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Review Article

A_1 receptor antagonists as diuretic/natriuretic agents

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CONTENTS

Abstract

Diuretics effectively relieve clinical symptoms and signs in patients with heart failure; however, diuretic resistance and intolerance and diuretic-induced potassium imbalances diminish the efficacy and safety of conventional diuretics. A_1 receptor antagonists prevent the ability of endogenous adenosine to enhance renal epithelial transport. A_1 receptor blockers also uncouple proximal tubular reabsorption from tubuloglomerular feedback. Preclinical and clinical studies demonstrate that in animals and humans with and without left ventricular dysfunction, A_1 receptor antagonists enhance sodium, but not potassium, excretion, increase glomerular filtration rate and attenuate the decline in glomerular filtration induced by loop diuretics. A_1 receptor antagonists also lessen acute renal failure induced by nephrotoxins, and adenosine receptor antagonists that block both A_1 and A_{2B} receptors may emerge as an important new class of "renal friendly" diuretics.

Introduction: the need for new diuretics

Role of diuretics in heart failure

Left ventricular dysfunction is an increasingly prevalent clinical condition (1) and the primary goals of treatment of heart failure are to relieve symptoms and prolong survival. Of the drugs currently available for heart failure, diuretics are therapeutically superior in their efficacy in relieving clinical symptoms and signs (2). When administered either i.v. or p.o., diuretics result in a substantial decrease in the elevated pulmonary vascular pressures. Moreover, diuretics reduce symptoms of breathlessness and signs of peripheral edema in patients with congestive heart failure in direct proportion to the degree of diuresis. In a very recent meta-analysis of randomized controlled trials, Faris *et al.* (3) report that diuretics in heart failure not only reduce the risk of worsening disease and improve exercise capacity, but also reduce the risk of death. In short, in many patients, diuretics substantially improve the patient's quality of life, economic capacity and survival. Not only do diuretics afford significant clinical improvements, diuretics are the most cost-effective treatment of any single drug class currently available for the treatment of patients with congestive heart failure. Thus, diuretic therapy is firmly established as the principle therapeutic modality for the treatment of edema, congestion and fluid overload in patients with heart failure (2). Indeed, nearly all heart failure patients will ultimately require diuretic therapy. Unfortunately, as heart failure progresses, diuretic resistance and intolerance becomes a major limiting factor to successful diuretic therapy in patients with severe heart failure (2, 4).

Diuretic resistance

Diuretic resistance is the inability of diuretics to sufficiently resolve edema because of adaptations that counteract the effectiveness of diuretics (5). In this regard, recent experimental work has revealed several ways in which kidneys adjust to chronic diuretic treatment. First, nephron segments "downstream" from the main

site-of-action of the diuretic compensate by increasing their ability to reabsorb sodium (5). Second, depletion of extracellular fluid volume triggers the release of circulating and local factors that increase sodium reabsorption (5). Third, the renal tubules undergo structural and functional changes that reduce their ability to excrete sodium and respond to diuretics (5). All of these adaptations increase the rate of sodium reabsorption and blunt the effectiveness of diuretic therapy. Experimental results indicate that addition of a second drug may act synergistically because the second diuretic inhibits the processes limiting the effectiveness of the first diuretic (6). Based on an appreciation of the mechanisms of diuretic resistance, new treatment modalities can be designed to block specific adaptive processes and improve overall diuretic effectiveness.

Diuretic intolerance

Poor renal function is an independent risk factor for mortality in patients with heart failure (7). In this regard, mortality risk approximately doubles when glomerular filtration rate (GFR) declines by one half (7). Patients hospitalized for heart failure are at high risk of worsening renal function (8), and patients with left ventricular dysfunction are sensitive to the adverse renal effects of diuretics (9). Angiotensin converting enzyme inhibitors also may augment diuretic intolerance in the setting of congestive heart failure (10). Studies by Krumholz *et al.* (8) demonstrate that worsening renal function is associated with a prolonged duration of hospitalization, higher in-hospital costs and an increased risk of in-hospital mortality. Importantly, in advanced heart failure, intravenous diuretic therapy sufficient to cause a weight loss of 2 kg or more is associated with worsening renal function in 21% of patients. In such patients, duration of hospitalization is increased from a median of 9-17 days and mortality is increased (relative risk = 5.2) (8). Clearly diuretics that improve rather than diminish renal function are badly needed. For reasons described below, selective A_1 receptors antagonists are being developed to overcome diuretic resistance and intolerance in patients with heart failure.

Renal pharmacology of A_1 receptors

Subtypes of adenosine receptors

There are 4 distinct subtypes of adenosine receptors, A_1, A_{2A}, A_{2B} and A_3. All of the adenosine receptor subtypes belong to the superfamily of G-protein coupled receptors and have 7 putative transmembrane spanning domains (for review see 11). Although the focus of this review is the A_1 receptor, for comparison, Table I summarizes the structure, genes, signal transduction

Table I: Characteristics of human adenosine receptors.

A_1 Receptor	A_{2A} Receptor	A_{2B} Receptor	A_3 Receptor
Receptor structure			
Heptahelical G-protein coupled receptor	Heptahelical G-protein coupled receptor	Heptahelical G-protein coupled receptor	Heptahelical G-protein coupled receptor
326 amino acids	412 amino acids	332 amino acids	318 amino acids
Mass = 36.5 kDa	Mass = 44.7 kDa	Mass = 36.3 kDa	Mass = 36.2 kDa
Gene structure			
Gene location is 1q32.1	Gene location is 22q11.2	Gene location is 17p11.2	Gene locations is 1p13.3
1 intron in gene	1 intron in gene	1 intron in gene	1 intron in gene
Signal transduction mechanisms			
Signals via G_i, G_o	Signals via G_s, G_{olf}, $p21^{ras}$	Signals via G_s, G_q	Signals via G_i, G_q
↑AC	↑AC	↑AC	↓AC
↓PLC		↑PLC	↑PLC
↑K^+			
↓Ca^{2+}			
Selective agonists			
CPA	CGS-21680	No selective	IB-MECA
CCPA	DPMA	agonists	CL-IB-MECA
Selective antagonists			
DPCPX	SCH-58261		MRS-1191
BG-9719	ZM-241385	MRS-1754	MRS-1220

AC, adenylyl cyclase; PLC, phospholipase C, K^+, potassium channels; Ca^{2+}, calcium channels; CPA, N^6-cyclopentyladenosine; CCPA, 2-chloro-*N*^{6}-cyclopentyladenosine; CGS-21680, 2-[*p*-(carboxyethyl)phenylethylamino]-5'-*N*-ethylcarboxamidoadenosine; DPMA, *N*-[2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)ethyl]adenosine; IB-MECA, 1-[6-[[3-iodophenyl)methyl]amino]-9*H*-purin-9-yl]-1-deoxy-*N*-methyl-β-D-ribofuranuronamide; CL-IB-MECA, 1-[2-chloro-6-[[3-iodophenyl)methyl]amino]-9*H*-purin-9-yl]-1-deoxy-*N*-methyl-β-D-ribofuranuronamide; DPCPX, 1,3-dipropyl-8-cyclopentylxanthine; BG-9719, (*S*)-1,3-dipropyl-8-[2-(5,6-epoxynorbornyl)xanthine; SCH-58261, 5-amino-7-(2-phenylethyl)-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine; ZM-241385, 4-(2-[7-amino-2-(2-furyl)[1,2,4]-triazolo-[2,3-*a*][1,3,5]triazin-5-ylamino]ethyl)phenol; MRS-1754, *N*-(4-cyanophenyl)-2-[4-(2,6-dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-phenoxy] acetamide; MRS-1191, 1,4-dihydro-2-methyl-6-phenyl-4-(pheynlethylnyl)-3,5-pyridinedicarboxylic acid, 3-ethyl 5-(phenylmethyl) ester; MRS 1220, *N*-[9-chloro-2-(2-furanyl)[1,2,4]triazolo[1,5-*c*]quinazolin-5-yl]benzeneacetamide; ↑, increases; ↓, decreases.

Table II: Adenosine A_1 receptor expression in the kidney.

