A1 Receptor Blockade Induces Natriuresis with a Favorable Renal Hemodynamic Profile in SHHF/Mcc-fa<sup>cp</sup> Rats Chronically Treated with Salt and Furosemide

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ABSTRACT

Our goal was to test the hypothesis that A1 receptor blockade induces diuresis/natriuresis with a favorable renal hemodynamic/cardiac profile in aged, lean SHHF/Mcc-fa<sup>cp</sup> rats, a rodent model of hypertensive dilated cardiomyopathy. Thirteen-month-old SHHF/Mcc-fa<sup>cp</sup> rats were pretreated for 72 h before experiments with furosemide (100 mg/kg by gavage 72, 48, and 24 h before experiments) to mimic the clinical setting of chronic diuretic therapy and were given 1% NaCl as drinking water to reduce dehydration/sodium depletion. Animals were instrumented for measurement of systemic and renal hemodynamics, renal excretory function, and cardiac performance, and baseline values were obtained during a 30-min clearance period. Animals then received either vehicle (n = 9), BG9719 [the S-enantiomer of 1,3-dipropyl-8-[2-(5,6-epoxynorbornyl)] xanthine (also called CVT-124)] (highly selective A1 receptor antagonist; 0.1 mg/kg bolus + 10 μg/kg/min; n = 9) or furosemide (loop diuretic; 30 mg/kg; n = 8) and measurements were repeated during four subsequent clearance periods. Both BG9719 and furosemide increased urine volume and absolute and fractional sodium excretion. BG9719 increased renal blood flow and glomerular filtration rate, but did not affect fractional potassium excretion. Furosemide decreased renal blood flow and glomerular filtration rate and increased fractional potassium excretion. Neither drug altered afterload; however, furosemide, but not BG9719, decreased preload (central venous pressure and ventricular end diastolic pressure). Neither drug altered systolic function (+dP/dt<sub>max</sub>; however, furosemide, but not BG9719, attenuated diastolic function (−dP/dt<sub>max</sub>, increased tau). In the setting of left ventricular dysfunction, chronic salt loading and prior loop diuretic treatment, selective A1 receptor antagonists are effective diuretic/natriuretic agents with a favorable renal hemodynamic/cardiac performance profile.

Regulation of excretory organs by purinergic receptors is as ancient as vertebrate evolution. For example, in the shark rectal gland, an organ system that evolved over 400 million years ago, primitive adenosine receptors strongly modulate the rate of salt transport (Forrest, 1996).

Modulation of sodium chloride excretion by purinergic receptors also occurs in mammals. In opossum kidney cells (proximal tubular phenotype), activation of A1 adenosine receptors increases Na<sup>+</sup>-phosphate and Na<sup>+</sup>-glucose symport (Coulson et al., 1991), and in rabbit proximal convoluted tubules, stimulation of A1 receptors accelerates Na<sup>+</sup>-3HCO<sub>3</sub><sup>-</sup> symport in the basolateral membrane (Takeda et al., 1993).

In healthy animals, selective antagonism of kidney A1 receptors induces a brisk diuretic/natriuretic response with little or no effects on potassium excretion. In normal rats, intravenous administration of either 1,3-dipropyl-8-cyclopentylxanthine (Knight et al., 1993) or 8-(dicyclopropylmethyl)-1,3-dipropylxanthine (Shimada et al., 1991), both selective A1 blockers, induces a diuretic/natriuretic response. Importantly, intravenous administration of equipotent doses of 8-cyclopentyl-1,3-dipropylxanthine and FK453, two highly potent and selective, but structurally dissimilar, A1 antagonists, induces similar increases in urine and sodium excretion, yet equivalent doses of FR113452, the less active enantiomer of FK453, do not change either sodium excretion or urine volume (Kuan et al., 1993). These latter experiments provide definitive proof that blockade of A1 receptors induces diuresis/natriuresis. BG9719, the S-enantiomer of 1,3-dipropyl-8-[2-(5,6-epoxynorbornyl)] xanthine (also called CVT-124), is one of the most selective and potent A1 blockers.

