Published in final edited form as:

Acta Physiol (Oxf). 2015 January ; 213(1): 242–248. doi:10.1111/apha.12413.

Adenosine, type 1 receptors: role in proximal tubule Na⁺ reabsorption

William J Welch, Ph.D.

Georgetown University, 4000 Reservoir Road, Building D-395, Washington, DC, 20007, USA

William J Welch: welchw@georgetown.edu

Abstract

Adenosine type 1 receptor (A₁-AR) antagonists induce diuresis and natriuresis in experimental animals and humans. Much of this effect is due to inhibition of A₁-ARs in the proximal tubule, which is responsible for 60–70% of the reabsorption of filtered Na⁺ and fluid. Intratubular application of receptor antagonists indicates that A₁-AR mediates a portion of Na⁺ uptake in PT and PT cells, via multiple transport systems, including Na⁺/H⁺ exchanger-3 (NHE3), Na⁺/PO₄³⁻ co-transporter and Na⁺-dependent glucose transporter, SGLT. Renal microperfusion and recollection studies have shown that fluid reabsorption is reduced by A₁-AR antagonists and is lower in A₁-AR KO mice., compared to WT mice. Absolute proximal reabsorption (APR) measured by free-flow micropuncture is equivocal, with studies that show either lower APR or similar APR in A₁-AR KO mice, compared to WT mice. Inhibition of A₁-ARs lowers elevated blood pressure in models of salt-sensitive hypertension, partially due to their effects in the proximal tubule.

Introduction

Adenosine activates 4 distinct receptors that mediate a variety of functional responses in several tissues, including neural, vascular and epithelial cells. In the kidney, adenosine targets primarily resistance vessels, which can alter renal blood flow and may be part of its regulation. However, adenosine can also promote Na⁺ transport in the nephron through activation of adenosine type 1 receptors (A₁-AR) and possibly type 2 receptors (A₂-AR). Caffeine and theophylline, components of coffee and tea respectively are methylxanthine compounds and act as non-selective adenosine receptor antagonists. Both can induce diuresis and natriuresis. These effects have recently been attributed to their action on adenosine type 1 receptors (A₁-AR), though they are not selective for A₁-AR. More information on the sites and diuretic actions has been derived from the use of a large family of recently formulated xanthine and non-xanthine antagonists, with a large range of specificity. Despite progress, there is less agreement on the nephron site(s) of action. Adenosine receptors are located along the nephron, yet the specific role(s) of the various receptors have not been well described. Here I will review the evidence that adenosine, produced in the proximal tubule acts locally and perhaps downstream to enhance Na⁺ uptake and contributes to the kidney's overall ability to maintain fluid and electrolyte balance.

Diuretic and natriuretic actions of caffeine and A₁-AR antagonists

Though there are not large, well-controlled studies, several observations support the concept that that caffeine and to a lesser extent, theophylline induces diuresis and natriuresis in humans (Jackson 2001), (Nussberger et al. 1990). Most retrospective studies have shown only modest differences in fluid volume and conclude that normal consumption of caffeine and theophylline does not alter fluid balance regulation (Reviewed in Maughan and Griffin (Killer et al. 2014, Maughan and Griffin 2003). However in studies in which subjects have avoided recent caffeine intake, single moderate-to-large doses induced short-term diuresis and natriuresis (Neuhauser et al. 1997, Rachima-Maoz et al. 1998) (Shirley et al. 2002). There were no health risks associated with these effects. Shirley et al (Shirley et al. 2002) showed that the caffeine diuresis they observed in healthy males also lowered PT reabsorption, measured by lithium clearance, suggesting the diuresis was partially due to changes in the proximal tubule. Similarly, caffeine in energy drinks induced a short-term diuresis when compared with other energy drinks not containing caffeine (Riesenhuber et al. 2006). However the diuretic actions of caffeine are more clearly shown in well-controlled animal studies. Rieg et al (Rieg et al. 2005) in an extensive series of experiments showed that acute caffeine led to a 25–30% increase in urine flow and Na⁺ excretion in wild type mice. The effects of caffeine were absent in adenosine, type 1-receptor KO mice.

