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Purinergic Signaling in Kidney Disease

Robert I. Menzies¹, Frederick W Tam², Robert J Unwin^{3,4} and Matthew A. Bailey¹

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

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Correspondence:

Robert Unwin
UCL Centre for Nephrology,
UCL Medical School
Royal Free Campus
Rowland Hill Street,
London NW3 2PF,
United Kingdom
Email: robert.unwin@ucl.ac.uk

1 **Abstract**

2 Nucleotides are key subunits for nucleic acids and provide energy for intracellular metabolism.
3 They can also be released from cells to act physiologically as extracellular messengers or
4 pathologically as danger signals. Extracellular nucleotides stimulate membrane receptors in the
5 P2 and P1 family. P2X are ATP-activated cation channels; P2Y and P1 are G-protein coupled
6 receptors activated by ATP, ADP, UTP and UDP or adenosine, respectively. Renal P2 receptors
7 influence both vascular contractility and tubular function. Renal cells also express
8 ectonucleotidases that rapidly hydrolyze extracellular nucleotides. These enzymes integrate this
9 multi-receptor purinergic-signaling complex by determining the nucleotide milieu, as well as
10 titrating receptor activation.

11 Purinergic signaling also regulates immune cell function by modulating the synthesis and release
12 of various cytokines such as IL1- β and IL-18 as part of inflammasome activation. Abnormal or
13 excessive stimulation of this intricate paracrine system can be pro- or anti-inflammatory, and is
14 also linked to necrosis and apoptosis. Kidney tissue injury causes a localized increase in ATP
15 concentration, and sustained activation of P2 receptors can lead to renal glomerular, tubular
16 and vascular cell damage. Purinergic receptors also regulate the activity and proliferation of
17 fibroblasts, promoting both inflammation and fibrosis in chronic disease.

18 In this short review we summarize some of the recent findings related to purinergic signaling in
19 the kidney. We focus predominantly on the P2X7 receptor, discussing why antagonists have so
20 far disappointed in clinical trials and how advances in our understanding of purinergic signaling
21 might help to reposition these compounds as potential treatments for renal disease.

22 Introduction

23 Since their discovery in the 1970s, P2 purinergic receptors (P2R) have evolved from an initially
24 contentious biological concept ¹, through to a progressive understanding of their complex
25 physiological actions, emerging now as attractive and 'druggable' targets for disease ^{2,3}. To date,
26 the most advanced potential therapeutic P2R targets are antagonists for P2Y₁₂R to inhibit
27 thrombosis ⁴, and P2X₇R for the treatment of chronic inflammatory diseases such as
28 rheumatoid arthritis ⁵ and COPD ⁶. Several P2X₇R antagonists have completed Phase 2 clinical
29 trials, but despite pre-clinical promise, these compounds have failed to deliver the expected
30 benefit and so interest in P2X₇R has declined. In this concise review we cover purinergic
31 signaling in the kidney and explore the contribution of this system to renal physiology and
32 disease. The main focus is on the role of P2X receptors, particularly P2X₇R, in renal injury and
33 disease. P2X₇R can orchestrate interactions between the immune and vascular systems, and
34 defining this complex interaction as inflammation and injury develop may help us unlock the
35 potential of P2X₇R antagonists as renal therapeutics.

36

37 P2 receptors and purinergic signaling in the kidney

38 Purinergic receptors are sub-classified as P1R that bind adenosine and P2R that are activated by
39 purine/pyrimidine nucleotides; P2R are in turn subdivided into P2YR and P2XR. The 8 P2YRs are
40 coupled to G-proteins and are activated with differing selectivity by adenosine triphosphate
41 (ATP), adenosine diphosphate (ADP), uridine triphosphate (UTP) and uridine diphosphate (UDP).
42 The 7 P2XRs are trimeric ligand-gated ion channels activated by ATP, but not, or only weakly, by
43 ADP or adenosine monophosphate (AMP). The molecular properties of these receptors and
44 their ligands are described in detail in the *IUPHAR/BPS Guide to Pharmacology*:
45 <http://www.guidetopharmacology.org>.