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ABBREVIATIONS: GFR, glomerular filtration rate; RBF, renal blood flow; PE, polyethylene; CVP, central venous pressure; PEG, polyethylene glycol; SBP, systolic blood pressure; DBP, diastolic blood pressure; MABP, mean arterial blood pressure; +dP/dt<sub>max</sub>, maximum rate of rise in intraventricular pressure during ventricular contraction; −dP/dt<sub>max</sub>, maximum rate of fall in intraventricular pressure during ventricular relaxation; VPSP, ventricular peak systolic pressure; τ, time constant for ventricular relaxation; VEDP, ventricular end diastolic pressure; VMDP, ventricular minimal diastolic pressure; LSD, least significant difference; 2-F-ANOVA, two-factor analysis of variance.
yet identified (Belardinelli et al., 1995). In conscious rats, BG9719 causes a marked diuresis and natriuresis, and the maximum diuretic/natriuretic effect of BG9719 is greater than that for hydrochlorothiazide (Gellai et al., 1998).

A1 blockers, such as BG9719, may be particularly useful in the setting of diuretic resistance and/or diuretic intolerance (i.e., renal dysfunction induced by diuretic therapy). For example, BG9719 potentiates the diuretic/natriuretic effect of furosemide without enhancing furosemide-induced kaliuresis (Gellai et al., 1998) and increases urine volume and salt excretion in sodium-loaded animals (Pfister et al., 1997). These findings suggest that drugs such as BG9719 may be useful in diuretic resistance states. With regard to diuretic intolerance, BG9719 prevents increases in distal delivery of NaCl from causing tubuloglomerular feedback-mediated reductions in glomerular filtration rate (GFR) and renal blood flow (RBF) by uncoupling proximal tubular reabsorption from single nephron glomerular filtration (Wilcox et al., 1999).

Left ventricular dysfunction is an increasingly prevalent clinical condition (Ghali et al., 1990) that is often associated with diuretic-resistant edema (Kramer et al., 1999). Moreover, patients with left ventricular dysfunction are sensitive to the adverse renal effects of diuretics (Lonn and McKelvie, 2000), particularly in patients receiving angiotensin-converting enzyme inhibitors (Mandal et al., 1994). Given the unique pharmacological profile of A1 adenosine blockers, it is possible that this class of drugs may prove useful in patients with left ventricular dysfunction. In heart failure, blockade of A1 receptors not only may improve renal excretory function but also may improve both renal hemodynamics and left ventricular function. In this regard, because the negative inotropic effects of A1 receptor agonists are enhanced in failing cardiomyocytes (Borst et al., 1999), it is conceivable that blockade of A1 receptors could improve heart performance in the setting of left ventricular dysfunction.

Little is known regarding the renal and cardiovascular effects of A1 antagonism in the setting of left ventricular dysfunction. Accordingly, the purpose of the present study was to assess the effects of A1 receptor antagonism with BG9719 on cardiac performance, renal hemodynamics, and renal excretory function in aged, lean SHHF/Mcc-fa<sup>fa</sup> rats, a well characterized rodent model of hypertensive dilated cardiomyopathy (Holycross et al., 1997; Khadour et al., 1997; Sharkey et al., 1998; Bergman et al., 1999; Carraway et al., 1999; Sharkey et al., 1999). Studies were conducted in SHHF/Mcc-fa<sup>fa</sup> rats pretreated with NaCl plus furosemide to mimic the common clinical situation of high salt intake combined with chronic diuretic therapy.

**Materials and Methods**

**Animals and Animal Housing.** Lean 13-month-old SHHF/Mcc-fa<sup>fa</sup> rats were obtained from Genetic Models, Inc. (Indianapolis, IN). In the context of SHHF nomenclature, “lean” refers to animals that have at least one normal leptin receptor gene (homozygous or heterozygous for a normal leptin receptor gene), whereas “obese” refers to animals that are homozygous for the mutated, nonfunctional leptin receptor gene. SHHF/Mcc-fa<sup>fa</sup> rats spontaneously, whether lean or obese, develop dilated cardiomyopathy. In this regard, cardiac hypertrophy is observable as early as 3 months of age and is fully developed by 6 months of age (McCune et al., 1994, 1995). Lean male SHHF/Mcc-fa<sup>fa</sup> die of congestive heart failure at 15 to 18 months of age, and the development of heart failure is associated with increased blood pressure (McCune et al., 1994, 1995) in a range similar to that observed in the spontaneously hypertensive rat (Pfeffer et al., 1979). However, SHHF/Mcc-fa<sup>fa</sup> rats are genetically predisposed to hypertensive heart failure at an incidence of 100%, whereas only some aged spontaneously hypertensive rats die of heart failure (Pfeffer et al., 1979).

