Purinergic Signaling in Kidney Disease

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Abstract

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 or adenosine, respectively. Renal P2 receptors influence both vascular contractility and tubular function. Renal cells also express ectonucleotidases that rapidly hydrolyze extracellular nucleotides. These enzymes integrate this multi-receptor purinergic-signaling complex by determining the nucleotide milieu, as well as titrating 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.

Introduction

Since their discovery in the 1970s, P2 purinergic receptors (P2R) have evolved from an initially contentious biological concept [1], through 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 COPD [6]. Several P2X7R antagonists have completed Phase 2 clinical trials, but despite pre-clinical promise, these compounds have failed to deliver the expected benefit and so interest in P2X7R has declined. In this concise 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 sub-classified as P1R that bind adenosine and P2R that are activated by purine/pyrimidine nucleotides; P2R are in turn subdivided into P2YR and P2XR. 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*: 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) 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, through 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 non-inflammatory disease states ranging from hypertension [14] to transplant rejection, to polycystic kidney disease [15]. However, more beguiling 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 expresses 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 intra-renal prostanoids [23]. The P2 receptor subtype(s) that mediates the vasodilatatory response to ATP is 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 pre-glomerular arteries and medullary microcirculation [14], which is discussed more below. Other P2 receptors can influence endothelial function, for example, vasodilatation caused by UDP is abolished in P2Y6R null mice [27]. The descending *vasa recta* are also affected by extracellular nucleotides, since infusion of ATP into the renal artery reduces medullary blood flow as a result of P2X1R activation [23], and ATP released from sympathetic nerves causes constriction of *vasa recta* pericytes [28].

Multiple P2R subtypes are expressed in glomerular cells (**Figure 2**). Under normal conditions, P2YR predominate [29] and extracellular nucleotides influence mesangial proliferation and contraction, as well as contraction of the parietal sheet [29]. In podocytes, P2Y1R is the dominant functional receptor as demonstrated by comprehensive pharmacological profiling and immunolocalization [30]; however, recently P2X4R has been shown to have a mechano-sensitive role affecting the podocyte actin cytoskeleton [31], although P2X4R knockout mice, while hypertensive, have no obvious gross glomerular phenotype and are not known to be proteinuric. In contrast, P2Y1R null mice are protected from acute nephrotoxic injury, showing preserved renal function, reduced capillary rarefaction and fibrosis, and enhanced survival [32]. P2Y1R activation may, therefore, contribute to glomerular injury. P2X7R expression also seems to be associated with glomerular injury, since it is increased in multiple glomerular cells types, including inflammatory cells, in models of severe hypertension, type 1 diabetes [33], and acute inflammatory glomerulonephritis [34]. Uncovering the primary role of this increased glomerular P2X7R expression remains an active area of research.

P2 receptors and renal tubular physiology

P2R exert a largely inhibitory effect on tubular electrolyte transport and this, together with expression in specific nephron segments, has been reviewed extensively elsewhere [35] and is summarized in **Figure 2**. The processes are best defined for sodium flux, which is tonically suppressed by P2R activation in several nephron segments [36]. It is likely that such paracrine control by extracellular nucleotides provides a route for rapid modulation of tubular transport that can link solute and fluid delivery to adaptive transport capacity, for example adenosine-mediated tubuloglomerular feedback is impaired in $CD73^{-/-}$ mice [37]. This form of control can integrate with more slowly adapting hormonal systems, for example the renin-angiotensin-

aldosterone system (RAAS) to regulate the phenomenon of aldosterone escape [38]. Indeed, ATP release by tubular cells, stimulated by increased flow, contributes to the control of extracellular fluid volume by the kidney, and blood pressure regulation, as discussed below.

Proximal tubule

The proximal tubule, which expresses apical P2Y1R and P2X5R, and basolateral P2Y4R and P2Y6R [39, 40], accounts for reabsorption of ~65% of the filtered sodium load. Extracellular nucleotides inhibit the major sodium transporters in this segment, NHE3 [41], NaPi2 [42] and Na,K-ATPase [43], and inhibition of transepithelial flux has been confirmed *in vivo* [44]. The ATP concentration in tubular fluid is unknown, although measurements in bulk fluid collected from the end of the proximal convoluted tubule (PCT) report concentrations of 100-300nmol/l [45]. The brush border membrane expresses ENPP3 (ectonucleotide pyrophosphatase/ phospodiesterase 3) and ecto-5'-nucleotidase (NT5E; CD73) [12] that should terminate physiological signaling. Microperfusion studies using nucleotide scavengers suggest that the 'ambient' concentration of the physiological purinergic ligand, most probably ADP, is ~10μmol/l, exerting a tonic inhibitory effect that may help to balance tubular sodium reabsorption with glomerular filtration [44].

