ATP, P2 receptors and the renal microcirculation

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Abstract Purinoceptors are rapidly becoming recognised as important regulators of tissue and organ function. Renal expression of P2 receptors is broad and diverse, as reflected by the fact that P2 receptors have been identified in virtually every major tubular/vascular element. While P2 receptor expression by these renal structures is recognised, the physiological functions that they serve remains to be clarified. Renal vascular P2 receptor expression is complex and poorly understood. Evidence suggests that different complements of P2 receptors are expressed by individual renal vascular segments. This unique distribution has given rise to the postulate that P2 receptors are important for renal vascular function, including regulation of preglomerular resistance and autoregulatory behaviour. More recent studies have also uncovered evidence that hypertension reduces renal vascular reactivity to P2 receptor stimulation in concert with compromised autoregulatory capability. This review will consolidate findings related to the role of P2 receptors in regulating renal microvascular function and will present areas of controversy related to the respective roles of ATP and adenosine in autoregulatory resistance adjustments.

Keywords Afferent arteriole · Autoregulation · Adenosine · P2X receptors · Hypertension · Tubuloglomerular feedback

Introduction

Understanding the physiology of renal purinoceptors has become a rapidly developing area of investigation. It is now recognised that extracellular ATP and adenosine are important signalling molecules regulating the renal microcirculation and tubular function and influencing renin secretion [1–20]. This review will summarise information defining the roles of extracellular ATP in regulating renal microvascular function and renal haemodynamics.

P2 receptors and their expression in the renal vasculature and glomeruli

P2 receptors are expressed by renal vascular, glomerular, mesangial and tubular epithelial cells [1–15]. P2X₁ receptors are expressed by vascular smooth muscle of arcuate and interlobular arteries and afferent arterioles but do not appear to be expressed by glomeruli or efferent arterioles [4, 12, 21]. P2X₁ receptor expression by afferent arteriole vascular smooth muscle has been confirmed by immunostaining and by western blot analysis [4, 11]. P2Y₁ receptors are found on afferent and efferent arterioles and P2X₂ receptor expression has been detected in larger intrarenal arteries and veins [6]. P2Y₁, P2Y₂ and P2X₇ receptors are expressed in rat mesangial cells [22, 23] and P2Y₂ receptors are found in podocytes [3, 6]. Mouse mesangial cells express P2X₂, P2X₄, P2X₇, P2Y₂ and P2Y₄ receptors. mRNA is detected for P2X₁ and P2X₃ receptors in murine mesangial cells but protein is not found by western blot analysis [9]. Considering the wide distribution of renal P2 receptor subtypes, it seems likely that purinoceptors play diverse roles in regulating renal vascular and tubular function.

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Purinoceptors and renal haemodynamics

The modulation of renal vascular resistance by nucleotides and nucleosides has been recognised since the 1920s, but only received widespread attention in recent decades [24–42]. The renal vascular response to infused ATP or other P2 agonists depends on many factors including the species, the type of agonist infused, ambient vascular tone and the experimental conditions. Intrarenal infusion of ATP into canine kidneys produces vasodilation by stimulating endothelial release of nitric oxide [43, 44]. Vasoconstriction is observed in isolated perfused rat kidneys under basal tone, and either vasoconstriction or vasodilation when renal vascular resistance is elevated [45–48]. Infusion of ATP or α β -methylene ATP (an effective P2X₁ receptor agonist, which also has some influence on P2X₃ and P2X₅ receptors) into the isolated perfused rat kidney induces a sustained concentration-dependent vasoconstriction under normal conditions [45–49], but ATP-mediated responses are less consistent under high-tone conditions [45, 48].

Suprarenal aortic infusion of ATP into anaesthetised rats that had been fed on a low salt diet led to nitric oxide-dependent vasodilation in the medullary circulation, whereas in rats fed a high salt diet, medullary blood flow decreased in response to ATP [50]. P2Y receptor agonists, such as 2-methylthio ATP, UTP or ATP- γ -S, produce nitric oxide-dependent vasodilation when infused at low concentrations but vasoconstriction at higher concentrations [45, 49]. Thus, endothelial P2Y receptors may mediate vasodilation due to nitric oxide-dependent relaxation of the renal microvasculature, which reverts to a pronounced vasoconstriction during inhibition of nitric oxide synthase [45]. P2X receptor-mediated vasoconstrictor responses are also augmented by inhibition of nitric oxide production [45].