Animals were housed in the University of Pittsburgh Animal Care Facility for 1 month before being used in this study. Temperature, relative humidity, and the light cycle were kept at 22°C, 55%, and 12 h (7:00 AM to 7:00 PM), respectively. Animals were fed Pro Lab RMI 3000 rodent diet (PMI Nutrition Inc., St. Louis, MO) containing 0.26% sodium and 0.82% potassium and water ad libitum. Institutional guidelines for animal welfare were followed and the protocol was approved by the Institutional Animal Care and Use Committee.

**Chronic Treatments.** Animals were given 1% saline as drinking water beginning 72 h before the acute experiments. In addition, animals also received furosemide (100 mg/kg; Sigma, St. Louis, MO) in 0.5% methylcellulose by gavage 72, 48, and 24 h before the experiment. The half-life of furosemide in rats is less than 1 h (Hammelund and Paalzow, 1982), so furosemide would be cleared from the animals in approximately 5 h, i.e., many hours before the acute experiment. The purpose of the furosemide plus 1% saline was to mimic the common clinical scenario of chronic diuretic therapy while preventing dehydration and sodium depletion.

**Animal Surgery.** On the day of the acute experiment, each animal was anesthetized with pentobarbital (45 mg/kg i.p.) and placed on a Deltaphase isothermal pad (Braintree Scientific, Braintree, MA). Body temperature was monitored with a rectal temperature probe (Physitemp Instruments, Clifton, NJ) and maintained at 37.0 ± 0.5°C by adjusting a heat lamp positioned above the rat. A short section of polyethylene (PE) tubing (PE-240) was placed in the trachea to facilitate respiration. Two PE-50 cannulas were inserted into the right jugular vein and an infusion of 5% dextrose at 50 μl/min was initiated via one cannula. The other jugular cannula was advanced to the right atrium and connected to a low-pressure analyzer (model LPA; Micro-Med Inc., Louisville, KY) for monitoring central venous pressure (CVP). A PE-50 cannula was placed in the right femoral vein and an infusion at 10 μl/min of the vehicle for BG9719 (20% ethanol, 30% PEG2000, 50% sterile water) was begun.

A PE-50 catheter was placed in the right femoral artery for blood sample collection and for measurement of systolic (SBP), diastolic (DBP), and mean arterial blood pressure (MABP) via a digital blood pressure analyzer (model BPA; Micro-Med Inc.). A PE-50 catheter filled with 10% heparin solution was advanced via the right carotid artery into the heart and secured with a plastic suture. A PE-10 catheter was inserted into the left ureter to facilitate collection of urine, and a flow probe (model 1RB; Transonic Systems, Inc., Ithaca, NY) was placed on the left renal artery and connected to a transit time flowmeter (model T206; Transonic System, Inc.) for determination of renal blood flow. Renal vascular resistance was calculated as arterial blood pressure divided by renal blood flow. Via the jugular line, insulin [carboxyl-<sup>14</sup>C] (0.5 μCi/ml bolus followed by 0.035 μCi/min infusion) was administered.

**Experimental Protocol.** After a 60-min rest period, baseline parameters were recorded in all animals during a 30-min renal clearance period. A midpoint blood sample (300 μl) for measurement of radioactivity was collected. Plasma and urine [<sup>14</sup>C]insulin radioactivity were measured, and renal clearance of [<sup>14</sup>C]insulin was calculated.
lated for estimation of GFR. Next, animals were randomly assigned to one of three groups, i.e., the time/vehicle control group, the BG9719 group, or the furosemide group. The time/vehicle control group received an intravenous bolus of 8% ethanolamine (0.1 ml/kg) plus an intravenous bolus of PEG400 (0.1 ml/kg) followed by an intravenous infusion at 10 μl/min of 20% ethanol, 30% PEG200, and 50% sterile water. The BG9719 group received an intravenous bolus of 8% ethanolamine (0.1 ml/kg) plus an intravenous bolus of BG9719 (0.1 mg/kg dissolved in PEG400 at 0.1 mg/0.1 ml) followed by an intravenous infusion of BG9719 at 10 μg/kg/min dissolved in 20% ethanol, 30% PEG200, and 50% sterile water and infused at 10 μl/min. BG9719 was synthesized and provided by Biogen, Inc. (Cambridge, MA). The furosemide group received an intravenous bolus of furosemide (30 mg/kg dissolved in 8% ethanolamine at 30 mg/0.1 ml) plus an intravenous bolus of PEG400 (0.1 ml/kg) followed by an intravenous infusion at 10 μl/min of 20% ethanol, 30% PEG200, and 50% sterile water. Four additional 30-min clearance periods were conducted after the treatments, during which all parameters were again measured. At the end of the protocol, 1.5 ml of blood was collected for plasma levels of sodium and potassium. Urinary and plasma sodium and potassium were measured by flame photometry (Instrumentation Laboratory, Lexington, MA).