The distal nephron

Increased fluid flow or changes in osmolality of the tubular fluid promotes nucleotide secretion in both the thick limb of Henle [46] and collecting duct [47], inhibiting transport in downstream nephron segments. In the thick ascending limb of Henle (TALH), ATP release is dependent on activation of the transient receptor potential cation channel TRPV4 osmosensor [48]. These nucleotides activate endothelial NO synthase (NOS3) in thick limb cells, and P2R signaling underpins the flow-dependent increase in NO production [49] and subsequent inhibition of apical

NKCC2 and basolateral Na,K-ATPase activity [50]. Studies in knockout mice suggest P2X4R and P2Y2R contribute to this signaling arc [51, 52].

Extracellular ATP has long been known to inhibit the epithelial sodium channel (ENaC), the rate-limiting step for sodium transport in the connecting tubule and collecting duct [53]. Studies in isolated segments show that ATP activates P2Y2R to reduce the open probability of ENaC [54-56]. P2yr2 null mice lack the tonic suppression of ENaC and are hypertensive [54]. Studies *in vivo* suggest that P2X4R activation also inhibits ENaC [53, 57] and our own pilot studies in a P2X4R null mouse suggest that this receptor may be important in the modulation of sodium transport by aldosterone (Craigie et al, unpublished).

P2R and blood pressure regulation

Hypertension is a major modifiable risk factor for cardiovascular and renal disease and is highly prevalent [58]. Human genetic studies have found an association between SNPs in P2XR encoding genes and blood pressure or cardiovascular disease. The loss of function variant rs28360472 in P2RX4 associates with increased pulse pressure [59], itself an important cardiovascular risk factor. An intronic SNP (rs591874) in the gene encoding P2X7R is associated with elevated blood pressure [60]. The loss of function variant rs3751143 is common (25% heterozygosity and up to 3% homozygosity) and protects against ischemic stroke [61]. The physiology of P2RX7 genetic variation is almost certainly subtle, if not complex. For example, rs3751143 does not associate with impaired endothelial dysfunction or vascular stiffness in essential hypertensives [62], but does confer a significantly reduced sensitivity to P2X7R antagonism [63].

Pressure-natriuresis is an important mechanism of long-term blood pressure control [64] and is modulated by paracrine factors that inhibit sodium transport in the renal proximal tubule, including extracellular nucleotides. Microdialysis experiments reveal a direct relationship between renal artery perfusion pressure and the concentration of ATP in the interstitial fluid of

the kidney cortex [65]. As mentioned earlier, extracellular nucleotides inhibit the key transporters in the proximal tubule [41-43]. This natriuretic effect is buttressed by inhibition of sodium transport in the distal nephron. Increased flow through the collecting duct promotes ATP secretion to inhibit ENaC. This ATP release is abolished in connexin 30 knockout mice, severely attenuating the pressure-natriuresis response [9]. Consistent with this, mice over-expressing human NTPDase1 (CD39), a cell surface enzyme that scavenges extracellular nucleotides, display a small impairment of the natriuretic response to a high sodium diet and concomitant aldosterone infusion [66]. It is assumed that P2Y2R mediates the inhibitory effect of ATP on distal tubule sodium transport. Receptor agonists have been considered as potential antihypertensives. P2yr2 null mice display enhanced ENaC activity and are hypertensive. Surprisingly, blood pressure is salt resistant [67] and endothelial dysfunction with impaired NO release may be causal. Recent studies also suggest that ATP can inhibit ENaC indirectly: in IMCD cells, activation of P2X7R promotes synthesis of endothelin-1, which is pro-natriuretic due to ETB-mediated inhibition of ENaC [68]. However, the significance of this cell line-based study is not clear, since acute P2X7R antagonism *in vivo* improves the pressure-natriuresis relationship [14].

