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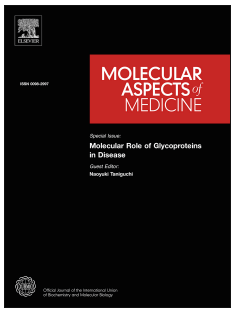

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Adenosine Contribution to Normal Renal Physiology and Chronic Kidney Disease

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Abstract

Adenosine is a nucleoside that is particularly interesting to many scientific and clinical communities as it has important physiological and pathophysiological roles in the kidney. The distribution of adenosine receptors has only recently been elucidated; therefore it is likely that more biological roles of this nucleoside will be unveiled in the near future. Since the discovery of the involvement of adenosine in renal vasoconstriction and regulation of local renin production, further evidence has shown that adenosine signaling is also involved in the tubuloglomerular feedback mechanism, sodium reabsorption and the adaptive response to acute insults, such as ischemia. However, the most interesting finding was the increased adenosine levels in chronic kidney diseases such as diabetic nephropathy and also in non-diabetic animal models of renal fibrosis. When adenosine is chronically increased its signaling via the adenosine receptors may change, switching to a state that induces renal damage and produces phenotypic changes in resident cells. This review discusses the physiological and pathophysiological roles of adenosine and pays special attention to the mechanisms associated with switching homeostatic nucleoside levels to increased adenosine production in kidneys affected by CKD.

Keywords: Chronic Kidney Disease; Adenosine Receptors; Nucleoside Transporters; Renal Fibrosis.

Contents

1. Introduction

The adenosine nucleoside was identified as a bioactive molecule when Drury and Szent-Györgyi (1929) discovered its influence in several physiological tasks such as cardiovascular function. The role of adenosine in renal physiology was first studied in the 60´s when it was discovered that infusion of adenosine to the renal artery increased renal vascular resistance (Hashimoto and Kumakura, 1965; Haddy and Scott, 1968). This evidence promptly lead to the notion that adenosine decreases glomerular filtration, and then its role on sodium excretion rates and influence on renin activity were characterized (Tagawa and Vander, 1970; Osswald, 1975).

Since the competitive nature of methylxanthines, including caffeine and theophylline, on the effects of adenosine in the heart (De Gubareff and Sleator, 1965) and brain (Sattin and Rall, 1970) were recognized, it was convincingly supported the idea that specific receptors for this nucleoside may exists (Cobbin et al., 1974). In the 90's adenosine receptors from human and mammals were cloned. There are four different adenosine receptors, named A_1, A_{2A}, A_{2B}, and A_3, belonging to the receptor family with seven transmembrane domains, coupled to diverse types of G proteins, which exhibit different affinities to their adenosine ligand (Fredholm et al. 2001, 2011). Since their identification, multiple studies have searched for the presence of adenosine receptors in renal cells, using diverse experimental approaches, to correlate their localization with a physiological function (see Table I). Knockout animal models of these receptors have recently been generated, some of which have been a valuable tool for evaluating the effects of adenosine in the kidney (Sun et al. 2001; Tak et al. 2014; Yang et al. 2016). Therefore, discovering the biochemical mechanisms that control adenosine extracellular availability and influence its biological

activity has been a daunting task. A series of ectoenzymes that metabolize precursor nucleotides to generate adenosine were also identified. Additionally, nucleoside transporter systems which control adenosine flux through the plasmatic membrane, involved in presenting the ligand to activate signalling via adenosine receptors, were also characterized (Quezada et al. 2013; Shirley et al. 2009).

In this review we will discuss the contribution of adenosine and its receptors to fundamental renal physiological functions. We will also present evidence that supports the role on adenosine in renal fibrosis progression, which is considered a common event during chronic kidney disease (CKD), independent of its origin, and which strongly correlates with progressive loss of renal function.

2. Adenosine metabolism in the kidney

The biological effects of adenosine are mediated by signaling via adenosine receptors in the plasma membrane. Adenosine is the main, if not exclusive, agonist of these receptors; therefore its bioactivity is dependent on its extracellular availability (Fredholm et al. 2011). Inosine could act as a partial agonist of the A_3AR subtype (Jin et al. 1997; Fredholm et al. 2001), but since this nucleoside is a catabolic product of adenosine it reinforces the physiological importance of adenosine generation at the tissular level. Adenosine is generated from intracellular synthetic pathways or in the extracellular compartment by catabolism of precursor nucleotides such as ATP or cyclic AMP (cAMP) (Jackson and Dubey, 2004; Vallon et al. 2009). The intracellular pathway of adenosine synthesis is mediated either by an intracellular 5'-nucleotidase, which dephosphorylates AMP (Schubert et al. 1979; Zimmermann et al. 1998), or hydrolysis of S-adenosyl-homocysteine

(Broch and Ueland, 1980). Intracellularly generated adenosine may be transported into the extracellular space mainly via specific bi-directional transporters by facilitated diffusion (Pastor-Anglada and Pérez-Torras, 2015). Some cells have an increased potential to release adenosine into the extracellular milleu (Fredholm et al. 1994; Parkinson et al. 2005), however, there is no evidence of renal cells having this potential. This is likely due to the fact that extracellular catabolism of precursor nucleotides is the major source of adenosine production at renal compartments during physiological or pathological responses.

Classical examples of adenosine generation from nucleotide precursors have been described along the nephron. ATP may be hydrolyzed enzymatically by ectonucleoside triphosphate diphosphohydrolase (CD39) generating AMP. AMP then converts into adenosine via the enzyme 5'-ectonucleotidase (CD73) (Vallon et al. 2009). In addition, alkaline phosphatase at the proximal tubule may also generate adenosine even though it has a high K_m for adenine nucleotides (Oyarzún et al. 2015). Prostatic acid phosphatase (PAP) is another enzyme that catalyzes AMP hydrolysis, however, its distribution and physiological roles in the kidney are poorly understood (Lam et al. 1989), although it has been attributed a role in renal carcinogenesis (Shibata et al. 2003). The types and distribution of ectonucleotidases along the rat nephron and murine renal substructures was previously described (Kishore et al. 2005; Shirley et al. 2009) and therefore is not included within this review.

In the rat glomerulus, the basal ATP release rate is approximately 0.30 pmol/min/1000 glomeruli (Karczewska et al. 2007). Exogenous ATP was rapidly degraded by the glomeruli suspension, with a $t_{1/2}$ decay of 2 min, indicating that efficient extracellular catabolism occurs at this compartment, an observation that is reinforced by the abundance of nucleotidases in the glomerulus (Kishore et al. 2005; Shirley et al. 2009). Addition of

ARL67156, an ecto-ATPase activity inhibitor, to the glomeruli suspension caused extracellular ATP to gradually increase. Following ATP hydrolysis the AMP concentration was higher than other hydrolysis derived products. Under normal conditions, glomeruli hydrolyze AMP to adenosine, although less efficiently than ATP to ADP and AMP (Karczewska et al. 2007), which allows adenosine signaling in glomerular cells or downstream signaling throughout the nephron.

Another example of adenosine signaling dependent on extracellular ATP hydrolysis is paracrine communication between macula densa cells and afferent arterioles. ATP released from cells in the macula densa is metabolized to adenosine in the extracellular space and mediates the vasocontractile response of afferent arterioles. Despite the fact that P_2 purinergic receptors are widely distributed throughout the kidney (Burnstock et al. 2014), only a minor role in afferent arteriolar vasoconstriction has been perceived (Schnermann, 2011; 2015), indicating that metabolic imbalance towards the degradation of released ATP is relevant to the production of the vasoactive effector adenosine. Indeed, the paracrine pathway and afferent arteriolar vasoconstriction was deficient in mice that do not express 5'-ectonucleotidase (Castrop et al. 2004).

In addition, adenosine may also be generated from extracellular cAMP degradation by an ecto-AMP phosphodiesterase, followed by AMP metabolism to adenosine by 5'-ectonucleotidase (Jackson and Dubey, 2004). This pathway for extracellular adenosine formation was demonstrated in *in vivo* proximal tubules, collecting tubules and in primary cultures of cells derived from these tubules (Jackson et al. 2003, 2006). Intriguingly, renal epithelial cells along the nephron can extracellularly metabolize non-conventional 2',3'-cAMP to 2'-AMP and 3'-AMP and further efficiently metabolize extracellular 2'-AMP and

3'-AMP to adenosine using non classical phosphodiesterases and ecto-5'-nucleotidase (Jackson and Gillespie, 2013). Since 2', 3'-cAMP may be released from cells upon injury, further research is needed to identify if there is a significant difference in 2', 3'-cAMP levels between the physiological and pathophysiological states.

