The Involvement of Purinergic Receptors in the Kidney: Physiological and Pathophysiologic Implications

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ABSTRACT

The studies on the purinergic system in the kidney clearly showed its role on the renal hemodynamics, glomerular filtration and tubular function. The effects of purinergic agonists on the mechanisms of tubuloglomerular feedback, and tubular transport of water and solutes, are well defined. In addition, several studies have documented the role of adenosine and specific ATP receptors on the processes of renal diseases, with special interest on the ischemia-reperfusion injury, renal cystic disease, glomerular and tubulointerstitial diseases. Therefore, the purinergic system has become a growing field for research in renal physiology and pathophysiology, leading to therapeutic possibilities of using specific agonists and antagonists.

Keywords: Purinergic receptors. Renal physiology. Renal diseases. ATP. Adenosine.

INTRODUCTION

The activation of purinergic receptors is involved in the mechanisms of several diseases, and has been increasingly a matter of recent investigations. Since the first study by Drury and Szent-Gyorgyi\(^1\) published in 1929, several investigators have done substantial contributions in the field of the “purinergic system”. In 1948, Buchthal and Folkow showed that acetylcholine-mediated skeletal muscle contraction was potentiated by ATP\(^2\). However it was only in the early seventies that Geoffrey Bunstock and his colleagues first documented the release of adenosine nucleotides at the level of non-adrenergic nerves in the gut, acting as a neurotransmitter\(^3,4\).

In the last nearly 40 years, the study of purinergic receptors has increasingly contributed to the knowledge of their implications in physiology and pathophysiology. So far, over 700 studies have been published implicating the role of purinergic receptors in diverse disease processes, and more than 600 papers have documented...
their presence in the kidney. This review introduces the nephrologist to the most relevant aspects of the purinergic system in the physiology and pathophysiology of the kidney.

THE PURINERGIC SYSTEM

Purinergic receptors bind nucleosides and specific nucleotides, with different subtypes and affinities for adenosine, AMP, ADP, ATP and UTP. There are essentially two major branches in the purinergic system: P1 and P2 receptors. P1, or most commonly called “A” receptors, are linked to adenosine and comprise four subtypes of G protein-coupled receptors: A1, A2A, A2B, and A3, according to the most recent nomenclature. Adenosine is mostly generated from the hydrolysis of ATP by cell energy generation, under the effect of endo- and ecto-nucleotidases. P2 receptors are more responsive to ATP, UTP and ADP. In 1985 Bunstock and Kennedy proposed a subclassification of P2 receptors into P2X and P2Y subtypes. P2Y family comprises metabotropic receptors, coupled to G protein. Presently, eight subtypes have been cloned: P2Y1, P2Y2, P2Y4, P2Y6, P2Y11, P2Y13, P2Y14. P2X receptors have two transmembrane (TM) regions with both N- and C-terminus located inside the cells. The exception is for P2X7 receptors, which has a longer C-terminus. P2Y presents seven TM with the N- and C-terminus in the extra and intracellular compartments, respectively. Figure 1 shows the topologies for P2X and P2Y receptors. P2X are ligand-gated ion channels for Na+, Ca+ and K+, known as ionotropic. Currently, seven subtypes of P2X receptors have been cloned and identified as P2X1-7.

Today, an increasing amount of information on the involvement of purinergic receptors on several disease models, are coming to knowledge. Adenosine has been implicated in chronic airway inflammation, Parkinson’s disease, cardioprotection, Huntington’s disease and bladder disfunction. Additionally, the polymorphic character of their structures is being characterized, and related to some diseases. Thus, P2X7 receptors are related to tuberculosis, chronic lymphocytic leukemia, human and murine lupus, and susceptibility for bipolar affective disorder, whereas a genetic variant of P2X5 has been related to chronic myeloid leukemia. In the case of P2Y receptors, P2Y1 may have an important role in cystic fibrosis and P2Y12 has been associated with risk for ischemic cerebrovascular events.

For nephrologists, a special interest has focused on the role of adenosine and adenosine nucleotides on the tubulo-glomerular feedback, and more recently in the beneficial effects of adenosine and its analogues on renal ischemia-reperfusion injury. Additionally, there are still much to be discovered in the area of purinergic P2 receptors, where special subtypes like P2X7 might be consistently involved in immunological and inflammatory processes of lesion to the glomerular and tubular renal cells and interstitial fibrosis.

