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REVIEW

Molecular insights into P2X signalling cascades in acute kidney injury

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Abstract

Acute kidney injury (AKI) is a critical health issue with high mortality and morbidity rates in hospitalized individuals. The complex pathophysiology and underlying health conditions further complicate AKI management. Growing evidence suggests the pivotal role of ion channels in AKI progression, through promoting tubular cell death and altering immune cell functions. Among these channels, P2X purinergic receptors emerge as key players in AKI pathophysiology. P2X receptors gated by adenosine triphosphate (ATP), exhibit increased extracellular levels of ATP during AKI episodes. More importantly, certain P2X receptor subtypes upon activation exacerbate the situation by promoting the release of extracellular ATP. While therapeutic investigations have primarily focused on $P2X_4$ and $P2X_7$ subtypes in the context of AKI, while understanding about other subtypes still remains limited. Whilst some P2X antagonists show promising results against different types of kidney diseases, their role in managing AKI remains unexplored. Henceforth, understanding the intricate interplay between P2X receptors and AKI is crucial for developing targeted interventions. This review elucidates the functional alterations of all P2X receptors during normal kidney function and AKI, offering insights into their involvement in AKI. Notably, we have highlighted the current knowledge of P2X receptor antagonists and the possibilities to use them against AKI in the future. Furthermore, the review delves into the pathways influenced by activated P2X receptors during AKI, presenting potential targets for future therapeutic interventions against this critical condition.

Keywords P2X receptors · Acute kidney injury · P2X receptor antagonists · Adenosine triphosphate · Ion channels

Introduction

Acute kidney injury (AKI) is a severe health condition resulting from various primary complications, including diabetes, surgery, organ transplant, and cardiovascular diseases. Globally, the incidence of AKI has been reported to be 21% of the population, with a hospital mortality rate of approximately 21% [1]. Importantly, ischemia-reperfusion injury (IRI) and sepsis are the leading cause of AKI in hospitalized patients. Importantly, mitochondrial dysfunction, observed during IRI and sepsis, is associated with a reduced synthesis of new ATP molecules. Therefore, the cell compensates for its energy demands by exporting stored ATP from the mitochondrial matrix to the cytoplasm via ATP/ADP carrier protein. This process elevates extracellular ATP levels. The increased extracellular ATP acts as a primary ligand and binds to P2X purinergic receptors that mediate various biological functions such as inflammation, tissue damage, cell proliferation, and graft vs. host response [2, 3]. P2X receptors are subdivided into various subunits with different functions; for example, the $P2X_1$ receptor responsible for autoregulation of kidney blood pressure gets disturbed during AKI [4]. $P2X_3$ receptors, crucial for sensory nerve transmission, get upregulated during IRI leading to removal or denervation of superior cervical ganglion, and consequently increased inflammation and tissue injury [5]. $P2X_4$ receptors, with high affinity for ATP, become significantly activated during AKI, triggering an increased inflammation response via activation of NLRP3 inflammasome [6]. Although $P2X_7$ receptor expressed in tubular epithelial cells in small amounts, their upregulation during AKI contributes to increased inflammation via various mechanisms

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[7]. Therefore, deepening our understanding of P2X receptors in AKI is crucial.

Consequently, targeting P2X receptors during AKI is an emerging concept. Available reports highlight the significant contribution of P2X receptors to inflammation and injury of the kidney, with these receptors being targeted in various kidney diseases such as diabetic nephropathy, chronic kidney disease, and glomerular nephritis [8, 9]. Numerous animal studies targeting P2X receptors in AKI have demonstrated efficacy in managing and controlling the condition. Despite these advancements, several aspects related to P2X receptors still need to be explored. Specifically, the role of different P2X receptor subunits in AKI remains to be fully elucidated, and the precise mechanism by which P2X receptors aggravate AKI is still unclear. This review seeks to address these knowledge gaps by discussing the distribution and function of various subunits of P2X in the kidney. Additionally, it aims to delineate their roles in AKI progression, and assess how therapies targeting P2X receptors can benefit AKI management.

P2X receptors: during normal physiology

P2X purinergic receptors are ligand-gated inotropic receptors expressed in the vascular, immune, and structural cells of the kidney and exert distinct physiological functions through binding of extracellular ATP [10]. There are seven subunits of P2X receptors, and each subunit display a unique function [11]. This section delves into the precise location and function of these P2X receptor subunits within the kidney.