46 P2 receptors are expressed in all segments of the nephron and renal cells often express multiple
47 receptor subtypes at both the apical and basolateral cell membranes ^{7,8}. Renal cells can also
48 release ATP and UTP into the extracellular space. This release is likely to be regulated and is
49 facilitated by several transport systems that involve vesicular or lysosomal exocytosis, or
50 channel-mediated release via connexins ⁹ or pannexins ¹⁰. Extracellular ATP and UTP have short
51 half-lives due to rapid catabolism by ectonucleotidases (**Figure 1**) that are also expressed by
52 renal cells ^{11,12}. Their immediate breakdown products, ADP and UDP, are potent agonists at
53 P2Y1R,12R,13R, and P2Y6R,14R, respectively. Further metabolism of ADP produces the 5'AMP
54 (through CD39) and eventually adenosine (through CD73), the agonist at P1R (A1,2A,2B,3) that
55 are also present in renal epithelia. Thus, the kidney has complex and regulated machinery for
56 hierarchical purinergic signaling integrated by the action of ectonucleotidases. Ascribing specific
57 physiological functions to a given receptor subtype has been challenging: available receptor
58 agonists are not sufficiently selective and are often unstable ¹¹. In contrast, selective and
59 specific receptor antagonists are providing a pharmacological means of assessing the function(s)
60 of this system *in vivo*.

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

67

68

69

70 **P2 receptors control renal vascular and microvascular function**

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

79 Direct renal artery infusion of ATP increases blood flow, causing vasodilation due to production
80 of nitric oxide (NO) by the endothelium ²² and also NO-independent vasodilatation induced by
81 intra-renal prostanoids ²³. The P2 receptor subtype(s) that mediates the vasodilatory response
82 to ATP is unknown. In human arterial endothelial cells and endothelial cells cultured from the
83 mouse pulmonary artery, P2X4R is the most abundantly expressed receptor, followed by P2X7R
84 ²⁴⁻²⁶. P2X4R mediates the release of NO in response to increased shear stress ²⁴. This response is
85 lost in P2X4R null mice, which have endothelial dysfunction and hypertension ²⁵. P2X7R
86 activation seems to promote a tonic vasoconstriction of both the pre-glomerular arteries and
87 medullary microcirculation ¹⁴, which is discussed more below. Other P2 receptors can influence
88 endothelial function, for example, vasodilatation caused by UDP is abolished in P2Y6R null mice
89 ²⁷. The descending *vasa recta* are also affected by extracellular nucleotides, since infusion of
90 ATP into the renal artery reduces medullary blood flow as a result of P2X1R activation ²³, and
91 ATP released from sympathetic nerves causes constriction of *vasa recta* pericytes ²⁸.

92

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

107

108 **P2 receptors and renal tubular physiology**

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

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

120

121 **Proximal tubule**

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

133

134 **The distal nephron**

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

141 NKCC2 and basolateral Na,K-ATPase activity⁵⁰. Studies in knockout mice suggest P2X4R and
142 P2Y2R contribute to this signaling arc^{51,52}.

143 Extracellular ATP has long been known to inhibit the epithelial sodium channel (ENaC), the rate-
144 limiting step for sodium transport in the connecting tubule and collecting duct⁵³. Studies in
145 isolated segments show that ATP activates P2Y2R to reduce the open probability of ENaC⁵⁴⁻⁵⁶.
146 P2yr2 null mice lack the tonic suppression of ENaC and are hypertensive⁵⁴. Studies *in vivo*
147 suggest that P2X4R activation also inhibits ENaC^{53,57} and our own pilot studies in a P2X4R null
148 mouse suggest that this receptor may be important in the modulation of sodium transport by
149 aldosterone (Craigie et al, unpublished).

150

151 **P2R and blood pressure regulation**

152 Hypertension is a major modifiable risk factor for cardiovascular and renal disease and is highly
153 prevalent⁵⁸. Human genetic studies have found an association between SNPs in P2XR encoding
154 genes and blood pressure or cardiovascular disease. The loss of function variant rs28360472 in
155 P2RX4 associates with increased pulse pressure⁵⁹, itself an important cardiovascular risk factor.
156 An intronic SNP (rs591874) in the gene encoding P2X7R is associated with elevated blood
157 pressure⁶⁰. The loss of function variant rs3751143 is common (25% heterozygosity and up to 3%
158 homozygosity) and protects against ischemic stroke⁶¹. The physiology of P2RX7 genetic
159 variation is almost certainly subtle, if not complex. For example, rs3751143 does not associate
160 with impaired endothelial dysfunction or vascular stiffness in essential hypertensives⁶², but
161 does confer a significantly reduced sensitivity to P2X7R antagonism⁶³.