In addition to the rate of extracellular synthesis, research has also focused on the mechanism that regulates external accumulation of adenosine; which is dependent on cell uptake (San Martin et al. 2009). Nucleoside transport in eukaryotic cells is mediated by two families of structurally-unrelated membrane proteins: the Na^+-independent facilitative equilibrative nucleoside transporters (ENTs) family and the Na^+-dependent concentrative nucleoside transporters (CNTs) family (Hyde et al. 2001; Gray et al. 2004; Kong et al. 2004; Baldwin et al. 2004). Each family of nucleoside transporters has several members, being CNT2, CNT3, ENT1, ENT2 and ENT3 capable of transporting adenosine (Pastor-Anglada and Casado, 2006). It is supposed that nucleoside transporters are widely distributed throughout all cells types, due to the implications that the uptake of bases and nucleobases has on energetic metabolism and salvage pathways. However, it is currently known that nucleoside transporters are expressed in specific cell type patterns and may have a selective subcellular distribution (Jennings et al. 2001; Govindarajan et al. 2007). In fact, ENT3 (Baldwin et al. 2005) and ENT2 isoforms (Grañé-Boladeras et al. 2016) were involved in nucleoside transport activity in intracellular compartments. A variety of physiological models have shown that pharmacological inhibition of ENT1 or ENT2 at the plasma membrane results in increased extracellular adenosine concentration, favoring a specific biological effect via adenosine receptor activation (Mubagwa and Flameng, 2001; Ackley et al. 2003; Sonoki et al. 2003; Choi et al. 2004; Riksen et al. 2005; Carrier et al.

2006; Farías et al. 2006; Grenz et al. 2012; Cárdenas et al. 2013). Also, there is increasing evidence of the distribution and probable function of nucleoside transporters in the kidney. In renal epithelial cells, hCNT1, hCNT2, and hCNT3 at the apical membrane and hENT1 and hENT2 at the basolateral membrane, apparently work together to mediate nucleoside reabsorption from the lumen to blood, driven by Na^+ gradients (Elwi et al. 2006). Additional evidence suggests that the distribution of the main equilibrative nucleoside transporter ENT1 may be present in both apical and basolateral membranes of renal epithelia and extratubular locations including the glomeruli, vascular smooth muscle and endothelial cells (Roa et al. 2009; Damaraju et al. 2007). Thus, extensive distribution of ENT1 throughout the kidney indicates that this transporter plays a role in facilitating adenosine signaling beyond nucleoside and nucleobase solute homeostasis (Roa et al. 2009; Damaraju et al. 2007; Elwi et al. 2006). An overview of the mechanisms involved in extracellular adenosine generation linked with cellular signaling is shown in figure 1.

3. Physiological functions of adenosine in the kidney

The kidney is a fundamental organ for corporal homeostasis. It is organized into functional units known as nephrons connected to a capillary network nourished by blood, which participates in the process of filtration, reabsorption and excretion of solutes, and blood back into the circulatory system. The basic nephron function is to purify the blood of waste products via filtration at the glomeruli, as well as remove solutes, ions and water from the filtrate at the tubular level to produce urine. Additionally, the kidney has a fundamental role in regulating blood volume and arterial pressure. It is also involved in the glucose metabolism and regulation of pH, ion levels and hormone synthesis. Thus, certain renal cells, such as tubular epithelial cells, are recognized as polyfunctional and therefore,

understanding their physiology is an interesting but hard task for clinical and basic researchers. Over the last years there has been special interest in the generation of factors that can locally affect renal cell function, among those the intrarenal Renin-Angiotensin System (RAS) and production of other vasoactive molecules (Zhuo et al. 2013). Equally, conclusive background information indicates that adenosine, produced as an autacoid, and its interaction with other effector systems, regulates renal function (Vallón et al. 2006). It is involved in the regulation of afferent and efferent artery vascular tone and crosstalk with the renin-angiotensin system (Navar et al. 1996; Peti-Peterdi and Harris, 2010; Weihprecht et al. 1994).

3.1 Involvement of adenosine in renal vascular tone regulation

Renin is an enzyme secreted by kidney juxtaglomerular cells (JG) where it is synthesized as a preproenzyme that later converts into prorenin; the mature but inactive form of the enzyme (Urushihara and Kagami, 2016; Morales, 2010). Once activated this enzyme is key to the renin-angiotensin system since it is in charge of transforming angiotensinogen to angiotensin I (Huang et al. 2016). Therefore the amount of renin is a limiting step during the production of Ang II, the main RAS component that acts as a critical element to the regulation of blood pressure and CKD pathogenesis. Renin release by JG cells is stimulated by cAMP and is inhibited by an increase in intracellular calcium levels (Ortiz-Capisano et al. 2013) and endothelins in a calcium dependent way (Ortiz-Capisano et al. 2014). cAMP is a second messenger used for the transduction of intracellular signals in diverse biological processes, having an important role in stimulating renin gene expression. Factors that increase cAMP levels stimulate renin expression in JG cell cultures (Lopez and Gomez, 2010; Gomez et al. 2009). The inhibitory effect of adenosine on renin release was

confirmed in dogs (Macias-Nunez et al. 1985; Arend et al. 1984), rats (Churchill and Bidani, 1987; Osswald et al. 1978), isolated rat kidney (Murray and Churchill, 1984, 1985), rat glomeruli (Skott and Baumbach, 1985) and in humans (Edlund et al. 1994). Adenosine produced at the macula densa inhibits renin release through activation of the A_1 adenosine receptor in mouse JG cells (Ortiz-Capisano et al. 2013). This has also been shown in vivo; when adenosine was injected into the renal artery in the canine kidney, a decrease in renin production was observed (Deray et al. 1989). Also, A_1 receptor knockout mice have a significantly increased renin plasmatic concentration compared to wild type mice (Schweda et al. 2005). Studies in JG mouse cells showed that A_1 receptor activation results in a calcium dependent inhibition of renin release via transient receptor potential canonical channels (TRPC)-mediated calcium entry (Ortiz-Capisano et al. 2013). The role of the adenosine A_2 subtype receptors in renin release is less clear. In the 80´s, studies using an A_1 receptor agonist (CHA) indicated that low concentrations were capable of inhibiting renin release, meanwhile elevated concentrations of CHA stimulated renin release (Churchill and Churchill, 1979, 1985), indicating the existence of an interaction between the agonist with other receptors. Later, in vivo studies in dogs showed that selective stimulation of A_2 receptors induced renin release (Miura et al. 1999).

The afferent and efferent arterioles are an important site for autoregulation of perfusion and glomerular filtration rate. In general, various vasoactive substances, such as thromboxane, superoxide and kinins, may modulate renal vasculature tone, reactivity and resistance. Importantly, studies have recognized the convergence of Angiotensin II activity (Ang II) and adenosine in the afferent arteriole to mediate tubuloglomerular feedback (TGF) (Franco et al. 2009; Persson et al. 2013). TGF indicates a negative relationship between NaCl

concentration at the macula densa and glomerular filtration rate or glomerular capillary pressure (Schnermann, 2015). Ang II induces renal microvasculature constriction via the AT_1 receptor (Harrison-Bernard et al. 2006). Meanwhile, adenosine produces different responses depending on the type of receptor with which it interacts; A_1 receptor regulates contraction (nM range) and A_2 receptor regulates dilation (nM range) (Lai et al. 2006a). At physiological concentrations Ang II increases the adenosine contractile response (Lai et al. 2006a). Correspondingly, low concentrations of adenosine significantly increase the response of the afferent arteriole to Ang II (Lai et al. 2006a). Many studies have shown that the adenosine A_1 receptor is important for the synergic interaction between adenosine and Ang II, however, other mechanisms can also contribute to this phenomenon (Lai et al. 2006b; Hansen et al. 2003; Gao et al. 2011). It was recently shown that arteriolar contraction induced by Ang II is decreased in A_1 receptor knockout mice; however administration of adenosine sensitizes the contractile response both in control and knockout mice (Gao et al. 2015). On the other hand, it was shown that temporary injection of Ang II elevates interstitial and tissue levels of adenosine (Franco et al. 2008). To explain this effect, it is assumed that vasoconstriction induced by Ang II leads to ischemia, producing de novo formation of adenosine. This is also explained by the fact that Ang II induces a significant decrease in adenosine deaminase (ADA) activity, as well as a decrease in mRNA and protein levels of this enzyme, which catabolize adenosine to inosine. In turn, increased adenosine concentration also directs downregulation of the A_{2A} receptor, allowing the adenosine vasoconstrictor effect to be preferentially regulated through A_1 receptor activation (Franco et al. 2008). In humans and animals with CKD, the circulating levels of Ang II are induced (Urushihara and Kagami, 2016). Interestingly, Dai et al. (2011) showed that renal fibrosis progression, in a model of Ang II infusion, was concurrent with

increased adenosine levels and could be blocked by using an adenosine A_{2B} receptor antagonist, highlighting the interaction between adenosine signalling and the RAS system.