PURINERGIC SIGNALING AND THE KIDNEY

This section will first approach the physiological and pathophysiological aspects of adenosine A receptors in the renal physiology, including tubuloglomerular feedback and their involvement on pathological conditions. Thereafter, the role of purinergic P2 receptors on the renal physiology, and their implications in various renal diseases will be addressed.

Adenosine and A receptors

Renal circulation and tubular physiology. In 1964, the studies by Thurau and also by Hashimoto et al. were the first to document a vasoconstrictive effect of adenosine on the renal circulation. In 1975, Osswald showed that this effect could be inhibited by theophylline. In addition, differential effects of intrarenal adenosine infusion were soon observed, in which a decrease in renal blood flow was detected in the more superficial areas of the cortex, whereas an increase was registered in deep portions of cortex and the medulla. The initial autoradiographic studies on adenosine receptors pointed to diverse localizations of A1 receptors. In the human kidney, a high density was measured over
the glomeruli. In the guinea-pig kidney the receptor sites were localized in the inner and outer medulla, although a low density of binding was also seen over the glomeruli. In 1991, Spielman and Arend reviewed the effects of adenosine in the hemodynamics of the kidney and on renal tubules, where adenosine was shown to increase fluid reabsorption. At the proximal tubule, it acts increasing the reabsorption of Na+ via A1 receptors. At more distal sites, however, a natriuretic effect predominates. In addition, a role in the metabolic control in certain circumstances, as in Na+ and water overload, has been suggested, decreasing oxygen consumption by inducing pre-glomerular vasoconstriction and decreasing GFR. In these conditions, there is increased luminal delivery of Na+, an increase in Na-K-ATPase activation, ATP hydrolysis and more adenosine released.

Tubuloglomerular feedback (TGF) and renin release. The investigation of the effectors of the TGF has pointed to adenosine as the mediator of afferent arteriole vasoconstriction. Further studies have better elucidated such mechanism. Vallon et al. have recently reviewed the effects of adenosine on kidney function, in which two aspects were emphasized; the vasoconstrictive effect of adenosine A1 receptors activation, and the inhibition of renin release. As a response to increased flow rate at the macula densa, which increases the concentration of luminal Na+ and Cl-, the increased influx of Na+, Cl- and K+ via the furosemide-sensitive co-transporter, stimulates both Na-K-ATPase and H-K-ATPase, which releases ADP, AMP and adenosine. The nucleotides are hydrolyzed to adenosine by ecto-nucleotidases. Adenosine then binds A1 receptors leading to increased intracellular calcium in extraglomerular mesangial cells. The results are increased vasoconstriction and decrease of renin secretion, respectively. Alternatively, it is noteworthy that substantial evidence points to ATP, acting on P2X1 receptors, as the main effector of TGF. Currently it is conceivable to consider both ATP and adenosine, effectors acting as mediators of TGF, with consistent involvement of A1 receptors. The effect of inhibition of renin release is mediated by A1 receptors, and it is thought that the activation of these receptors is required for inhibition of renin release after NaCl loading. Figure 2 illustrates the effector mechanisms proposed for the vasoconstrictive response of the TGF.

Implications in ischemia-reperfusion injury (IRI) and nephrotoxicity. The initial findings on the effect of adenosine were mostly described by Lee, Emala and others. Their studies using theophylline, the unselective adenosine receptor antagonist, in experimental acute renal failure, first suggested that the activation of these receptors might be deleterious to the renal parenchyma. However, pre-treatment with adenosine, A1 receptor activation, or A2A activation, clearly have resulted in improvement of the histological aspect after reperfusion. By contrast, studies on the A3 receptors stimulation evidenced that they indeed aggravate renal IRI. These findings may be related to the pro-apoptotic effect of A3 activation, observed on renal cells. Another unresolved issue is the paradoxical effect of theophylline, since it inhibits both A1 and A2A receptors, known to attenuate renal IRI when activated. Different experimental designs or even some as yet other unknown effects of theophylline that could overcome its effect on A1 and A2A receptors might explain this behaviour. More recent studies by Okusa and co-workers, have demonstrated the benefits of specific adenosine A2A agonists on IRI. The protective effect of ATL146e, a selective A2A agonist, was observed as a reduction of nearly 70% of renal damage when this drug was administered to rats after IRI.

