phenyltheophylline, or the adenosine uptake inhibitor, *S*-(*p*-nitrobenzyl)-6-thioinosine (90), suggesting that P2 receptors are directly activated by ATP, rather than requiring ATP to be converted to adenosine with subsequent stimulation of P1 receptors. This is consistent with observations made using blood-perfused juxtamedullary afferent arterioles, in which the afferent arteriolar vasoconstriction to ATP was enhanced by addition of the adenosine receptor antagonist (86). In contrast, micropuncture studies showed an attenuated TGF response by inhibition of adenosine formation with 5′-nucleotidase blocker, α,β-methylene adenosine diphosphate (102), and by deletion of ecto-5′-nucleotidase (CD73) gene expression (122, 123).

Taken together, these findings reveal the complexity of the renal autoregulatory mechanism. It is difficult to reconcile the experimental differences between the adenosine and ATP hypotheses; however, differences in nephron population may be involved. The majority of studies supporting adenosine as the mediator of TGF have been conducted using nephrons located in the superficial kidney surface, whereas much of the work directed at ATP and P2 receptors has been performed using inner cortical juxtamedullary nephrons. Although functional differences exist between superficial and juxtamedullary nephrons (64, 124, 125), current evidence clearly demonstrates that ATP rather than adenosine is released from macula densa cells in response to stimulation of TGF (114–116, 126). The important question remaining is whether or not ATP released from macula densa cells acts directly through stimulation of P2 receptors or is hydrolyzed to adenosine, which evokes TGF responses through activation of A_1 receptors on afferent arterioles.

Purinoceptors in Hypertension

Hypertension is very common in patients with chronic renal failure. Experimental evidence shows that in some hypertensive animal models renal autoregulation is impaired or the efficiency of the renal autoregulatory response is reduced (117, 127–133). Considering the role of purinoceptors in regulation of renal microvascular function and autoregulation, modulation of hemodynamic function, and tubular transport, it seems reasonable to question the role of purinoceptors in the pathophysiology of hypertension. Accumulating evidence indicates that purinoceptors may play a role in functional adaptations in development of hypertension and contribute to the pathophysiology of hypertension (128, 134, 135). Increased expression of $P2X_7$ receptor immunoreactivity is reported in mesangial cells collected from Ren-2 transgenic hypertensive rat kidney (135). The ATP-stimulated intracellular Ca^{2+} was significantly increased in glomerular mesangial cells in spontaneously hypertensive rats, and the P2Y receptors in mesangial cells could be resensitized by nitric oxide in Wistar Kyoto rats but not spontaneously hypertensive rats (136). Other studies (137) investigated the role of purinoceptors in a hypertensive rat model induced by chronic infusion of angiotensin II. This model exhibits a markedly impaired autoregulatory capability (128, 138), and Zhao and colleagues (137) found that afferent arteriolar vasoconstrictor responses to ATP and β,γ-methylene ATP were significantly attenuated. The response to adenosine was unchanged. $P2X_1$ receptor–mediated impairment of afferent arteriolar vasoconstriction is associated with a reduction of agonist-induced elevation of intracellular Ca^{2+} in freshly isolated microvascular smooth muscle cells. In this regard, the reduced ATP-mediated vasoconstriction of rat afferent arterioles may contribute to hypertension-induced renal injury by impairment of renal autoregulatory efficiency, resulting in increased glomerular capillary pressure. The relationship of reduced P2 receptor–mediated vasoconstriction to autoregulatory impairment and hypertension-induced renal injury remains to be clarified.

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