More studies have focused on the effects of A₁-AR antagonists, which have shown promise as potassium-sparing diuretics, since a major target of these drug is the proximal tubule (PT, as discussed later). Infusion of A₁-AR antagonists in anesthetized rats (Barrett and Wright 1994), (Collis et al. 1991), (Gellai et al. 1998), (Knight et al. 1993), (Kost et al. 2000), (Pfister et al. 1997), (Wilcox et al. 1999), (Yao et al. 1994), anesthetized dogs (Aki et al. 1997), anesthetized pigs (Lucas et al. 2002) and conscious dogs (Kobayashi et al. 1993) induced powerful diuresis and natriuresis. Several studies have treated human subjects and patients in clinical trials to evaluate the diuretic and natriuretic effects of several A₁-AR antagonists. In two groups of patients with diminished renal function (1: GFR > or = 71 ml/min or 2: GFR 30–71 ml/min), FK453, a non-xanthine A₁-AR antagonist, increased urine flow and Na⁺ excretion (Balakrishnan et al. 1996). Other studies have been limited to acute iv injection due to the lack of orally active formulations. A₁-AR antagonists have been used in models of congestive heart failure in dogs and pigs to induce diuresis. Acute treatment with the A₁-AR antagonist BG9719 increased urine flow and Na⁺ excretion in dogs, but also increased GFR (Lucas et al. 2002). The authors suggested that A₁-AR blockade of tubuloglomerular feedback might have contributed to the observed diuresis. In a subsequent study, this group observed that GFR returned to normal after 30 minutes and that during these later periods, the diuresis and natriuresis persisted (Lucas et al. 2002). In the initial clinical trials with BG9719, this compound enhanced the diuretic effect of furosemide, yet preserved GFR and avoided K⁺ losses (Gottlieb et al. 2000), (Gottlieb et al. 2002). Both of these results point to a renal tubular effect, but do not provide direct evidence of a proximal tubular effect. A₁-AR receptor antagonists also induced diuresis and natriuresis in other non-CHF related edematous patients (Stanley et al. 1998), (Lochan et al. 1998). The early positive results with A₁-AR antagonists led to Phase III clinical trials. The A₁-AR antagonist induced natriuresis and diuresis in heart failure (HF) patients in a Phase 1

trial without an effect on creatinine clearance (Ponikowski et al. 2010). These results were the basis of a Phase III clinical trial, PROTECT tested in acute HF patients. In 2033 AHF patients there were no significant differences in renal function (creatinine clearance) after 14 days and renal function decline in these patients treated with rolofylline or placebo (Voors et al. 2011). Subsequent follow-up in this patients showed slight benefits linked to fluid balance over 180 days (Cleland et al. 2014). In a separate analysis (Cleland et al. 2014, Givertz et al. 2014), rolofylline led to reduced post-hospitalization fluid balance and renal function events, related to improved renal function. The exact benefit and any changes in proximal tubular function were not determined. At present there is no clear clinical application for this class of drugs on renal function and fluid balance.

Nephron sites of adenosine receptor expression

Adenosine receptors and their genes are expressed in nearly all segments of the nephron (Palacios et al. 1987, Smith et al. 2001) (Smith et al. 1999, Yamaguchi et al. 1995). Protein expression of A₁, A_{2a} and A_{2b} have been described in the thick limb of the loop of Henle (TALH) and collecting duct. (Smith et al. 1999). Other studies have found little expression of A₁-AR in the proximal tubule, but mRNA for A₁-AR was identified (Vitzthum et al. 2004). We measured both protein and mRNA expression of A₁-AR in microdissected rat proximal tubules (Heyne et al. 2004) and showed different levels of salt intake affected the quantity of expression (Figure 1). Other studies that have tested functional responses to various agonists or antagonists measured A₁-AR expression in cultured PT cells (Pingle et al. 2004), baby hamster kidney cells (Mittal et al. 1999), A6 distal nephron cells (Ma and Ling 1996), MDCK cells (Saunders et al. 1996) and human kidney cells (Lee and Emala 2002). A_{2b} receptors are expressed in IMCD cells (Rajagopal and Pao 2010) and in isolated glomeruli (Valladares et al. 2008).

Functional evidence for proximal tubule actions of adenosine receptors

In vitro

Often in the absence of confirmation of protein expression, functional A₁-ARs have been demonstrated in isolated PTs or PT cells. In one of the first functional studies, Takeda et al showed that A₁-AR blockers reduced bicarbonate uptake in isolated rabbit PT segments (Takeda et al. 1993). Since bicarbonate is linked to active Na⁺ uptake in this segment, the authors concluded that the actions of the blocker impacted Na⁺ transport. In cultured PT cells, two different A₁-AR antagonists inhibited Na⁺ uptake (Cai et al. 1994). These authors showed that this effect was primarily mediated by phosphate-dependent Na⁺ uptake and not by glucose-dependent uptake. Na⁺-phosphate transport was also stimulated by A₁-AR agonists in human kidney cells (HK), (Tang and Zhou 2003). However, a separate study showed that glucose-dependent transport was blocked by the A₁-AR antagonist cyclopentyltheophylline (CPT) (Coulson et al. 1996) in PT cells.