Although P2X7R activation contributes to the physiological control of blood pressure by the kidney, sustained activation of the receptor, which does not de-sensitize with repeated exposure to ATP, promotes hypertensive renal injury. Thus, prophylactic P2X7R antagonism [69] or 'knock-out' of the murine P2X7k transcript [70], which leaves several functional P2RX7 transcripts intact [71], protects against the injury associated with salt-sensitive hypertension. P2X7R antagonism/deletion reduced albuminuria and interstitial fibrosis, lowered blood pressure and reduced the infiltration of T and B cells, macrophages and leucocytes. The mechanisms underpinning these effects are not known, as discussed further below. Our data suggest that P2X7R in the renal vasculature and microvasculature may impair blood pressure regulation by the kidney [14]. We identified elevated renal expression of P2X7R (and P2X4R) as a candidate gene

for hypertensive renal vascular injury in rats [72]. P2X7R localized to the vascular and microvascular endothelium down to afferent arterioles. The selective P2X7R antagonist AZ11657312 increased renal medullary perfusion and improved tissue oxygenation in angiotensin II–treated rats [14]; these beneficial effects were partially dependent on NO synthesis. Overall, activation of P2X7R induces microvascular dysfunction and regional hypoxia, particularly under high angiotensin II tone. These effects are pro-inflammatory and may contribute to progression of renal injury. In the next section, we discuss the role of P2X7R in renal injury and disease and assess the potential for antagonists as renal therapeutics.

P2XR and renal injury

There is consistent pre-clinical evidence supporting a role for P2X7R in inflammation (**Figure 3**), and, as already mentioned, P2X7R antagonists have been explored as a treatment target in rheumatoid arthritis [5], COPD [6], and IBD [73], but with mixed or generally disappointing results. This has caused interest in the receptor to wax and wane. However, it is likely that an improved understanding of the biological roles of P2X7R, including its unique two-stage ability to induce membrane permeability to large (>900 Da) molecules, rather than cations alone, as well as the regulation and function of the main splice variants, will provide a fresh impetus to the clinical testing of antagonists.

In the normal kidney P2X7R is typically only present at low levels, often undetectable by RNA analysis in whole kidney extracts. The receptor is normally localized to certain compartments, particularly the vasculature and microvasculature, at least in the rat [7, 14, 72]. A wealth of data shows that injury/inflammation increases expression in renal cells. For example, TNFα can induce expression of P2X7R in cultured mesangial cells [74]. In renal biopsy material from patients with lupus nephritis, increased expression of P2X7R protein has been found [75]. Nevertheless, it

remains to be investigated whether the extent of P2X7R expression correlates with the severity of clinical disease and a more detailed study with larger patient numbers is needed.

Glomerulonephritis

A more detailed characterization of the expression and potential function of P2X7R have been carried out in rodent models of nephrotoxic nephritis (NTN) [75]. In a mouse model of accelerated NTN, increased expression of P2X7R was co-localized to glomerular macrophages as well as intrinsic glomerular cells. In NTN in WKY rats, onset P2X7R expression coincided with onset of proteinuria. The inflamed glomeruli are infiltrated by macrophages showing the NLRP3 inflammasome activation [76]. The WKY strain of rat is known to be more susceptible to developing severe and progressive glomerulonephritis when compared with the resistant LEW rat strain. WKY and LEW rats have identical MHC genes, but have distinct genetic differences and differences in their expression of P2X7R and the NLRP3 inflammasome [76]. More specifically, bone marrow derived (BMD) macrophages from WKY rats have increased expression of P2X7R protein and mRNA associated with increased expression of multiple genes of the NLRP3 inflammasome pathway, even in their basal state *in vitro*, again when compared with BMD macrophages from LEW rats. Following priming with endotoxin and stimulation with extracellular ATP, compared with LEW rats, macrophages from WKY rats have higher levels of caspase-1 activation and secretion of more mature IL-1β and IL-18. Thus, strain differences in expression of P2X7R and subsequent downstream activation of the inflammasome may be responsible for the difference in susceptibility to experimental glomerulonephritis.