Citation	Technique	Species	Renal tissue	Findings
Freissmuth *et al.,* 1987 (12)	Receptor binding using HPIA	Rabbit	Cortical glomeruli and microvessels	Detection of A_1 receptor binding
Weber *et al*., 1998 (13)	Autoradiography using HPIA	Guinea pig	Renal medulla	Detection of A_1 receptor binding
Weaver and Reppert 1992 (14)	*In situ* hybridization	Rat	Kidney	Detection of A_1 receptor mRNA in collecting ducts and juxtaglomerular apparatus
Toya *et al*., 1993 (15)	Receptor-binding using CCPA	Human	Glomeruli	Detection of A_1 receptor binding
Yamaguchi *et al*., 1995 (16)	RT-PCR	Rat	Micro-dissected nephron segments	Detection of A_1 receptor mRNA in glomeruli, collecting duct, thick ascending limb and proximal tubules
Kreisberg *et al*., 1997 (17)	RT-PCR	Rat	Micro-dissected outer medullary descending vasa recta	Detection of A_1 receptor mRNA
Gould *et al*., 1997 (18)	Receptor binding using DPCPX and CGS-21680	Rat	Kidney membranes	Detection of A_1 receptor binding
	Northern blots		Kidney	Detection of A_1 receptor mRNA
Smith *et al*., 1999 (19)	Ligand binding using DPCPX	Rat	Kidney membranes	Detection of A_1 receptor binding
	Autoradiography using DPCPX		Kidney	Detection of A_1 receptor binding in inner medullary collecting ducts
	Northern blots		Kidney	Detection of A_1 receptor mRNA
Halimi *et al*., 1999 (20)	RT-PCR	Rat	Kidney	Detection of A_1 receptor mRNA
Zou *et al*., 1999 (21)	Western blotting	Rat	Kidney cortical and medullary membranes	Detection of A_1 receptors
Smith *et al*., 2000 (22)	Autoradiography using DPCPX	Rat	Kidney	Detection of A_1 receptor binding in glomeruli and medulla
Smith *et al*., 2001 (23)	Immunocytochemistry	Rat	Kidney	Detection of A_1 receptors in afferent arterioles, mesangial cells, proximal tubules and collecting ducts
Jackson *et al*., 2002 (24)	Western blotting	Rat	Isolated preglomerular microvessels	Detection of A_1 receptors

mechanisms, selective agonists and selective antagonists for all 4 adenosine receptor subtypes.

Distribution of A_1 receptors within the kidney

Using a variety of techniques including radioligand binding, RT-PCR, Northern blotting, Western blotting, autoradiography, immunocytochemistry and *in situ* hybridization, investigators report the detection of A_1 receptor mRNA, protein and binding in the kidneys from rats, rabbits, guinea pigs and humans (Table II). In the kidneys, A_1 receptors are strongly expressed in the preglomerular microcirculation (24), glomeruli (12, 15, 22), juxtaglomerular apparatus (14) and collecting tubules (14, 16, 19, 23). Surprisingly, it is difficult to detect A_1 receptors in the proximal tubule, a major site of action of adenosine on renal epithelial transport (see below). However, RT-PCR detects A_1 receptor mRNA expression in the proximal tubule, thick ascending limb (16) and the outer medullary descending vasa recta (17).

Role in the regulation of preglomerular vascular resistance

A_1 receptor stimulation causes vasoconstriction in both superficial and deep nephrons (25), and most of the

increase in vascular resistance mediated by A_1 receptors is in the preglomerular microvessels (26-28); however, vasoconstriction of the outer medullary descending vasa recta contributes to the decrease in blood flow of deeper nephrons (29). Importantly, A_1 receptor-mediated preglomerular vasoconstriction is modulated by nitric oxide and angiotensin II. Nitric oxide reduces A_1 receptor-mediated preglomerular vasoconstriction (30), and angiotensin II enhances A_1 receptor-mediated preglomerular vasoconstriction (31-37). It is important to note that in heart failure, nitric oxide-mediated renal vasodilation is impaired (38), the renin-angiotensin system is activated (39) and circulating levels of adenosine are increased (40). Moreover, oxidative stress is increased in heart failure (41), and oxidative stress increases renal adenosine levels by activating 5'-nucleotidase (42). Therefore, in heart failure, the A_1 receptor may importantly contribute to renal vascular resistance, and this is in part the rationale for using A_1 receptor antagonists as "renal friendly" diuretics in heart failure patients.

Role in tubuloglomerular feedback

When single nephron glomerular filtration rate (SNGFR) is greater than the reabsorptive capacity of the proximal tubule, the concentration of NaCl bathing the macula densa increases and the macula densa releases a chemical signal to the afferent arteriole of that nephron causing the afferent arteriole to constrict. Constriction of the afferent arteriole decreases glomerular capillary pressure, single nephron blood flow and SNGFR, thus completing a negative feedback mechanism termed tubuloglomerular feedback (TGF).

Osswald and coworkers propose (43, 44) that TGF is mediated as follows: increased NaCl deliver to the macula densa → accelerated NaCl reabsorption by the macula densa → enhanced energy utilization and ATP breakdown in the macula densa → augmented rate of adenosine production from AMP in the macula densa → diffusion of adenosine from the macula densa to the nearby afferent arteriole → afferent arteriolar vasoconstriction mediated by A_1 receptor activation.

In support of the aforementioned hypothesis, studies show that increasing renal energy demand by the kidney increases adenosine, while diminishing ATP, levels in the kidney (43). Moreover, hypertonic saline stimulates adenosine release from mouse thick ascending limbs (45), and a high sodium diet increases renal interstitial levels of adenosine by approximately 18-fold (46) and increases total tissue adenosine levels in the renal cortex and medulla by approximately 2-fold (21). The hypothesis that adenosine mediates TGF is supported further by the observations that nonselective adenosine receptor antagonists, such as theophylline (43) (Fig. 1) and DPSPX (47) (Fig. 1), as well as selective A_1 receptor antagonists, such as DPCPX (48) (Fig. 1) and FK-838 (49) (Fig. 1), block TGF, as does exogenous adenosine deaminase, the enzyme that metabolizes adenosine to inosine (43). The reduction in renal blood flow induced by intrarenal infusions of hypertonic saline, a model of TGF, is blocked by adenosine receptor antagonists (34, 50, 51). Also, inhibition of adenosine transport with dipyridamole or inhibition of adenosine deaminase with erythro-9-(2-hydroxy-3-nonyl)adenine augments TGF (52), and intraluminal (47) or peritubular (48) infusions of selective A_1 agonists decrease glomerular hydrostatic pressure. Finally, recent studies demonstrate that TGF responses are completely absent in mice lacking A_1 receptors (53, 54).

An alternative, but not mutually exclusive, hypothesis to explain the mechanism of TGF posits that enhanced transport of NaCl in the macula densa causes depolarization of the basolateral membrane of the macula densa cell leading to release of ATP through an ATP channel (55). This alternative hypothesis further proposes that ATP is metabolized extracellularly to adenosine by the sequential actions of ecto-ATPase, ecto-ADPase and ecto-5′-nucleotidase and that adenosine then mediates TGF via A_1 receptors (55). Indeed, studies by Nishiyama and colleagues demonstrate that renal interstitial levels of ATP change as predicted by this hypothesis (56), and studies by Thomson and coworkers demonstrate that ecto-5′-nucleotidase is essential for a full TGF response (57). Regardless of how the macula densa generates extracellular adenosine, it is primarily the A_1 receptor that ultimately mediates the majority of TGF response, a conclusion with important implications for the use of A_1 receptor antagonists as diuretic/natriuretic agents (see below).

Role in the regulation of tubular transport

A_1 receptors augment transport in proximal tubular epithelial cells. For example, in cultured OK cells which have a proximal phenotype, activation of A_1 receptors enhanced both Na^+-glucose symport and Na^+-phosphate symport (58), and in microperfused proximal convoluted tubules stimulation of A_1 receptors increased basolateral Na^+-$3HCO_3$- symport (59). KW-3902 (Fig. 1), an A_1 receptor selective antagonist, decreased sodium-dependent phosphate transport in renal proximal tubular cells via increasing cyclic AMP (60, 61).

Numerous *in vivo* studies describe the effects of adenosine receptor agonists on renal excretory function. In this regard, i.v. infusions of either nonselective or selective A_1 agonists decreased urine volume and sodium excretion (62-67). For example, in humans, i.v. infusions of adenosine reduced the renal excretion of sodium, lithium, phosphate, uric acid, chloride and urea (68). In animals, infusions of nonselective A_1 receptor agonists either into the renal artery or in the suprarenal aorta usually (26, 31, 69-73) reduced sodium excretion and urine volume. The rat is an exception since infusions of adenosine or selective A_1 receptor agonists increased rather than decreased sodium excretion and urine volume (74-76). This is probably because in the rat renal levels of adenosine saturate renal epithelial A_1 receptors so that administration of exogenous adenosine receptor agonists