$P2X_1$ receptors

$P2X_1$ receptor is expressed in renal microvessels and maintains the hemodynamics of the kidney. At the molecular level, $P2X_1$ is activated by extracellular α, β-methylene ATP, and normal ATP. In the rat kidney α, β-methylene ATP display higher potency to activate $P2X_1$ than normal ATP [12, 13]. $P2X_1$ receptors maintain renal blood flow (RBF), vascular resistance, and blood pressure. Additionally, they contribute to tubuloglomerular feedback by sensing changes in NaCl concentration via macula densa cells [4]. $P2X_1$ receptors exhibit unique properties including rapid desensitization to ATP, swift internalization, and a high fractional calcium current, underscoring their role in bladder muscle contraction [14–16]. However, $P2X_1$ ablation does not necessarily guarantee a change in vasoconstriction. For example, in $P2X_1$ knockout mice, ATP still promotes vasoconstriction of afferent arterioles [17]. This suggests that the presence of other subtypes of P2X may contribute to the observed vasoconstriction. Also, beyond P2X receptors ATP still promote vasoconstriction via activation of smooth muscle cell P2Y receptors [18]. Though the vasoconstrictor response to alpha, beta-methylene ATP is dependent on the pH, as a lower pH decreases the response of P2X receptors [19]. Therefore, further clarification is still needed to fully understand the role of the $P2X_1$ receptor in the hemodynamics of the kidney.

$P2X_2$ receptor

$P2X_2$ receptors are immunolocalized in smooth muscles of large veins and arteries and are also present intracellularly in collecting ducts [20]. Their activation by ATP leads to the internalization of the aquaporin 2 (AQP2) gene responsible for forming AQP2 proteins, which permeates water transport by forming channels on the apical membrane in response to hyperosmolality in serum. Consequently, the internalization of the AQP2 gene results in reduced water permeability via the apical membrane to tubules, thus preventing water loss from the body [21, 22].

$P2X_3$ receptor

$P2X_3$ receptors transmit nociceptive stimuli from sensory nerves to the brain and spinal cord. They are expressed by neurons of the superior cervical ganglion (SCG), facilitating signal transmission from the central nervous system to the sympathetic nervous system in the kidney. Activation occurs in response to kidney injury or inflammation. The SCG can adjust levels of norepinephrine released in response to stress. Therefore, the SCG serves as an integrator of signals from both the sympathetic and central nervous systems to the kidney [5].

$P2X_4$ receptor

$P2X_4$ receptors regulate vascular resistance in renal arteries, primarily within the renal vascular endothelium. They control vascular structure and endothelial vasodilation in response to changes in renal blood flow. $P2X_4$ expression has been identified in proximal convoluted tubules and in thin ascending and thin descending segments as well as in collecting ducts. Additionally, P2X4 regulates the activity of epithelial sodium channels in cells of the collecting duct. Under normal Na + levels, the activation of both $P2X_4$ and P2Y leads to a modest inhibition of epithelial sodium channels (ENaC), responsible for Na + reabsorption. However, under conditions of reduced Na + levels, a more potent inhibition of ENaC activity occurs [23].

$P2X_5$ receptor

The $P2X_5$ receptor is expressed in the pars recta of proximal convoluted tubules and the collecting duct [24]. However, its specific role in kidney function remains undiscovered. Beyond its presence in the kidney, it plays an essential role in regulating the maturation of osteoclasts and hypermultinucleation [25]. Furthermore, studies indicate its involvement in inflammation by regulating levels of IL-1β IL-18 through the promotion of caspase 1 cleavage [26]. Investigating the impact of $P2X_5$ on kidney physiology and AKI could provide insights into its potential as a target for AKI treatment.

$P2X_6$ receptor

The $P2X_6$ receptor is expressed within the basolateral membrane of the proximal convoluted tubule, distal convoluted tubule, and collecting duct, with additional immunolocalization observed in the thin ascending limb loop of Henle [27, 28]. It was hypothesized that $P2X_6$ receptors are involved in magnesium reabsorption through magnesium channels on the apical membrane of DCT. However, an animal study aimed at determining the role of $P2X_6$ in the kidney revealed no significant changes in the concentration of Mg2 + ions or any other ions in P2XR KO mice [29]. Thus, more research is still required to understand the role of $P2X_6$ in kidney physiology.