The functional importance of P2X7R was investigated in gene knockout mice and with systemic treatment by a small molecule P2X7R antagonist [34]. Using the model of accelerated NTN, the P2X7R knockout mice had lower urinary monocyte chemoattract-1 (CCL2), fewer infiltrating

glomerular macrophages, less glomerular fibrin deposition and less proteinuria than in wild-type mice. In NTN rats, treatment with the P2X7R antagonist A438079 significantly reduced glomerular expression of CCL2, glomerular macrophage infiltration, glomerular fibrinoid necrosis and proteinuria compared with vehicle-treated rats. However, exactly how P2X7R is involved in antibody-mediated glomerulonephritis is unclear. Typically, extracellular ATP binds to P2X7R in endotoxin-primed macrophages, resulting in inflammasome activation and release of mature IL-1βand IL-18 [77], yet endotoxin or other bacterial products are not involved in the induction of NTN in WKY rats [34]. The interaction between immune complex stimulation and P2X7R needs further investigation and to ascertain whether treatment with the P2X7R antagonist after the onset of disease is effective in reducing the severity of glomerulonephritis. There is also recent evidence in lupus prone mice that treatment with a P2X7R antagonist can decrease the severity of renal injury and levels of dsDNA antibodies [78].

Acute kidney injury

Renal ischemia-reperfusion injury (IRI) is a common occurrence in many clinical settings from sepsis to major surgery, including renal transplantation. There is increased expression of P2X7R, mainly in the renal tubules, in a mouse model of renal IRI; treatment with A438079 reduced renal expression of chemokines (MCP-1 and RANTES), p-ERK, NGAL, renal tubular injury and cell death [79].

As well as the mentioned increase in P2X7R in a rat model of type 1 diabetes [33], in a mouse model of high fat diet-induced metabolic disease, proteinuria and albuminuria developed in the wild-type mice, but not in P2X7a variant knockout mice [80]. In the high fat diet fed mice there was also increased renal expression of P2X7R and components of the NLRP3 inflammasome that were attenuated in the high fat diet fed P2X7R knockout mice, as was renal expression of

chemokine CCL2, macrophage infiltration and expression of extracellular matrix protein. Moreover, increased expression of P2X7R and inflammasome components were found in renal tissue from patients with glomerulonephritis [75].

Fibrosis

Purinergic signaling is involved in tissue remodeling (**Figure 3**) and several studies in various tissues suggest that these pathways may also drive tissue fibrosis in chronic injury, one feature of which is a sustained increase in ambient concentrations of ATP, ADP, UTP and UDP [81]. Tissue fibroblasts express multiple P2R subtypes and respond to extracellular nucleotides by activating key pathways for the production of extracellular matrix. In cardiac fibroblasts, for example, P2Y2R activation is strongly pro-fibrotic [82], and activation of P2X4R and P2X7R promotes ERK1/2-dependent fibroblast proliferation [83]. This cluster of P2Rs is also relevant to the kidney in which fibroblasts and mesangial cells mainly determine ECM deposition. In this context, P2Y2R activation increases mesangial cell proliferation [74] and P2X7R activation increases matrix production by mesangial cells [84].

The role of P2 receptors in renal fibrosis has been investigated in the unilateral ureteral obstruction (UUO) model [85]. Transient expression of P2X7R was detected only in tubular epithelial cells 7 days after induction of UUO in wild-type mice. The renal tubular expression of TGF-β1, macrophage infiltration, tubular apoptosis and tubulointerstitial fibrosis were reduced in P2X7R knockout mice compared with wild-type mice by day 14. The role of the inflammasome in this model has also been investigated. Knockout of apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) in mice results in reduced UUO-mediated tubulointerstitial fibrosis, together with fewer infiltrating inflammatory cells and reduced renal expression of mRNA for IL-1β, CCL2, TGFβ1 and collagen I; however, it is not clear

how P2X7R may regulate TGF-β1 expression [86]. While there is a well-established relationship between stimulation of P2X7R and activation of the inflammasome, it is not known what the priming signal is in the sterile UUO model and what triggers fibrogenesis.