We have shown that a portion of ²²Na uptake in response to either adenosine antagonists or agonists in cultured PT cells is linked to NHE3 activation (personal communication). A₁-R antagonists inhibited Na⁺ uptake in A6 cells (Ma and Ling 1996), and when blockers were exposed to the apical membranes they prevented Na⁺ uptake, which was specifically

dependent on amiloride-sensitive Na⁺ channels (Hayslett et al. 1995, Macala and Hayslett 2002)

In vivo

Mizumoto and Karasawa (Mizumoto and Karasawa 1993) used KW-3902 in anesthetized rats to demonstrate a powerful diuretic effect. They showed most of the effect of this drug was in the proximal tubule by measuring greater clearance of Na (CNa) than clearance of exogenous lithium (CLi), which is reabsorbed primarily in the PT. In two subsequent micropuncture studies we showed that A₁-AR blockade had effects in the PT. Systemic infusion of BG9719 increased UNaV and UV and lowered absolute (26.1±3.2 to 20.4±2.0 nl/min, p<0.05) and fractional (60±3 to 46±4%, p<0.05 proximal reabsorption (APR) in anesthetized rats (Wilcox et al. 1999). In a follow-up study we (Kulick et al. 2008) microperfused the A₁-AR antagonist into the PT of rats maintained on 3 levels of salt intake and recollected fluid downstream to assess fluid uptake over the surface S-2 segment of the PT. Since only the S-2 segment is accessible for microperfusion studies, data collected at this site have limitations, since most of Na⁺ reabsorption occurs in the S-1 segment. The A₁-AR antagonist directly lowered fluid reabsorption (Jv), regardless of salt intake, although its greatest effect was in animals maintained on low salt (LS) intake. (Figure 1). We measured greater expression of A₁-ARs in microdissected PT in LS rats compared to those maintained on normal salt and high salt, suggesting that low salt stimulated A₁-AR expression and enhanced reabsorption. We subsequently microperfused adenosine deaminase (ADA) into the PT in these groups. ADA metabolizes and therefore inactivates adenosine and therefore reduced Jv similarly in all groups. We concluded that A₁-AR antagonists inhibited the action of locally PT generated adenosine. Figure 2 shows the effects of the A₁-AR antagonist, BG9719 and adenosine deaminase (ADA) perfused directly into the PT, with recollection of fluid downstream. This shows that under these conditions inhibition of both adenosine and A₁-ARs reduces Jv in the PT.

Most previous studies cited above, used systemically administered A₁-AR antagonists and showed diuretic effects, yet did not specifically identify the source of adenosine. Filtered adenosine, reported in the 100–200 pmol range (Jackson 2001) might act in the PT to facilitate Na⁺ uptake. Heyne et al (Heyne et al. 2004) determined that urinary excretion of adenosine in humans was approximately 50% of the filtered adenosine. Other studies that suggest urinary adenosine is partially derived from extracellular cAMP (Jackson et al. 2007), which presumably could also be filtered. In addition to binding to various adenosine receptors along the nephron, adenosine is also reabsorbed by the nephron, especially in the proximal tubule. This occurs by cellular uptake by apical concentrating nucleoside transporters (CNT), which are sodium dependent and transport to the interstitium by basolateral equilibrating nucleoside transporters (ENT) (Elwi et al. 2009). The appearance of adenosine in urine suggests that the nephron apparently does not recover all of the filtered adenosine or that there is production and release of adenosine along the nephron. However, there is no direct evidence or clear mechanism for the addition of adenosine into the lumen. Our studies were the first to suggest that locally produced adenosine acts on A₁-ARs to promote reabsorption in the PT. Indeed, the possible production of adenosine may have effects even further downstream than the PT. Beach and Good (Beach and Good 1992)

showed that adenosine or an A₁-AR agonist reduced transport (measured by changes in JHCO₃⁻) in isolated segments of the TALH. This was reversed by an A₁-AR antagonist, suggesting opposite effect of A₁AR in the PT versus the TALH. Vallon et al (Vallon et al. 2006) provides a comprehensive review of adenosine effects in other segments of the nephron.