162 Pressure-natriuresis is an important mechanism of long-term blood pressure control⁶⁴ and is
163 modulated by paracrine factors that inhibit sodium transport in the renal proximal tubule,
164 including extracellular nucleotides. Microdialysis experiments reveal a direct relationship
165 between renal artery perfusion pressure and the concentration of ATP in the interstitial fluid of

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

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

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

199

200 **P2XR and renal injury**

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

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

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

217

218 *Glomerulonephritis*

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

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

239 glomerular macrophages, less glomerular fibrin deposition and less proteinuria than in wild-type
240 mice. In NTN rats, treatment with the P2X7R antagonist A438079 significantly reduced
241 glomerular expression of CCL2, glomerular macrophage infiltration, glomerular fibrinoid
242 necrosis and proteinuria compared with vehicle-treated rats. However, exactly how P2X7R is
243 involved in antibody-mediated glomerulonephritis is unclear. Typically, extracellular ATP binds
244 to P2X7R in endotoxin-primed macrophages, resulting in inflammasome activation and release
245 of mature IL-1 β and IL-18⁷⁷, yet endotoxin or other bacterial products are not involved in the
246 induction of NTN in WKY rats³⁴. The interaction between immune complex stimulation and
247 P2X7R needs further investigation and to ascertain whether treatment with the P2X7R
248 antagonist after the onset of disease is effective in reducing the severity of glomerulonephritis.
249 There is also recent evidence in lupus prone mice that treatment with a P2X7R antagonist can
250 decrease the severity of renal injury and levels of dsDNA antibodies⁷⁸.

251

252 *Acute kidney injury*

253 Renal ischemia-reperfusion injury (IRI) is a common occurrence in many clinical settings from
254 sepsis to major surgery, including renal transplantation. There is increased expression of P2X7R,
255 mainly in the renal tubules, in a mouse model of renal IRI; treatment with A438079 reduced
256 renal expression of chemokines (MCP-1 and RANTES), p-ERK, NGAL, renal tubular injury and cell
257 death⁷⁹.

258 As well as the mentioned increase in P2X7R in a rat model of type 1 diabetes³³, in a mouse
259 model of high fat diet-induced metabolic disease, proteinuria and albuminuria developed in the
260 wild-type mice, but not in P2X7a variant knockout mice⁸⁰. In the high fat diet fed mice there
261 was also increased renal expression of P2X7R and components of the NLRP3 inflammasome that
262 were attenuated in the high fat diet fed P2X7R knockout mice, as was renal expression of

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

266

267 *Fibrosis*

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

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

287 how P2X7R may regulate TGF- β 1 expression ⁸⁶. While there is a well-established relationship
288 between stimulation of P2X7R and activation of the inflammasome, it is not known what the
289 priming signal is in the sterile UUO model and what triggers fibrogenesis.

290 P2X4R is closely related to P2X7R and there has been ongoing controversy over whether P2X4R
291 and P2X7R can form heterotrimers ^{87, 88}. The potential importance of P2X4R in renal fibrosis has
292 been investigated in the UUO model. Surprisingly, the P2X4R knockout mice showed increased
293 renal fibrosis following induction of UUO associated with increased expression of TGF β 1 and
294 connective tissue growth factor (CTGF, also known as CCN2), and increased amounts of type I
295 collagen ⁸⁹. These results suggest that P2X7R is pro-fibrotic in this model and that P2X4R may
296 have an anti-fibrotic role through its regulation of pro-fibrotic growth factors.

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

306

307 **What now for P2X7R antagonists?**

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

311 Glu496Ala) can impair P2X7R function ^{95,96}: ATP-dependent IL-1 β 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*?

326

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671 **Disclosures**

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679 **Figure 1: The autocrine / paracrine purinoceptor system**

680 A range of stimuli including cellular stretch, trauma, or agonist binding triggers ATP release into
681 the extracellular space. Ectonucleotidases located on the plasma membrane catalyse sequential
682 hydrolysis of ATP to ADP, 5'AMP and adenosine. P1 receptors recognize adenosine while P2
683 receptors bind di- and tri-phosphate nucleotide molecules. P2X receptors are non-selective
684 cation channels with 3 protein subunits that may form homo- or heteromeric arrangements; all
685 bind ATP. P2Y receptors are 7 transmembrane-spanning domain G-protein-coupled receptors;
686 agonist preferences span adenosine and uracil di- and tri- nucleotides. NTPDase: ectonucleoside
687 triphosphate diphosphohydrolase.