P2 receptors and the renal microcirculation: single vessel studies

Extracellular ATP is an important autocrine and paracrine regulator of preglomerular vascular responses, mediated by P2X and P2Y receptor activation [21, 33, 41, 51–55]. Infusion of relatively low doses of α β -methylene ATP into the rabbit renal artery produces renal cortical and medullary vasoconstriction as indicated by decreases in regional blood flow [56, 57]. Exposure of microperfused rabbit afferent arterioles (with attached glomeruli) to ATP or β γ -methylene ATP reduced arteriolar diameter [41]. Afferent arterioles also express adenosine-sensitive A₁ receptors, raising the question of whether the vasoconstriction is induced by ATP-dependent activation of P2 receptors, or whether the ATP is hydrolyzed to adenosine which vasoconstricts arterioles by activating A₁ receptors. The fact that ATP vasoconstricts afferent arterioles directly was estab-

lished by demonstrating that ATP-mediated vasoconstriction persists even during adenosine receptor blockade and that the sustained vasoconstriction to ATP is eliminated during P2 receptor blockade [21, 33, 51].

Renal vascular resistance is regulated primarily by adjusting afferent arteriolar resistance, with only lesser resistance contributions being made by the upstream arterial segments [58]. Of the three preglomerular vascular segments evaluated for responsiveness to P2 receptor activation by ATP, only the afferent arteriole exhibits sustained ATP-mediated vasoconstriction at concentrations as low as 100 nM (Fig. 1) [21]. Arcuate arteries respond to 10 and 100 μ M ATP with a transient vasoconstriction that quickly subsides [21]. Interlobular arteries respond to 100 μ M ATP with a sustained vasoconstriction, but lower concentrations produce no significant response [21]. Importantly, efferent arterioles are completely unresponsive to ATP concentra-

Fig. 1 ATP concentration–response relationship for the intrarenal pre- and postglomerular vascular segments. Average segmental diameter responses evoked by ATP applied to the adventitial surface of arcuate arteries (*top panel*), interlobular arteries (*second panel*), afferent arterioles (*third panel*) and efferent arterioles (*bottom panel*). After the control period (*Con*), increasing concentrations of ATP (0.1, 1.0, 10 and 100 μ M) were applied at 5-min intervals as indicated by the dotted lines. Each protocol ended with a 5-min recovery period (*Rec*) while bathed with control solution. Each data point represents diameter measurements taken at 12-s intervals and normalised as a percentage of the control diameter. Data were modified from an earlier report [21]

tions as high as 100 μM [21]. These observations support the hypothesis that extracellular ATP could serve as a paracrine regulator whose actions primarily influence afferent arteriolar vascular smooth muscle and thus arteriolar diameter [8, 12, 55, 59].

As indicated above, P2Y receptors modulate vascular resistance by stimulating the synthesis and release of endothelium-derived relaxing factors [45, 49, 60]. Direct assessment of the contribution of endogenous nitric oxide in single intrarenal arteries reveals that the transient ATP-mediated vasoconstriction of rat arcuate arteries reverts to a sustained vasoconstriction in kidneys pretreated with L-NAME [60]. This observation is consistent with whole-kidney studies where inhibition of nitric oxide synthase with L-NAME greatly reduces the renal vasodilation evoked by ATP or P2Y receptor agonists such as 2-methylthio ATP or UTP [46, 61]. Furthermore, intrarenal infusion of ATP into the canine kidney in vivo produces a rapid vasodilation under basal conditions; whereas during inhibition of nitric oxide synthase, ATP produces sustained renal vasoconstriction [43]. Thus, nitric oxide is responsible for P2 receptor-mediated renal vasodilation, suggesting that regulation of renal vascular tone by ATP may involve a complex interplay between segmental vasoconstrictor and vasodilatory signals arising from selective, paracrine activation of P2 receptors on renal vascular smooth muscle cells and endothelial cells. This idea is supported by observations in other renal and nonrenal vascular beds demonstrating that nitric oxide and endothelium-derived hyperpolarizing factor(s) contribute to the vasodilation produced by P2Y receptor activation [61–66].

P2 receptor-mediated second messenger systems in the renal microcirculation

Preglomerular vascular smooth muscle cells and glomerular mesangial cells respond to α β -methylene ATP, or ATP, with a rapid, biphasic increase in intracellular calcium concentration. This calcium response involves calcium release from intracellular stores and/or influx of calcium from the extracellular fluid (see example traces shown in Fig. 2 and Fig. 5b) [9, 11, 59, 67–74]. Low concentrations of ATP ($\leq 1.0 \mu\text{M}$) vasoconstrict afferent arterioles by activation of L-type Ca^{2+} channels. Higher concentrations of ATP ($> 1.0 \mu\text{M}$) vasoconstrict afferent arterioles by combining calcium influx and calcium release to increase the intracellular calcium concentration [11, 59, 73–76]. Calcium influx, and afferent arteriolar vasoconstriction, induced by ATP is markedly attenuated by blockade of voltage-gated L-type Ca^{2+} channels [59, 72–76]. Removal of extracellular calcium merely blunts the peak increase in calcium induced by 10 μM ATP, while the sustained elevation of calcium is abolished (Fig. 2b vs. d) [59, 73,

74]. Thus, release of calcium from intracellular stores is a major contributor to the peak calcium response to ATP, while calcium influx supports the sustained increase in intracellular calcium concentration [59, 72–76]. Interestingly, the sustained concentration-dependent afferent arteriolar vasoconstriction by ATP is attenuated, or eliminated, by superfusion with calcium-free medium or blockade of L-type Ca^{2+} channels [75, 76].