Nitric oxide (NO) is another vasoactive molecule relevant to renal function. Bioactivity of adenosine receptors produces different effects on NO levels, generating diverse results at the vascular beds. Activation of the A_1 receptor regulates renal vasoconstriction through both decreased NO generation and increased production of vasoconstrictor compounds from the COX (cyclooxygenases) pathway of arachidonic acid metabolism (Barrett and Droppleman, 1993; Walkowska et al. 2007). On the other hand, activation of the A_{2A} and A_{2B} receptors produces vasodilator effects through stimulation of NO production (El-Gowelli et al. 2013; Carroll et al. 2006). While A_{2A} and A_{2B} receptors are expressed in afferent arterioles, studies indicate that the vasodilator actions occur essentially by activation of the A_{2B} receptor (Feng and Navar, 2010). However, Carlström and colleagues showed that stimulation of the A_{2A} receptor attenuated tubuloglomerular feedback responses by stimulating endothelial nitric oxide synthase (eNOS), presumably at the afferent arteriole (Carlstrom et al. 2011). It is also known that intravenous administration of adenosine causes vasodilatation mediated by the activation of the A_{2A} receptor/NO axis, since dilatation induced by adenosine was abolished in eNOS knockout mice and in wild type mice treated with l-NAME, suggesting that eNOS is a source of NO that mediates the vascular effects of A_{2A} activation (Hansen et al. 2005). The potential role of the adenosine A_{2A} receptor in mediating eNOS activation and modulating vascular tone has been shown in rat aorta, carotid artery (Ray and Marshall, 2006; Teng et al. 2008) and human fetal endothelium, which is named the ALANO pathway in the latter system (San Martín and Sobrevia, 2006).

Lastly, studies on the effects of A_3 receptor activation on vascular control at the renal beds are scarce. Evidence indicates that activation of the A_3 receptor dilated the norepinephrine-preconstricted afferent arterioles and blunted the vasoconstrictive effect of adenosine A_1 receptor activation (Lu et al. 2015). The effects of A_3 receptor activation in other vascular niches are contradictory to date (Hinschen et al. 2003; Ansari et al. 2007).

3.2 Roles of adenosine in ions balance

Many studies have characterized the role of adenosine in absorption and excretion of ions in the tubular renal system. The first correlation between adenosine and balance of the Na^+ and Cl^- secretory and absorptive pathways in the kidney come from Siragy and Linden, (1996) who described that increased NaCl intake could modulate adenosine production in the kidney. In experiments performed with rats maintained with a low salt diet (0.15%), adenosine concentration significantly decreased (23.3 ± 3 nM cortex; 55.5 ± 5 nM medulla). Meanwhile when salt intake increased (4%), adenosine renal production also increased (418 ± 43 nM cortex; 1040 ± 37 nM medulla), being higher in the medulla than in the renal cortex (Siragy and Linden, 1996) and indicating that NaCl concentration and adenosine production in the kidney is tightly regulated, especially in the medullar zone. As described above, TGF is regulated by adenosine through the A_1 and A_{2A} adenosine receptors. This mechanism directly relates tubular NaCl concentration in the ascending limb of Henle's loop and afferent arteriolar tone (Hansen and Schnermann, 2003; Huang et al. 2006). The best characterized adenosine function in solute reabsorption in the kidney is related to tubular Na^+ reabsorption. The A_1 adenosine receptor regulates Na^+ reabsorption in the proximal tubule (responsible for reabsorbing 60-70% of filtrated Na^+) effecting the activity of multiple transporter systems, including the Na^+/H^+ exchanger-3 (NHE3), the

Na^+/PO_4^- cotransporter and Na^+-dependent glucose transporter (SGLT) (Welch, 2015). It was shown that administration of selective A_1 receptor antagonists cause diuresis and natriuresis, reaffirming that this receptor mediates sodium and water reabsorption (Vallon et al. 2009). Also, the use of an antagonist or deletion of the A_1 receptor decreased hypertensive outcomes in different animal models (Welch, 2015). Other studies have demonstrated a relationship between salt intake and adenosine A_1 receptor expression. Increased salt intake in rats decreased adenosine A_1 receptor expression in the collector duct, meanwhile in the proximal tubule, decreased salt intake increased A_1 receptor expression thus increasing Na^+ reabsorption (Siragy and Linden, 1996; Zou et al. 1999; Kulick et al. 2008). These changes could be part of a feedback mechanism that allows increased Na^+ reabsorption in the proximal tubule when faced with low salt intake and high excretion under high salt consumption, apparently functioning through modulation of A_1 receptor expression and activity (Rajagopal and Pao, 2010; Zou et al. 1999). Although many researchers have shown a relation between NaCl homeostasis and regulation via activity of the adenosine A_1 receptor in the kidney, evidence is still needed to understand the intracellular mechanisms that regulate this feedback.

One proposed mechanism of NaCl homeostasis through the adenosine A_{2A} receptor involves stimulation of K^+ channels and Cl^- secretion (Wang et al. 2011; Gu et al. 2007). Most potassium is reabsorbed in the proximal tubule by passive transport and solvent drag. 25% is reabsorbed in the loop of Henle by the Na^+-K^+-$2Cl^-$ symporter and the remaining potassium reaches the distal nephron and can be reabsorbed or eliminated in the urine (Stone et al. 2016, Palmer, 2015). Adenosine activates K^+ channels in the basolateral membrane of cells of the ascending limb of the Henle´s loop in the rat kidney, dependent

on A_{2A} receptor activation of PKA (Gu et al. 2007). K^+ channels participate in epithelial transport in cells from the thick ascending limb of the loop of Henle, which is responsible for the absorption of 20-25% of filtered Na^+ (Gu et al. 2007; Hebert, 1998). The apical K^+ channels are essential for K^+ recycling, maintaining $Na^+/Cl^-/K^+$ cotransporter function (Simon et al. 1996), meanwhile basolateral K^+ channels participate in generating the membrane potential in the thick ascending limb of the Henle´s loop (Hebert et al. 2005). Thus, the A_{2A} adenosine receptor could increase signalling through the cAMP-PKA pathway, activating K^+ basolateral channels and leading to synchronized entrance of apical Cl^- and basolateral exit, which indirectly increase $Na^+/Cl^-/K^+$ cotransporter activity at the apical membrane, favouring Na^+ reabsorption (Wang et al. 2011; Gu et al. 2007). The A_{2B} receptor mediates a different signalling pathway to regulate NaCl secretion through the collecting duct when faced with excessive salt intake (Rajagopal and Pao, 2010). Studies in mIMCD-K2 cells (renal inner medullary collecting duct) show that adenosine activates the apical A_{2B} receptor when its concentration reaches the micromolar range, stimulating Cl^- secretion through CFTR (cystic fibrosis transmembrane conductance regulator), via a cAMP/PKA dependent signalling pathway (Rajagopal and Pao, 2010).

Studies also indicate that adenosine could regulate the transport of other ions in the kidney. Adenosine modulates Mg^{2+} uptake in distal convoluted tubule cells via A_1 and A_2 receptors and a volume sensitive-like chloride conductance in the rabbit distal convoluted tubule cell line (DC1) (Kang et al. 2001; Rubera et al. 2001).

To mediate these physiological tasks fine-tuned regulation of extracellular levels and clearance of adenosine is required. Basal extracellular adenosine levels are estimated to be between 30 to 200 nM (Ballarín et al. 1991). This amount is sufficient to activate some of

the high affinity adenosine receptors under physiological conditions and can result from equilibrative transport of adenosine through ENTs, balancing intracellular production with extracellular nucleoside generation. From this baseline level, adenosine can increase substantially when extracellular formation increases as a result of adenine nucleotide release from cells as described above. In addition, extracellular accumulation of adenosine may occur due to decreased cell uptake through ENTs. Both these mechanisms may have a role in dysregulation of adenosine levels in CKD models. Indeed, *ent1-/-* mice exhibit elevated interstitial and plasmatic adenosine levels affecting renal physiology and renal cell function (Li et al. 2013).

