Purinergic signaling in kidney disease

Robert I. Menzies[1], Frederick W. Tam[2], Robert J. Unwin[3,4] and Matthew A. Bailey[1]

[1]British Heart Foundation Centre for Cardiovascular Science, The University of Edinburgh, Edinburgh, Scotland, UK; [2]Imperial College Renal and Transplant Centre, Department of Medicine, Imperial College London, UK; [3]Cardiovascular and Metabolic Diseases Biotech Unit, AstraZeneca Gothenburg, Sweden; and [4]UCL Centre for Nephrology, University College London, London, UK

Nucleotides are key subunits for nucleic acids and provide energy for intracellular metabolism. They can also be released from cells to act physiologically as extracellular messengers or pathologically as danger signals. Extracellular nucleotides stimulate membrane receptors in the P2 and P1 family. P2X are ATP-activated cation channels; P2Y and P1 are G-protein coupled receptors activated by ATP, ADP, UTP, and UDP in the case of P2 or adenosine for P1. Renal P2 receptors influence both vascular contractility and tubular function. Renal cells also express ectonucleotidases that rapidly hydrolyze extracellular nucleotides. These enzymes integrate this multireceptor purinergic-signaling complex by determining the nucleotide milieu to titrate receptor activation. Purinergic signaling also regulates immune cell function by modulating the synthesis and release of various cytokines such as IL1-β and IL-18 as part of inflammasome activation. Abnormal or excessive stimulation of this intricate paracrine system can be pro- or anti-inflammatory, and is also linked to necrosis and apoptosis. Kidney tissue injury causes a localized increase in ATP concentration, and sustained activation of P2 receptors can lead to renal glomerular, tubular, and vascular cell damage. Purinergic receptors also regulate the activity and proliferation of fibroblasts, promoting both inflammation and fibrosis in chronic disease. In this short review we summarize some of the recent findings related to purinergic signaling in the kidney. We focus predominantly on the P2X7 receptor, discussing why antagonists have so far disappointed in clinical trials and how advances in our understanding of purinergic signaling might help to reposition these compounds as potential treatments for renal disease.

Kidney International (2016) ■, ■–■; http://dx.doi.org/10.1016/j.kint.2016.08.029

KEYWORDS: adenosine; ATP; inflammation; kidney; P2X; P2Y; renal tubule; vasculature

Correspondence: *Robert Unwin, UCL Centre for Nephrology, UCL Medical School, Royal Free Campus, Rowland Hill Street, London NW3 2PF, UK. E-mail: robert.unwin@ucl.ac.uk*

Received 19 May 2016; revised 10 August 2016; accepted 15 August 2016

Since their discovery in the 1970s, P2 purinergic receptors (P2Rs) have evolved from an initially contentious biological concept[1] to a progressive understanding of their complex physiological actions, emerging now as attractive and "druggable" targets for disease.[2,3] To date, the most advanced potential therapeutic P2R targets are antagonists for P2Y12R to inhibit thrombosis,[4] and P2X7R for the treatment of chronic inflammatory diseases such as rheumatoid arthritis[5] and chronic obstructive pulmonary disease.[6] Several P2X7R antagonists have completed phase 2 clinical trials, but despite preclinical promise, these compounds have failed to deliver the expected benefit and interest in P2X7R has consequently declined. In this limited review we cover purinergic signaling in the kidney and explore the contribution of this system to renal physiology and disease. The main focus is on the role of P2X receptors, particularly P2X7R, in renal injury and disease. P2X7R can orchestrate interactions between the immune and vascular systems, and defining this complex interaction as inflammation and injury develop may help us unlock the potential of P2X7R antagonists as renal therapeutics.

P2 receptors and purinergic signaling in the kidney

Purinergic receptors are subclassified as P1Rs that bind adenosine and P2Rs that are activated by purine/pyrimidine nucleotides; P2Rs are in turn subdivided into P2YRs and P2XRs. The 8 P2YRs are coupled to G-proteins and are activated with differing selectivity by adenosine triphosphate (ATP), adenosine diphosphate (ADP), uridine triphosphate (UTP), and uridine diphosphate (UDP). The 7 P2XRs are trimeric ligand-gated ion channels activated by ATP but not, or only weakly, by ADP or adenosine monophosphate (AMP). The molecular properties of these receptors and their ligands are described in detail in the IUPHAR/BPS Guide to Pharmacology available online at http://www.guidetopharmacology.org.

P2 receptors are expressed in all segments of the nephron, and renal cells often express multiple receptor subtypes at both the apical and basolateral cell membranes.[7,8] Renal cells can also release ATP and UTP into the extracellular space. This release is likely to be regulated and is facilitated by several transport systems that involve vesicular or lysosomal exocytosis, or channel-mediated release via connexins[9] or pannexins.[10] Extracellular ATP and UTP have short half-lives due to rapid catabolism by ectonucleotidases (Figure 1) that are also expressed by renal cells.[11,12] Their immediate breakdown products, ADP and UDP, are potent agonists at P2Y1R,12R,13R and P2Y6R,14R, respectively. Further metabolism of ADP produces the 5′AMP (through CD39)