P2X4R is closely related to P2X7R and there has been ongoing controversy over whether P2X4R and P2X7R can form heterotrimers [87, 88]. The potential importance of P2X4R in renal fibrosis has been investigated in the UUO model. Surprisingly, the P2X4R knockout mice showed increased renal fibrosis following induction of UUO associated with increased expression of TGFβ1 and connective tissue growth factor (CTGF, also known as CCN2), and increased amounts of type I collagen [89]. These results suggest that P2X7R is pro-fibrotic in this model and that P2X4R may have an anti-fibrotic role through its regulation of pro-fibrotic growth factors.

More recent studies show that nucleotidases may also contribute to fibrosis by regulating the half-life of ATP. ENTPD1 (CD39)-null mice are more sensitive to ischemic tissue injury than wild-type mice [90], because ATP persists and its hydrolysis to protective adenosine is blunted. Similarly, these null mice have more pronounced renal injury in the IRI model [91, 92]; although in this setting the role of adenosine is less certain, since the deletion of CD73, the enzyme that converts AMP to adenosine, was also protective [93]. Overall, these data suggest that enzymes involved in terminating P2R signaling may be less tractable as therapeutic targets than the receptors themselves. Recent studies indicate that CD39 expression by T-reg lymphocytes is essential for their pro-reparative role in response to chronic renal injury [94].

What now for P2X7R antagonists?

P2X7R antagonists may have failed because of significant gaps in our knowledge about the complex processing and diverse roles of *P2XR7* gene products and the implications this may have for P2X7R in disease. Single nucleotide polymorphisms (SNPs) such as rs3751143 (causing

311 Glu496Ala) can impair P2X7R function [95,96]: ATP-dependent IL-1β release from lymphocytes is
312 significantly suppressed in individuals carrying this SNP [97]. Alternative splicing can produce novel
313 protein isoforms that are emerging as important factors in disease pathogenesis, as well as in
314 determining the right treatment target [98].

315 Human P2X7R has at least 10 splice isoforms, the functions of which have not been unraveled;
316 however, in rodents, the common 'k variant' of P2X7R is much more sensitive to ATP than the
317 original full-length 'a variant' [99]. Pre-clinical data suggest that genetic variation in P2X7R will
318 increase the population wide variance of both agonist and antagonist binding affinities,
319 suggesting that we need to re-evaluate or redefine clinical trials on the basis of the P2X7R
320 "fingerprint". The tissue distribution, regulation and function of these splice isoforms in the
321 healthy kidney is just beginning to be explored; the pharmacogenomics of P2X7R and the impact
322 of disease is largely unknown. The next phase of research will define these key biological
323 processes involving P2X7R, which may not all be 'bad' [100], and provide a better understanding of
324 how isoform-specific receptor antagonists should be deployed in kidney disease. Is this *P2X7R*
325 *Redux*?

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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 non-selective cation channels with 3 protein subunits that may form homo- or heteromeric arrangements; all bind ATP. 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.

Figure 2: P2 Receptors in the kidney

P2Y and P2X receptor expression along the nephron: vasculature, glomeruli and tubules.

Figure 3: P2XR related inflammation in (diabetic) kidney disease

Local production of chemokines, adhesion molecules and inflammatory cytokines are upregulated under chronic stimulation of factors including hyperglycemia. Macrophages are the main infiltrating inflammatory cell type (expressing P2X7R) in both the glomerular and tubulointerstitial compartments where they contribute to extracellular matrix (ECM) secretion, amplification of the inflammatory cascade and eventually fibrosis.