4. Pathogenesis of Chronic kidney disease

Chronic kidney disease (CKD) is defined by most clinical and genetic epidemiological studies as a condition with an estimated GFR (eGFR) of < 60 $ml \cdot min^{-1} \cdot 1.73\ m^{-2}$, irrespective of the presence or absence of any additional kidney damage (Levey et al. 2005). Pathological features of CKD are inflammatory infiltration, tubular atrophy, capillary rarefaction, podocyte depletion and fibrosis (Duffield et al. 2013; Campanholle et al. 2013). CKD remains an incurable disease and it is estimated to affect 8-16% of the world's population (Jha et al. 2013). Two of the most prevalent causes of CKD are hypertension and diabetes. CKD severely affects patient's quality of life, their lifetime productivity and is a source of mortality due to cardiovascular events (Go et al. 2004). As diabetic and nondiabetic CKD progresses, the costs associated with patient care considerably increase too, due to the requirement of organ replacement therapies (Jha et al. 2013). Patient management includes prescribing antidiabetics, antidyslipidemics and antihypertensives. Over the last decades, the use of RAS blockers, including angiotensin-

converting enzyme inhibitors or angiotensin II type I receptor antagonists, affected only modestly CKD progression (Jha et al. 2013). Therefore, searching for new therapies and tools to assist in the early diagnosis of CKD continues to be a challenge.

Among the pathogenic mechanisms involved in CKD in diabetes, the best described mechanism is how hyperglycemia alters renal cell functions. Hyperglycemia activates several protein kinase C isoforms, a step that is required for local production of growth factors such as the transforming growth factor-β (TGF-β) and the vascular endothelial growth factor (VEGF) (Ziyadeh, 2008). Also involved in this pathology are metabolic factors, such as advanced glycosylation end products (AGE) (Forbes et al. 2003), oxygen reactive species (Ha et al. 2008) and aldose reductase/polyol pathways (Dunlop, 2000). Further CKD of diabetic and non-diabetic origin presents common alterations to renal synthesis and the activity of some hemodynamic factors such as RAS (Mezzano et al. 2003), endothelins (ETs) (Sorokin and Kohan, 2003) and a decrease in nitric oxide (NO) bioavailability (Nakagawa, 2009). Production of pro-inflammatory mediators, as well as monocytes/macrophages interstitial invasion, may also contribute to renal injury and fibrosis (Fornoni et al. 2008). Recently, the pathogenic role of adenosine signaling in mediating glomerulopathy (Quezada et al. 2013; Cárdenas et al. 2013) and renal fibrosis (Kretschmar et al. 2016; Roberts et al. 2014) has emerged as a new player contributing to CKD as discussed below.

The fibrotic process is crucial in CKD since it generates irreversible organ scaring as a result of extracellular matrix (ECM) deposition in renal structures such as the capillary glomerular wall, arterioles, mesangial and tubule-interstitial space (Duffield, 2014; Liu, 2005), leading to progressive loss of renal function and end stage renal disease (ESRD)

(Duffield, 2013). Briefly, renal fibrosis pathogenesis is commonly represented by an initial and repetitive tissue insult (toxic, metabolic, infectious, ischemic or immunologic) leading resident kidney cells, such as tubular epithelial cells and mesangial cells (Campanholle et al. 2013), to trigger an inflammatory processes, secreting a series of pro-inflammatory cytokines and chemokines such as, TNF-α, IL1-β, MCP-1, among others (Hirschberg, 2005; Meng et al. 2014). These signaling molecules induce platelet coagulation and infiltration of the immune cells such as neutrophils, dendritic cells, T lymphocytes and monocytes/macrophages in the glomeruli and interstitium (Duffield, 2013). Inflammatory cells produce harmful molecules, such as reactive oxygen species, inflammatory and fibrogenic cytokines: TGF-β, PDGF, CTGF, FGF-2, IL-13 (Bondi, 2010; Chen, 2011, Strutz et al. 2000). These factors stimulate resident cells, where part of these cells undergo apoptosis (Song, 2007) and the other portion directly or indirectly contribute to the accumulation of interstitial myofibroblasts in the interstitium, around the blood vessels or in the glomerulus (Grande et al. 2015; Humphreys et al. 2010; LeBleu et al. 2013; Lin et al. 2008; Wu et al. 2013). Myofibroblasts are defined as cells with contractile properties that expresses α-SMA, type II intermediate filaments desmin and vimentin, and secrete proteins and ECM proteoglycans (fibrillar collagen type I, III, IV, fibronectin, laminin, perlecan and heparin). Although this is a simplified view of the processes that occurs during renal fibrogenesis, several of these steps occur simultaneously and can be much more complex. However, accumulation of myofibroblasts is a key event that persistently generates an increase in ECM deposits (Duffield et al. 2013, Duffield, 2014), which finally leads to disorganization of the kidney parenchyma and irreversible loss of kidney function. In this scenario, phenotypic transformation of resident and infiltrating cells in the kidney gives a clue about the origins of myofibroblasts. Recent evidence (Humphreys et al. 2010; Lovisa

et al. 2015) showed that injured tubule epithelial cells acquire a mesenchymal-like phenotype, losing their properties to protect functional kidney parenchyma through cell cycle–dependent proliferation, dedifferentiation and repairing (Lovisa et al. 2015). Additionally, reprogramed epithelial cells acquire a profibrotic secretome that orchestrates transdifferentiation and recruitment of cells that contribute to accumulation of interstitial myofibroblasts (Grande et al. 2015; Wu et al. 2013; Lovisa et al. 2015; Grgic et al. 2012). Several sources of myofibroblasts have been identified by using cell-tracer fate *in vivo* in animal models of fibrosis. Among the identified contributors are local resident pericytes and fibroblasts (Chen et al.. 2011; Lin et al. 2008; Wu et al. 2013; Grgic et al. 2012; Smith et al. 2012), cells recruited from the bone marrow and from endothelial to mesenchymal cell transition (EndMT) (LeBleu et al. 2013) and macrophages via their transition to myofibroblasts in a process termed macrophage-myofibroblast transition (MMT) (Nikolic-Paterson et al. 2011; Wang et al. 2016). Another pathological characteristic of CKD is glomerulosclerosis. Many studies have described that mesangial cells may acquire a myofibroblast-like phenotype that generates excessive ECM accumulation and increase TIMP (tissue inhibitors of metalloproteinases) levels (Riser et al. 2000; Bollineni and Reddi, 1993; Kagami et al. 1994; Ziyadeh et al. 2000). Recent evidence shows that loss of podocytes is a feature observed in numerous studies involving patients with CKD (Kim and Cheigh, 2001; Asanuma, 2015, Reiser and Sever, 2013). Injury and consequent depletion of podocytes using transgenic mice, provoked glomerulosclerosis with evident mesangial expansion, collapse of glomerular capillaries, and decreased kidney function (Wiggins et al. 2005, Wharram et al. 2005). Finally, other authors indicated that podocyte injury led to a phenotypic change to myofibroblasts, expressing collagen1a1. This indicates that podocytes

and parietal epithelial cells also generate basal membrane thickening and capillary obstruction contributing to kidney fibrosis (Campanholle et al. 2013; Duffield, 2014).

Interestingly, the first evidence that correlates adenosine with kidney fibrosis comes from studies performed by Ratech and col. (1985) in adenosine deaminase (ADA) deficient patients. Examination of renal tissue in cases of ADA deficiency showed mesangial sclerosis. Congenital ADA deficiency generates severe combined immune deficiency (SCID) (Booth and Gaspar, 2009) which is a consequence of impeded nucleotide metabolism containing adenine as nitrogenized base. The nucleotide dATP acts as an inhibitor of overall ribonucleotide reductase activity, affecting the immune system response. Furthermore, adenosine is also increased in this pathology. Recreation of adenosine imbalance in *ada-/-* KO mice leads to renal accumulation of collagen and proteinuria that can be blocked by using an adenosine receptor antagonist (Dai et al. 2011). The involvement of altered adenosine in fibrotic processes is not restricted to the kidney. ADA deficiency leads to the spontaneous development of pulmonary (Chunn et al. 2006) and skin (Fernandez et al. 2008) fibrosis in mice. In these models, increased collagen deposition was accompanied by increased levels of key fibrosis mediators, including TGF-β, connective tissue growth factor and interleukin-13 (Ackley et al. 2003). In addition, pulmonary inflammation and fibrosis in wild-type mice subjected to bleomycin-induced lung injury also showed upregulated adenosine signaling (Sun et al. 2006). Another study showed induction of liver fibrosis in mice treated with CCl4, ethanol or TAA with extracellular adenosine levels two- to three-fold higher (Peng et al. 2008). Thus, gaining knowledge about the biochemical events that control local extracellular adenosine levels is

fundamental to the understanding of homeostatic kidney function and the pathogenic signaling pathways that mediate renal alterations.