P2X receptors are ligand-gated channels that allow influx of extracellular Ca^{2+} and Na^{+} ions. Activation of P2X receptors on afferent arterioles leads to calcium influx, whereas calcium mobilisation from intracellular stores occurs in response to P2Y receptor activation (Fig. 2) [59, 74]. Thus, P2X₁ receptor-mediated vasoconstriction and calcium signalling responses are eliminated in calcium-free bathing solutions (Fig. 2a vs. c) and during blockade of voltage-gated, L-type Ca^{2+} channels [59, 72–76]. In contrast, P2Y receptor-mediated vasoconstriction and calcium signalling responses persist during calcium channel blockade or in calcium-free conditions (Fig. 2d), indicating differing signalling mechanisms between the two receptor families [59, 72–76]. In summary, activation of voltage-dependent calcium channels and calcium influx are important signalling elements for ATP-mediated vasoconstriction of afferent arterioles through P2X₁ receptor activation, whereas calcium release mechanisms predominate in vascular responses evoked by P2Y receptor activation.

Studies also indicate that cytochrome P450 metabolites, such as 20-hydroxyecosatetraenoic acid (20-HETE), may play an important role as second messengers facilitating P2 receptor activation in renal microvascular smooth muscle. 20-HETE is an important modulator of L-type Ca^{2+} channel function, K^{+} channel function and renal vascular autoregulatory responses [77–80]. In one study, afferent arteriolar responses to ATP, α β -methylene ATP and UTP were determined before and after treatment with the selective CYP450 hydroxylase inhibitor, *N*-methylsulfonyl-12,12-dibromododec-11-enamide (DDMS), or the 20-HETE antagonist, 20-hydroxyecos-6(Z),15(Z)-dienoic acid (20-HEDE) [81]. The sustained vasoconstriction normally observed in response to α β -methylene ATP under control conditions was eliminated during 20-HETE inhibition with DDMS or 20-HEDE [81]. In contrast, afferent arteriolar vasoconstriction induced by P2Y₂ receptor activation with UTP was unaffected by inhibition of 20-HETE [81]. The ATP-induced increase in intracellular calcium concentration in preglomerular microvascular smooth muscle cells was significantly attenuated by 20-HEDE [82]. Similarly, 20-HETE inhibition attenuated the increase in intracellular calcium concentration induced by α β -methylene ATP, but responses evoked by UTP were unchanged [82]. These results demonstrate that 20-HETE plays a significant role in the renal vascular vasoconstrictor response and the eleva-

Fig. 2 The effect of α β -methylene ATP and ATP on intracellular calcium concentration in preglomerular smooth muscle cells. Response of intracellular calcium concentration evoked by α β -methylene ATP or ATP (each 10 μ M) in the presence of 1.8 mM extracellular calcium (**a** and **b**), in the absence of extracellular calcium (**c** and **d**) and during P2X₁ receptor blockade with NF-279 (**e**). The periods of exposure to α β -methylene ATP or ATP administration are indicated by the *black bars*. The periods of exposure to calcium-free medium or NF-279 are shown by a second set of *black bars*. Calcium measurements were performed using fura 2. Data were modified from an earlier report [74]

tion of intracellular calcium concentration in response to P2X₁ receptor activation.

Rho-kinase and its multiple co-factors modulate vascular tone, purportedly by increasing calcium sensitivity and thus smooth muscle contractility [83–85]. The Rho-kinase system also influences P2 receptor-mediated vasoconstriction, 20-HETE-dependent intracellular signaling, renal microvascular function and myogenic autoregulatory behaviour [86–93]. Inhibition of Rho-kinase in

hydronephrotic kidneys vasodilates virtually every pre- and postglomerular artery/arteriole and attenuates endothelin- or adenosine-induced renal microvascular vasoconstriction [87]. Similarly, preliminary in vitro studies revealed that Rho-kinase inhibition with Y-27632 produced a rapid, concentration-dependent afferent arteriolar vasodilation and inhibited renal autoregulatory responses [88]. In addition, vasoconstrictor responses induced by angiotensin II or ATP were markedly attenuated [88]. Interesting-

ly, the afferent arteriolar vasoconstriction elicited by α β -methylene ATP was completely eliminated [88]. Thus, P2 receptor activation involves induction of a number of second messenger systems that also play major roles in regulating pressure-dependent renal perfusion.