In a subsequent study we showed that absolute and fractional proximal reabsorption were lower in the A₁-AR KO mice compared to WT (Barrett and Wright 1994). We also microperfused the PT over a physiological range of 2–10 µl/min and found that fluid reabsorption (J_v) was lower in the KO mice at each perfusion rate (Bell et al. 2010). Further, we concluded that the reduced APR contributed to the more rapid elimination of a moderate saline-volume load in A₁-AR KO mice compared to WT. In addition, the proximal tubule regulatory mechanism, glomerular-tubular balance (GTB) was impaired in A₁-AR KO mice, and this defect perhaps also contributed to the more rapid excretion of the saline-volume load. However, in separate micropuncture studies **Vallon** et al (Vallon et al. 2004) failed to see any differences in fluid or Na⁺ or fluid delivery at the end of the proximal tubule.

Consequences of proximal tubule adenosine receptor activity

Fluid balance

Evidence cited above suggests that adenosine receptors contribute to regulation of Na⁺ and fluid balance, primarily in the proximal tubule, but perhaps also in the distal nephron by promoting a portion of Na⁺ uptake in these segments. Therefore activation of adenosine receptors under normal conditions may participate in excess Na⁺ and fluid retention under fluid challenges, such as high salt intake, hypertension and elevated angiotensin II. Therefore targeting these receptors may be therapeutic. A₁-AR antagonists have in fact been used to correct edematous states such as congestive heart failure (CHF), ascites and excess fluid used in treating infants with extracorporeal membrane oxygenation with varying success (Gottlieb et al. 2002, Gottlieb et al. 2000, Lochan et al. 1998, Stanley et al. 1998). Clinical trials to develop this family of drugs have not yet been successful. Experimentally, we showed that an acute saline volume load was excreted more rapidly in A₁-AR KO mice (Bell et al. 2010), suggesting that A₁-ARs participate in reabsorption of excess fluid and electrolytes in the PT under normal conditions.

Blood Pressure regulation

Due to the powerful diuretic and natriuretic effects of A₁-AR antagonism, targeting this receptor in models of hypertension, especially salt and volume-dependent forms of hypertension have been tested. Salt-sensitive hypertension is often the result of excess Na⁺ and fluid retention. The effects of 3 related studies are shown in Figure 3. One of the earliest studies showed that A₁-AR antagonist prevented full blood pressure response to high salt intake in Dahl salt-sensitive rats (Nomura et al. 1995). Na⁺ and fluid excretions were higher in A₁-AR antagonist-treated rats. Further, lithium clearance was higher in treated rats, suggesting that the effects of the antagonists were partially mediated in the PT. More recently we showed that BP increases to Ang II infusion for 2 weeks were abrogated in A₁-AR KO mice (Lee et al. 2012). There were no differences in GFR in these mice, yet A₁-AR

KO mice excreted more Na⁺, phosphate and fluid over the first week of Ang II infusion, suggesting the mechanism to lower BP was linked to less Na⁺ retention in these mice, possibly due to inhibition of Na-phosphate transport. NaPi-2, the major Na⁺-PO₄ transporter in the proximal tubule was suppressed by 3-fold in A₁-AR KO mice compared to WT mice, treated with Ang II. This study concluded that A₁-AR mediated Na⁺ uptake in the PT was linked to NaPi-2 expression and activity. We have also shown that DOCA-salt loading, a more distinct form of SS hypertension was less effective in A₁-AR KO mice (personal communication, Figure 3). The A₁-AR mice had lower absolute proximal reabsorption (APR) before treatment, compared to WT mice, but APR was reduced to similar levels in both strains following 3 weeks of DOCA-salt treatment. We concluded that absence of A₁-ARs in more distal nephron segments might have prevented the excess Na⁺ and fluid retention in this model seen in WT mice.

Summary

I have summarized substantial evidence that A₁-ARs are involved in Na⁺ and fluid retention and that much of this is due to receptors in the proximal tubule. Drugs targeting A₁-ARs induce diuresis and natriuresis in experimental animals and humans. Direct perfusion of the PT with A₁-ARs antagonists and adenosine deaminase inhibits uptake in experimental settings. Proximal reabsorption is reduced in A₁-AR KO mice. Salt-sensitive hypertension and Ang II infused hypertension are less effective in mice deficient of A₁-ARs. A₁-ARs in the nephron, as well as other subtypes of adenosine receptors contribute to Na⁺ and fluid uptake; thus should be considered as part of fluid and Na⁺ balance regulation.