5. Adenosine and renal pathology

5.1 Dysregulated adenosine levels

Due to several limitations when quantifying adenosine in biological samples, such as its short half-life due to widely distributed metabolizing enzymes and the necessity of complex equipment such as mass spectrometers or high resolution chromatographers, clinical research that associates dysregulated adenosine levels with diseases is scarce. However, conclusive evidence collected from patients affected by diabetic CKD exists. Xia and col showed that disparate adenosine levels and the catabolic products inosine and uric acid, in the plasma of patients with diabetic nephropathy (DN) is concurrent with disease progression; meanwhile healthy and diabetic patients without renal repercussion have adenosine levels within the basal range (Xia et al. 2009, 2010). Further, using an integrated biomarker system to find a predictor of diabetic nephropathy in humans, it was found that the plasmatic adenosine-derived metabolite inosine could be useful as a prognosis tool (Huang et al. 2013).

Additionally, several animal models have been used to correlate dysregulated adenosine homeostasis with the CKD pathogenesis. Among these, experimental diabetes induced in murines recapitulates glomerular alterations and incipient stages of fibrotic activation of cells, while animals with unilateral ureteral obstruction, hypertension or infused Ang II resemble glomerular and tubulointerstitial alterations leading to fibrosis and loss of renal function.

Studies in animal models of diabetic kidney disease reproduced the observations found in diabetic nephropathy patients. Firstly, experiments in streptozotocin-induced diabetic rats indicated that adenosine levels were significantly increased in renal vein plasma (Angielski et al. 1989). Roa et al. (2009) using this same animal model showed that isolated glomeruli from diabetic rats contained significantly higher external adenosine compared to controls (37.4 ± 3.1 v/s 6.0 ± 0.6 nM, corrected per µg of total glomeruli protein). Further, it was recently demonstrated that renal injury progression in diabetic rats develops with increased adenosine levels, having a strong correlation with the profibrotic marker α-SMA (Kretschmar et al. 2016), meanwhile increased urinary adenosine excretion could be detected during early onset of kidney dysfunction (Oyarzún et al. 2016). These observations highlight the fact that locally generated adenosine contributes to setting the pathological milieu of CKD progression. The biochemical events associated with increased local adenosine generation in diabetic kidney disease models was recently elucidated. ENT1 and ENT2 activity was examined to define the mechanisms associated with changes in adenosine levels at the glomerulus (Roa et al. 2009; Quezada et al. 2013). At this compartment, sodium-independent uptake activity was significantly decreased in diabetic rat glomeruli, in particular ENT1 activity was inhibited to 50%; these being the main engines for increasing extracellular adenosine accumulation that drives diabetic glomerulopathy (Roa et al. 2009). Recent evidence demonstrated inhibition of ENT1 activity in the proximal tubules of rat diabetic kidney (Kretschmar et al. 2016), thus probably also contributing to increased external adenosine. Indeed, in *ent1-/-* mice or animals with pharmacologically inhibited ENT activity, the levels of interstitial and plasmatic adenosine were elevated (Li et al. 2013), highlighting the critical role of ENTs in adenosine homeostasis. This is of a major relevance because histological examination of

ent1-/- mice revealed increased interstitial collagen deposition and α-SMA in the kidney (Guillén-Gómez et al. 2012; Kretschmar et al. 2016). Some studies indicated that insulin and elevated glucose levels can regulate ENT activity in some cell types (Sakowicz et al. 2004; Pawelczyk et al. 2003; Muñoz et al. 2006; Westermeier et al. 2011). There is strong evidence of the inhibitory effect of high glucose concentration on ENT1 and ENT2 activity in human endothelial cells and murine lymphocytes and podocytes (Sakowicz et al. 2004; Pawelczyk et al. 2003; Muñoz et al. 2006; Westermeier et al. 2011; Karczewska et al. 2011). Some studies have identified the effect of insulin on restoring decreased nucleoside uptake activity. Recovery of basal adenosine extracellular levels in glomeruli exposed to high glucose, which have increased nucleoside accumulation, was attained by the addition of insulin via upregulation of ENT2 activity; suggesting that this transporter is a target of insulin in the kidney (Alarcón et al. 2015). This opposing modulation of ENT activity by glucose and insulin may allow maintenance of homeostatic adenosine levels during fluctuating physiological conditions. The impact of insulin on controlling renal cell function is of great interest. In fact, mice with podocyte-targeted deletion of the insulin receptor develop significant albuminuria together with histological features that recapitulate diabetic nephropathy (Welsh, 2010). Importantly, insulin resistance and poor glycemic control is present in both type 1 and type 2 diabetic patients, being a risk factor for the development of diabetic kidney disease (Ekstrand et al. 1998; Groop et al. 1993). Mima et al. (2011) analyzed both a type 1 diabetes model, generated by STZ treatment, and a type 2 diabetes model, using the Zucker fatty strain, and observed a loss of insulin signaling in the kidney, suggesting that renal cells are susceptible to developing insulin resistance. Similar findings were described in podocytes (Tejada et al. 2008). We also recently demonstrated downregulation of the insulin receptor protein in kidney cortex from STZ induced diabetic

rats and diabetic patients (Gatica et al. 2013). Thus, lack of insulin responsiveness may permit kidney injury progression even when hormonal replacement therapy is performed. Also, this condition may affect adenosine handling by downregulating nucleoside uptake and leading to chronically increased levels of adenosine.

In regards to adenosine metabolizing enzyme activity in diabetic CKD, information is available only from experimental models. In STZ-diabetic rats slight changes in 5'-ectonucleotidase activity were observed in glomeruli (Roa et al. 2009), however a significant increase was also observed in the proximal tubules of diabetic rats (Oyarzún et al. 2015), indicating that compartmentalized extracellular adenosine generation may be differentially regulated. Interestingly, Oyarzún et al. (2015) demonstrated that AMPase activity mediated by 5'-ectonucleotidase, CD73, was increased during early diabetic renal injury. Since CD73 activity can be measured in urine, its use as a clinical tool for evaluating diabetic renal alterations affecting proximal tubules was proposed by the authors (Oyarzún et al. 2015). Studies of tubular damage biomarkers, such as KIM-I, NGAL, L-FABP and Cystatin C are inconsistent, with larger studies in humans showing no additional value to traditional prediction models (Lim, 2014). Thus CD73 would be useful as a marker of CKD progression in the clinic. Other studies on adenosine metabolizing enzymes found that the expression of ecto-adenosine kinase (adenosine → AMP) was significantly lower in diabetic rat kidney (Pawelczyk et al. 2000; Sakowicz and Pawelczyk, 2002).

Using different experimental approaches, it was suggested that increased adenosine and signaling is a common event in the pathogenesis of non-diabetic chronic kidney disease (Dai et al. 2011). Such is the case of renal fibrosis in mice generated by Ang II infusion and unilateral ureteral obstruction (UUO) (Dai et al. 2011; Lee et al. 2013). Also, Dai et al.

(2011) observed development of renal fibrosis in *ada-/-* animals. Whereas failed adenosine uptake due to insulin deficiency was observed in the diabetic model, however the mechanism leading to chronically high adenosine levels in these other CKD models is unknown. Genetic and pharmacological studies in mice revealed that CD73-mediated excess renal adenosine activates the A_{2B} receptor and signaling, contributing to Ang II-induced hypertension (Zhang et al. 2013). Also, CD73 and adenosine A_{2B} receptor levels were significantly increased in the kidneys of human CKD patients compared with normal individuals and were further elevated in hypertensive CKD (Zhang et al. 2013). Thus, altered adenosine handling by cells or extracellular generation may contribute to chronic adenosine levels in CKD. In turn, future research should focus on establishing the alterations that occur to cellular functions mediated by adenosine and its receptors.

5.2 Pathogenic functions of adenosine receptors in CKD

In addition to the physiological functions attributed to adenosine receptors described in section 3, some of these receptors are linked to both protective and deleterious effects on kidney cells and renal function. Using pharmacological approaches and genetic deletion of receptors, the involvement of adenosine in acute and CKD was identified. As depicted in Table 2, the protective role of A_1 and A_{2A} receptor subtypes signaling was recognized by using the renal ischemia and reperfusion model where neutrophils infiltration and necrosis of proximal tubules was reduced. These findings could be applied to the creating of new therapies for acute renal disease.