688

689 **Figure 2: P2 Receptors in the kidney**

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

691

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

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

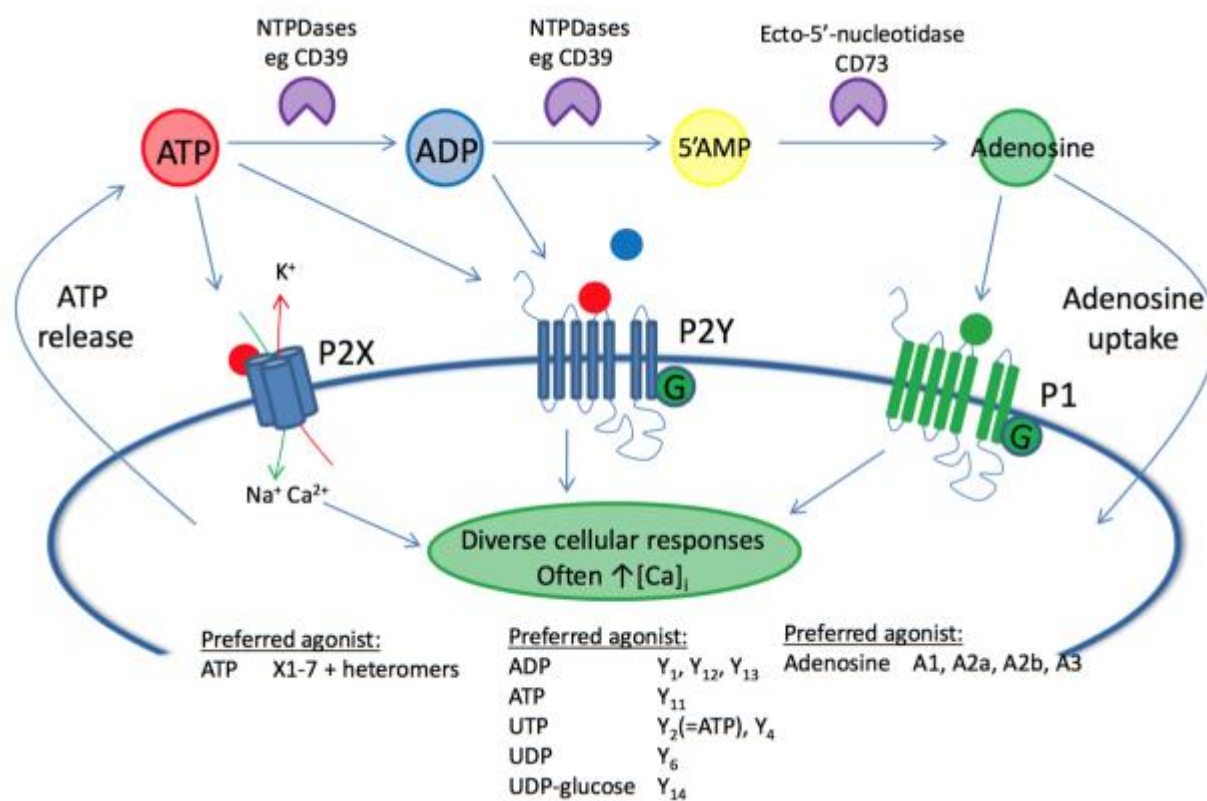
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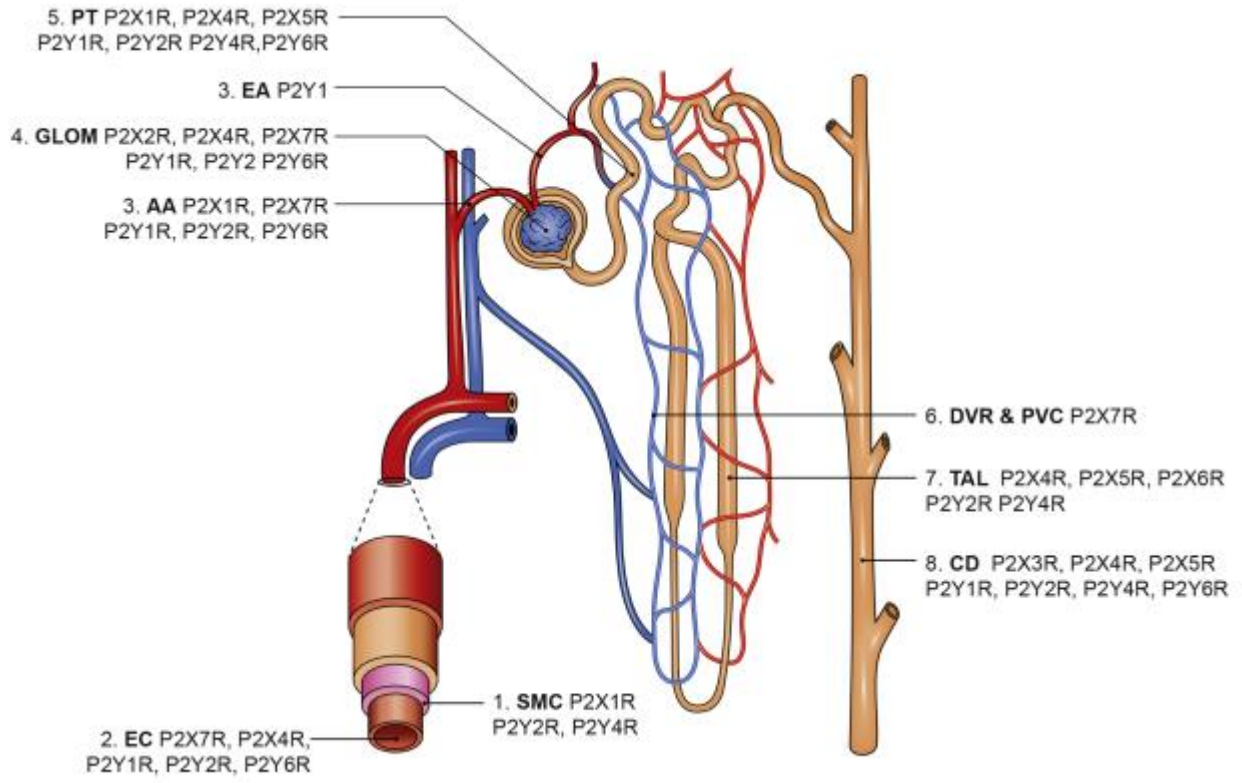
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704 **Figure 1**