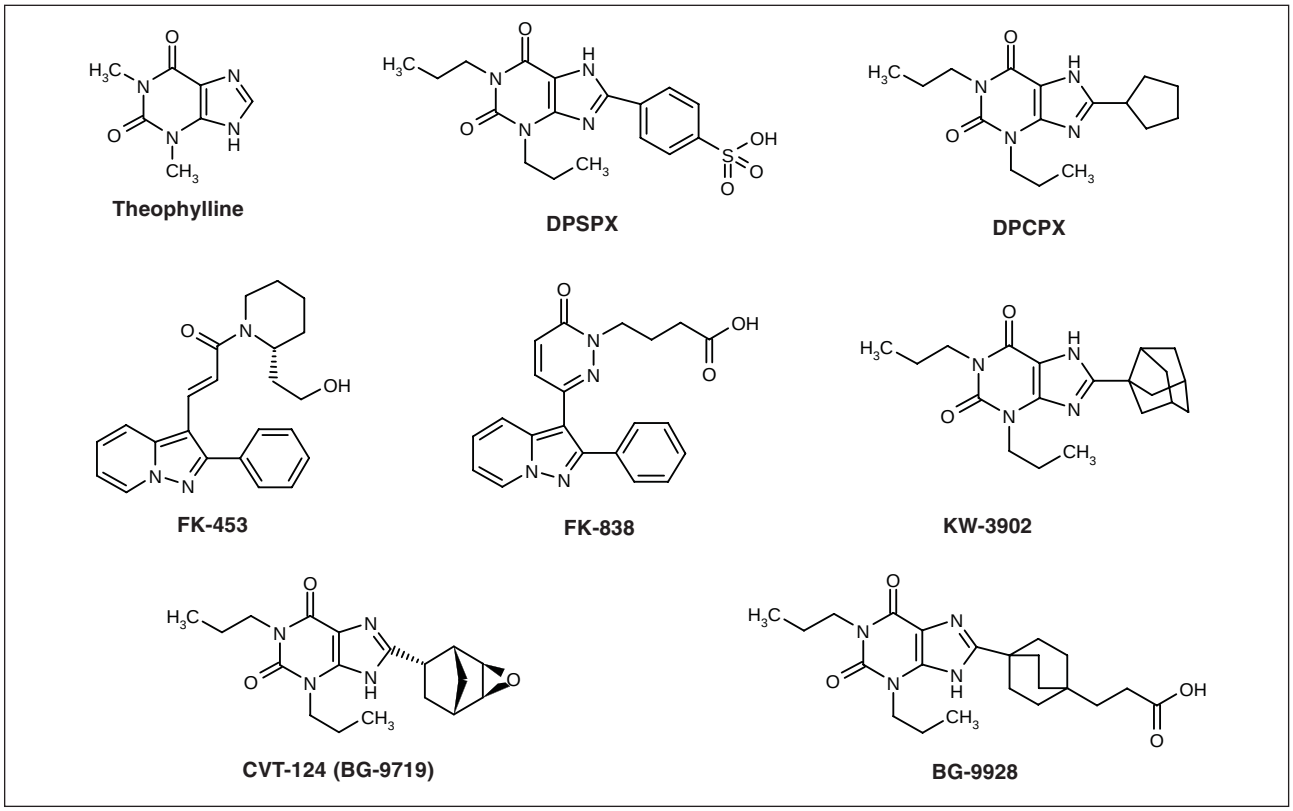

Fig. 1.

cannot further increase epithelial transport.

Diuretic/natriuretic effects of A_1 receptor antagonists

Preclinical studies in animals without left ventricular dysfunction

Selective blockade of renal A_1 receptors rapidly (within min) and markedly (3- to 10-fold) increases urinary sodium excretion in normal animals without significantly altering potassium excretion, systemic hemodynamics or renal blood flow. Most studies do not detect a change in GFR induced by selective blockade of renal A_1 receptors in normal animals; however, Wilcox and coworkers reported a significant increase in GFR induced by CVT-124 (BG-9719) (Fig. 1) (77). Treatment of rats with either DPCPX (78,79) or 8-(dicyclopropylmethyl)-1,3-dipropylxanthine (80), both highly selective A_1 receptor antagonists, increased sodium, but not potassium, excretion. DPCPX and FK-453 (Fig. 1), structurally dissimilar and highly potent and selective A_1 antagonists, stimulated sodium, but not potassium, excretion and increased urine volume in rats (81). Importantly, the diuretic/natriuretic effects of DPCPX versus FK-453 are of a similar magnitude when these agents were administered at doses that cause an equivalent degree of blockade of A_1 receptors (81). Moreover, FR-113452, the enantiomer of FK-453 which has little affinity for A_1 receptors, did not change sodium excretion or urine volume (81) when administered at a dose matching a diuretic/natriuretic dose of FK-453 (81). The head-to-head comparison of DPCPX, FK-435 and FR-113452 virtually clinches the conclusion that A_1 receptor blockade increases sodium excretion and urine volume (81). In addition, Gellai *et al.* (82) report that CVT-124 (BG-9719) (Fig. 1), one of the most selective and potent A_1 receptor antagonists yet discovered, caused diuresis and natriuresis, without affecting potassium excretion, in conscious rats. The maximum diuretic/natriuretic effect of CVT-124 was twice that of hydrochlorothiazide, and CVT-124 augmented the diuretic/natriuretic response to furosemide, yet did not potentiate furosemide-induced potassium excretion. CVT-124 also increased sodium but not potassium, excretion and urine volume in sodium-loaded animals (83), and racemic 1,3-dipropyl-8-[2-(5,6-epoxynorbornyl)] xanthine, like CVT-124, was also diuretic/natriuretic (84). A second generation molecule, BG-9928 (Fig. 1), has similar pharmacologic properties to CVT-124 (BG-9719), but has increased affinity for A_1 receptors, and is orally available, more stable and more soluble (85). The binding affinity (K_i) for the human A_1 receptor is 7.4 nM. The compound is orally active, with a dose of 0.3 mg/kg p.o. achieving full

natriuretic response in rats and with i.v. doses as low as 0.03 mg/kg showing activity in nonhuman primates. The renal protective effects of BG-9928 are demonstrated by coadministration with the loop diuretic furosemide. In rats, administration of BG-9928 effectively attenuated the reductions in GFR that usually occur with the administration of furosemide. In dogs, direct intrarenal infusions of KW-3902, another potent and selective A_1 receptor antagonist, increased urine flow and sodium, but not potassium excretion (86).

Clinical studies in humans without left ventricular dysfunction

The effects of selective A_1 receptor antagonists in humans are similar to the effects of this class of drugs in animals. Balakrishnan *et al.* (87) report that in healthy, male subjects oral administration of FK-453 slightly increased GFR, did not change blood pressure or renal blood flow, did not alter the excretion of glucose or amino acids, but caused a marked increase in urine flow rate, osmolar clearance and absolute and fractional excretions of sodium, phosphate, bicarbonate, chloride, magnesium and uric acid.

In patients with essential hypertension, FK-453 caused a 3-fold increase in sodium excretion within 1 h of administration without affecting renal blood flow or GFR (88). The increase in sodium excretion induced by FK-453 was accompanied by increases in the fractional excretion of lithium, phosphate and uric acid and by an enhanced urinary excretion of calcium and magnesium. In hypertensive patients, FK-453 decreased arterial blood pressure, but not until several hours after administration of FK-453 when the natriuresis was complete. With sustained administration, blood pressure returned to normal.

In patients with mild to moderate renal impairment, FK-453 did not alter arterial blood pressure, renal blood flow or GFR, but significantly increased urine volume, osmolar clearance and absolute and fractional excretion of sodium, phosphate, bicarbonate, lithium, uric acid, magnesium and chloride (89).

Studies in healthy subjects with single oral capsules of BG-9928 in doses ranging over 1800-fold indicate that the pharmacokinetic and pharmacodynamic properties of BG-9928 are consistent with once a day dosing (90). In this regard, a dose-response is observed for natriuresis, whereas in healthy subjects BG-9928 did not affect potassium excretion, creatinine clearance, heart rate, blood pressure or ECG. Single oral doses of BG-9928 are generally safe and well tolerated. Phase II studies are underway with this compound.

Preclinical studies in animals with left ventricular dysfunction

As described above, there is a significant medical need for diuretic/natriuretic drugs that do not reduce renal function in patients with left ventricular dysfunction. It is conceivable that A_1 receptor antagonists might be useful in this regard; however, until recently, very little was known regarding the effects of A_1 antagonism on the kidneys and cardiovascular system in the setting of left ventricular dysfunction. In a recent publication, we report the effects of A_1 receptor antagonism with BG-9719 on heart performance and renal hemodynamics and excretory function in aged, lean SHHF/Mcc-fa^{cp} rats (91). Lean SHHF/Mcc-fa^{cp} rats spontaneously develop dilated cardiomyopathy and cardiac hypertrophy is observable as early as 3 months of age and is fully developed by 6 months of age (92, 93). Lean male SHHF/Mcc-fa^{cp} expire of congestive heart failure at between 15 and 18 months of age, and the occurrence of heart failure is associated with increased arterial blood pressure (92, 93) in a range similar to that observed in the spontaneously hypertensive rat.

Furosemide is a loop diuretic that is frequently used chronically and in high doses in patients with congestive heart failure. An inappropriately high dietary salt ingestion is also commonly observed in heart failure patients and importantly contributes to diuretic resistance (6). Moreover, a high NaCl intake markedly increases renal adenosine levels (21, 46), a phenomenon that could influence the diuretic/natriuretic efficacy of A_1 antagonists. Accordingly, in our study, we describe the effects of BG-9719 in 13-month-old SHHF/Mcc-fa^{cp} rats pretreated 72, 48 and 24 h before the experiments with a high dose of furosemide and provided with 1% NaCl as drinking water to mimic the clinical situation of an inappropriate dietary salt intake in the presence of a loop diuretic.