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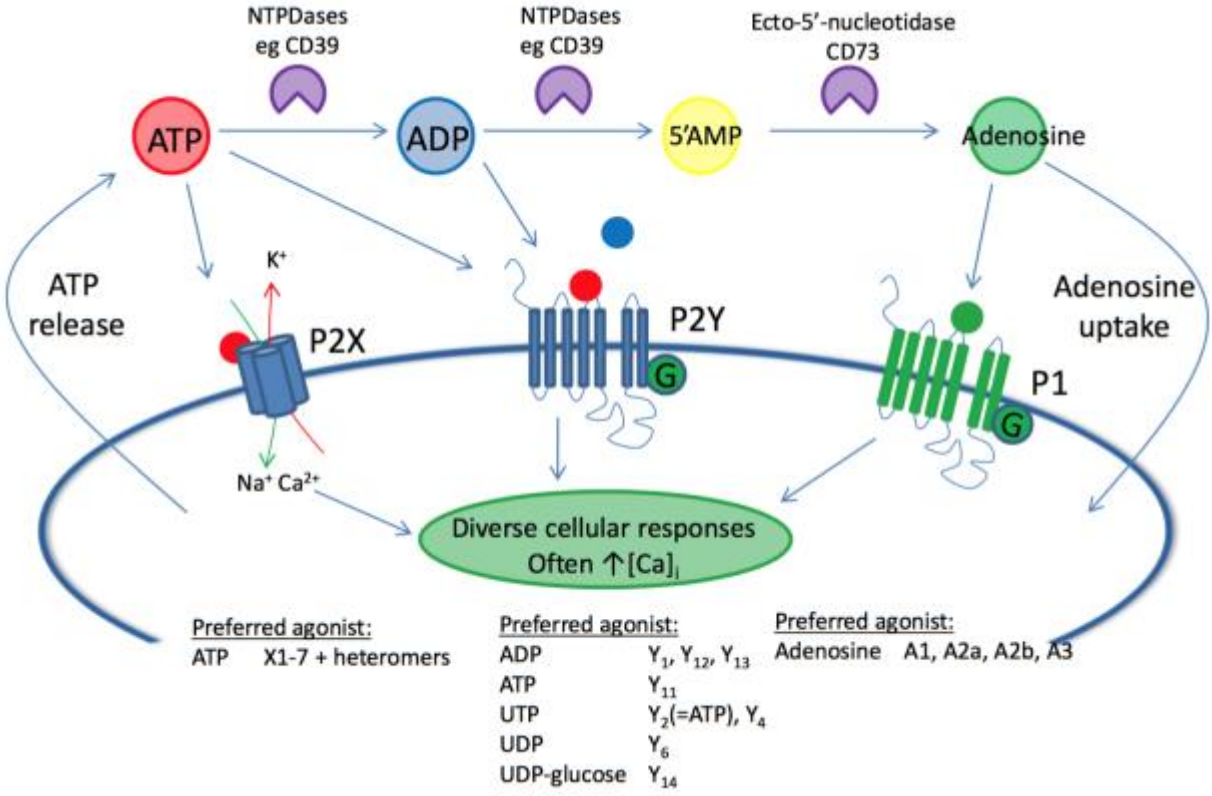
704 **Figure 1**



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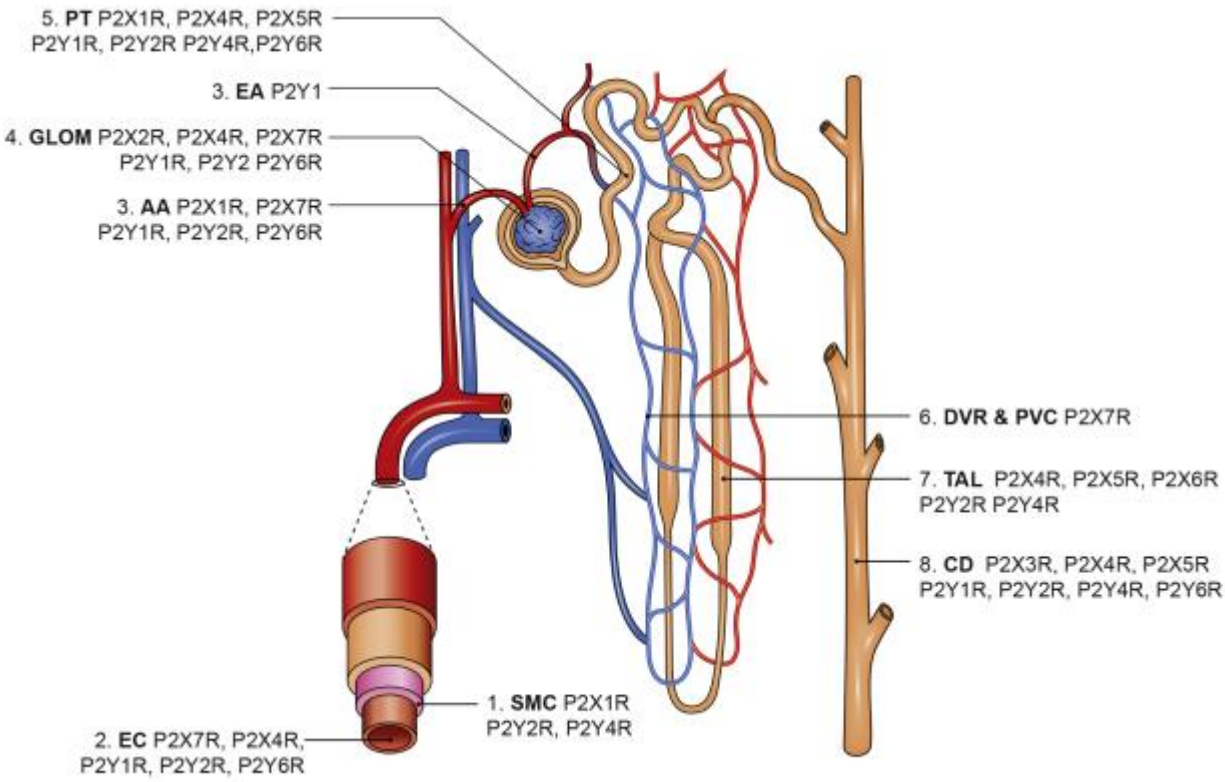
707 **Figure 2**