$P2X_7$ receptor

The $P2X_7$ receptor is present in the collecting duct's epithelial cells and the glomerulus's mesangial cells. Normally expressed at low levels under physiological conditions, $P2X_7$ receptors exhibit upregulation in the presence of proinflammatory cytokines such as TNF-α, IL-18, and IL-1α, leading to increased cell apoptosis [30]. Predominantly expressed in immunological cells. $P2X_7$ is involved in mediating apoptosis and inflammation by activating interleukin converting enzyme and promoting the release of interleukin-1β by macrophages. During conditions such as ischemia, diabetes, and injury, the expression of $P2X_7$ receptors increases [8]. Indeed available data supporting non-selectivity of $P2X_7$ suggest that activated $P2X_7$ receptor further promotes nonselective permeability pathway consisting of a sizeable pore-like structure responsible for permeation of molecules of less than 1KDa molecular mass [31, 32]. However, study conducted on human embryonic kidney (HEK) and macrophage-like cell (RAW) cells revealed two distinct and selective pathways in HEK and RAW cells responsible for ion permeation, challenging the previous hypothesis [33]. UUO model using $P2X_7$ receptor knockouts demonstrated various effects, including an increase in interstitial macrophages, reduced myofibroblasts, decreased collagen deposition, and diminished expression of TGF-β in the kidney interstitium. Moreover, reduction in cell apoptosis indicated the involvement of the $P2X_7$ receptor in this physiological process [34]. Apart from these, $P2X_7$ and $P2X_4$ receptors have also been identified on vascular endothelial cells, enabling the regulation of renal vascular contractility by increasing vasoconstriction and levels of vasodilators. Therefore, they are responsible for maintaining renal vascular resistance [35].

Different subclass of P2X receptors and their role in AKI progression

In the earlier section, we outlined the function of P2X receptors during normal physiology in the kidney. Emerging data shows that the function of the P2X receptor during pathophysiological conditions such as AKI gets changed, which ultimately initiates complex signalling pathways and protein synthesis involved in the progression of kidney malfunction.

$P2X_1$ in AKI

As discussed earlier, the $P2X_1$ receptor is involved in the autoregulation of the kidney. In rats with IRI-induced AKI, IRI resulted in a decrease in the baseline diameter of afferent arterioles. The impairment of afferent arteriolar autoregulation led to an attenuation of $P2X_1$ receptor-mediated vasoconstriction in afferent arteriole. Consequently, IRI-induced AKI leads to a loss of kidney autoregulation resulting in a decrease in renal blood flow (RBF) and glomerular filtration rate (GFR), along with increase in renal microvascular resistance due to the IRI-induced elevation of reactive oxygen species and a reduction in $P2X_1$ receptor-mediated afferent arteriolar vasoconstriction via the agonist β, γ-mATP, which is essential for maintaining kidney autoregulation (Fig. 1) [36, 37]. However, it is noteworthy that the blockade of the $P2X_1$ receptor doesn't entirely stop renal autoregulation as other receptors such as adenosine A1 receptors, Angiotensin II, and KCl are also involved in autoregulation of the kidney. Nevertheless, the silencing or blockade of $P2X_1$ receptors leads to loss of pressure-dependent autoregulation in the kidney [38]. Another in-vivo study showed that the $P2X_1$ receptor is a primary receptor involved in pressure autoregulation of kidneys. Inhibiting adenosine A1 receptors didn't significantly alter renal autoregulation, while inhibition of the $P2X_1$ receptor resulted in a considerable reduction in the autoregulation response inducing hypertension and kidney damage (Table 1) [12]. Inhibition of $P2X_1$ results in the loss of autoregulation; thus, $P2X_1$ agonists