Our study indicates that in SHHF/Mcc-fa^{cp} rats pretreated as described above, acute administration of either BG-9719 or furosemide dramatically (> 10-fold) increases urinary sodium excretion. Even more noteworthy are the observations that BG-9719 enhanced renal blood flow and GFR, but did not affect fractional potassium excretion, whereas furosemide reduced renal blood flow and GFR and enhanced the fractional excretion of potassium. Although neither BG-9719 nor furosemide altered afterload or left ventricular systolic function, furosemide but not BG-9719, significantly reduced both preload and left ventricular diastolic performance. These results indicate that A_1 receptor antagonists may have unique pharmacological properties in left ventricular dysfunction to enhance renal blood flow and GFR while preserving left ventricular diastolic function. This is in stark contrast to the effects of furosemide which decreased renal blood flow and GFR and compromised left ventricular diastolic performance.

Lucas *et al.* report the effects of 0.1 mg/kg BG-9719 (94) and 1 mg/kg BG-9719 (95) on systemic hemodynamics and indices of renal function in pigs with pacing-induced congestive heart failure. In pigs paced for 3 weeks at 240 beats/min, 0.1 mg/kg BG-9719 increased urine output by 2-fold and sodium excretion by 3-fold; whereas, 1 mg/kg BG-9719 increased urine output by 6-fold and sodium excretion by 10-fold. Both doses of

BG-9719 markedly increased creatinine clearance and 1 mg/kg BG-9719 reduced pulmonary vascular resistance by 38%. Thus, as in SHHF/Mcc-fa^{cp} rats, these studies in pacing-induced congestive heart failure in pigs indicate that the diuresis/natriuresis associated with A_1 receptor blockade is associated with an improvement in renal function.

Clinical studies in humans with left ventricular dysfunction

Gottlieb and coworkers (96) report that in a small group of patients with congestive heart failure, a single dose of BG-9719 increased sodium excretion rate approximately 5-fold without significantly altering renal blood flow or GFR, whereas furosemide markedly reduced GFR from 84 to 63 ml/min/1.73 m^2. In a larger group of heart failure patients, Gottlieb *et al.* (97) found that BG-9719 alone caused an increase in urine output and sodium excretion and improved GFR. Furosemide administration resulted in a reduction in GFR. BG-9719 plus furosemide blocked the furosemide-induced decline in GFR and had an additive effect on sodium excretion, when compared to furosemide alone.

Sites and mechanisms of diuretic/natriuretic action

In epithelial cells with a proximal tubular phenotype, activation of A_1 receptors enhanced apical Na^+-glucose symport and Na^+-phosphate symport (58), and in microperfused proximal convoluted tubules stimulation of A_1 receptors increased basolateral Na^+-$3HCO_3$- symport (59). Blockade of endogenous adenosine/A_1 receptor interactions would therefore be expected to inhibit reabsorption in the proximal tubule. In support of this conclusion, A_1 receptor antagonists increase lithium and phosphate clearances (87, 89), findings that strongly support the proximal tubule as the dominant diuretic site of action of A_1 receptor antagonists.

The ability of A_1 receptor antagonists to inhibit transport in the proximal tubule is most likely due to reversal of G_i protein-mediated inhibition of adenylyl cyclase. The evidence for this conclusion is: 1) A_1 receptors are coupled to G_i proteins and inhibit adenylyl cyclase (11); 2) cyclic AMP inhibits apical H^+-Na^+ antiport and basolateral Na^+-$3HCO_3$- symport in the proximal tubule (98); 3) the diuretic/natriuretic effects of A_1 receptor antagonists are abolished in animals pretreated with pertussis toxin (99), a toxin that ADP ribosylates and inactivates G_i proteins.

Although the proximal tubule is a major diuretic site of action of A_1 receptor antagonists, A_1 receptors are more highly expressed in the collecting duct compared with the proximal tubule (16, 23). Indeed, our work demonstrates (24) that A_1 receptors are much more highly expressed in the renal medulla –the site of the medullary collecting duct– compared with the renal cortex –the site of the proximal tubule. This relatively rich expression of A_1 receptors in the collecting duct suggests that A_1 receptor antagonists may exert a diuretic action at this site as well as in the proximal tubule. In support of this hypothesis, A_1 receptor antagonists do not increase potassium excretion despite the fact that sodium excretion is increased. Since all classes of diuretics that act proximal to the collecting duct increase potassium excretion (100), the fact that A_1 receptor antagonists do not increase potassium excretion, combined with the high level of expression of A_1 receptors in the collecting duct (16, 23), suggests that the collecting duct is a secondary site of action of A_1 receptor antagonists. However, the biochemical mechanisms by which A_1 receptor antagonists inhibit transport in the collecting duct and prevent the expected enhanced potassium excretion are presently unclear.

The afferent arteriole represents yet a third diuretic site of action. Normally, blockade of reabsorption in the proximal tubule triggers a TGF response due to increased delivery of NaCl to the macula densa (101). Activation of TGF decreases GFR and renal blood flow and thereby reduces the excretory function of the kidneys. Since adenosine mediates TGF by activating A_1 receptors in the preglomerular microcirculation (see above), blockade of A_1 receptors would prevent TGF-induced reductions in sodium excretion. Indeed, Wilcox *et al.* (77) report that CVT-124 (BG-9719) uncoupled proximal tubular reabsorption from SNGFR, and our recent studies demonstrated that the preglomerular microcirculation was relatively enriched in A_1 receptors (24). As mentioned earlier, the ability of A_1 receptor antagonists to block TGF in part explains the “renal friendly” pharmacology of this class of diuretics.

Renal indications for A_1 receptor antagonists

Congestive heart failure

Diuretics contribute importantly to the health and well-being of patients with congestive heart failure (see above). However, diuretic resistance and diuretic intolerance limit the benefits of currently available diuretic drugs (see above). By providing an alternative mechanism of action, A_1 receptor antagonists when combined with loop or thiazide diuretics, may diminish diuretic resistance without enhancing potassium loss. Equally important, by blocking A_1 receptors in the preglomerular microcirculation, A_1 receptor antagonists may improve GFR and prevent reductions in GFR frequently caused by loop diuretics.

Radiocontrast media-induced nephropathy

Administration of radiocontrast media often induces a decrease in GFR that can cause in susceptible patients acute renal failure (ARF) (102-104). This phenomenon is most likely mediated by activation of TGF secondary to increased delivery of NaCl to the macula densa. Since A_1

receptors mediate TGF (see above), A_1 receptor antagonists may be useful to prevent radiocontrast media-induced nephropathy.

In support of this conclusion, Arend *et al.* (105) and Deray *et al.* (106) reported that theophylline, an adenosine receptor antagonist, inhibited radiocontrast-induced decreases in GFR in dogs, and Erley *et al.* (107), Katholi *et al.* (108) and Huber *et al.* (109) reported that theophylline attenuated radiocontrast-induced changes in GFR in patients. Studies by Erley *et al.* (110, 111), Yao *et al.* (112) and Oldroyd *et al.* (113) demonstrate that selective blockade of A_1 receptors with either DPCPX or KW-3902 prevented radiocontrast-induced reductions GFR in rat kidneys. In dogs KW-3902 prevented radiocontrast-induced reductions in GFR (114, 115).

Acute renal failure

Endogenous adenosine/A_1 receptor interactions may contribute to acute renal failure (ARF). In this regard, the nonselective antagonists aminophylline (116), theophylline (117) and 8-phenyl theophylline (118) and several highly A_1 receptor selective antagonists such as DPCPX (119, 120), 8-(dicyclopropylmethyl)-1,3-dipropylxanthine (80), FK-453 (121, 122) and KW-3902 (123) reduced glycerol-induced ARF. Aminophylline (124), DPCPX (125), FK-453 (122) and KW-3902 (123, 126) inhibited cisplatin-induced ARF. FK-453 (122) and KW-3902 (127) reduced gentamicin-induced ARF. KW-3902 blocked the accumulation of gentamicin in proximal tubules (128) and KW-3902 (129) attenuated cephaloridine-induced nephrotoxicity. Theophylline (130-134) and KW-3902 (135) attenuated ischemia/hypoxia-induced ARF. DPCPX (136) and KW-3902 (137) attenuated somewhat endotoxin-induced ARF.

Type 2 diabetes

Both loop and thiazide diuretics worsen glycemic control (100). A_1 receptor antagonists may improve glucose uptake in skeletal muscle (138, 139) and A_{2B} receptor antagonists may reduce hepatic glucose production (140). Therefore, A_1 receptor antagonists with A_{2B} receptor blocking activity may provide a unique diuretic class that increases sodium excretion while improving glucose control. In this regard, we report (141) the effects of long-term administration (6 months) of an orally active dual A_1/A_{2B} receptor antagonist (BG-9928; Fig. 1) in an animal system (ZSF1 rats) designed to model the complex pathology that characterizes, with increasing frequency, the modern cardiac patient. The ZSF1 rat is a model of the metabolic syndrome that expresses obesity, hypertension, type 2 diabetes, dyslipidemia, dilated cardiomyopathy and severe nephropathy. After 6 months of administration, BG-9928 (10 mg/kg/day) reduced urinary glucose excretion and attenuated worsening of the oral glucose tolerance test and improves fasting plasma glucose and insulin levels. Moreover, BG-9928 blocked the age-related increase in plasma triglycerides and significantly reduced focal segmental glomerulosclerosis and cardiac vasculitis/inflammation, degenerative ischemic changes and necrosis. Thus, in dilated cardiomyopathy complicated by the metabolic syndrome and renal disease, chronic dual A_1/A_{2B} receptor blockade improves type 2 diabetes, lowers plasma triglycerides and attenuates renal and cardiac histopathology.