References

- Aki Y, Tomohiro A, Nishiyama A, Kiyomoto K, Kimura S, Abe Y. Effects of KW-3902, a selective and potent adenosine A₁ receptor antagonist, on renal hemodynamics and urine formation in anesthetized dogs. *Pharmacology*. 1997; 55:193–201. [PubMed: 9396079]
- Balakrishnan VS, Coles GA, Williams JD. Functional role of endogenous adenosine in human chronic renal disease. *Exp Nephrol*. 1996; 4:26–36. [PubMed: 8788597]
- Barrett RJ, Wright KF. A selective adenosine A₁ receptor antagonist attenuates renal dysfunction during controlled hypotension with adenosine in rats. *Anesth Analg*. 1994; 79:460–5. [PubMed: 8067549]
- Beach RE, Good DW. Effects of adenosine on ion transport in rat medullary thick ascending limb. *Am J Physiol*. 1992; 32:F482–F487. [PubMed: 1415577]
- Bell TD, Luo Z, Welch WJ. Glomerular tubular balance is suppressed in adenosine type 1 receptor-deficient mice. *Am J Physiol Renal Physiol*. 2010; 299:F1158–63. [PubMed: 20810615]
- Cai H, Batuman V, Puschett DB, Puschett JB. Effect of KW-3902, a novel adenosine A₁ receptor antagonist, on sodium-dependent phosphate and glucose transport by the rat renal proximal tubular cell. *Life Sci*. 1994; 55:839–845. [PubMed: 8072380]
- Cleland JG, Chiswell K, Teerlink JR, Stevens S, Fiuzat M, Givertz MM, Davison BA, Mansoor GA, Ponikowski P, Voors AA, Cotter G, Metra M, Massie BM, O'Connor CM. Predictors of postdischarge outcomes from information acquired shortly after admission for acute heart failure: a report from the Placebo-Controlled Randomized Study of the Selective A₁ Adenosine Receptor Antagonist Rolofylline for Patients Hospitalized With Acute Decompensated Heart Failure and Volume Overload to Assess Treatment Effect on Congestion and Renal Function (PROTECT) Study. *Circ Heart Fail*. 2014; 7:76–87. [PubMed: 24281134]