P2 receptors and their roles in renal autoregulation and tubuloglomerular feedback

The phenomenon of autoregulation is a critical renal microvascular control mechanism that serves to assure and protect normal renal function [55, 94–98]. The autoregulatory mechanism maintains a stable renal blood flow (Fig. 3a), glomerular capillary pressure and glomerular filtration rate by buffering acute changes in renal perfusion pressure with precise adjustments in preglomerular vascular resistance [36, 55, 94–98]. Whole-kidney autoregulation integrates the combined influences of two major regulatory systems. They include an intrinsic myogenic mechanism operating along the preglomerular vascular tree, and the tubuloglomerular feedback mechanism, which regulates tone in the distal afferent arteriole through an interaction between the arteriole and the macula densa region in the thick ascending limb of the loop of Henle of the same nephron [55, 95, 97–101]. The signalling mechanism(s) by which changes in transmural pressure and/or stimulation of tubuloglomerular feedback produce precise adjustments in afferent arteriolar resistance remain somewhat controversial and are under intensive study. This section of the review will try to highlight the important controversies in the area but will not go into exhaustive detail. The interested reader is referred to some excellent reviews, which devote more attention to the subject [8, 12, 28, 95, 97, 98, 102–106] (plus, see article by Bell et al [107] in this Special Issue). Instead, this review will focus on the critical data leading to the postulate that ATP transduces haemodynamic and tubular information into autoregulatory adjustments in afferent arteriolar diameter by stimulating P2 receptors.

It is generally agreed that autoregulation is mediated by a locally generated paracrine messenger molecule, or molecules, linking myogenic and tubuloglomerular feedback signals from the macula densa with highly precise adjustments in afferent arteriolar resistance to modulate glomerular capillary pressure and glomerular filtration rate [36, 55, 95, 97, 98]. Recently, most attention has focused on the respective roles of adenosine versus its precursor, ATP, as direct effectors of autoregulatory vascular responses [33, 55, 95, 97, 98, 101]. The evidence for adenosine in modulating tubuloglomerular feedback arises mainly from micropuncture studies in rat kidneys. Pharmacological blockade of A_1 receptors blunts tubuloglomerular feedback-dependent changes in proximal tubule stop-flow pressure stimulated by increasing distal tubular

Fig. 3 Postulated signalling mechanisms for ATP-mediated autoregulatory adjustments in afferent arteriolar diameter. Panel **a** illustrates a normal profile for autoregulation of renal blood flow (solid black line) and the autoregulatory profiles that might be observed under conditions of moderate and severe autoregulatory dysfunction (modified from [171]). **b** ATP might be released from afferent arteriole smooth muscle cells in response to an increase in renal perfusion pressure. This ATP released into the perivascular interstitial fluid could act upon $P2X_1$ receptors to evoke autoregulatory vasoconstrictor responses. ATP released from the macula densa, in response to an increase in distal tubular fluid NaCl concentration, can traverse the interstitial fluid of the juxtaglomerular apparatus to act on afferent arterioles and induce tubuloglomerular feedback-mediated vasoconstriction. Alternatively, released ATP could be degraded to adenosine in the interstitial fluid prior to activating afferent arteriolar adenosine A_1 receptors to regulate afferent arteriolar resistance

NaCl concentration [108–110]. Tubuloglomerular feedback responses are also inhibited by suppression of adenosine formation from ATP using an antagonist of ecto-5'-nucleotidase (the enzyme that catalyses the final reaction in the formation of adenosine), either alone or in combination with continuous administration of an A_1 receptor agonist (cyclohexyladenosine) to “clamp” endogenous adenosine levels [109, 111]. Furthermore, mice deficient in ecto-5'-nucleotidase have attenuated tubuloglomerular feedback responses [111, 112]. These observations support a primary

role for A₁ receptors in mediating tubuloglomerular feedback responses (Fig. 3b). Alternatively, the activation of vasodilatory A₂ receptors on afferent arterioles could blunt autoregulatory and tubuloglomerular feedback responses under conditions where A₁ receptors are genetically deleted/inactivated or they are blocked pharmacologically [54, 102, 113]. Nevertheless, renal blood flow autoregulation is inhibited by approximately 40% in adenosine A₁ receptor-deficient mice and tubuloglomerular feedback responses are attenuated [114, 115]. Interestingly, glomerular filtration rate is normal in these mice. Collectively, these data support the hypothesis that adenosine is an important paracrine signalling molecule for transmitting tubuloglomerular feedback and contribute to whole-kidney autoregulatory responses [114–118].