Conclusions

A_1 receptor antagonists are a new class of "renal friendly" diuretics that work by blocking A_1 receptors in the proximal tubule, collecting duct and preglomerular microcirculation. These drugs may enhance traditional diuretic therapy in patients with congestive heart failure by overcoming diuretic resistance, by increasing GFR and by preventing loop diuretic-induced decreases in GFR. Pretreatment of patients with A_1 receptor antagonists may also reduce the risk of radiocontrast media-induced nephropathy and may attenuate the decline in renal function induced by a number of nephrotoxic drugs. Finally, dual A_1/A_{2B} receptors may be a unique class of diuretics that have a favorable effect on glucose and triglycercide levels.

References

1. Nohria, A., Lewis, E., Stevenson, L.W. *Medical management of advanced heart failure*. JAMA 2002, 287: 628-40.

2. Taylor, S.H. *Diuretic therapy in congestive heart failure*. Cardiol Rev 2000, 8: 104-14.

3. Faris, R., Flather, M., Purcell, H., Henein, M., Poole-Wilson, P., Coats, A. *Current evidence supporting the role of diuretics in heart failure: A meta analysis of randomised controlled trials*. Int J Cardiol 2002, 82: 149-58.

4. Weinfeld, M.S., Chertow, G.M., Stevenson, L.W. *Aggravated renal dysfunction during intensive therapy for advanced chronic heart failure*. Am Heart J 1999, 138: 200-2.

5. Ellison, D.H. *Diuretic resistance: Physiology and therapeutics*. Sem Nephrol 1999, 19: 581-97.

6. Kramer, B.K., Schweda, F., Riegger, G.A. *Diuretic treatment and diuretic resistance in heart failure*. Am J Med 1999, 106: 90-6.

7. Hillege, H.L., Girbes, A.R., de Kam, P.J., Boomsma, F., de Zeeuw, D., Charlesworth, A., Hampton, J.R., van Veldhuisen, D.J. *Renal function, neurohormonal activation, and survival in patients with chronic heart failure*. Circulation 2000, 102: 203-10.

8. Krumholz, H.M., Chen, Y.T., Vaccarino, V., Wang, Y., Radford, M.J., Bradford, W.D., Horwitz, R.I. *Correlates and impact on outcomes of worsening renal function in patients > or =65 years of age with heart failure*. Am J Cardiol 2000, 85: 1110-13.

9. Weinfeld, M.S., Chertow, G.M., Stevenson, L.W. *Aggravated renal dysfunction during intensive therapy for advanced chronic heart failure*. Am Heart J 1999, 138: 200-2.

10. Cataliotti, A., Boerrigter, G., Chen, H.H., Jougasaki, M., Costello, L.C., Tsuruda, T., Lee, S.C., Malatino, L.S., Burnett, J.C. Jr. *Differential actions of vasopeptidase inhibition versus angiotensin-converting enzyme inhibition on diuretic therapy in experimental congestive heart failure*. Circulation 2002, 105: 639-44.

11. Olah, M.E., Stiles, G.L. *The role of receptor structure in determining adenosine receptor activity*. Pharmacol Therap 2000, 85: 55-75.

12. Freissmuth, M., Hausleithner, V., Tuisl, E., Nanoff, C., Schutz, W. *Glomeruli and microvessels of the rabbit kidney contain both A_1- and A_2-adenosine receptors*. Naunyn-Schmiedebergs Arch Pharmacol 1987, 335: 438-44.

13. Weber, R.G., Jones, C.R., Palacios, J.M., Lohse, M.J. *Autoradiographic visualization of A_1-adenosine receptors in brain and peripheral tissues of rat and guinea pig using ^{125}I-HPIA*. Neurosci Lett 1988, 87: 215-20.

14. Weaver, D.R., Reppert, S.M. *Adenosine receptor gene expression in rat kidney*. Am J Physiol 1992, 263: F991-5.

15. Toya, Y., Umemura, S., Iwamoto, T., Hirawa, N., Kihara, M., Takagi, N., Ishii, M. *Identification and characterization of adenosine A_1 receptor-cAMP system in human glomeruli*. Kidney Int 1993, 43: 928-32.

16. Yamaguchi, S., Umemura, S., Tamura, K., Iwamoto, T., Nyui, N., Ishigami, T., Ishii, M. *Adenosine A_1 receptor mRNA in microdissected rat nephron segments*. Hypertension 1995, 26: 1181-5.

17. Kreisberg, M.S., Silldorff, E.P., Pallone, T.L. *Localization of adenosine-receptor subtype mRNA in rat outer medullary descending vasa recta by RT-PCR*. Am J Physiol 1997, 272: H1231-8.

18. Gould, J., Morton, M.J., Sivaprasadarao, A., Bowmer, C.J., Yates, M.S. *Renal adenosine A_1 receptor binding characteristics and mRNA levels during the development of acute renal failure in the rat.* Brit J Pharmacol 1997, 120: 947-53.

19. Smith, J.A., Whitaker, E.M., Yaktubay, N., Morton, M.J., Bowmer, C.J., Yates, M.S. *Regulation of renal adenosine A_1 receptors: Effect of dietary sodium chloride*. Eur J Pharmacol 1999, 384: 71-9.

20. Halimi, G., Sampol, J., Clot-Faybesse, O., Mercier, L., Devaux, C., Berland, Y., Dussol, B., Rochat, H., Guieu, R. *Cyclosporine A and purinergic receptors in rat kidney*. Life Sci 1999, 65: 2801-13.

21. Zou, A.P., Wu, F., Li, P.L., Cowley, A.W. Jr. *Effect of chronic salt loading on adenosine metabolism and receptor expression in renal cortex and medulla in rats*. Hypertension 1999, 33: 511-6.

22. Smith, J.A., Whitaker, E.M., Bowmer, C.J., Yates, M.S. *Differential expression of renal adenosine A_1 receptors induced by acute renal failure*. Biochem Pharmacol 2000, 59: 727-32.

23. Smith, J.A., Sivaprasadarao, A., Munsey, T.S., Bowmer, C.J., Yates, M.S. *Immunolocalisation of adenosine A_1 receptors in the rat kidney.* Biochem Pharmacol 2001, 61: 237-44.

24. Jackson, E.K., Zhu, C., Tofovic, S.P. *Expression of adenosine receptors in the preglomerular microcirculation*. Am J Physiol 2002, 283: F41-51.

25. Agmon, Y., Dinour, D., Brezis, M. *Disparate effects of adenosine A_1- and A_2-receptor agonists on intrarenal blood flow*. Am J Physiol 1993, 265: F802-6.

26. Osswald, H., Spielman, W.S., Knox, F.G. *Mechanism of adenosine-mediated decreases in glomerular filtration rate in dogs*. Circ Res 1978, 43: 465-9.

27. Murray, R.D., Churchill, P.C. *Effects of adenosine receptor agonists in the isolated, perfused rat kidney.* Am J Physiol 1984, 247: H343-8.

28. Murray, R.D., Churchill, P.C. *Concentration dependency of the renal vascular and renin secretory responses to adenosine receptor agonists*. J Pharmacol Exp Ther 1985, 232: 189-93.

29. Sildorff, E.P., Kreisberg, M.S., Pallone, T.L. *Adenosine modulates vasomotor tone in outer medullary descending vasa recta of the rat*. J Clin Invest 1996, 98: 18-23.

30. Barrett, R.J., Droppleman, D.A. *Interactions of adenosine A_1 receptor-mediated renal vasoconstriction with endogenous nitric oxide and ANG II*. Am J Physiol 1993, 265: F651-9.

31. Osswald, H., Schmitz, H.J., Heidenreich, O. *Adenosine response of the rat kidney after saline loading, sodium restriction and hemorrhage*. Pflügers Arch 1975, 357: 323-33.

32. Spielman, W.S., Osswald, H. *Blockade of postocclusive renal vasoconstriction by angiotensin II antagonist: Evidence for an angiotensin-adenosine interaction*. Am J Physiol 1979, 237: F463-7.

33. Hall, J.E., Granger, J.P. *Adenosine alters glomerular filtration control by angiotensin II*. Am J Physiol 1986, 250: F917-23.

34. Deray, G., Sabra, R., Herzer, W.A., Jackson, E.K., Branch, R.A. *Interaction between angiotensin II and adenosine in mediating the vasoconstrictor response to intrarenal hypertonic saline infusions in the dog.* J Pharm Exp Ther 1990, 252: 631-5.