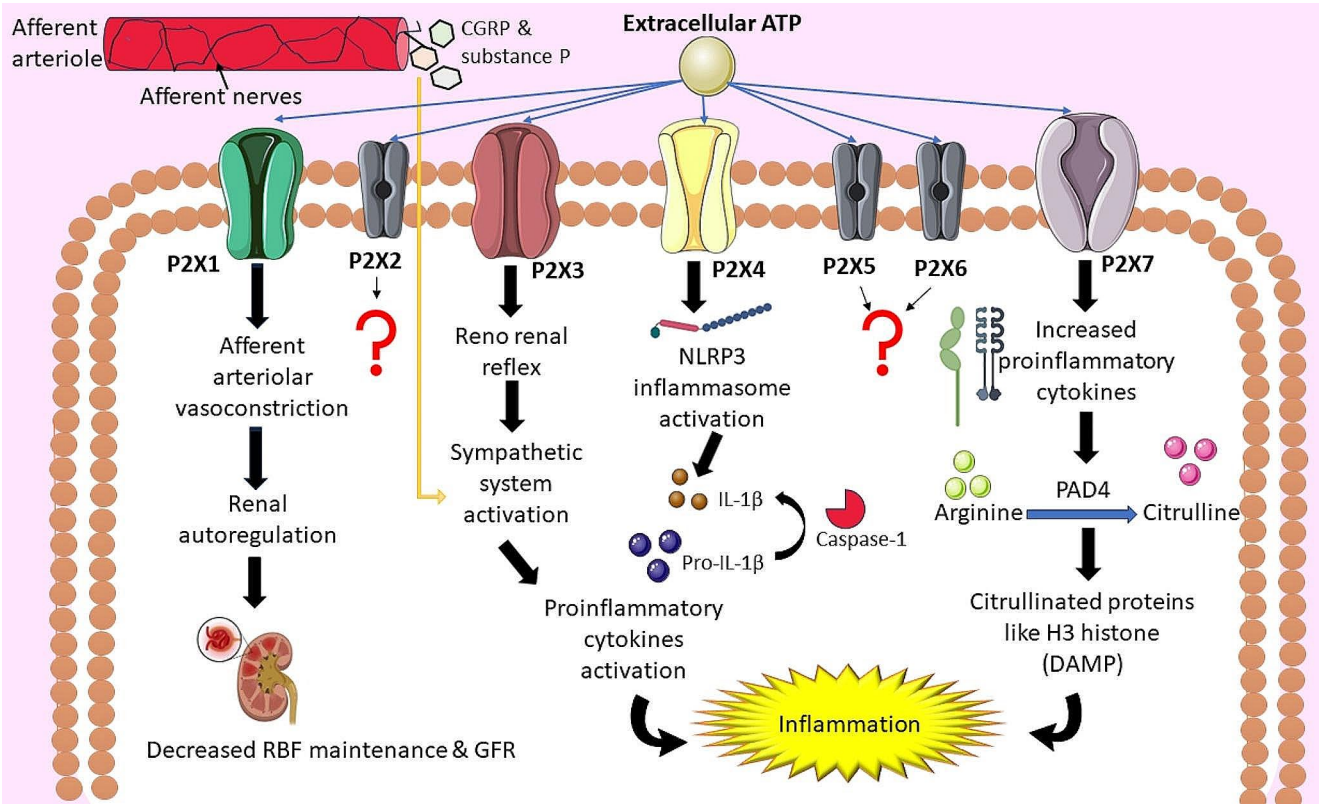

Fig. 1 P2X receptors family in AKI pathophysiology
During an AKI episode, the inhibition of afferent arteriolar vasoconstriction decreases renal autoregulation. Moreover, the activation of renal reflex due to which CGRP & substance P results in activation of the sympathetic system, activating proinflammatory cytokines. $P2X_4$ receptor activation results in the activation of NLRP3 inflammasome activation. The $P2X_7$ receptor gets upregulated and activated during AKI and leads to activation of PAD4, which is responsible for the citrullination of proteins that acts as damage-associated molecular pattern (DAMPs) and activates further immune reactions, thereby inflammation and apoptosis. $P2X_2$, $P2X_5$, and $P2X_6$ receptors in AKI or any other kidney disease have not yet been studied and remain immense areas of research
Abbreviations: RBF: renal blood flow; GFR: glomerular filtration rate; PAD4: Protein arginine deaminase; CGRP: calcitonin gene-related peptide

such as α, β methylene ATP, β, and γ-methylene ATP can be administered to restore kidney autoregulation [39, 40].

$P2X_3$ in AKI

Due to neuropathic injury, $P2X_3$ receptors in sensory neurons activate the NF-κB mediated signalling pathway, leading to an increased production of inflammatory cytokines and chemokines [41]. $P2X_3$ activation also increases phosphorylation of extracellular signal-regulated kinases (ERK1/2), contributing to inflammatory pain induction (Fig. 1) [42]. In the context of IRI-induced AKI, damage associated with ischemia triggers the "reno renal" reflex [43]. This reflex involves the activation of primary afferent nerve terminals, leading to the release of calcitonin gene-related peptide (CGRP), substance P, and other factors. These signals are transmitted to the central nervous system (CNS) and hypothalamus, where sympathetic activity is integrated via superior cervical ganglion (SCG) (Table 1). This results in a sympathetic signal to the kidney, increasing inflammatory activity by elevating levels of proinflammatory cytokines such as IL-6 and TNF-α. During AKI, chemical mediators, such as CGRP, activate $P2X_3$ receptors in the SCG, leading to hyperactivation of the sympathetic nervous system. This, in turn, increases the release of norepinephrine, further inducing the activation of proinflammatory cytokines and escalating inflammation and tissue damage [5, 42]. These findings have been confirmed by animal studies, in which denervation of the SCG has resulted in the upregulation of $P2X_3$ receptors and increased ischemia-induced injury [5, 44]. To counteract the detrimental effects of $P2X_3$ receptors, inhibition is necessary. This can be achieved by administering $P2X_3$ antagonists to help control AKI. Various $P2X_3$ antagonists suitable for AKI conditions are discussed below.