**Statistical Analysis.** The effects BG9719 and furosemide on the measured parameters were assessed with a repeated measures two-factor analysis of variance (2-F-ANOVA) in which one factor was treatment (vehicle versus BG9719 or vehicle versus furosemide) and the second factor was clearance period (repeated measures factor). Post hoc comparisons of clearance periods 2 through 5 to the basal clearance period 1 were performed with a Fisher’s LSD test. Statistical comparisons were performed with the Number Cruncher Statistical System (Kaysville, UT), and the criterion for significance was P < 0.05. All data in tables, figures, and text are means ± S.E.M.

**Results**

BG9719 and furosemide significantly (P < 0.0001; 2-F-ANOVA) increased urine volume (Fig. 1). BG9719 and furosemide also significantly (P < 0.0001; 2-F-ANOVA) increased absolute and fractional sodium excretion (Figs. 2 and 3, respectively). The effects of both drugs on renal excretory function were greatest during the first 30-min clearance period and waned somewhat during the ensuing 90 min. However, renal excretory parameters were significantly elevated during all postdrug clearance periods (Figs. 1–3). Furosemide significantly (P < 0.0001; 2-F-ANOVA) increased the fractional excretion of potassium, whereas BG9719 was potassium-neutral, i.e., fractional excretion of potassium was unchanged (Fig. 4).

BG9719 significantly (P = 0.0029; 2-F-ANOVA) increased glomerular filtration rate (Fig. 5) and nearly significantly (P = 0.0507; 2-F-ANOVA) increased renal blood flow (Fig. 6). Indeed, when analyzed with Fisher’s LSD test, the renal blood flows during clearance periods 2 through 5 (during BG9719) were significantly (P < 0.05) different compared with the basal period (before BG9719). In contrast, furosemide significantly (P = 0.0029; 2-F-ANOVA) decreased glomerular filtration rate (Fig. 5) and significantly (P < 0.0001; 2-F-ANOVA) decreased renal blood flow (Fig. 6). The changes in glomerular filtration rate and renal blood flow induced by BG9719 and furosemide were observed during the first 30-min clearance period and were maintained for the duration of the experiment. BG9719 tended to reduce renal vascular resistance, but this effect was not significant (Fig. 7). Furosemide, on the other hand, significantly (P = 0.0171; 2-F-ANOVA) increased renal vascular resistance, an effect
Fig. 2. Line graph of the effects of intravenous administration of vehicle (top), BG9719 (0.1 mg/kg + 10 µg/kg/min; middle), and furosemide (30 mg/kg; bottom) on sodium excretion rate in lean, male 14-month-old SHHF/Mcc-fa<sup>α</sup> rats that were pretreated 72, 48, and 24 h before the acute experiment with furosemide (100 mg/kg by gavage) and with 1% saline as drinking water. Values indicate means ± S.E.M. P values refer to interaction term in two-factor analysis of variance. a, indicates P < 0.05 compared with the first clearance period (Fisher's LSD test).