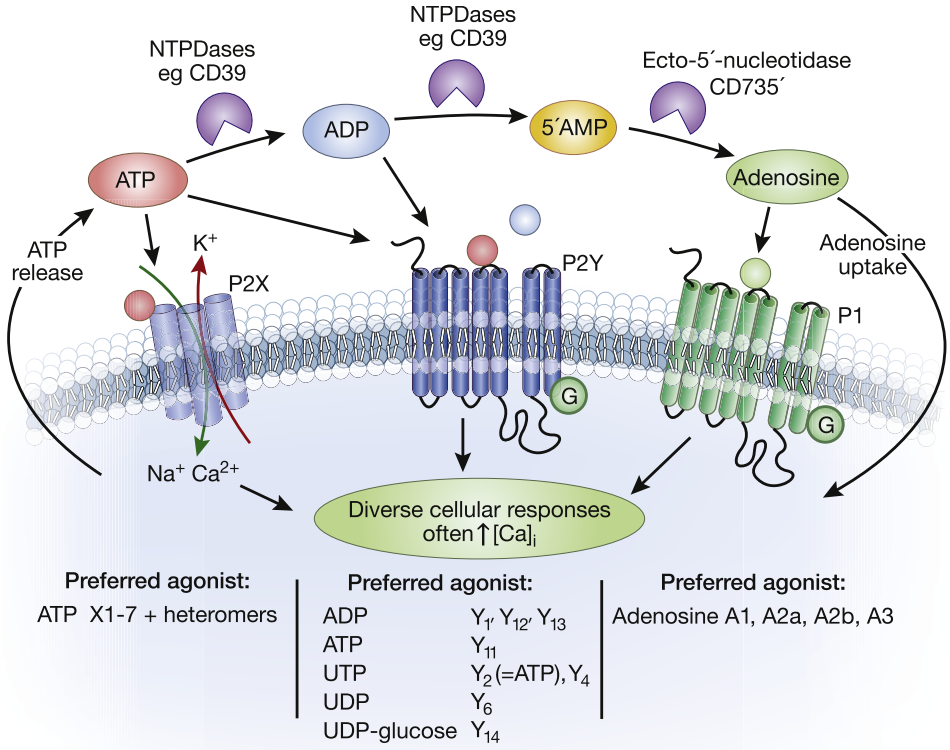

Figure 1 | The autocrine/paracrine purinoceptor system. A range of stimuli including cellular stretch, trauma, or agonist binding triggers ATP release into the extracellular space. Ectonucleotidases located on the plasma membrane catalyse sequential hydrolysis of ATP to ADP, 5′AMP, and adenosine. P1 receptors recognize adenosine, while P2 receptors bind di- and tri-phosphate nucleotide molecules. P2X receptors are nonselective cation channels with 3 protein subunits that may form homo- or heteromeric structures that can all bind ATP. P1 and P2Y receptors are 7 transmembrane-spanning domain G-protein-coupled receptors; agonist preferences span adenosine and uracil di- and tri- nucleotides. NTPDase, ectonucleoside triphosphate diphosphohydrolase.

and eventually adenosine (through CD73), the agonist at P1R (A1,2A,2B,3) that are also present in renal epithelia. Thus, the kidney has complex and regulated machinery for hierarchical purinergic signaling integrated by the action of ectonucleotidases. Ascribing specific physiological functions to a given receptor subtype has been challenging; available receptor agonists are not sufficiently selective and are often unstable.[11] In contrast, selective and specific receptor antagonists are providing a pharmacological means of assessing the function(s) of this system *in vivo*.

Extracellular nucleotides can influence a range of physiological functions, from cell proliferation and growth to energy metabolism and transepithelial solute flux. These functions have been reviewed in depth recently[13] and we can provide only a brief overview. It is evident that abnormal P2R activity can occur in various inflammatory and noninflammatory disease states including hypertension,[14] transplant rejection, and polycystic kidney disease.[15] However, more intriguing is the therapeutic potential for P2XR antagonists in chronic kidney disease (CKD).

P2 receptors control renal vascular and microvascular function

P2 receptors are expressed throughout the vasculature and microvasculature (Figure 2) and strongly influence vessel function.[16] The renal vasculature and microvasculature also express NTPDase1 (CD39) that hydrolyses ATP to ADP and AMP, and thereby rapidly curtail purinergic signaling.[17] P2X1R is the dominant receptor in vascular smooth muscle, and application of ATP to the adventitia evokes contraction in the pre-glomerular vasculature.[18,19] P2X1R null mice display an attenuated pressure-induced constriction of the afferent arteriole,[20] and targeted deletion of NTPDase1 prolongs the half-life of extracellular ATP, enhancing the vascular response to increased pressure.[21]

Direct renal artery infusion of ATP increases blood flow, causing vasodilation due to production of nitric oxide (NO) by the endothelium[22] and also NO-independent vasodilatation induced by intrarenal prostanoids.[23] The P2 receptor subtype or subtypes that mediate the vasodilatatory response to ATP are unknown. In human arterial endothelial cells and endothelial cells cultured from the mouse pulmonary artery, P2X4R is the most abundantly expressed receptor, followed by P2X7R.[24–26] P2X4R mediates the release of NO in response to increased shear stress.[24] This response is lost in P2X4R null mice, which have endothelial dysfunction and hypertension.[25] P2X7R activation seems to promote a tonic vasoconstriction of both the preglomerular arteries and medullary microcirculation,[14] which is discussed further below. Other P2 receptors