$P2X_3$ antagonists

Gefapixant (MK-7264/AF-219) Gefapixant, a recently USFDA-approved selective and reversible $P2X_3$ receptor antagonist, exhibits efficacy in refractory chronic cough. It

Table 1 Different P2X receptors in AKI as drug targets

P2X receptor subtype	Mechanism in AKI	Agonist/antagonist	Reference
$P2X_1$	Decrease in baseline diameter of afferent arterioles leading and attenuation of $P2X_1$ mediated afferent arteriole vasoconstriction, thus resulting in compromised renal autoregulation post-IRI.	α, β methyl ATP	[40]
$P2X_3$	Reno renal reflex activation resulting in the release of CGRP and substance P leading to sympathetic system activation via superior cervical ganglion and increased inflammatory activity via proinflammatory cytokines (IL6, TNF-α) activation.	Gefapixant, Eliapixant, Filapixant, camlipixant	[49, 53]
$P2X_4$	NLRP3 inflammasome activation leads to the activation of the apoptosis-associated speck-like protein, activating proinflammatory cytokines and increasing inflammation.	5-BDBD, NP-1815-PX, NC-2s600, PSB-15,417	[61, 62]
$P2X_7$	NLRP3 inflammasome activation increases IL-1β synthesis and activation, resulting in inflammation. Increased PAD4 activation leads to increased citrullination of histone protein and chromatin condensation, resulting in nuclear damage and death.	$P2X_7$ antagonists: A804591, A438079, Probenecid	[64, 65]

Abbreviations: ATP: adenosine triphosphate, NLRP3: NOD like receptor protein 3, IL-1β: interleukin 1β, PAD4: peptidyl arginine deaminase, CGRP: calcitonin gene-related protein

binds to the allosteric site of the $P2X_3$ receptor on primary afferent neurons and prevents its activation via ATP released by damaged tissues, thus inhibiting sensory nerves activation and subsequent signalling to the CNS (Table 2) [45–47]. In an animal study exploring gefapixant's potential as a therapy for myocardial infarction, it has been found that the compound inhibits NLRP3 inflammasome and cleaved caspase 1. This regulatory effect on interleukins (IL-1β & IL-18) helps in suppressing cardiac inflammation [48, 49]. While research on gefapixant's application in kidney disease is yet to be conducted, existing data suggests significant renal excretion. In one clinical study, an increased half-life of the gefapixant was found in renal-impaired patients, influencing the drug's duration of action [50]. Therefore, reducing the clinical dose can achieve the desired therapeutic effect in patients with kidney disease. In a study involving concurrent administration of pyrimethamine with gefapixant, plasma exposure to gefapixant increased by 24%, accompanied by a 30% reduction in renal clearance (Table 2) [50]. Notably, renal or urological adverse events are infrequent in patients with idiopathic pulmonary fibrosis. However, no adverse effects were reported in renal cell carcinoma and refractory cough patients [51]. Discrepancies in renal-related events were noted between the placebo group and those administered gefapixant [52]. This evidence will be instrumental in informing future research endeavours on the application of this drug in kidney disease, including AKI.

Eliapixant (BAY-18,170,800) Eliapixant is a $P2X_3$ receptor antagonist currently under clinical trials for various medical conditions, including overactive bladder syndrome in phase IIa, diabetic nephropathy (phase IIa), endometriosis (phase IIb), and refractive chronic cough (phase IIb). Its efficacy lies in preventing neurogenic inflammation and inhibiting nociceptive action mediated by the $P2X_3$ receptor. Nevertheless, the exact molecular mechanism remains enigmatic [53]. Information regarding the use of eliapixant in kidney disease is currently unavailable, though renal-impaired patients were excluded in a specific clinical trial involving this drug [54]. Interestingly, despite the lack of data on its application in kidney conditions, eliapixant demonstrates minimal urinary excretion and does not induce renal adverse effects, indicating a favorable renal safety profile. However, further research is required in this context.