Fig. 3. Line graph of the effects of intravenous administration of vehicle (top), BG9719 (0.1 mg/kg + 10 µg/kg/min; middle), and furosemide (30 mg/kg; bottom) on fractional sodium excretion in lean, male 14-month-old SHHF/Mcc-fa<sup>α</sup> rats that were pretreated 72, 48, and 24 h before the acute experiment with furosemide (100 mg/kg by gavage) and with 1% saline as drinking water. Values indicate means ± S.E.M. P values refer to interaction term in two-factor analysis of variance. a, indicates P < 0.05 compared with the first clearance period (Fisher's LSD test).
Fig. 4. Line graph of the effects of intravenous administration of vehicle (top), BG9719 (0.1 mg/kg + 10 μg/kg/min; middle), and furosemide (30 mg/kg; bottom) on fractional potassium excretion in lean, male 14-month-old SHHF/Mcc-fa<sup>®</sup> rats that were pretreated 72, 48, and 24 h before the acute experiment with furosemide (100 mg/kg by gavage) and with 1% saline as drinking water. Values indicate means ± S.E.M. P values refer to interaction term in two-factor analysis of variance. a, indicates P < 0.05 compared with the first clearance period (Fisher’s LSD test).

Fig. 5. Line graph of the effects of intravenous administration of vehicle (top), BG9719 (0.1 mg/kg + 10 μg/kg/min; middle), and furosemide (30 mg/kg; bottom) on glomerular filtration rate in lean, male 14-month-old SHHF/Mcc-fa<sup>®</sup> rats that were pretreated 72, 48, and 24 h before the acute experiment with furosemide (100 mg/kg by gavage) and with 1% saline as drinking water. Values indicate means ± S.E.M. P values refer to interaction term in 2-factor analysis of variance. a, indicates P < 0.05 compared with the first clearance period (Fisher’s LSD test).
Fig. 6. Line graph of the effects of intravenous administration of vehicle (top), BG9719 (0.1 mg/kg + 10 μg/kg/min; middle), and furosemide (30 mg/kg; bottom) on renal blood flow in lean, male 14-month-old SHHF/Mcc-fa<sup>−/−</sup> rats that were pretreated 72, 48, and 24 h before the acute experiment with furosemide (100 mg/kg by gavage) and with 1% saline as drinking water. Values indicate means ± S.E.M. P values refer to interaction term in two-factor analysis of variance. a, indicates P < 0.05 compared with the first clearance period (Fisher’s LSD test).

Fig. 7. Line graph of the effects of intravenous administration of vehicle (top), BG9719 (0.1 mg/kg + 10 μg/kg/min; middle), and furosemide (30 mg/kg; bottom) on renal vascular resistance in lean, male 14-month-old SHHF/Mcc-fa<sup>−/−</sup> rats that were pretreated 72, 48, and 24 h before the acute experiment with furosemide (100 mg/kg by gavage) and with 1% saline as drinking water. Values indicate means ± S.E.M. P values refer to interaction term in two-factor analysis of variance. a, indicates P < 0.05 compared with the first clearance period (Fisher’s LSD test).
that was observed during the first 30-min clearance period and maintained for the duration of the experiment (Fig. 7).

Neither BG9719 nor furosemide markedly affected SBP, DBP, or MABP (Table 1). In this regard, BG9719 did not significantly affect DBP or MABP (Table 1). Compared with the vehicle control group, BG9719 slightly, but significantly ($P = 0.0212$; 2-F-ANOVA), reduced SBP. However, compared with the predrug clearance period, this effect of BG9719 was not significant. Compared with the vehicle control group, furosemide slightly, but significantly, decreased SBP and MABP ($P = 0.0352$ and $P = 0.0060$, respectively; 2-F-ANOVA); however, only in the case of MABP during the last clearance period was this effect significantly different from the predrug clearance period.

BG9719 did not significantly alter CVP, VEDP, VMDP, or VPSP (Table 2). Furosemide did not alter VPSP, but significantly reduced CVP, VEDP, and VMDP (Table 2; $P = 0.0239$, $P = 0.0259$, and $P < 0.0001$, respectively; 2-F-ANOVA).

Neither BG9719 nor furosemide significantly altered $dP/dt_{\text{max}}$ (Table 3), and BG9719 did not alter $-dP/dt_{\text{max}}$, duration of relaxation, half-time of relaxation, or $\tau$. In contrast, furosemide significantly decreased $-dP/dt_{\text{max}}$ ($P = 0.0046$; 2-F-ANOVA), duration of relaxation ($P = 0.0008$; 2-F-ANOVA), and half-time of relaxation ($P = 0.0238$; 2-F-ANOVA) and nearly significantly ($P = 0.0519$; 2-F-ANOVA) increased the time constant of relaxation (Table 3).