- Collis MG, Shaw G, Keddie JR. Diuretic and saluretic effects of 1,3-dipropyl-8-cyclopentylxanthine, a selective A1-adenosine receptor antagonist. *J Pharm Pharmacol.* 1991; 43:138–9. [PubMed: 1672902]
- Coulson R, Proch PS, Olsson RA, Chalfant CE, Cooper DR. Upregulated renal adenosine A1 receptors augment PKC and glucose transport but inhibit proliferation. *Am J Physiol.* 1996; 270:F263–74. [PubMed: 8779886]
- Elwi AN, Damaraju VL, Kuzma ML, Mowles DA, Baldwin SA, Young JD, Sawyer MB, Cass CE. Transepithelial fluxes of adenosine and 2'-deoxyadenosine across human renal proximal tubule cells: roles of nucleoside transporters hENT1, hENT2, and hCNT3. *Am J Physiol Renal Physiol.* 2009; 296:F1439–51. [PubMed: 19297449]
- Gellai M, Schreiner GF, Ruffolo RR Jr, Fletcher T, DeWolf R, Brooks DP. CVT-124, a novel adenosine A1 receptor antagonist with unique diuretic activity. *J Pharm Exp Therapeu.* 1998; 286:1191–1196.
- Givertz MM, Postmus D, Hillege HL, Mansoor GA, Massie BM, Davison BA, Ponikowski P, Metra M, Teerlink JR, Cleland JG, Dittrich HC, O'Connor CM, Cotter G, Voors AA. Renal function trajectories and clinical outcomes in acute heart failure. *Circ Heart Fail.* 2014; 7:59–67. [PubMed: 24281137]
- Gottlieb SS, Brater DC, Thomas I, Havranek E, Bourge R, Goldman S, Dyer F, Gomez M, Bennett D, Ticho B, Beckman E, Abraham WT. BG9719 (CVT-124), an A1 adenosine receptor antagonist, protects against the decline in renal function observed with diuretic therapy. *Circulation.* 2002; 105:1348–1353. [PubMed: 11901047]
- Gottlieb SS, Skettino SL, Wolff A, Beckman E, Fisher ML, Freudenberger R, Gladwell T, Marshall J, Cines M, Bennett D, Liittschwager EB. Effects of BG9719 (CVT-124), an A1-adenosine receptor antagonist, and furosemide on glomerular filtration rate and natriuresis in patients with congestive heart failure. *J Am Coll Cardiol.* 2000; 35:56–9. [PubMed: 10636259]
- Hayslett JP, Macala LJ, Smallwood JJ, Kalghatgi L, Gasalla-Herraiz J, Isaacs C. Adenosine stimulation of Na⁺ transport is mediated by an A1 receptor and a [Ca²⁺]_i-dependent mechanism. *Kidney Int.* 1995; 47:1576–84. [PubMed: 7643526]
- Heyne N, Benohr P, Muhlbauer B, Delabar U, Risler T, Osswald H. Regulation of renal adenosine excretion in humans--role of sodium and fluid homeostasis. *Nephrol Dial Transplant.* 2004; 19:2737–41. [PubMed: 15385638]
- Jackson EK, Mi Z, Dubey RK. The extracellular cAMP-adenosine pathway significantly contributes to the in vivo production of adenosine. *J Pharmacol Exp Ther.* 2007; 320:117–23. [PubMed: 17028245]
- Killer SC, Blannin AK, Jeukendrup AE. No evidence of dehydration with moderate daily coffee intake: a counterbalanced cross-over study in a free-living population. *PLoS One.* 2014; 9:e84154. [PubMed: 24416202]
- Knight RJ, Bowmer CJ, Yates MS. The diuretic action of 8-cyclopentyl-1,3-dipropylxanthine, a selective A1 adenosine receptor antagonist. *Br J Pharmacol.* 1993; 109:271–7. [PubMed: 8495243]
- Kobayashi T, Mizumoto H, Karasawa A. Diuretic effects of KW-3902 (8-(noradamantan-3-yl)-1,3-dipropylxanthine), a novel adenosine A1 receptor antagonist, in conscious dogs. *Biol Pharm Bull.* 1993; 16:1231–5. [PubMed: 8130772]
- Kost CK, Herzer WA, Rominski BR, Mi Z, Jackson EK. Diuretic response to adenosine A₁ receptor blockade in normotensive and spontaneously hypertensive rats: role of pertussis toxin-sensitive G-proteins. *J Pharmacol Exp Ther.* 2000; 292:752–760. [PubMed: 10640315]
- Kulick A, Panico C, Gill P, Welch WJ. Low salt intake increases adenosine type 1 receptor expression and function in the rat proximal tubule. *Am J Physiol Renal Physiol.* 2008; 295:F37–41. [PubMed: 18480183]
- Lee DL, Bell TD, Bhupatkar J, Solis G, Welch WJ. Adenosine A1-receptor knockout mice have a decreased blood pressure response to low-dose ANG II infusion. *Am J Physiol Regul Integr Comp Physiol.* 2012; 303:R683–8. [PubMed: 22874421]
- Lee HT, Emala CW. Characterization of adenosine receptors in human kidney proximal tubule (HK-2) cells. *Exp Nephrol.* 2002; 10:383–92. [PubMed: 12381923]