Filapixant (BAY-1,902,607) Filapixant is a selective $P2X_3$ inhibitor closely related to Eliapixant. It is currently under Phase II of clinical investigation for the treatment of refractive chronic cough. It possesses a strong affinity for the *in-vitro* $P2X_3$ receptor and exhibited effectiveness against refractive chronic cough; however, the precise mechanism of its action is still unknown (Table 2) [55]. The impact of Filapixant on kidney function and its safety profile in this regard are currently elusive and necessitate thorough investigation.

Camlipixant (BLU-5937) BLU-5937 is a selective, homotrimeric $P2X_3$ receptor antagonist. It is currently in phase III of a clinical trial for refractive chronic cough treatment. Nota-

Table 2 P2X receptor targeting drugs in clinical trials

Sr. No.	Receptor subtype	Drug	Clinical trial	Disorder/Dysfunction	Study objective	Study status
1.	$P2X_3$	Gefapixant	NCT05813223 NCT01432730	Refractory chronic cough	To study the effect of Gefapixant on patients with chronic cough	Completed successfully. Gefapixant is found to be effective in reducing refractory chronic cough
2.	$P2X_3$	BLU-5937	NCT03979638	Chronic refractory cough	To study the effect of BLU-5937 on patients having refractory chronic cough by observing the change in awake frequency of cough	Completed (results awaited)
3.	$P2X_3$	BLU-5937	NCT04693195	Chronic pruritis, Atopic dermatitis	To study the effect on Atopic dermatitis patients for treatment of chronic pruritis	Completed (results awaited)
4.	$P2X_3$	BAY1718080	NCT04562155	Refractory chronic cough/unexplained cough	To determine the optimum dose required for patients having long-standing cough	Completed (results awaited)
5	$P2X_7$	JHJ-54,175,446	NCT02587819	Inflammation-induced depression	To test its effect in preventing inflammation-induced depression	Ongoing (phase II)
6	$P2X_7$	Memantine	NCT03918616	Parkinsons disease	To test its effect on the treatment of parkinson's disease	Completed (results awaited)

bly, it has demonstrated a favorable profile with fewer or no taste-related adverse effects compared to other $P2X_3$ inhibitors (Table 2) [56, 57]. Despite its promising characteristics, data concerning the renal safety and pharmacokinetics of BLU-5937 are currently unavailable and must be studied.

$P2X_4$ in AKI

In proximal convoluted kidney tubules, activation of the $P2X_4$ receptor occurs during post-ischemic conditions, leading to an increase in apoptosis and inflammation. This effect is attributed to the activation of NLRP3 inflammasome signalling [58]. Post-ischemic injury related damage of tubular cells induces the assembly of proteins into the NLRP3 inflammasome, initiating apoptosis-associated speck-like protein recruitment. This recruitment leads to the conversion of caspase 1 to its active form, further triggering the maturation of proinflammatory cytokines such as IL-18 and IL-1β, exacerbating inflammation and tissue damage and worsening the kidney condition (Table 1) [59]. Animal studies have demonstrated that inhibition of $P2X_4$ has been effective in reducing ischemic AKI-induced injury and inflammation. Notably, caspase 1 activation also results in pyroptosis [6]. Consequently, $P2X_4$ antagonists present a promising avenue for preventing and reducing injury and inflammation of tubular cells. The subsequent section provides a detailed overview of various $P2X_4$ antagonists.

$P2X_4$ antagonists

5-(3-bromophenyl)-1,3-dihydro-2 H-benzofuro(3,2-e)-1,4-diazepin-2-one 5-(3-bromophenyl)-1,3-dihydro-2 H-benzofuro(3,2-e)-1,4-diazepin-2-one (5-BDBD) is a benzodiazepine derivative $P2X_4$ selective antagonist. In an animal study aimed at determining the role of the $P2X_4$ receptor in AKI, pretreatment of mice with the $P2X_4$ antagonist 5-BDBD showed significant reduction in AKI biomarkers such as neutrophil gelatinase-associated lipocalin (NGAL) and blood urea nitrogen (BUN). Moreover, a reduction in apoptosis, inflammation, and necrosis was also observed in 5-BDBD treated mice [6]. Furthermore, in another animal study, focused on the activity of 5-BDBD on HEK cells, the compound was identified as a potent antagonist. Importantly, it exhibited no antagonist activity against $P2X_2$ receptors or $P2X_7$ receptors, which is usually observed in the case of $P2X_4$ receptor antagonism [60].

NP-1815-PX NP-1815-PX is a $P2X_4$ selective antagonist. The effects of NP-1815-PX were determined in an animal model of 2,4-dinitrobenzene sulfonic acid-induced colitis in rats. The results demonstrated its effectiveness in preventing inflammation. Notably, NP-1815-PX was found to neutralize the increase in levels of IL-1β by inhibiting the NLRP3 inflammasome pathway, which is responsible for the release

and maturation of IL-1β, thus preventing apoptosis and inflammation of kidney epithelial cells [61].