### Discussion

The major findings of the present study are that in a rat model of dilated cardiomyopathy in which animals were pre-treated with a loop diuretic and high sodium intake to mimic common clinical scenario, BG9719, a highly potent and selective $\alpha_1$ blocker, increased urine volume, fractional sodium excretion, GFR, and RBF, without increasing fractional potassium excretion or adversely affecting systolic/diastolic cardiac performance. Although furosemide, a loop diuretic often used in patients with left ventricular dysfunction, also increased urine volume and fractional sodium excretion, furosemide caused a significant decrease in GFR, RBF, and diastolic function and significantly increased potassium excretion. To our knowledge, this is the first study to 1) examine the effects of an $\alpha_1$ blocker on renal function in animals with left ventricular dysfunction; 2) examine the effects of an $\alpha_1$ blocker on cardiac function in the setting of cardiomyopathy; 3) compare head-to-head the renal effects of an $\alpha_1$ blocker versus a loop diuretic in cardiomyopathy; and 4) compare head-to-head the cardiac effects of an $\alpha_1$ blocker versus a loop diuretic in the setting of left ventricular dysfunction. Our results suggest that selective $\alpha_1$ blockers possess unique pharmacological properties that may translate into improved therapy for patients with impaired left ventricular function.

These studies were conducted in aged SHHF/Mcc-fa\(^{\text{op}}\) rats, an animal model of spontaneous heart failure developed by McCune et al. (1994, 1995). Although ventricular hypertrophy and dysfunction are observable at younger ages, lean, male SHHF/Mcc-fa\(^{\text{op}}\) rats usually die between 15 and 18 months of age of rapid-onset, severe heart failure (McCune et al., 1994, 1995). This time course of slowly progressive left ventricular dysfunction followed by a short period of rapidly developing and fatal severe heart failure presents a difficult challenge for protocol design. In this regard, the younger the animals are studied, the less severe is left ventricular dysfunction but the more robust is the model system, whereas the older the animals are studied, the greater is the extent of left ventricular dysfunction and therefore the greater is the likelihood of death of research animals either before or during acute experiments. Also, because animals achieve end stage heart disease at different ages, waiting too long results in unacceptable between-animal variability. These factors must be balanced in deciding at what age to study the animals.

In this study, the animals were approximately 14 months old at the time of the acute experiments and were not yet expressing fulminating heart failure. Even so, the 14-month-old SHHF/Mcc-fa\(^{\text{op}}\) rats used in our study had evidence of left ventricular dysfunction. Recently, we found that in 9-month-old spontaneously hypertensive rats and 9-month-old lean, male SHHF/Mcc-fa\(^{\text{op}}\) rats, $-dP/dt_{\text{max}}$ was 14.6 and 13.1 mm Hg/ms, respectively (Tofovic et al., 1999). In these same animals, $-dP/dt_{\text{max}}$ was 9.0 and 7.8 mm Hg/ms, respectively. In the present study, in 14-month-old lean, male SHHF/Mcc-fa\(^{\text{op}}\) rats, $-dP/dt_{\text{max}}$ and $dP/dt_{\text{max}}$ were approximately 9.2 and 5.7 mm Hg/ms, respectively. Thus, compared with 9-month-old spontaneously hypertensive rats and 9-month-old SHHF/Mcc-fa\(^{\text{op}}\) rats, the 14-month-old SHHF/Mcc-fa\(^{\text{op}}\) rats used in the present study were beginning to demonstrate systolic/diastolic dysfunction.

#### Table 1

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*Indicates significantly different from clearance period 1 (Fisher's LSD).
Responses to A1 blockers may be influenced by salt intake (Zou et al., 1999). Therefore, renal adenosine levels are markedly increased by the effects of BG9719 in animals ingesting a high NaCl intake is often used in high doses and chronically in patients with heart failure. Inappropriately high NaCl intake is common in patients being treated with diuretics and contributes to diuretic resistance (Kramer et al., 1999). Therefore, in the present study, rats were provided 1% NaCl as drinking water to mimic the clinical scenario of diuretic therapy plus inappropriate NaCl intake. Another reason for examining the effects of BG9719 in animals ingesting a high NaCl intake is that renal adenosine levels are markedly increased by chronic salt loading (Sirag and Linden, 1996; Zou et al., 1999) and salt loading profoundly down-regulates A1 receptors in the renal cortex (Zou et al., 1999). Therefore, renal responses to A1 blockers may be influenced by salt intake because both the endogenous ligand and its receptor are altered. Yet another reason for administering 1% NaCl was to prevent sodium and volume depletion, which could have prevented diuretic and natriuretic effects of both acute furosemide and BG9719.