However, other results suggest that adenosine and A₁ receptors are less important for overall autoregulatory resistance adjustments in both in vivo and in vitro settings [30, 54, 113, 119]. Only modest inhibition of tubuloglomerular feedback responses was observed during peritubular perfusion with adenosine receptor antagonists or saturating concentrations of adenosine [54], and autoregulation of renal blood flow and glomerular filtration rate remains normal in the canine kidney during adenosine receptor blockade [30, 119]. Micropuncture studies revealed that blockade of adenosine A₁ receptors dilated rat afferent arterioles and their upstream arterial segments, but tubuloglomerular feedback responses remained intact [34, 54]. Pressure-mediated vasoconstriction of rat juxta-medullary afferent arterioles is unaffected by blockade of A₁ receptors [33, 120]. During saturation of adenosine receptors with high doses of adenosine, marked vasodilation and loss of afferent autoregulatory responses occurred via an A₂ receptor mechanism [113]. Blockade of A₂ receptors alone or combined A₂ and A₁ receptor antagonism restored afferent arteriolar autoregulatory capability [113]. Collectively, these studies suggest that adenosine modulates autoregulatory responses via selective activation of A₁ and A₂ receptors, but is not essential for the manifestation of autoregulatory responses.

An alternative hypothesis is that extracellular ATP serves as the primary signalling molecule mediating renal autoregulatory and tubuloglomerular feedback responses (Fig. 3b) [33, 43, 51, 54, 95, 97, 120–125]. Microdialysis of the renal cortical interstitium reveals that the concentration of ATP in the cortical interstitial fluid increases as renal arterial pressure increases and as autoregulatory adjustments in renal vascular resistance occur. Interstitial adenosine concentrations do not change during this period [36, 120, 124, 125]. Renal cortical interstitial ATP concentration also increases when tubuloglomerular feedback responses are induced by increasing distal volume delivery by means of acetazolamide treatment [120, 125]. Conversely, inter-

stitial ATP concentration decreases when tubuloglomerular feedback responses are inhibited with furosemide [120, 125]. These manipulations of tubuloglomerular feedback signals do not alter renal interstitial adenosine concentrations. In addition, pressure- and tubular flow-mediated increases in renal cortical interstitial ATP concentration were maintained in kidneys treated with the L-type Ca²⁺ channel blocker, nifedipine, which completely prevented autoregulatory adjustments in renal vascular resistance, indicating that changes in interstitial ATP concentrations precede changes in renal vascular resistance [124]. Thus, interstitial ATP concentrations are directly correlated with conditions that evoke tubuloglomerular feedback and myogenic autoregulatory responses, consistent with extracellular ATP mediating autoregulatory adjustments in preglomerular vascular resistance (Fig. 3b).

As indicated above, afferent arterioles represent the primary preglomerular renal microvascular element that sets and regulates renal vascular resistance. Traditionally, direct assessment of afferent arteriolar responses to vasoactive stimuli has been accomplished either using isolated, cannulated arterioles held on perfusion pipettes in vitro or using hydronephrotic kidneys in vitro or in vivo. The in vitro blood-perfused juxtamedullary nephron preparation presents a unique opportunity to directly observe renal microvascular function without separating the vascular element from upstream or downstream vascular segments and while maintaining the association of the blood vessel with the tubular structures it serves [126–129]. Accordingly, this preparation provides a useful tool for observing myogenic and tubuloglomerular feedback contributions to renal autoregulation [99, 130]. With this preparation, pressure-mediated afferent arteriolar vasoconstriction is markedly attenuated by P2 purinoceptor desensitisation, by pharmacological blockade of P2 receptors or by genetic deletion of P2X receptors [12, 33, 51, 131]. As shown in Fig. 4, pressure-mediated afferent arteriolar autoregulatory vasoconstriction was inhibited by non-selective P2 receptor blockade with suramin or PPADS or by more selective P2X receptor blockade with NF-279 [33, 51]. Furthermore, mice lacking P2X₁ receptors exhibit impaired pressure-mediated afferent arteriolar vasoconstriction [33]. While it was clear that deletion or inactivation of P2X₁ receptors inhibited autoregulatory behaviour, it was not clear if this inhibition reflected loss of myogenic behaviour or tubuloglomerular feedback influences. In order to try to identify which component of autoregulation was influenced by P2X₁ receptor inactivation, experiments were performed to delete the tubuloglomerular feedback component. The rationale was that if tubuloglomerular feedback responses were already absent in the P2X₁ knockout mice, then interventions designed to inhibit tubuloglomerular feedback responses would have no effect on the autoregulatory