NC-2600 NC-2600 is a $P2X_4$ receptor antagonist that operates through the same mechanism as NP-1815-PX. Inhibition of $P2X_4$ by NC-2600 prevents inflammation and apoptosis of renal epithelial cells. This protective effect is achieved through the inhibition of NLRP3 inflammasome, thus obstructing release and activation of IL-1β [61].

PSB-15,417 In a study investigating the presence of $P2X_4$ receptor in the dorsal root ganglion during diabetes-induced neuropathic hyperalgesia in streptozotocin-induced diabetic rats, the administration of the $P2X_4$ antagonist PSB-1517 led to reversal of diabetes-induced neuropathic hyperalgesia and an overall improvement in the condition. These findings indicate the involvement of the $P2X_4$ receptor, and suggest that there is an overexpression of the $P2X_4$ receptor occurs in cases of diabetic nephropathic hyperalgesia [62].

$P2X_7$ receptor

Under hypoxic conditions, $P2X_7$ receptor expression is found to increase under in the extracellular compartment of tubular epithelial cells. Importantly, there is a concurrent elevation in the release of ATP by tubular epithelial cells, while ATP synthase expression remains unchanged. Treatment with ATPase has been shown to improve condition and downregulate IL-1β levels (Table 1). In in-vitro studies, inhibiting the $P2X_7$ receptor in tubular epithelial cells led to the inhibition of NLRP3 activation and reduced increased levels of IL-1β expression. Previous research demonstrated that pre-treatment with oxidized ATP before IRI led to decreased levels of BUN and serum creatinine as compared to the PBS-IRI. Additionally, it increased regulatory-T cells (Tregs), inhibiting both innate as well as adaptive immune effects due to injury [63]. Peptidyl arginine deaminase 4 (PAD4), responsible for the conversion of arginine to citrulline (citrullination), is implicated in AKI. Increased activation of PAD4 during IRI, driven by elevated levels of proinflammatory cytokines, chemokines, and toll-like receptor ligands, leads to enhanced activation of PAD4 and consequently increased citrullination of proteins such as H3 histone. This, in turn, acts as a damage-associated molecular pattern (DAMP) that causes chromatin condensation, resulting in cellular and nuclear damage and death. In a study using a $P2X_7$ agonist (BzATP) administration of the compound resulted in AKI in PAD4 competent mice but not in PAD4 non-competent mice, showing that $P2X_7$ receptor-dependent PAD4 activation leads to AKI [64]. On the other hand, $P2X_7$ inhibition decreases IL-1β expression in kidney tubular cells and reduces serum creatinine levels [65]. $P2X_7$ activation by extracellular ATP triggers potassium efflux from cells, leading to caspase 1 activation, which converts proinflammatory cytokines IL-1β and IL-18 into mature cytokines, intensifying inflammation and damage. Consequently, the $P2X_7$ receptor is an essential target for preventing inflammation and damage to the kidney. There are various $P2X_7$ receptor antagonists available for clinical use that may be beneficial in cases of AKI.

$P2X_7$ antagonists

A438079 In the aforementioned study, the administration of A438079 in rats post-IRI led to a significant decrease in serum creatinine, BUN levels and a reduction in renal damage [63].

A804598 In the previously discussed study on $P2X_7$ and PAD4-related AKI, it was observed that the administration of the $P2X_7$ receptor antagonist A804598 prevented AKI in PAD4-competent mice. Activation of $P2X_7$ receptor induces the production of PAD4 in tubular cells either directly or by increasing intracellular calcium influx, resulting in inflammation and death of tubular cells [64].

Probenecid Probenecid (PBN), a molecule approved for gout treatment has shown potential benefits in treating AKI. PBN blocks pannexin 1 channels in injured kidney tissues, resulting in $P2X_7$ receptor inhibition, further resulting in NLRP3 inflammasome signalling inhibition, consequently reducing inflammatory response and, thus, damage of the kidney tissue. PBN also leads to the activation of Tregs, thus decreasing the elevated immune system response due to AKI [66]. On the other side, probenecid also inhibits different organic anion transporters (OATs) in the kidney and increases the plasma concentrations of several drugs [67].