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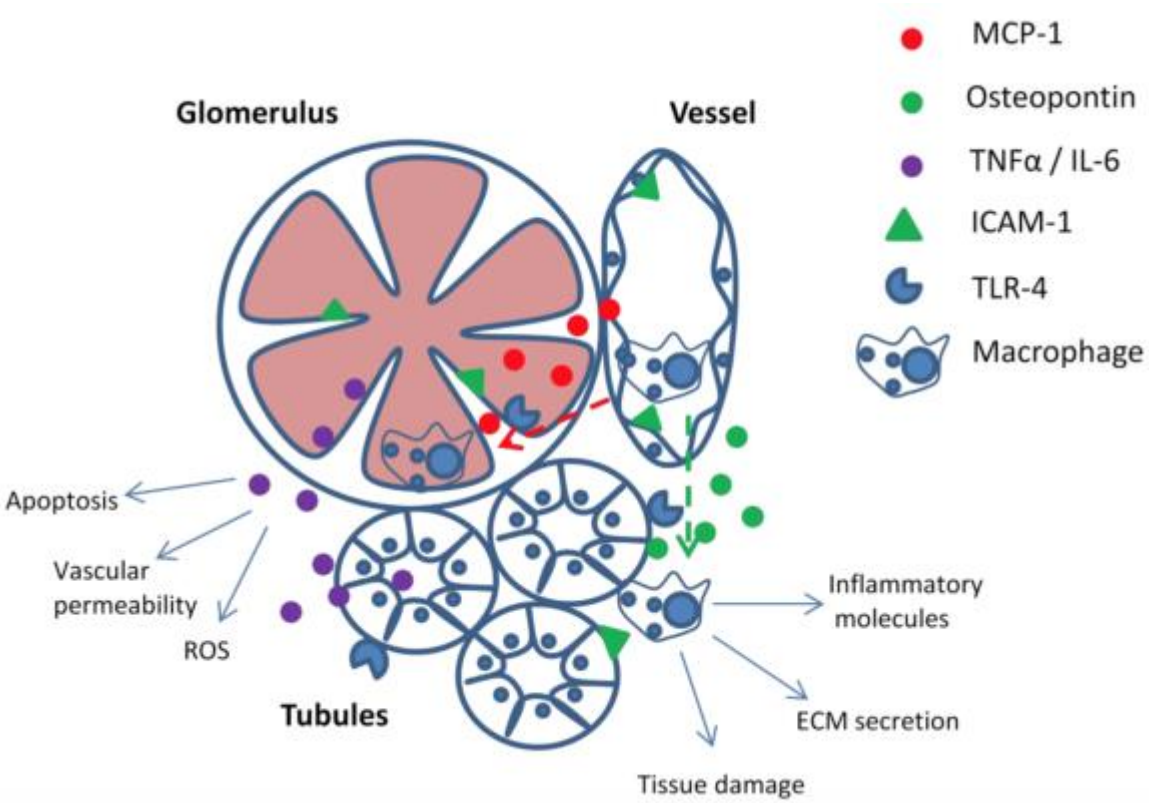
710 **Figure 3**



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