Fig. 4 Effect of P2 receptor blockade on the afferent arteriolar autoregulatory response induced by an increase in perfusion pressure. Pressure-mediated afferent arteriolar autoregulatory responses are depicted before and during P2 receptor blockade with NF-279 (*top panel*), PPADS (*centre panel*) and suramin (*bottom panel*). Autoregulatory responses were induced by increasing perfusion pressure in 30 mmHg increments at 5-min intervals. The data are expressed as a percentage of the control diameter and each data point represents the average diameter response over the last 2 min of each period. Control responses are shown by the black symbols and the response during P2 receptor blockade are shown by the grey symbols. At the end of each protocol, perfusion pressure was returned to 100 mmHg to determine recovery for the pressure stimulus. Data are modified from earlier reports [33, 51]

response in these mice, whereas the same interventions would attenuate overall autoregulatory responses in normal wild-type mice by subtracting the tubuloglomerular feedback contribution from the overall autoregulatory response. Accordingly, tubuloglomerular feedback responses were inhibited by either by resection of the loops of Henle (papillectomy) or by exogenous administration of furosemide. Acute papillectomy interrupts the flow of distal tubular fluid past the macula densa and minimises tubuloglomerular feedback-dependent influences on afferent arteriolar function [33, 99, 132–134], while furosemide administration has been used to inhibit tubuloglomerular feedback responses by inhibiting the NKCC-2 transporter in the apical membrane of the macula densa cells [33, 125,

132, 133, 135–141]. Using these strategies, it was clear that pressure-mediated autoregulatory responses were significantly blunted in wild-type mice, whereas furosemide or papillectomy had no effect on the autoregulatory response observed in P2X₁ receptor knockout mice [33]. This observation suggests that the tubuloglomerular feedback component of the autoregulatory response is already absent in mice lacking P2X₁ receptors. Further support for the P2X₁ receptor hypothesis arises from the observation that afferent arterioles from P2X₁ knockout mice vasoconstrict during A₁ receptor stimulation with the A₁ receptor agonist, N⁶-cyclopentyl adenosine, and A₁ receptor-mediated vasoconstriction of rat afferent arterioles was not affected by P2X₁ receptor blockade with NF-279 [33]. Thus, P2X₁ receptor knockout mice exhibit blunted autoregulatory behaviour despite retaining a functional adenosine receptor system. These data suggest that ATP-sensitive P2X₁ receptors are essential signalling components of pressure-mediated autoregulatory behaviour and for translating macula densa signals into tubuloglomerular feedback-mediated vasoconstriction of afferent arterioles.

That ATP is a mediator of tubuloglomerular feedback signals is supported by recent studies showing that macula densa cells release ATP in response to conditions known to evoke tubuloglomerular feedback responses [121–123]. Using a microdissected glomerular preparation with an attached macula densa, Bell et al [121] determined that ATP was released from the basolateral aspect of the macula densa in response to a tubuloglomerular feedback stimulus. They used biosensor cells overexpressing P2X receptors to monitor cellular responses by whole-cell patch clamp. When the biosensor was placed in close proximity to the basolateral surface of the macula densa, a tubuloglomerular feedback stimulus stimulated an increase in the intracellular calcium concentration in the biosensor cell. The calcium concentration in the biosensor cell also increased when exposed to ATP directly, or when the NaCl concentration in the distal tubular fluid was increased. The calcium response was prevented if the biosensor was moved away from the macula densa. These data argue that ATP, or another purinergic substance, is released from the basolateral aspect of the macula densa into the surrounding fluid.

Studies using the isolated perfused rabbit juxtaglomerular apparatus, combined with confocal fluorescence imaging, demonstrate propagation of a calcium signal from the macula densa toward the proximal afferent arteriole, the adjacent glomerulus and intraglomerular cells, in response to increasing tubular flow past the macula densa [142]. Propagation of the calcium signal and afferent arteriolar vasoconstriction are inhibited by P2 receptor blockade but not by P1 receptor blockade. Accordingly, ATP appears to represent a key signalling molecule linking the macula

densa with tubuloglomerular feedback-mediated afferent arteriolar vasoconstriction [143].

Interestingly, increases in tubular fluid flow rate in isolated perfused mouse thick ascending loops of Henle result in P2 receptor-mediated increases in epithelial cell cytosolic calcium concentration [144]. This flow-induced elevation of intracellular calcium concentration was almost completely blocked when the non-selective P2 receptor antagonist, suramin, or the ATP scavenger, apyrase, was added to the bath solution. These data suggest that increasing tubular fluid flow and/or tubular distention stimulate ATP release from the basolateral aspect of the renal tubule into the adjacent bathing medium. Addition of suramin or apyrase to the luminal perfusate also significantly blunted the perfusion-induced increase in intracellular calcium concentration in the tubular epithelial cells. The change in fluid flow is postulated to be detected by mechanical stimulation of a primary cilium extending into the tubular lumen from the apical membrane of the epithelium. This study suggests that mouse thick ascending loop of Henle tubular epithelial cells are capable of detecting changes in tubular distention and/or tubular fluid flow rate and respond by releasing ATP into the tubular fluid and into the “interstitial fluid” adjacent to the basolateral surface of the tubule. Interestingly, similar observations have also been made for other renal tubular epithelia. Mechanical stimulation of apical monocilia has been found to regulate ATP release from cultured mouse collecting duct principal cells [145]. Flow-induced ATP release was robust in cilium-competent monolayers of epithelial cells but significantly blunted in cilium-deficient monolayers [145]. This may represent an important sensory system used by macula densa cells to monitor tubular fluid flow and thereby influence macula densa ATP release. Macula densa cells extend a single cilium from the apical membrane into the tubular lumen [146]. Thus, to extrapolate from observations made in other renal epithelia to the macula densa, mechanical stimulation of the macula densa cilia may trigger ATP release from the basolateral membrane into the interstitial fluid adjacent to the basolateral surface of the macula densa plaque and initiate tubuloglomerular signals to the afferent arteriole.