JHJ-54,175,446 JHJ-54175446, a $P2X_7$ inhibitor, is currently undergoing phase II evaluation in a multicenter, randomized, double-masked, placebo-controlled clinical trial for testing its effect on inflammation-induced depression. This clinical trial is guided by the hypothesis that inhibition of $P2X_7$ results in decreased inflammation. Thus, it can be beneficial in managing depression induced by inflammation (NCT02587819). Considering this, it could also be

explored in the context of AKI to determine whether it can help decrease inflammation associated with this condition.

Memantine Memantine, recognized as an anti-Alzheimer's drug and an NMDA antagonist, exhibit indirect inhibition of the $P2X_7$ receptor [68]. Moreover, the memantine also provide protection via antioxidant, anti-aggregating, or acetyl choline esterase (AChE) and monoaminoxidase inhibition [69]. A study has explored its potential as a$P2X_7$ inhibitor in Parkinson's disease, grounded in the hypothesis that activation of α-synuclein in microglial cells results in activation of NLRP3 inflammasome. This cascade leads to neuroinflammation, thus resulting in degeneration of dopaminergic neuron and the onset of Parkinson's. (NCT03918616). As activation of NLRP3 inflammasome is also one of the pathomechanisms of AKI, the effects of Memantine could be investigated in this context.

Various P2X receptor subclasses in alternative conditions that may serve as potential targets for addressing AKI

$P2X_5$ receptor

$P2X_5$ receptor has been involved in various processes including tumor development, inflammasome activation, and inflammatory bone loss. The $P2X_5$ receptor is reported to regulate osteoclast maturation and multinucleation in inflammatory conditions. Although the precise mechanism of multinucleation is not well-defined, it is hypothesized that $P2X_5$ receptor activation leads to inflammasome activation, resulting in the production of Il-1α [25]. $P2X_5$ is well recognized to be involved in the activation of inflammasome in bone-marrow developed macrophages during *L. monocytogenes* infection. Consequent caspase-1 activation usually triggers an immune response against the pathogen, however, this response was not observed in $P2X_5$ deficient mice [70]. Additionally, the involvement of a crucial cofactor methylosome protein (MEP50) has been identified. MEP50 binds to the C-terminal of the $P2X_5$ receptor and initiates the maturation of osteoclasts. This highlights the essential role of the arginine methyltransferase 5 and methylosome protein complex in osteoclast maturation [71]. Consequently, future studies could explore whether $P2X_5$ is also involved in AKI's inflammasome activation and inflammatory response.

$P2X_6$ receptor

It has been observed that among bladder cancer patients, those with high expression of the $P2X_6$ receptor showed a higher survival rate. This suggests that upregulation of the $P2X_6$ receptor could be beneficial in managing bladder cancer [72]. Furthermore, high expression of the $P2X_6$ receptor has also been observed in renal carcinoma cells. Activation of the $P2X_6$ receptor leads to alteration in calcium influx and ERK1/2 phosphorylation, thus perceived as responsible for metastasis and invasion of cancer cells [73]. Given that ERK1/2 is also an essential regulator in AKI as it has been shown to reduce the NAD + enzyme, it becomes pertinent to study the effects of the $P2X_6$ receptor in the context of AKI [74].

Conclusion & future perspectives

P2X receptors play essential roles in the normal physiology of the kidney. Specific P2X receptors, including $P2X_3$, $P2X_4$, and $P2X_7$, gets activated in the kidney during ischemic conditions and initiates complex inflammatory and apoptotic signaling. This eventually interferes with normal kidney autoregulation. Therefore, the P2X receptor may represent a potential target for the management of AKI. AQP2 plays detrimental role in the water transports in collecting ducts. P2X receptors are known for altering the functioning of AQP2 in the kidney. However, the effect of inhibition of P2X receptors by AQP2 knock-out or using a molecule that inhibit AQP2 is still unknown in AKI. In future, such studies need to be carried out in AKI condition. Inhibitors targeting $P2X_3$, $P2X_4$, and $P2X_7$ receptors have potential to reduce inflammatory conditions in tubular cells and thus protect the kidney from further damage. Therefore, clinical studies focusing on these P2X inhibitors are much required in the future to understand their effectiveness in AKI patients. However, certain P2X receptor subunits like $P2X_2$, $P2X_5$, and $P2X_6$ have not been thoroughly investigated in terms of their roles in the normal kidney functioning and progression of kidney disease including AKI. Available reports of $P2X_2$, $P2X_5$, and $P2X_6$ in other kidney diseases confirms them as a potential therapeutic target. Future studies need to be focused on understanding the role and regulation of $P2X_2$, $P2X_5$, and $P2X_6$ in kidney and effect of their agonists/antagonists on the progression of AKI.

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Declarations

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