The involvement of adenosine receptors in CKD (see Table 2) will be discussed with an emphasis on fibrotic process. Evidence indicates that the adenosine A_{2A} receptor has a protective effect during podocyte injury, which was evaluated using toxicity assays in cells *in vitro* and by inhibiting monocyte/macrophage adhesion to endothelium in an experimental model of diabetic kidney disease (Persson et al. 2015; Awad et al. 2006, 2008). However, how this protection is overwhelmed in CKD and is poorly understood. Apparently, adenosine signaling properties may be altered by the chronic increase of ligand due to the different pharmacological characteristics of the receptors, thus effecting different cellular responses (Fredholm et al. 2011; Roberts et al. 2014). Also, adenosine receptors may change their number and distribution during CKD. Analysis of the distribution and abundance of adenosine receptors in the human kidney and changes during diabetic CKD are underway. The distribution and abundance of A_1, A_{2B} and A_3 receptors in the healthy kidney and in the kidney of diabetic nephropathy patients is shown in Figure 1. Histochemistry analysis shows changes in the expression of the adenosine receptors with the progression of the disease. There is evident loss of A_1 receptor at the glomerulus, with increased staining at the tubules. One of the most noticeable alterations in the diabetic CKD was A_{2B} receptor expression induced at the glomerular cells and increased abundance at the tubules. Interestingly, A_{2B} receptor expression in non-diabetic CKD exhibited a similar upregulated pattern (Zhang et al. 2013). For the A_3 receptor the most remarkable feature was receptor distribution at the tubules and interstitium during the advanced state of the diabetic kidney disease, which was recently recognized by Kretschmar et al. (2016). Overall, it is expected that these receptor distribution patterns contribute to their correlation with functional consequences at the cellular level. Indeed, the induction of the glomerular A_{2B} receptor can be linked to increased TGF-β release and VEGF overproduction at the

glomerulus as previously described in the pathogenesis of diabetic renal injury in rats (Valladares et al. 2008; Roa et al. 2009; Cárdenas et al. 2013). Several groups have reinforced the probable pathogenic role of the adenosine A_{2B} receptor, showing that this receptor subtype directs processes associated with CKD progression such as unbalance of the VEGF-NO axis and endothelin-1 induction in a hypoxia-inducible factor-α–dependent manner (Wilkinson et al. 2016; Patel et al. 2014; Zhang et al. 2013). Remarkably, a strong association between A_{2B} receptor and renal fibrosis has been observed. Dai et al. (2011) demonstrated that development of renal fibrosis generated in all three models, *ada-/-* mice, Ang II infused animals and ureteral unilateral obstructed (UUO) animals, can be avoided when using an A_{2B} receptor antagonist. A_{2B} receptor mediated renal fibrosis was related to interleukin-6 induction (Dai et al. 2011). Additionally, a role for the A_{2B} receptor in renal fibrosis could come from its capacity to mediate profibrotic activation of renal fibroblasts (Wilkinson et al. 2016). Cardenas et al. (2013) observed decreased induction of α-SMA in diabetic rats with pharmacological antagonism of the A_{2B} receptor, probably resulting from attenuated profibrotic activation of resident or infiltrating cells. Complementary pathogenic effects were attributed to adenosine signaling via the adenosine A_3 receptor in renal fibrosis. Signaling via A_3 receptor is involved in the transition of tubular epithelial cells to a mesenchymal-like phenotype that leads to tubulointerstitial parenchyma remodeling and extracellular matrix deposition as described in section 4 (Kretschmar et al. 2016; Lee et al. 2013). Interestingly, these studies suggest crosstalk between the A_3 receptor and TGF-β fibrotic cascade in proximal tubule cells (Kretschmar et al. 2016; Lee et al. 2013). Furthermore, TGF-β induced CD73 in these cells (Oyarzún et al. 2015) reinforcing a positive feedback mechanism for directing kidney fibrosis. In vivo antagonism of the A_3 receptor attenuated fibrosis markers in diabetic rats and fibrosis in UUO animals

(Kretschmar et al. 2016; Lee et al. 2013). Thus common mechanisms of renal fibrosis pathogenesis in diabetic and non-diabetic CKD can be interfered through adenosine receptor antagonism.

Several questions have arisen about the role of adenosine due to alterations in receptor profiles in diabetic and non-diabetic CKD. One of these refers to the mechanisms associated with A_{2B} receptor induction or A_3 receptor distribution in the kidney. Studies have demonstrated induction of A_{2B} receptor mediated by the proinflammatory mediator TNF-α and hypoxia (Kolachala et al. 2010; St Hilaire et al. 2008; Yang et al. 2010; Koeppen et al. 2011). Interestingly these two factors are associated with CKD progression (Carrero et al. 2009; Fu et al. 2016). Ang II may also mediate A_{2B} receptor induction (Dai et al. 2011). Thus a major task will be to solve the way that A_{2B} receptor, with the lowest ligand affinity, may be upregulated concurrently with increased adenosine. Another interesting aspect is the tubulointerstitial distribution of the A_3 receptor in diabetic CKD, resembling the myofibroblast pattern in renal fibrosis. Since the A_3 receptor is also expressed in bone marrow derived cells (Haskó et al. 2008), the expression pattern in diabetic CKD could represent a pool of myofibroblasts from non-resident cells. Thus, contribution of this receptor subtype to myofibroblast generation or maintenance must be solved to be able to understand adenosine signaling integrated into the fibrotic process.

6. Concluding remarks

- One of the most astounding characteristics in experimental models of chronic renal disease of diabetic and non-diabetic origin is the increase in extracellular adenosine and of its catabolic products, which indicates that the pathogenesis of these renal conditions is

from a common axis. In humans, clinical studies confirm this characteristic in patients affected by diabetic nephropathy.

- The biochemical mechanisms associated with increased adenosine levels involve both higher ectonucleotidase activity, which degrades precursor nucleotides, and lower extracellular adenosine uptake through equilibrative nucleoside transporter systems; although the series of events that underlie these alterations are unknown. In diabetes, metabolic and hormonal unbalance may influence lower ENT activity.

- Cell signaling properties are altered due to a chronic increase of adenosine. Even more, changes in the amount and distribution of the adenosine receptors in chronic renal disease of diabetic and non-diabetic origin have been described.

- The A_{2B} and A_3 adenosine receptors emerge as possible targets for pharmacological intervention due to their role in the development of renal fibrosis during chronic renal disease. However, the intervention possibilities are still limited. The development of a clinically useful A_{2B} receptor selective antagonist is underway for the treatment of asthma and pulmonary disease. In fact, CVT-6883 has progressed to phase I clinical studies in humans, however it still requires further research. In the case of A_3 receptor antagonists only preclinical studies has been performed. Some of the antagonists may be selected for clinical phase studies based on their pharmacological and chemical properties (Baraldi et al. 2012). Interestingly, continuous administration of adenosine deaminase (ADA) decreased adenosine levels and precluded CKD in some animal models. Further, ADA replacement therapy is an effective therapy for ADA deficient patients (Booth and Gaspar, 2009; Wen et

al. 2010), although the cost of this product and its limitations in immune competent patients limits its implementation in the CKD population.

- Further researches to resolve how the chronic adenosine cascade is founded to trigger pathogenic effects leading to CKD are underway. Also, validation of clinical tools that detect induction of the adenosine axis in CKD are needed. Measuring CD73 activity in the urine of at risk patients may be useful for detecting CKD, in addition to current standard procedures. This analysis will provide improved knowledge about the pathological processes that occur in human CKD and will help us observe when interception of adenosine signaling might be useful for opportune intervention.

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8. References

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Legend of the Figures

Figure 1. Adenosine metabolism and cellular effects in the kidney. A. Homeostatic extracellular adenosine levels are generated from hydrolysis of the precursor nucleotides ATP and cAMP which are released by kidney cells. ATP is metabolized to AMP by the cell surface enzyme ecto-nucleoside triphosphate diphosphohydrolase 1 (ENTPD1 or CD39) and then to adenosine by ecto-5′-nucleotidase (NT5E or CD73). Extracellular cAMP is the substrate of phosphodiesterase (PDE) and consecutively AMP is dephosphorylated by CD73 to generate adenosine. The mechanisms involved in cell-mediated release of precursor nucleotides may include nucleotide permeable channels or extrusion mediated by vesicles, but this remains to be determined in kidney compartments. Intracellularly, adenosine is produced from S-adenosylhomocysteine (SAH) and converted to AMP by adenosine kinase (AK) or metabolized by the salvage pathway (SP) of purine nucleosides and nucleobases. The Equilibrative Nucleoside Transporters (NT) balances extracellular levels of the nucleoside and adenosine in the cells. In addition NT activity may be decreased to mediate extracellular accumulation of adenosine. B. Finely regulated extracellular levels of adenosine signal through four G-protein-coupled receptors A_1, A_{2A},

A_{2B}, and A_3 in the healthy kidney to mediate physiological tasks such as tubuloglomerular feedback (TGF), renin release and ions balance. In contrast, chronic kidney disease models show persistently high levels of adenosine thus affecting cellular functions. The most remarkable consequences of unbalanced adenosine signaling in diabetic and non-diabetic CKD are glomerulopathy and renal fibrosis.