- Lochan SR, Adeniyi-Jones S, Assadi FK, Frey BM, Marcus S, Baumgart S. Coadministration of theophylline enhances diuretic response to furosemide in infants during extracorporeal membrane oxygenation: a randomized controlled pilot study. *J Pediatr*. 1998; 133:86–9. [PubMed: 9672516]
- Lucas DG, Hendrick JW, Sample JA, Mukherjee R, Escobar GP, Smits GJ, Crawford FA, Spinale FG. Cardiorenal effects of adenosine subtype 1 (A1) receptor inhibition in an experimental model of heart failure. *J Am Coll Surg*. 2002; 194:603–609. [PubMed: 12022600]
- Ma H, Ling BN. Luminal adenosine receptors regulate amiloride-sensitive Na⁺ channels in A6 distal nephron cells. *Am J Physiol*. 1996; 270:F798–805. [PubMed: 8928841]
- Macala LJ, Hayslett JP. Basolateral and apical A1 adenosine receptors mediate sodium transport in cultured renal epithelial (A6) cells. *Am J Physiol Renal Physiol*. 2002; 283:F1216–25. [PubMed: 12388417]
- Maughan RJ, Griffin J. Caffeine ingestion and fluid balance: a review. *J Hum Nutr Diet*. 2003; 16:411–20. [PubMed: 19774754]
- Mittal RA, Tan CH, Khoo HE. Evidence for A1 and A2B adenosine receptors in baby hamster kidney (BHK) cells. *Biofactors*. 1999; 10:25–33. [PubMed: 10475587]
- Mizumoto H, Karasawa A. Renal tubular site of action of KW-3902, a novel adenosine A1-receptor antagonist, in anesthetized rats. *Jpn J Pharmacol*. 1993; 61:251–3. [PubMed: 8483302]
- Neuhauser B, Beine S, Verwied SC, Luhrmann PM. Coffee consumption and total body water homeostasis as measured by fluid balance and bioelectrical impedance analysis. *Ann Nutr Metab*. 1997; 41:29–36. [PubMed: 9194998]
- Nomura H, Nagashima K, Kusaka H, Karasawa A. Antihypertensive effects of KW-3902, an adenosine A1-receptor antagonist, in Dahl salt-sensitive rats. *Jpn J Pharmacol*. 1995; 68:389–96. [PubMed: 8531413]
- Nussberger J, Mooser V, Maridor G, Juillerat L, Waeber B, Brunner HR. Caffeine-induced diuresis and atrial natriuretic peptides. *J Cardiovasc Pharmacol*. 1990; 15:685–91. [PubMed: 1692926]
- Palacios JM, Fastbom J, Wiederhold KH, Probst A. Visualization of adenosine A₁ receptors in the human and the guinea-pig kidney. *Euro J Pharmacol*. 1987; 138:273–276.
- Pfister JR, Belardinelli L, Lee G, Lum RT, Milner P, Stanley WC, Linden J, Baker SP, Schreiner G. Synthesis and biological evaluation of the enantiomers of the potent and selective A₁-adenosine antagonist 1,3-dipropyl-8-[2-(5,6-epoxy)norbonyl]xanthine. *J Med Chem*. 1997; 40:1773–1778. [PubMed: 9191953]
- Pingle SC, Mishra S, Marcuzzi A, Bhat SG, Sekino Y, Rybak LP, Ramkumar V. Osmotic diuretics induce adenosine A1 receptor expression and protect renal proximal tubular epithelial cells against cisplatin-mediated apoptosis. *J Biol Chem*. 2004; 279:43157–67. [PubMed: 15272017]
- Ponikowski P, Mitrovic V, O'Connor CM, Dittrich H, Cotter G, Massie BM, Givertz MM, Chen E, Murray M, Weatherley BD, Fujita KP, Metra M. Haemodynamic effects of rolofylline in the treatment of patients with heart failure and impaired renal function. *Eur J Heart Fail*. 2010; 12:1238–46. [PubMed: 20823097]
- Rachima-Maoz C, Peleg E, Rosenthal T. The effect of caffeine on ambulatory blood pressure in hypertensive patients. *Am J Hypertens*. 1998; 11:1426–32. [PubMed: 9880124]
- Rajagopal M, Pao AC. Adenosine activates a2b receptors and enhances chloride secretion in kidney inner medullary collecting duct cells. *Hypertension*. 2010; 55:1123–8. [PubMed: 20308611]
- Rieg T, Steigle H, Schnermann J, Richter K, Osswald H, Vallon V. Requirement of intact adenosine A1 receptors for the diuretic and natriuretic action of the methylxanthines theophylline and caffeine. *J Pharmacol Exp Ther*. 2005; 313:403–409. [PubMed: 15590766]
- Riesenhuber A, Boehm M, Posch M, Aufricht C. Diuretic potential of energy drinks. *Amino Acids*. 2006; 31:81–3. [PubMed: 16847703]
- Saunders C, Keefer JR, Kennedy AP, Wells JN, Limbird LE. Receptors coupled to pertussis toxin-sensitive G-proteins traffic to opposite surfaces in Madin-Darby canine kidney cells. A1 adenosine receptors achieve apical and alpha 2A adrenergic receptors achieve basolateral localization. *J Biol Chem*. 1996; 271:995–1002. [PubMed: 8557716]
- Shirley DG, Walter SJ, Noormohamed FH. Natriuretic effect of caffeine: assessment of segmental sodium reabsorption in humans. *Clin Sci (Lond)*. 2002; 103:461–6. [PubMed: 12401118]