The models of nephrotoxic renal damage have been used to demonstrate differential effects of adenosine receptors activation and inhibition. Cisplatin-induced

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**Figure 2.** Mechanisms of adenosine and ATP release as effectors of the TGF. Luminal Na+ and Cl- stimulate furosemide-sensitive Na-K-2Cl co-transport in the epithelial cells of the TAL, activating Na-K-ATPase and H-K-ATPase which generates ADP and AMP. These nucleotides are also hydrolyzed to adenosine by ecto-nucleotidases, which then binds to A1 receptors in the SM cells, promoting vasoconstriction. ATP may be released from cells through anion channels, and acts increasing intracellular calcium in mesangial cells probably by P2Y2 receptors, and promoting vasoconstriction by P2X1 in SM cells. Calcium activation is transmitted among cells through gap junctions, decreasing renin secretion by granular cells of the juxtaglomerular apparatus. [Ado: adenosine; 5'-NT: ecto-5'-nucleotidase; SM cells: smooth muscle cells].
epithelial lesion can be attenuated by osmotic diuresis, which increases A_1 receptors expression. Cisplatin is likely acting via activation of A_1 adenosine receptors, or increasing their expression in renal tissue. On the other hand, the nephrotoxicity of calcineurin inhibitors, widely used in organs transplantation, seems to be mediated by adenosine receptors since theophylline attenuated the renal lesion in rats after tacrolimus infusion. In addition, A_1 receptor selective antagonism can prevent radiocontrast media-induced acute renal failure. This has been documented in several studies using animals and even in humans.

**Purinergic P2 receptors**

Both P2X and P2Y receptors have been described in the kidney. Elegant reviews by Schwiebert in 2001 and Burnstock in 2006 have extensively addressed the distribution of P2 receptors subtypes and signaling along the nephron. In this review, however, we present some of the most relevant topics regarding the presence and effects of these receptors in the kidney, emphasizing what has been documented in renal diseases in the last twenty years.

**Effects on transepithelial transport.** In the early 80s, purinergic P2 receptors have been documented by Simmons in MDCK cells, a kidney-derived epithelial cell line, where ATP stimulates chloride transport. Subsequent studies demonstrated that extracellular ATP increases intracellular calcium in proximal tubule and collecting duct. The effects of extracellular ATP on tubular epithelial cells have been attributed to activation of P2 receptors, acting as a co-transmitter of sympathetic nerves. This idea has emerged joining new concepts of purinergic regulation of diverse physiological transport processes in the renal tubules. In 1994 we documented the functional presence of P2Y_2 receptors, so called P2U, in cortical collecting ducts of the rabbits. That study showed that their activation increases intracellular calcium and inhibits the antidiuretic effect of vasopressin. These receptors were also implicated in the secretion of prostaglandin E2 in medullary collecting ducts. In addition to the study of Simmons, other studies showed that kidney epithelial cells respond to ATP with chloride secretion, and inhibition of PTH-mediated phosphate secretion in proximal tubules. The effects of P2Y_2 stimulation were also characterized from the luminal side of collecting ducts. An interesting review by Leipziger, published in 2003, addressed the effects of ATP on luminal P2Y_2 receptors. The CFTR (cystic fibrosis transmembrane conductance regulator) and ABC (ATP binding cassette) transporters were suggested as pathways for ATP release into the lumen; however, a secretion pathway for ATP has not been confirmed so far. Nevertheless, ATP or UTP can be secreted onto the luminal side of the cells, bind P2 receptors, activate chloride secretion, and inhibit sodium absorption. An in vivo study by Shirley et al. reported the effect of P2 activation on inhibition of sodium transport in collecting duct, as a non P2Y_2-mediated event.

The effects of P2X receptors are characterized as ATP-gated, calcium-mediated cation channels. They have been demonstrated in LLC-PK1 cells, a renal epithelial cell line, and in mouse distal convoluted tubule cells (MDCT), where ATP inhibits the transport of magnesium through a P2X receptor. Activation of P2X receptors are likely responsible for inhibition of sodium transport. The effects of P2X_1 and P2X_6 receptors were related to inhibition of EBC, the amiloride-sensitive epithelial sodium channel, largely responsible for sodium reabsorption along the nephron.