In our study, blockade of A1 receptors with BG9719 caused on average an 8-fold increase in sodium excretion without increasing fractional excretion of potassium. Although we (Kuan et al., 1993) and others (Shimada et al., 1991; Knight et al., 1993) have reported similar findings following A1 receptor blockade in normal rats, our study establishes that the natriuretic response to A1 receptor blockade without increasing potassium excretion is preserved in the setting of left ventricular dysfunction with prior high-dose loop diuretic administration and high NaCl intake. These data comple-

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<td>−6.7 ± 2.0</td>
<td>−6.6 ± 2.0</td>
<td></td>
</tr>
<tr>
<td>Furosemide</td>
<td>−2.2 ± 2.0</td>
<td>−4.0 ± 1.7</td>
<td>−4.7 ± 2.1</td>
<td>−5.2 ± 2.1</td>
<td>−4.6 ± 2.1</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

* indicates significantly different from clearance period 1 (Fisher’s LSD).

TABLE 3
Systolic and diastolic heart performance
Values represent means ± S.E.M.

<table>
<thead>
<tr>
<th>Group</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Period × Treatment Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>148 ± 9</td>
<td>157 ± 8</td>
<td>155 ± 9</td>
<td>159 ± 8</td>
<td>154 ± 10</td>
<td></td>
</tr>
<tr>
<td>BG9719</td>
<td>161 ± 4</td>
<td>165 ± 3</td>
<td>162 ± 4</td>
<td>160 ± 6</td>
<td>160 ± 7</td>
<td></td>
</tr>
<tr>
<td>Furosemide</td>
<td>153 ± 8</td>
<td>156 ± 10</td>
<td>161 ± 11</td>
<td>158 ± 14</td>
<td>152 ± 15</td>
<td></td>
</tr>
</tbody>
</table>

* indicates significantly different from clearance period 1 (Fisher’s LSD).

For clinical relevance, SHHF/Mcc-fa<sup>88</sup> rats were pretreated with high doses of furosemide. Furosemide is a diuretic that is often used in high doses and chronically in patients with heart failure. Inappropriately high NaCl intake is common in patients being treated with diuretics and contributes to diuretic resistance (Kramer et al., 1999). Therefore, in the present study, rats were provided 1% NaCl as drinking water to mimic the clinical scenario of diuretic therapy plus inappropriate NaCl intake. Another reason for examining the effects of BG9719 in animals ingesting a high NaCl intake is that renal adenosine levels are markedly increased by chronic salt loading (Sirag and Linden, 1996; Zou et al., 1999) and salt loading profoundly down-regulates A1 receptors in the renal cortex (Zou et al., 1999). Therefore, renal responses to A1 blockers may be influenced by salt intake because both the endogenous ligand and its receptor are altered. Yet another reason for administering 1% NaCl was to prevent sodium and volume depletion, which could have prevented diuretic and natriuretic effects of both acute furosemide and BG9719.

In our study, blockade of A1 receptors with BG9719 caused on average an 8-fold increase in sodium excretion without increasing fractional excretion of potassium. Although we (Kuan et al., 1993) and others (Shimada et al., 1991; Knight et al., 1993) have reported similar findings following A1 receptor blockade in normal rats, our study establishes that the natriuretic response to A1 receptor blockade without increasing potassium excretion is preserved in the setting of left ventricular dysfunction with prior high-dose loop diuretic administration and high NaCl intake. These data comple-
ventricular relaxation and the increase in the time constant evidenced by the reduction in maximum rate of decrease in RBF and reduced the ability of the left ventricle to relax, as performance. In contrast, furosemide decreased GFR and GFR and RBF and did not adversely affect diastolic cardiac performance. The striking aspect of the present study is the contrast between the effects of BG9719 and furosemide on renal hemodynamics and cardiac diastolic function. BG9719 increased GFR and RBF and did not adversely affect diastolic cardiac performance. In contrast, furosemide decreased GFR and RBF and reduced the ability of the left ventricle to relax, as evidenced by the reduction in maximum rate of decrease in intraventricular pressure, the decrease in the duration of ventricular relaxation and the increase in the time constant of relaxation.