- Smith JA, Sivaprasadarao A, Munsey TS, Bowmer CJ, Yates MS. Immunolocalisation of adenosine A(1) receptors in the rat kidney. *Biochem Pharmacol.* 2001; 61:237–44. [PubMed: 11163338]
- Smith JA, Whitaker EM, Yaktubay N, Morton MJ, Bowmer CJ, Yates MS. Regulation of renal adenosine A(1) receptors: effect of dietary sodium chloride. *Eur J Pharmacol.* 1999; 384:71–9. [PubMed: 10611422]
- Stanley AJ, Forrest EH, Dabos K, Bouchier IA, Hayes PC. Natriuretic effect of an adenosine-1 receptor antagonist in cirrhotic patients with ascites. *Gastroenterology.* 1998; 115:406–11. [PubMed: 9679046]
- Takeda M, Yoshitomi K, Imai M. Regulation of Na(+)-3HCO₃⁻ cotransport in rabbit proximal convoluted tubule via adenosine A1 receptor. *Am J Physiol.* 1993; 265:F511–9. [PubMed: 8238380]
- Tang Y, Zhou L. Characterization of adenosine A1 receptors in human proximal tubule epithelial (HK-2) cells. *Receptors Channels.* 2003; 9:67–75. [PubMed: 12916467]
- Valladares D, Quezada C, Montecinos P, Concha II, Yanez AJ, Sobrevia L, San Martin R. Adenosine A(2B) receptor mediates an increase on VEGF-A production in rat kidney glomeruli. *Biochem Biophys Res Commun.* 2008; 366:180–5. [PubMed: 18060864]
- Vallon V, Muhlbauer B, Osswald H. Adenosine and kidney function. *Physiol Rev.* 2006; 86:901–40. [PubMed: 16816141]
- Vallon V, Richter K, Huang DY, Rieg T, Schnermann J. Functional consequences at the single-nephron level of the lack of adenosine A1 receptors and tubuloglomerular feedback in mice. *Pflugers Arch.* 2004; 448:214–221. [PubMed: 14767772]
- Vitzthum H, Weiss B, Bachleitner W, Kramer BK, Kurtz A. Gene expression of adenosine receptors along the nephron. *Kidney Int.* 2004; 65:1180–90. [PubMed: 15086457]
- Voors AA, Dittrich HC, Massie BM, DeLuca P, Mansoor GA, Metra M, Cotter G, Weatherley BD, Ponikowski P, Teerlink JR, Cleland JG, O'Connor CM, Givertz MM. Effects of the adenosine A1 receptor antagonist rolofylline on renal function in patients with acute heart failure and renal dysfunction: results from PROTECT (Placebo-Controlled Randomized Study of the Selective Adenosine A1 Receptor Antagonist RoloFylline for Patients Hospitalized with Acute Decompensated Heart Failure and Volume Overload to Assess Treatment Effect on Congestion and Renal Function). *J Am Coll Cardiol.* 2011; 57:1899–907. [PubMed: 21545947]
- Wilcox CS, Welch WJ, Schreiner GF, Belardinelli L. Natriuretic and diuretic actions of a highly selective adenosine A₁ receptor antagonist. *J Am Soc Nephrol.* 1999; 10:714–720. [PubMed: 10203354]
- Yamaguchi S, Umemura S, Tamura K, Iwamoto T, Nyui N, Ishigami T, Ishii M. Adenosine A1 receptor mRNA in microdissected rat nephron segments. *Hypertension.* 1995; 26:1181–5. [PubMed: 7498992]
- Yao K, Kusaka H, Sano J, Sato K, Karasawa A. Diuretic effects of KW-3902, a novel adenosine A₁-receptor antagonist, in various models of acute renal failure in rats. *Jpn J Pharmacol.* 1994; 64:281–288. [PubMed: 8057529]

Figure 1. Protein and mRNA expression in microdissected rat proximal tubules. Expression was higher after short-term treatment with low salt (LS) intake. (From Kulick et al, 2008)

Figure 2. Proximal tubule fluid reabsorption (J_v) in *in vivo* microperfused and recollected S-2 segments in rats; J_v was reduced by the A₁-AR antagonist BG9719 and by adenosine deaminase (AD). Time controls with vehicle had no effect on J_v . Data calculated from Kulick et al (2008)

Figure 3.

Inhibition or deletion of A1-AR prevented increased blood pressure in three experimental models of hypertension: Dahl salt-sensitive (DSS) rats (Nomura et al); Ang II infused WT and A1-AR KO mice (Lee et al); DOCA-salt treated WT and A1-AR KO mice (personal communication, adult male mice, n=6–8, 3 weeks after treatment).