35. Weihprecht, H., Lorenz, J.N., Briggs, J.P., Schnermann, J. *Synergistic effects of angiotensin and adenosine in the renal microvasculature*. Am J Physiol 1994, 266: F227-39.

36. Munger, K.A., Jackson, E.K. *Effects of selective A_1 receptor blockade on glomerular hemodynamics: Involvement of renin-angiotensin system*. Am J Physiol 1994, 267: F783-90.

37. Traynor, T., Yang, T., Huang, Y.G., Arend, L., Oliverio, M.I., Coffman, T., Briggs, J.P., Schnermann, J. *Inhibition of adenosine-1 receptor-mediated preglomerular vasoconstriction in AT_{1A} receptor-deficient mice*. Am J Physiol 1998, 275: F922-27.

38. Abassi, Z.A., Gurbanov, K., Mulroney, S.E., Potlog, C., Opgenorth, T.J., Hoffman, A., Haramati, A., Winaver, J. *Impaired nitric oxide-mediated renal vasodilation in rats with experimental heart failure: Role of angiotensin II*. Circulation 1997, 96(10): 3655-64.

39. Garg, R., Yusuf, S. *Overview of randomized trials of angiotensin-converting enzyme inhibitors on mortality and morbidity in patients with heart failure: Collaborative Group on ACE Inhibitor Trials.* JAMA 1995, 273: 1450-6.

40. Funaya, H., Kitakaze, M., Node, K., Minamino, T., Komamura, K., Hori, M. *Plasma adenosine levels increase in patients with chronic heart failure.* Circulation 1997, 95: 1363-5.

41. Sharma, R., Davidoff, M.N. *Oxidative stress and endothelial dysfunction in heart failure.* Congestive Heart Failure 2002, 8: 165-72.

42. Chen, Y.F., Li, P.L., Zou, A.P. *Oxidative stress enhances the production and actions of adenosine in the kidney* Am J Physiol 2001, 28: R1808-16.

43. Osswald, H., Nabakowski, G., Hermes, H. *Adenosine as a possible mediator of metabolic control of glomerular filtration rate*. Int J Biochem 1980, 12: 263-7.

44. Osswald, H., Vallon, V., Mühlbauer, B. *Role of adenosine in tubuloglomerular feedback and acute renal failure*. J Autonomic Pharmacol 1996, 16: 377-80.

45. Baudouin-Legros, M., Badou, A., Paulais, M., Hammet, M., Teulon, J. *Hypertonic NaCl enhances adenosine release and hormonal cAMP production in mouse thick ascending limb*. Am J Physiol 1995, 269: F103-9.

46. Siragy, H.M., Linden, J. *Sodium intake markedly alters renal interstitial fluid adenosine.* Hypertension 1996, 27: 404-7.

47. Franco, M., Bell, P.D., Navar, L.G. *Effect of adenosine A_1 analogue on tubuloglomerular feedback mechanism*. Am J Physiol 1989, 257: F231-6.

48. Schnermann, J., Weihprecht, H., Briggs, J.P. *Inhibition of tubuloglomerular feedback during adenosine$_1$ receptor blockade*. Am J Physiol 1990, 258: F553-61.

49. Ren, Y., Arima, S., Carretero, O.A., Ito, S. *Possible role of adenosine in macula densa control of glomerular hemodynamics*. Kidney Int 2002, 61: 169-76.

50. Gerkens, J.F., Heidemann, H.T., Jackson, E.K., Branch, R.A. *Aminophylline inhibits renal vasoconstriction produced by intrarenal hypertonic saline*. J Pharmacol Exp Ther 1983, 225: 611-5.

51. Callis, J.T., Kuan, C.J., Branch, K.R., Abels, B.C., Sabra, R., Jackson, E.K., Branch, R.A. *Inhibition of renal vasoconstriction induced by intrarenal hypertonic saline by the nonxanthine adenosine antagonist CGS 15843A*. J Pharmacol Exp Ther 1989, 248: 1123-9.

52. Osswald, H., Hermes, H., Nabakowski, G. *Role of adenosine in signal transmission of tubuloglomerular feedback*. Kidney International 1982, 22:S136-42.

53. Sun, D., Samuelson, L.C., Yang, T., Huang, Y., Paliege, A., Saunders, T., Briggs, J., Schnermann, J. *Mediation of tubuloglomerular feedback by adenosine: Evidence from mice lacking adenosine 1 receptors*. Proc Natl Acad SciUS 2001, 98: 9983-8.

54. Brown, R., Ollerstam, A., Johansson, B., Skott, O., Gebre-Medhin, S., Fredholm, B., Persson, A.E. *Abolished tubuloglomerular feedback and increased plasma renin in adenosine A_1 receptor-deficient mice*. Am J Physiol 2001, 281: R1362-7.

55. Thomson, S.C. *Adenosine and purinergic mediators of tubuloglomerular feedback*. Curr Opin Nephrol Hypertens 2002, 11: 81-6.

56. Nishiyama, A., Majid, D.S., Walker, M., 3rd., Miyatake, A., Navar, L.G. *Renal interstitial ATP responses to changes in arterial pressure during alterations in tubuloglomerular feedback activity*. Hypertension 2001, 37: 753-9.

57. Thomson, S., Bao, D., Deng, A., Vallon, V. *Adenosine formed by 5'-nucleotidase mediates tubuloglomerular feedback*. J Clin Invest 2000, 106: 289-98.

58. Coulson, R., Johnson, R.A., Olsson, R.A., Cooper, D.R., Scheinman, S.J. *Adenosine stimulates phosphate and glucose transport in opossum kidney epithelial cells*. Am J Physiol 1991, 260: F921-8.

59. Takeda, M., Yoshitomi, K., Imai, M. *Regulation of Na^+-3HCO_3 cotransport in rabbit proximal convoluted tubule via adenosine A_1 receptor*. Am J Physiol 1993, 265: F511-9.

60. Cai, H., Batuman, V., Puschett, D.B., Puschett, J.B. *Effect of KW-3902, a novel adenosine A_1 receptor antagonist, on sodium-dependent phosphate and glucose transport by the rat renal proximal tubular cell*. Life Sci 1994, 55: 839-45.

61. Cai, H., Puschett, D.B., Guan, S., Batuman, V., Puschett, J.B. *Phosphate transport inhibition by KW-3902, an adenosine A_1 receptor antagonist, is mediated by cyclic adenosine monophosphate*. Am J Kidney Dis 1995, 26: 825-30.

62. Churchill, P.C. *Renal effects of 2-chloroadenosine and their antagonism by aminophylline in anesthetized rats*. J Pharmacol Exp Ther 1982, 222: 319-23.

63. Cook, C.B., Churchill, P.C. *Effects of renal denervation on the renal responses of anesthetized rats to cyclohexyladenosine*. Canadian Journal of Physiology and Pharmacology 1984, 62: 934-8.

64. Churchill, P.C., Bidani, A.K., Churchill, M.C., Prada, J. *Renal effects of 2-chloroadenosine in the two-kidney goldblatt rat*. J Pharmacol Exp Ther 1984, 230: 302-6.

65. Churchill, P.C., Bidani, A. *Renal effects of selective adenosine receptor agonists in anesthetized rats*. Am J Physiol 1987, 252: F299-303.

66. Levens, N., Beil, M., Jarvis, M. *Renal actions of a new adenosine agonist, CGS 21680A selective for the A_2 receptor*. J Pharmacol Exp Ther 1991, 257: 1005-12.

67. Edlund A., Sollevi, A. *Renal effects of i.v. adenosine infusion in humans*. Clinical Physiology 1993, 13(4): 361-71.

68. Balakrishnan, V.S., Coles, G.A., Williams, J.D. *Effects of intravenous adenosine on renal function in healthy human subjects*. Am J Physiol 1996, 271: F374-81.

69. Tagawa, H., Vander, A.J. *Effects of adenosine compounds on renal function and renin secretion in dogs*. Circ Res 1970, 26: 327-38.

70. Oßwald, H. *Renal effects of adenosine and their inhibition by theophylline in dogs*. Naunyn-Schmiedeberg's Arch Pharmacol 1975, 288: 79-86.

71. Spielman, W.S. *Antagonistic effect of theophylline on the adenosine-induced decrease in renin release*. Am J Physiol 1984, 247:F246-51.

72. Deray, G., Branch, R.A., Herzer, W.A., Ohnishi, A., Jackson, E.K. *Adenosine inhibits β-adrenoceptor but not DBcAMP-induced renin release.* Am J Physiol 1987, 252: F46-F52.

73. Deray, G., Branch, R.A., Ohnishi, A., Jackson, E.K. *Adenosine inhibits renin release induced by suprarenal-aortic constriction and prostacyclin*. Naunyn-Schmiedeberg's Arch Pharmacol 1989, 339: 590-5.