The ability of BG9719 to increase GFR and RBF is noteworthy. A1 receptors on the preglomerular microvessels cause renal vasoconstriction (Jackson, 1997, 2001) and facilitate the ability of angiotensin II to constrict preglomerular microvessels (Jackson, 1997, 2001). As mentioned above, a high salt intake increases renal adenosine levels (Siragy and Linden, 1996; Zou et al., 1999). Blockade of renal A1 receptors, therefore, would be expected to decrease preglomerular renal vascular resistance, thus leading to an increase in RBF. Also, a reduction in preglomerular renal vascular resistance would be expected to increase glomerular capillary pressure and, consequently, increase GFR. Surprisingly, most studies with selective A1 blockers failed to demonstrate changes in RBF or GFR (Jackson, 1997, 2001). Nonetheless, Wilcox et al. (1999) observed a positive effect of BG9719 on GFR in a micropuncture study in rats, and Gottlieb et al. (2001) reported that in heart failure patients on a 30-mEq sodium intake, BG9719 increased creatinine clearance. The significance of the present study is that it clearly demonstrates in left ventricular dysfunction plus chronic loop diuretic treatment plus NaCl loading that BG9719 increases, rather than decreases, RBF and GFR.

In contrast to BG9719, furosemide decreased RBF and GFR. This acute effect of furosemide on RBF and GFR has been noted by others (Tucker and Blantz, 1984); the mechanism, although, remains ill defined. Inasmuch as the adverse effects of loop diuretics on renal hemodynamics are not observed in the isolated, perfused kidney (Johnsson and Haraldsson, 1992) and can be ameliorated by administration of fluids (Tucker and Blantz, 1984), it seems likely that the adverse effects of furosemide on renal hemodynamics are mediated by systemic changes, such as activation of the sympathetic nervous system. It is important to note that furosemide lowered GFR and RBF and increased renal vascular resistance during the first clearance period after administration of furosemide and that this response did not increase over the subsequent clearance periods. Because volume depletion would be minimal just after furosemide administration and would increase during subsequent clearance periods, there appears to be no relationship between volume depletion and the renal hemodynamic effects of furosemide. Therefore, other mechanisms, such as direct preload reduction due to increased venous compliance, may account for the adverse effects of furosemide on renal hemodynamics. Indeed, in the present study, furosemide, but not BG9719, significantly decreased all indices of preload including CVP, VEDP, and VMDP. Thus, when it is desirable to avoid rapid changes in preload, antagonism of A1 receptors may be a rational alternative to loop diuretics.

Patients hospitalized for heart failure are at high risk of worsening renal function. Moreover, studies by Krumholz et al. (2000) 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 to 17 days and mortality is increased (relative risk = 5.2) (Weinfeld et al., 1999). Thus, it is conceivable that A1 blocker therapy in hospitalized patients with heart failure may reduce morbidity/mortality relative to standard diuretic therapy by mobilizing edema while increasing, rather than decreasing, renal function.

The effects of drugs on diastolic cardiac performance has not received the same level of scrutiny as drug action on systolic performance. In particular, we are not aware of any studies examining the effects of either loop diuretics or A1 blockers on diastolic cardiac function. Because catecholamines increase cardiac relaxation (Walsh, 1990) and furosemide activates the sympathetic nervous system (Petersen and DiBona, 1995), we did not anticipate that furosemide would reduce the maximum rate of decrease in intraventricular pressure, decrease the duration of cardiac relaxation, and increase the time constant for relaxation. We do not know the mechanism of this effect; however, the marked contrast between furosemide and BG9719 in this regard could have important clinical implications, particularly in patients with diastolic, rather than systolic, left ventricular dysfunction.

In conclusion, the present study demonstrates in an animal model of left ventricular dysfunction and in the setting of chronic loop diuretic administration plus a high salt intake that antagonism of A1 receptors effectively increases sodium excretion without significantly increasing potassium excretion. Moreover, in contrast to loop diuretics, the natriuretic effect of A1 receptor antagonism is accompanied by an increase, rather than a decrease, in GFR and RBF and by a preservation of preload and diastolic cardiac performance.

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