Figure 2. Distribution of adenosine receptors in human diabetic CKD. Immunohistochemical detection of A_1, A_{2B} and A_3 adenosine receptors (AR) in human kidney sections from non-diabetic normal tissue and biopsies from diabetic nephropathy patients. In the glomerular compartment there is evident induction of the A_{2B} receptor subtype in diabetic CKD. Further, A_3 receptor distribution to the tubulointerstitium was observed in advanced stages of diabetic nephropathy. The A_1 receptor subtype was decreased in glomeruli but increased in tubular epithelial cells as the disease progresses. Selected images show representative progressive stages of renal injury probed by the content of α-smooth muscle actin (α-SMA) and pathological analysis. Original magnification 200x. Scale bars 50µm.

Table 1. Adenosine receptor distribution in the kidney

Adenosine receptor subtype and localization	Specie/Cell culture	Method	References
Adenosine A_1 receptor			
Podocytes and distal convoluted tubules	Rat	Immunohistochemistry	Pawelczyk et al., 2005
Isolated kidney glomeruli and podocytes	Rat/cultured podocytes	RT-PCR	Valladares et al., 2008
Thin limbs of Henle, collecting duct system and to a lesser extent in the medullary thick ascending limb.	Mouse/rat	RT-PRC	Vitzthum et al., 2004
Microdissected afferent arteriole	Mouse	RT-PCR	Lu et al., 2015
Microdissected efferent arteriole	Mouse	RT-PCR	Al-Mashhadi et al., 2009
Juxtaglomerular cells	Primary cultures Mouse	Immunolabeling/confocal microscopy/RT-PCR	Ortiz-Capisano et., 2013
Mesangial cells	SV40 transformed mouse mesangial cell line	RT-PCR	Zhao et al., 2002
Proximal tubule cells	Human papillomavirus 16 (HPV-16) transformed HK-2 cells	Real time RT-PCR	Tang and Zhou, 2003
Adenosine A_{2A} receptor			
Glomeruli	Mouse/Rat	RT-PCR	Vitzthum et al., 2004

Conditionally immortalized podocyte cell line	Mouse	RT-PCR/ Immunocytochemistry	Awad et al., 2008
Juxtaglomerular cells	Primary cultures of isolated mouse juxtaglomerular cells.	RT-PCR	Ortiz-Capisano et al., 2013
Juxtamedullary afferent arterioles	Rat	In vitro blood-perfused juxtamedullary nephron technique combined with videomicroscopy.(Functional assay)	Feng and Navar, 2010
Microdissected efferent arterioles	Mouse	RT-PCR	Al-Mashhadi et al., 2009
Proximal tubule cells	Human papillomavirus 16 (HPV-16) transformed HK-2 cells	Real time RT-PCR	Tang and Zhou, 2003
Adenosine A_{2B} receptor			
Cortical thick ascending limb of Henle and in the distal convoluted tubule	Mouse /rat	RT-PCR	Vitzthum et al., 2004
Glomeruli and tubules	Human	Immunohistochemistry	Zhang et al., 2013
Juxtaglomerular cells	Mouse	Primary cultures of isolated mouse juxtaglomerular cells/RT-PCR	Ortiz-Capisano et al., 2013
Juxtamedullary afferent arterioles.	Rat	In vitro blood-perfused juxtamedullary nephron technique combined with videomicroscopy.	Feng and Navar, 2010
Renal inner medullary collecting duct	Immortalized cell line mIMCD-K2/murine model.	Western	Rajagopal and Pao, 2010
Isolated glomeruli and podocytes	Rat/cultured podocytes	RT-PCR/ Immunohistochemistry	Valladares et al., 2008

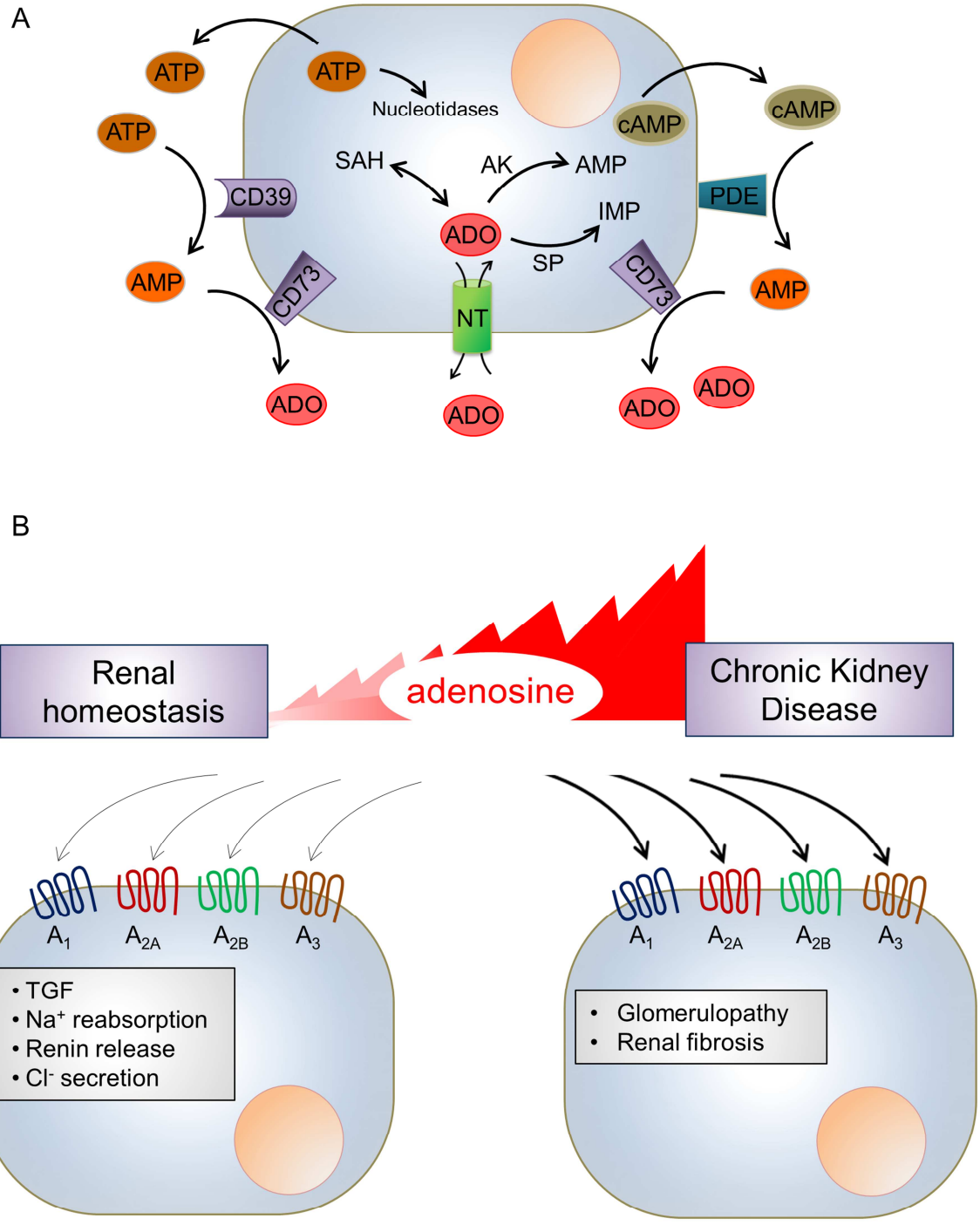
Microdissected efferent arterioles.	Mice	RT-PCR	Al-Mashhadi et al., 2009
Mesangial cells	SV40 transformed mouse mesangial cell line from adult male Wistar-Kyoto rats	RT-PCR/Inhibition assay with MRS1754	Zhao et al., 2002 Jackson et al., 2010; Jackson et al., 2011
Fibroblast	Rat cell line NRK-49F	RT-PCR	Wilkinson et al., 2016
Initial segment of the inner medullary collecting duct	Murine cell line mIMCD-K2	Western blotting	Rajagopal et al., 2010
Adenosine A_3 receptor			
Microdissected isolated afferent arteriole	Mouse	RT-PCR	Lu et al., 2015
Microdissected efferent arterioles	Mouse	RT-PCR	Al-Mashhadi et al., 2009
Mesangial cells	SV40 transformed mouse mesangial cell line	RT-PCR	Zhao et al., 2002
Glomeruli and tubules	Human	Immunohistochemistry	Kretschmar et al. 2016
Proximal tubules	Human papillomavirus 16 (HPV-16) transformed HK-2 cells and rat purified proximal tubules	Western blot	Kretschmar et al. 2016