74. Miyamoto, M., Yagil, Y., Larson, T., Robertson, C., Jamison, R.L. *Effects of intrarenal adenosine on renal function and medullary blood flow in the rat*. Am J Physiol 1988, 255: F1230-4.

75. Yagil, Y. *The effects of adenosine on water and sodium excretion*. J Pharmacol Exp Ther 1994, 268: 826-35.

76. Fransen, R., Koomans, H.A. *Adenosine and renal sodium handling: Direct natriuresis and renal nerve-mediated antinatriuresis*. J Am Soc Nephrol 1995, 6: 1491-7.

77. Wilcox, C.S., Welch, W.J., Schreiner, G.F., Belardinelli, L. *Natriuretic and diuretic actions of a highly selective adenosine A_1 receptor antagonist*. J Am Soc Nephrol 1999, 10: 714-20.

78. Collis, M.G., Shaw, G., Keddie, J.R. *Diuretic and saliuretic effects of 1,3-dipropyl-8-cyclopentylxanthine, a selective A_1-adenosine receptor antagonist*. J Pharm Pharmacol 1991, 43: 138-9.

79. Knight, R.J., Bowmer, C.J., Yates, M.S. *The diuretic action of 8-cyclopentyl-1,3-dipropylxanthine, a selective A_1 adenosine receptor antagonist*. Br J Pharmacol 1993, 109: 271-7.

80. Shimada, J., Suzuki, F., Nonaka, H., Karasawa, A., Mizumoto, H., Ohno, T., Kubo, K., Ishii, A. *8-(Dicyclopropylmethyl)-1,3-dipropylxanthine: A potent and selective adenosine A_1 antagonist with renal protective and diuretic activities*. J Med Chem 1991, 34: 466-9.

81. Kuan, C.J., Herzer, W.A., Jackson, E.K. *Cardiovascular and renal effects of blocking A_1 adenosine receptors*. J Cardiovascular Pharmacol 1993, 21: 822-8.

82. Gellai, M., Schreiner, G.F., Ruffolo, R.R. Jr., Fletcher, T., DeWolf, R., Brooks, D.P. *CVT-124, a novel adenosine A_1 receptor antagonist with unique diuretic activity*. J Pharmacol Exp Ther 1998, 286: 1191-6.

83. Pfister, J.R., Belardinelli, L., Lee, G., Lum, R.T., Milner, P., Stanley, W.C., Linden, J., Baker, S.P., Schreiner, G. *Synthesis and biological evaluation of the enantiomers of the potent and selective A_1-adenosine antagonist 1,3-dipropyl-8-[2-(5,6-epoxynorbonyl)]-xanthine*. J Med Chem 1997, 40: 1773-8.

84. Oberbauer, R., Schreiner, G.F., Meyer, T.W. *Natriuretic effect of adenosine A_1-receptor blockade in rats*. Nephrology Dialysis and Transplantation 1998, 13: 900-3.

85. Ticho, B. et al. Drug Dev Res, in press.

86. Aki, Y., Tomohiro, A., Nishiyama, A., Kiyomoto, K., Kimura, S., Abe, Y. *Effects of KW-3902, a selective and potent adenosine A_1 receptor antagonists, on renal hemodynamics and urine formation in anesthetized dogs*. Pharmacol 1997, 55: 193-201.

87. Balakrishnan, V.S., Coles, G.A., Williams, J.D. *A potential role for endogenous adenosine in control of human glomerular and tubular function*. Am J Physiol 1993, 265: F504-10.

88. van Buren, M., Bijlsma, J.A., Boer, P., van Rijn, H.J.M., Koomans, H.A. *Natriuretic and hypotensive effect of adenosine-1 blockade in essential hypertension*. Hypertension 1993, 22: 728-34.

89. Balakrishnan, V.S., Coles, G.A., Williams, J.D. *Functional role of endogenous adenosine in human chronic renal disease.* Experimental Nephrology 1996, 4: 26-36.

90. B. Ticho *et al.* 7th Int Symp Adenosine Adenine Nucleotides (May 26-31, Gold Coast, Australia) 2002, 46.

91. Jackson, E.K., Kost, C.K. Jr., Herzer, W.A., Smits, G.J., Tofovic, S.P. *A_1 receptor blockade induces natriuresis with a favorable renal hemodynamic profile in SHHF/Mcc-fa^{cp} rats chronically treated with salt and furosemide*. J Pharmacol Exp Ther 2001, 299: 978-87.

92. McCune, S.A., Radin, J.M., Jemkins, J.E., Chu, Y.-Y., Park, S., Peterson, R. *SHHF/Mcc-fa^{cp} rat model. Effects of gender and genotype on age of expression of metabolic complication and congestive heart failure and on response to drug therapy*. In: Frontiers in diabetes research: lesson from animal diabetes V. E. Shafrir (Ed.). pp. 255-270, Smith-Gorden, London 1994.

93. McCune, S.A., Park, S., Radin, J.M., Jurin, R.R. *The SHHF/Mcc-fa^{cp}: A genetic model of congestive heart failure*. In: Mechanisms of heart failure. P.K. Singal, I.M.C. Dixon, R.E. Beamish and Dhalla, M.S. (Eds.). pp. 91-106, Kluwer Academic Publishers, Boston 1995.

94. Lucas, D.G. Jr., Patterson, T., Hendrick, J.W., Holder, J., Sample, J.A., Joffs, C., Davis, J. et al. *Effects of adenosine receptor subtype A_1 on ventricular and renal function*. J Cardiovasc Pharmacol 2001, 38: 618-24.

95. Lucas, D.G. Jr., Hendrick, J.W., Sample, J.A., Mukherjee, R., Escobar, G.P., Smits, G.J., Crawford, F.A. Jr., Spinale, F.G. *Cardiorenal effects of adenosine subtype 1 (A_1) receptor inhibition in an experimental model of heart failure*. Journal of the Am Coll Surg 2002, 194:603-9.

96. Gottlieb, S.S., Skettino, S.L., Wolff, A., Beckman, E., Fisher, M.L., Freudenberger, R., Gladwell T. et al. *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 College Cardiol 2000, 35: 56-9.

97. Gottlieb, S.S., Brater, D.C., Thomas, I., Havranek, E., Bourge, R., Goldman, S., Dyer, F. et al. *BG9719 (CVT-124), an A_1 adenosine receptor antagonist, protects against the decline in renal function observed with diuretic therapy*. Circulation 2002, 105: 1348-53.

98. Feraille, E., Doucet, A. *Sodium-potassium-adenosinetriphosphatase-dependent sodium transport in the kidney: hormonal control*. Physiol Rev 2001, 81: 345-418.

99. Kost, C.K., Jr., Herzer, W.A., Rominski, B.R., Mi, Z., Jackson, E.K. *Diuretic response to adenosine A_1 receptor blockade in normotensive and spontaneously hypertensive rats: Role of pertussis toxin-sensitive G-proteins*. J Pharmacol Exp Ther 2000, 292: 752-60.

100. Jackson, E.K. Chapter 29: *Diuretics*. Goodman and Gilman's The Pharmacological Basis of Therapeutics (10th edition), Edited by J.G. Hardman et al., McGraw-Hill, New York, pages 757-787, 2001.

101. Persson, A.E., Wright, F.S. *Evidence for feedback mediated reduction of glomerular filtration rate during infusion of acetazolamide*. Acta Physiologica Scandinavica 1982, 114: 1-7.

102. Chou, C.C., Hook, J.B., Hsieh, C.P., Burns, T.D., Dabney, J.M. *Effects of radiopaque dyes on renal vascular resistance*. J Lab Clin Med 1971, 78: 705-12.

103. Ansari, Z., Baldwin, D.S. *Acute renal failure due to radiocontrast agents*. Nephron 1976, 17: 28-40.

104. Katzberg, R.W., Schulman, G., Meggs, L.G., Caldicott, W.J.H., Damieno, M.M., Hollenberg, N.K. *Mechanism of renal response to contrast media in dogs: Decrease in renal function due to hypertonicity*. Invest Radiol 1983, 18: 74-80.

105. Arend, L.J., Bakris, G.L., Burnett, J.C. Jr., Megerian, C., Spielman, W.S. *Role for intrarenal adenosine in the renal hemodynamic response to contrast media*. J Lab Clin Med 1987, 110: 406-11.

106. Deray, G., Martinez, F., Cacoub, P., Baumelou, B., Baumelou, A., Jacobs, C. *A role for adenosine, calcium and ischemia in radiocontrast-induced intrarenal vasoconstriction*. Am J Nephrol 1990, 10: 316-22.

107. Erley, C.M., Duda, S.H., Schlepckow, S., Koehler, J., Huppert, P.E., Strohmaier, W.L., Bohle, A., Risler, T., Osswald, H. *Adenosine antagonist theophylline prevents the reduction of glomerular filtration rate after contrast media application*. Kidney Int 1994, 45: 1425-31.

108. Katholi, R.E., Taylor, G.J., McCann, W.P., Woods, W.T. Jr., Womach, K.A., McCoy, C.D., Katholi, C.R. et al. *Nephrotoxicity from contrast media: attenuation with theophylline*. Radiology 1995, 195: 17-22.