Metabolism of extracellular ATP involves ectonucleotidases that are highly expressed in the kidney [7, 147–153] (plus see article by Shirley, Vekaria & Sévigny [154] in this Special Issue). Ectonucleotidases cleave ATP to AMP and 5'-nucleotidase hydrolyzes AMP to adenosine. These ectoenzymes may represent a mechanism by which the interstitial adenosine concentration is regulated through systematic degradation of ATP in the renal interstitial fluid. Thus, ectonucleotidases may catabolize ATP released from the macula densa to yield adenosine that can activate P1 receptors. To distinguish P2 receptor-mediated

effects from P1 receptor effects, various pharmacological, biochemical and molecular tools have been used, including metabolically stable P2 receptor agonists, P1 and P2 receptor antagonists, adenosine uptake inhibitors and ectonucleotidase-deficient mice. In isolated perfused rat kidneys, the vascular response to ATP is unaltered by the adenosine receptor antagonist, 8-phenyltheophylline or the adenosine uptake inhibitor, *S*-(*p*-nitrobenzyl)-6-thioinosine [45]; indicating that P2 receptors are directly activated by ATP. In juxtamedullary nephrons, ATP-mediated vasoconstriction of afferent arterioles is enhanced during adenosine receptor blockade [21]. However, Takenaka et al reported that enhanced production of adenosine from AMP by intrarenal infusion of 5'-nucleotidase improved autoregulation in Thy-1 nephritic rats, which are a model of mesangial cell ablation pathology associated with impaired autoregulation [153]. Moreover, tubuloglomerular feedback stop-flow pressure responses are attenuated by inhibition of adenosine formation with the 5'-nucleotidase blocker, α β -methylene ADP [109], and by deletion of the gene coding for ecto-5'-nucleotidase (CD73) expression [111, 112]. However, if ATP is not metabolised and its concentration increases in the interstitial fluid, it is possible that it could desensitize P2 receptors and thereby lead to reduced tubuloglomerular feedback reactivity.

Studies were recently performed to assess the ability of the cells making up the glomeruli to catabolize ATP to its main degradation products: ADP, AMP and adenosine [151]. Incubation of isolated glomeruli under control conditions revealed that basal extracellular ATP concentrations in the bathing medium reached 1.0 nM and increased approximately 6.5-fold when ecto-ATPase activity was inhibited with 6-*N*, *N*-diethyl- β - γ -dibromomethylene-D-adenosine-5-triphosphate (ARL67156) [151]. In the absence of ecto-ATPase inhibitor, exogenous ATP added to these isolated glomeruli was almost completely hydrolyzed within 20 min, with AMP being the major catabolic product. AMP has little effect on afferent arteriole diameter [151]. Mechanical perturbation of isolated glomeruli during inhibition of ecto-ATPase activity increases the ATP concentration in the bathing medium by approximately 35%. These data demonstrate that ATP is constitutively released from isolated rat glomeruli and that this release is enhanced by mechanical stimulation or glomerular deformation. Glomeruli express detectable ecto-5'-nucleotidase activity which can be modulated by dietary salt [152].

A recent report suggests a potential interaction between ATP- and adenosine-mediated autoregulatory influences and connexins 37 and 40 [155]. Blockade of connexins 37 and 40 attenuated autoregulatory behaviour by approximately 50%. Blunting of the autoregulatory response with connexin blockers was augmented by A₁ receptor blockade but not by P2 receptor blockade. However, when A₁ and P2

receptor blockers were used together, the impact of connexin inhibitors was significantly enhanced. These data suggest that A₁ receptors and P2 receptors may interact with each other and with connexins 37 and 40 to transduce purinergic autoregulatory signals intended to regulate preglomerular resistance. The authors concluded that A₁ and P2 receptors are needed to achieve complete whole-kidney autoregulatory behaviour and suggested that A₁ and P2 receptors interact in that response.