**Effects on renal vessels.** The hemodynamic effects of P2 activation were clearly defined by Churchill and Ellis attributing the vasoconstriction to activation of P2X, and vasodilation to P2Y receptors. These authors and others have addressed the effect of P2 receptors, particularly P2Y_11, on the stimulation of renin secretion. On the other hand, the effects of P2X activation were further defended by Mitchell and Navar, relating this action to the modulation of the tubuloglomerular feedback, an idea supported by Inscho et al. In that study using knock out mice for P2X_1 receptors, the authors suggested this subtype as the one implicated in this hemodynamic regulatory process. The study by Ren et al. proposed that ATP is released from the macula densa and acts on P2X_ receptors. A recent review by Castrop has focused on a coordinated action of both ATP and adenosine on the TGF. ATP is broken to AMP which is degraded by ecto-5′-nucleotidases to form adenosine which then acts on A_1 receptors to induce vasoconstriction.

**P2 receptors and renal diseases.** Nucleotides can be released during cell lesion, which allows their binding to purinergic receptors in surrounding cells. Several studies have been addressing the role of P2 receptors activation on glomerular diseases. It has been demonstrated that extracellular nucleotides stimulate growth of cultured mesangial cells. In 1998, Schulze-Lohoff and associates demonstrated that P2X_ receptors cause apoptosis and necrosis of mesangial cells in culture. These findings were further examined in rat glomerular mesangial cells by Harada et al. They demonstrated that P2Y_2 and P2Y_4 are proliferative, whereas P2X_ receptors cause apoptotic cell death. Further studies from Vonend...
et al\textsuperscript{84} and Solini et al\textsuperscript{85} identified the increased expression of P2X\textsubscript{7} receptors in cells from the glomeruli, suggesting its role on the diabetic glomerular disease. A more recent study by Turner et al also pointed to a role of P2X\textsubscript{7} in the development of experimental and human lupus glomerulonephritis\textsuperscript{86}, although the group of Solini has recently emphasized a more important role of P2X\textsubscript{4} in human glomerular disease\textsuperscript{87}. Taken together, these studies showed that the purinergic system, acting through diverse P2 receptors, could actively participate on the processes of glomerular diseases.

Purinergic receptors are also involved in cystic diseases of the kidney. During normal organogenesis, P2X\textsubscript{7} is expressed in renal mesenchymal cells. In addition, collecting ducts express P2X\textsubscript{7} during cystogenesis in the first 3 weeks of postnatal life in the cpk/cpk mouse, a model of autosomal recessive polycystic kidney disease (ARPKD)\textsuperscript{88}. In a previous study by Wilson et al\textsuperscript{89}, ATP was detected in cysts from human autosomal dominant PKD (ADPKD) kidneys. In that study it was hypothesized that extracellular ATP released into the cysts from human epithelia may contribute to the gradual expansion of cyst fluid volume. However, the study by Hillman et al\textsuperscript{90} has demonstrated that P2X\textsubscript{7} activation inhibits cyst growth in a murine model of PKD. Furthermore, a more recent finding of Turner et al\textsuperscript{91}, showing that activation of a P2Y receptor stimulates cyst growth, raises the possibility of a balanced effect by ATP, acting detrimentally through an as yet unknown P2Y receptor, and being inhibitory through activation of P2X\textsubscript{7} receptors. Therefore, P2Y antagonists might constitute a promising therapeutic alternative to halt the progression of human PKD.

Finally, using P2X\textsubscript{7} knock out mice submitted to unilateral ureteral obstruction, a model of tubulo-interstitial disease, we have recently documented a role of P2X\textsubscript{7} receptors in the development of renal interstitial inflammation, fibrosis, and apoptosis of renal epithelial cells\textsuperscript{92}. In that study, P2X\textsubscript{7} was expressed in epithelial cells in an early phase of interstitial inflammation, suggesting that it can be expressed in certain disease states, as has been observed with models of diabetes and hypertension\textsuperscript{84,85}.

**CONCLUSIONS**

Purinergic receptors are being increasingly investigated, and their implications on several diseases are moving clinicians towards laboratories around the world. Present discoveries are opening new perspectives that enable scientists to pursue the detailed involvements of the purinergic system in the fields of renal physiology and renal diseases. The expectation of new drugs, serving as agonists and antagonists of specific purinergic receptors, constitute promising valuable tools for the treatment of several diseases. Nonetheless, there are still much to be investigated on the aspects of the involvement of extracellular nucleotides and nucleosides on the various processes of kidney diseases.

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