109. Huber, W., Ilgmann, K., Page, M., Hennig, M., Schweigart, U., Jeschke, B., Lutilsky, L., Weiss, W., Salmhofer, H., Classen, M. *Effect of theophylline on contrast material-nephropathy in*

patients with chronic renal insufficiency: Controlled, randomized, double-blinded study. Radiology 2002, 223: 772-9.

110. Erley, C.M., Heyne, N., Burgert, K., Langanke, J., Risler, T., Osswald, H. *Prevention of radiocontrast-induced nephropathy by adenosine antagonists in rats with chronic nitric oxide deficiency*. J Am Soc Nephrol 1997, 8: 1125-32.

111. Erley, C.M., Heyne, E.N., Rossmeier, S., Vogel, T., Risler, T., Osswald, H. *Adenosine and extracellular volume in radiocontrast media-induced nephropathy*. Kidney Int 1998, 54: S-192-4.

112. Yao, K., Heyne, N., Erley, C.M., Risler, T., Osswald, H. *The selective adenosine A_1 receptor antagonist KW-3902 prevents radiocontrast media-induced nephropathy in rats with chronic nitric oxide deficiency*. Eur J Pharmacol 2001, 414: 99-104.

113. Oldroyd, S.D., Fang, L., Haylor, J.L., Yates, M.S., El Nahas, A.M., Morcos, S.K. *Effects of adenosine receptor antagonists on the responses to contrast media in the isolated rat kidney*. Clin Sci 2000, 98: 303-11.

114. Arakawa, K., Suzuki, H., Naitoh, M., Matsumoto, A., Hayashi, K., Matsuda, H., Ichihara, A., Kubota, E., Saruta, T. *Role of adenosine in the renal responses to contrast medium*. Kidney Int 1996, 49: 1199-206.

115. Nagashima, K., Yamagata, T., Ushiki, J., Yamaguchi, K., Nishiyama, A., Abe, Y., Karasawa, A. *Role for renal interstitial adenosine in the contrast medium-induced renal dysfunction*. J Am Soc Nephropathy 1998, 9: 428A.

116. Bidani, A.K., Churchill, P.C. *Aminophylline ameliorates glycerol-induced acute renal failure in rats*. Can J Physiol Pharmacol 1983, 61: 567-71.

117. Bidani, A.K, Churchill, P.C., Packer, W. *Theophylline-induced protection in myoglobinuric acute renal failure: Further characterization*. Can J Physiol Pharmacol 1987, 65: 42-5.

118. Bowmer, C.J., Collis, M.G., Yates, M.S. *Effect of the adenosine antagonist 8-phenyltheophylline on glycerol-induced acute renal failure in the rat*. Br J Pharmacol 1986, 88: 205-12.

119. Kellett, R., Bowmer, C.J., Collis, M.G., Yates, M.S. *Amelioration of glycerol-induced acute renal failure in the rat with 8-cyclopentyl-1,3-dipropylxanthine*. Br J Pharmacol 1989, 98:1066-74.

120. Panjehshahin, M.R., Munsey, T.S., Collis, M.G., Bowmer, C.J., Yates, M.S. *Further characterization of the protective effect of 8-cyclopentyl-1,3-dipropylxanthine on glycerol-induced acute renal failure in the rat*. J Pharm Pharmacol 1991, 44: 109-13.

121. Ishikawa, I., Shikura, N., Takada, K. *Amelioration of glycerol-induced acute renal failure in rats by an adenosine A_1 receptor antagonist (FR-113453)*. Renal Fail 1993, 15:1-5.

122. Andoh, T.F., Terai, T., Nakano, K., Horiai, H., Mori, J., Kohsaka, M. *Protective effects of FR113453, an adenosine A_1-receptor antagonist, on experimental acute renal failure in rats*. In: Proceed Int Symp Acute Renal Failure; October 2-4, 1991; University of North Carolina at Chapel Hill; page 92.

123. Suzuki, F., Shimada, J., Mizumoto, H., Karasawa, A., Kubo, K., Nonaka, H., Ishii, A., Kawakita, T. *Adenosine A_1 antagonists. 2. Structure-activity relationships on diuretic activities and protective effects against acute renal failure*. J Med Chem 1992, 35: 3066-75.

124. Heidemann, H.T., Müller, S., Mertins, L., Stepan, G., Hoffmann, K., Ohnhaus, E. *Effect of aminophylline on cisplatin nephrotoxicity in the rat*. Br J Pharmacol 1989, 97: 313-8.

125. Knight, R.J., Collis, M.G., Yates, M.S., Bowmer, C.J. *Amelioration of cisplatin- induced acute renal failure with 8-cyclopentyl-1,3-dipropylxanthine*. Br J Pharmacol 1991, 104: 1062-8.

126. Nagashima, K., Kusaka, H., Karasawa, A. *Protective effects of KW-3902, an adenosine A_1-receptor antagonist, against cisplatin-induced acute renal failure in rats*. Jpn J Pharmacol 1995, 67: 349-57.

127. Yao, K., Kusaka, H., Sato, K., Karasawa, A. *Protective effects of KW-3902, a novel adenosine A_1-receptor antagonist, against gentamicin-induced acute renal failure in rats*. Jpn J Pharmacol 1994, 65: 167-70.

128. Yao, K. *Effect of KW-3902, a selective adenosine A_1-receptor antagonist, on accumulation of gentamicin in the proximal renal tubules in rats*. Yakugaku Zasshi - J Pharmaceut Soc Jpn 2000, 120:801-805.

129. Nagashima, K., Kusaka, H., Sato, K., Karasawa, A. *Effects of KW-3902, a novel adenosine A_1-receptor antagonist, on cephaloridine-induced acute renal failure in rats*. Jpn J Pharmacol 1994, 64: 9-17.

130. Osswald, H., Schmitz, H.J., Kemper, R. *Tissue content of adenosine, inosine and hypoxanthine in the rat kidney after ischemia and postischemic recirculation.* Pflügers Arch 1977, 371: 45-9.

131. Sakai, K., Akima, M., Nabata, H. *A possible purinergic mechanism for reactive ischemia in isolated, cross-circulated rat kidney*. Jpn J Pharmacol 1979, 29: 235-42.

132. Lin, J.J., Churchill, P.C., Bidani, A.K. *Effect of theophylline on the initiation phase of postischemic acute renal failure in rats*. J Lab Clin Med 1986, 108: 150-4.

133. Gouyon, J.B., Guignard, J.P. *Theophylline prevents the hypoxemia-induced renal hemodynamic changes in rabbits*. Kidney International 1988, 33: 1078-83.

134. Lin, J.J., Churchill, P.C., Bidani, A.K. *Theophylline in rats during maintenance phase of post-ischemic acute renal failure*. Kidney Int 1988, 33:24-8.

135. Nishiyama, A., Miyatake, A., Aki, Y., Fukui, T., Rahman, M., Kimura, S., Abe, Y. *Adenosine A_1 receptor antagonist KW-3902 prevents hypoxia-induced renal vasoconstriction*. J Pharmacol Exp Ther 1999, 291: 988-93.

136. Knight, R.J., Bowmer, C.J., Yates, M.S. *Effect of the selective A_1 adenosine antagonist 8-cyclopentyl-1,3-dipropylxanthine on acute renal dysfunction induced by Escherichia Coli endotoxin in rats*. J Pharm Pharmacol 1993, 45: 979-84.

137. Yao, K., Ina, Y., Nagashima, K., Ohno, T., Karasawa, A. *Effect of the selective adenosine A_1-receptor antagonist KW-3902 on lipopolysaccharide-induced reductions in urine volume and renal blood flow in anesthetized dogs*. Jpn J Pharmacol 2000, 84: 310-5.

138. Xu, B., Berkich, D.A., Crist, G.H., LaNoue, KF. *A_1 adenosine receptor antagonism improves glucose tolerance in Zucker rats*. Am J Physiol 1998, 274: E271-9.

139. Crist, G.H., Xu, B., Lanoue, K.F., Lang, CH. *Tissue-specific effects of in vivo adenosine receptor blockade on glucose uptake in Zucker rats*. FASEB J 1998, 12: 1301-8.

140. Harada, H., Asano, O., Kawata, T., Inoue, T., Horizoe, T., Yasuda, N., Nagata, K., Murakami, M., Nagaoka, J., Kobayashi,

S., Tanaka, I., Abe, S. *2-Alkynyl-8-aryladenines possessing an amide moiety: Their synthesis and structure-activity relationships of effects on hepatic glucose production induced via agonism of the A_{2B} adenosine receptor*. Bioorg Med Chem 2001, 9(10): 2709-26.

141. Tofovic, S.P., Salah, E.M., Smits, G., Whalley, E., Ticho, B., Jackson, E.K. *Chronic A1 and A2b adenosine receptor blockade improves diabetes and attenuates renal and cardiac histopathology in animals with the metabolic syndrome*. Abstr accepted for presentation at Am Coll Cardiol 2003.