Purinoceptors in hypertension

Hypertension is frequently associated with progressive renal injury that develops through a number of poorly understood factors [156, 157]. Compromised renal autoregulatory efficiency can be a contributing factor that results in chronic elevation of glomerular capillary pressure and subsequent glomerular injury [96]. Renal autoregulation is impaired in many experimental models of hypertension [106, 158–165]. Given that purinoceptors are important for regulation of renal microvascular function and autoregulation, and for modulation of haemodynamic function and tubular transport, it is reasonable to examine the relationship between purinoceptor function and hypertension-related renal injury. Studies indicate that purinoceptors contribute to the functional adaptations in the development of hypertension and may contribute to the renal pathophysiology of hypertension [162, 166, 167]. P2X₇ receptor immunoreactivity is increased in mesangial cells from Ren-2 transgenic hypertensive rats [166]. Renal injury in Ang-II-infused hypertensive rats is ameliorated by treatment with the P2Y₁₂ receptor antagonist, clopidogrel, as well as the non-selective P2 receptor blocker, PPADS, without reducing arterial pressure [168]. Pressure-mediated afferent arteriolar autoregulatory responses are attenuated in Ang-II-infused hypertensive rats [162, 169]. Consistent with impaired autoregulatory behaviour, afferent arteriolar vasoconstrictor responses to ATP and β γ -methylene ATP are markedly attenuated compared with those seen in normotensive controls (Fig. 5a), supporting the hypothesis that P2 receptors are essential elements in the autoregulatory response. Interestingly, afferent arteriolar responses to the P1 receptor agonist, adenosine, were unchanged [11]. P2X₁ receptor-mediated impairment of afferent arteriolar vasoconstriction is associated with impaired calcium signalling responses to ATP or β γ -methylene ATP in freshly isolated preglomerular smooth muscle cells from hypertensive rats compared to normotensive controls (Fig. 5b) [11]. Reduction of the calcium signalling response could account for the impaired afferent arteriolar autoregulatory efficiency and thus contribute to hypertension-induced renal and glomerular injury. Attenuated responsiveness to P2

Fig. 5 Effect of ANG-II hypertension on the afferent arteriolar diameter and calcium signalling responses to ATP. Panel a presents the changes in afferent arteriolar diameter in kidneys from normotensive rats (black symbols) and from Ang-II hypertensive rats (grey symbols). Data are expressed as a percentage of the control diameter. ATP was administered in concentrations of 0.1–100 μM. Values represent average diameters measured over the last 2 min of each treatment period and plotted as means ± SE. **P* < 0.05, significant difference from control diameter; #*P* < 0.05 significant difference from normotensive controls. **b** Presents the change in intracellular calcium concentration (detected by fura 2) induced by 10 μM ATP in single preglomerular smooth muscle cells taken from normotensive (black trace) and ANG II hypertensive rats (grey trace). The period of ATP administration is depicted by the black bar. Data are modified from an earlier report [11]

receptor stimulation in this model of hypertension is counter to responses observed with other vasoconstrictor agonists. For example, afferent arteriolar responses to angiotensin II are enhanced in Ang-II-infused hypertensive rats whereas the response to noradrenaline and adenosine are unchanged [160, 170]. Simultaneous loss of autoregulatory responsiveness and responsiveness to P2X₁ receptor activation is interesting and supports the argument that normally functioning P2X₁ receptors are essential for normal autoregulatory behaviour to occur.

Perspectives

P2 receptors in the control of renal tubular and haemodynamic function remain an emerging and exciting field of study. Evidence that P2 receptors are ubiquitously expressed throughout the kidney but that the receptor expression profile is different between different nephron segments and structures argues that each serves an important physiological function. Studies have not progressed far enough to definitively assign function to receptors expressed in each kidney region. However, compelling data are accumulating that implicate P2 receptors as contributing importantly to both physiological and pathophysiological processes. There is much to be learned about this class of receptors and there is much excitement ahead as their respective roles are defined.

It is clear that P1 and P2 receptors are effective modulators of renal microvascular function. P2X₁ receptors are important for pressure-dependent autoregulatory adjustments in renal vascular resistance. The debate continues on whether or not they are important for tubuloglomerular feedback adjustments in afferent arteriole resistance. Many other questions remain to be answered. What is(are) the actual messenger molecule(s) responsible for autoregulatory changes in renal vascular resistance? Is it ATP, adenosine or another yet unidentified substance? Are the myogenic and the tubuloglomerular feedback components of the whole-kidney autoregulatory response mediated by the same messenger molecule? What is the role of renal ectonucleotidases in the physiology of the purine signalling pathways described in this review? Is autoregulatory impairment directly responsible for hypertension-related kidney injury or are there other more important intermediates involved? If failure of the renal vascular purinoceptor system is responsible for some aspects of hypertensive renal injury, could it also be involved in renal injury in other conditions such as diabetes, obesity or immunosuppressive therapies? Answers to these and other questions will help push development of this field and will contribute greatly to our understanding of why these tissue-specific renal purinoceptor receptor systems are distributed the way they are. Time and better investigational tools will help to clarify the physiology of these receptors. Nevertheless, the data generated to date clearly establish the renal P2 receptor system as an important regulatory system for maintaining renal vascular and tubular function.

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