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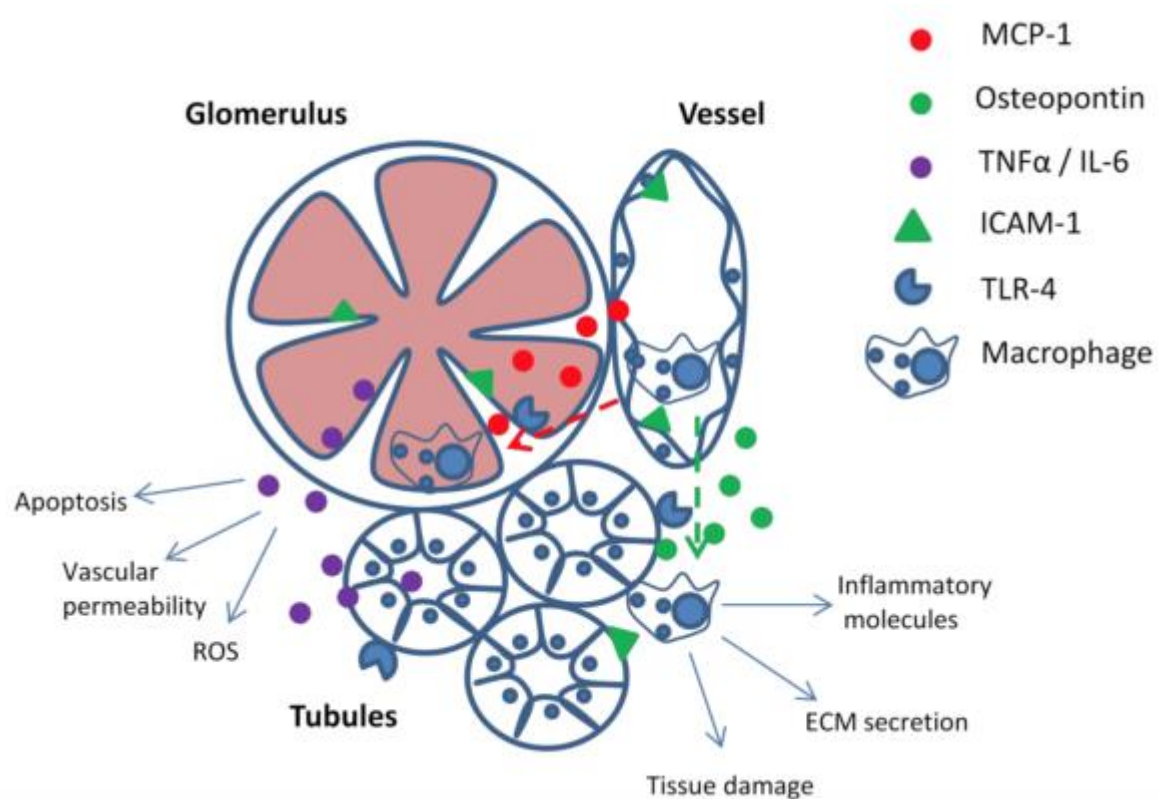
707 **Figure 2**



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710 **Figure 3**



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