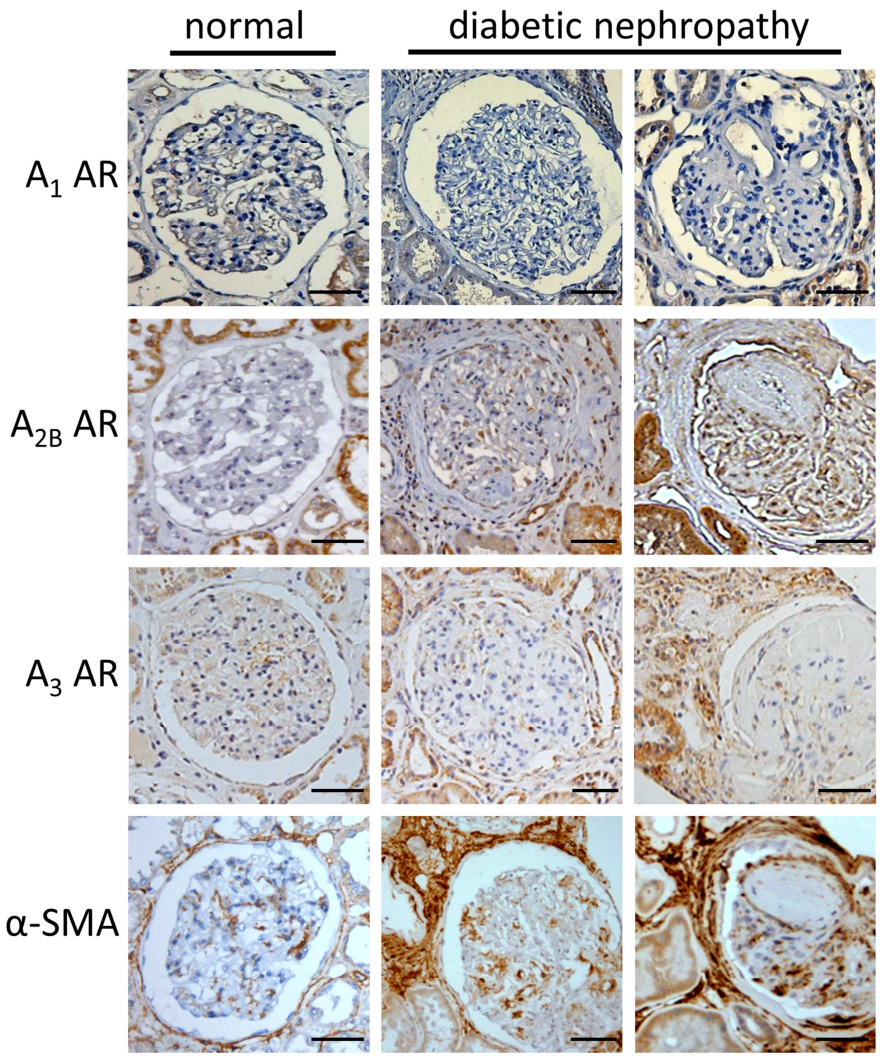
Table 2. Involvement of adenosine receptors in acute and chronic kidney diseases

Adenosine receptor	Model of kidney injury	Interventional strategy	Effect	Reference
A_1	Ischemia and reperfusion	CCPA agonist	Restores renal function, attenuates the expression of inflammation markers ICAM-1, IL-1β and TNF-α, necrosis and apoptosis	Lee et al. 2004a
	Ischemia and reperfusion	DPCPX antagonist	Increases renal dysfunction, tubular necrosis, inflammation and apoptosis	Lee et al. 2004a
	Ischemia and reperfusion	*A_1AR-/-* mice	Increases the expression of inflammation markers ICAM-1, IL-1β and TNF-α. Augments neutrophils infiltration	Lee et al. 2004b
	Ischemia and reperfusion	CCPA agonist	Reduces necrosis in proximal tubules, neutrophils infiltration, inflammation and apoptosis in IL-11 receptor KO mice	Kim et al. 2013
	Ischemia and reperfusion	DPCPX antagonist	Enhances lymphocyte infiltration and TNF-α production	Najafi et al. 2016
	Alloxan-induced diabetes	*A_1AR-/-* mice	Increases glomerular filtration rate	Sällström et al. 2007
	Diabetic nephropathy	*A_1AR-/-* mice	Increases renal injury and glomerular filtration rate	Faulhaber-Walter et al. 2008
A_{2A}	Ischemia and reperfusion	DWH146e antagonist	Inhibits inflammation, reduces renal damage	Okusa et al. 1999
	Ischemia and reperfusion	ZM241385 antagonist	Blockades the renoprotector effects of DWH146e	Okusa et al. 1999
	Ischemia and reperfusion	DWH 146e agonist	Reduces neutrophil infiltration in renal cortex and medulla. Decreases ICAM-1 expression	Okusa et al. 2000
	Diabetic nephropathy	ATL146e o ATL313 agonists	Attenuates the fibrotic marker fibronectin and reduces macrophages infiltration in glomeruli	Awad et al. 2006
	Glomerulonephritis	CGS21680 agonist	Reduces macrophages infiltration and collagen type I, II and IV deposition. Restores expression of E-cadherin and decreases the expression of α-	García et al. 2011

			SMA in interstitium and the glomerulus	
	unilateral ureteral obstruction	*A_{2A}AR-/-* mice	Increases progression of tubulointerstitial fibrosis	Xiao et al. 2013
	unilateral ureteral obstruction	CGS21680 agonist	Significantly reduces collagen deposition, TGF-β and lymphocytes T CD4+ infiltration	Xiao et al. 2013
	Diabetic nephropathy	CGS21680 agonist	Prevents glomerular damage by inhibition of the inflammatory pathway. Reverses proteinuria and decreases urinary excretion of TNF-α.	Persson et al. 2015
A_{2B}	unilateral ureteral obstruction	*A_{2B}AR-/-* mice	Attenuates IL-6 induction	Dai et al. 2011
	Diabetic nephropathy	MRS1754 antagonist	Restores nephrin and attenuates VEGF expression and α-SMA induction in glomeruli	Cárdenas el al. 2013
	Diabetic nephropathy	MRS1754 antagonist	Reduces VEGF expression and restores nitrite levels in the kidney tissue	Patel et al. 2014
	Diabetic nephropathy	*A_{2B}AR-/-* mice	Increases glomerular filtration rate and albumin excretion	Tak et al. 2014
	Cyclosporine A-induced Nephropathy	MRS1754 antagonist	Reverses the increase of VEGF induced by the non-selective agonist NECA of adenosine receptors	Patel et al. 2015
A_3	Ischemia and reperfusion	*A_3AR-/-* mice	Attenuates renal injury and restores renal function	Lee et al. 2003
	Ischemia and reperfusion	MECA agonist	Favors renal damage	Lee et al. 2003
	unilateral ureteral obstruction	LJ1888 antagonist	Inhibits the expression of fibronectin and collagen I. Reduces interstitial collagen	Lee et al. 2013
	Diabetic nephropathy	MRS1220 antagonist	Reduces α-SMA	Kretschmar et al. 2016
	Adriamycin-induced	LJ1888 antagonist	Restores nephrin and decreases collagen type IV, NF-κB, NOX4,	Min et al. 2016

	nephropathy		TLR4, TNFα, IL-1β and IFN-γ expression	


A
ATP
ATP
ATP
Nucleotidases
cAMP
cAMP
SAH
AK
AMP
CD39
PDE
IMP
ADO
SP
AMP
CD73
NT
CD73
AMP
ADO
ADO
ADO
ADO
B
Renal homeostasis
adenosine
Chronic Kidney Disease
A1
A2A
A2B
A3
• TGF
• Na+ reabsorption
• Renin release
• Cl- secretion
A1
A2A
A2B
A3
• Glomerulopathy
• Renal fibrosis


normal
diabetic nephropathy
A1 AR
A2B AR
A3 AR
α-SMA