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Dual A_1/A_{2B} Receptor Blockade Improves Cardiac and Renal Outcomes in a Rat Model of Heart Failure with Preserved Ejection Fraction

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ABSTRACT

Heart failure with preserved ejection fraction (HFpEF) is prevalent and often accompanied by metabolic syndrome. Current treatment options are limited. Here, we test the hypothesis that combined A_1/A_{2B} adenosine receptor blockade is beneficial in obese ZSF_1 rats, an animal model of HFpEF with metabolic syndrome. The combined A_1/A_{2B} receptor antagonist 3-[4-(2,6-dioxo-1,3-dipropyl-7*H*-purin-8-yl)-1-bicyclo[2.2.2]octanyl]propanoic acid (BG9928) was administered orally (10 mg/kg/day) to obese ZSF_1 rats ($n = 10$) for 24 weeks (from 20 to 44 weeks of age). Untreated ZSF_1 rats ($n = 9$) served as controls. After 24 weeks of administration, BG9928 significantly lowered plasma triglycerides (in mg/dl: control group, 4351 ± 550; BG9928 group, 2900 ± 551) without adversely affecting plasma cholesterol or activating renin release. BG9928 significantly decreased 24-hour urinary glucose excretion (in mg/kg/day: control group, 823 ± 179; BG9928 group, 196 ± 80) and improved oral glucose tolerance, polydipsia, and polyuria. BG9928 significantly augmented left ventricular diastolic function in association with a reduction in cardiac vasculitis and cardiac necrosis. BG9928 significantly reduced 24-hour urinary protein excretion (in mg/kg/day: control group, 1702 ± 263; BG9928 group, 1076 ± 238), and this was associated with a reduction in focal segmental glomerulosclerosis, tubular atrophy, tubular dilation, and deposition of proteinaceous material in the tubules. These findings show that, in a model of HFpEF with metabolic syndrome, A_1/A_{2B} receptor inhibition improves hyperlipidemia, exerts antidiabetic actions, reduces HFpEF, improves cardiac histopathology, and affords renal protection. We conclude that chronic administration of combined A_1/A_{2B} receptor antagonists could be beneficial in patients with HFpEF, in particular those with comorbidities such as obesity, diabetes, and dyslipidemias.

Introduction

A_1 receptor antagonists may be useful diuretics in heart failure with reduced ejection fraction (HFrEF). For example, in salt-loaded and loop diuretic–treated obese rats with spontaneously hypertensive heart failure (a rat model of HFrEF), selective A_1 receptor antagonism induces diuresis and natriuresis while exerting favorable effects on renal and cardiac hemodynamics (Jackson et al., 2001). In patients with HFrEF due to dilated cardiomyopathy or ischemic heart disease, A_1 blockade increases sodium excretion without worsening renal function (Gottlieb et al., 2011). In a large phase III clinical trial in patients with acute heart failure and renal impairment, although an A_1 receptor antagonist did not significantly affect the primary endpoint, the percentage of patients experiencing treatment success was significantly increased (Massie et al., 2010), and a subsequent analysis of this study (Metra et al., 2011) demonstrated an increase in the proportion of patients showing early relief from dyspnea and with a numerically lower mortality at 14 and 30 days, largely because of reduced heart failure mortality.

Although A_1 receptor antagonism appears to be useful in patients with HFrEF, whether this class of drugs is beneficial in patients with heart failure with preserved ejection fraction (HFpEF; also known as diastolic dysfunction) is unknown.

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ABBREVIATIONS: ATL-801, (*N*-[5-(1-cyclopropyl-2,6-dioxo-3-propyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-pyridin-2-yl]-*N*-ethyl-nicotinamide; BG9719, *S*)-1,3-dipropyl-8-[2-(5,6-epoxynorbornyl)] xanthine; BG9928, 3-[4-(2,6-dioxo-1,3-dipropyl-7*H*-purin-8-yl)-1-bicyclo[2.2.2]octanyl]propanoic acid; DBP, diastolic blood pressure; dP/dt, maximum rate of rise in intraventricular pressure during ventricular contraction; –dP/dt, maximum rate of fall in intraventricular pressure during ventricular relaxation; GFR, glomerular filtration rate; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; MABP, mean arterial blood pressure; OGTT, oral glucose tolerance test; SBP, systolic blood pressure; Tau, time constant of ventricular relaxation; VEGF, vascular endothelial growth factor.;

Because HFpEF is detected in more than 50% of all heart failure patients, and because the prevalence of HFpEF is growing relative to HFrEF (Owan et al., 2006; Borlaug and Paulus, 2011), it is important to evaluate the role of A_1 receptor antagonists in preclinical models of HFpEF. Therefore, one goal of the present study was to examine the effects of A_1 receptor antagonism in an animal model of HFpEF.

Another goal of the present study was to investigate the unique concept that combining A_1 and A_{2B} receptor antagonism could yield additional beneficial effects in heart failure patients with metabolic syndrome. The rationale for this objective is that A_{2B} receptors modulate insulin resistance and gluconeogenesis (Yasuda et al., 2003; Figler et al., 2011) and thus have an impact on glucose homeostasis (Rusing et al., 2006). Blockade of A_{2B} receptors may result in increased insulin sensitivity, decreased glucose production, and improved control of diabetes mellitus. Although A_{2B} receptors can induce coronary vasodilation, under most circumstances, A_{2A} receptors are predominant in this regard (Mustafa et al., 2009). Therefore, blocking A_{2B} receptors should have little adverse effect on coronary flow, particularly in the presence of A_1 receptor antagonism. Also, although acute A_{2B} receptor stimulation inhibits growth of cardiac fibroblasts and vascular smooth muscle cells (Dubey et al., 1996, 1997, 1998a,b), long-term A_{2B} receptor stimulation promotes inflammation and fibrosis (Mustafa et al., 2007; Dai et al., 2011; Zhang et al., 2013). Thus, long-term antagonism of A_{2B} receptors could improve target organ histology.

To achieve our twin goals, in the present study, we examined the chronic effects of 3-[4-(2,6-dioxo-1,3-dipropyl-7*H*-purin-8-yl)-1-bicyclo[2.2.2]octanyl]propanoic acid (BG9928) on cardiac and renal structure/function and metabolic status in aging, obese ZSF_1 rats. BG9928 is a high-affinity A_1 receptor antagonist that also blocks A_{2B} receptors (Kiesman et al., 2006), and obese ZSF_1 rats are genetically disposed to develop HFpEF, chronic renal insufficiency, vascular endothelial dysfunction, hypertension, hypertriglyceridemia, hypercholesterolemia, hyperglycemia, and insulin resistance (Tofovic and Jackson, 2003; Bilan et al., 2011; Hamdani et al., 2013; Leite et al., 2015a,b).

Materials and Methods

The experimental procedures and protocols used in this study were reviewed and approved by the Animal Care and Use Committee of the University of Pittsburgh School of Medicine. The investigation conforms with the *Guide for the Care and Use of Laboratory Animals 8th Edition 2011* published by the National Research Council.

Animals and Treatments. Studies were conducted in 20-week-old, male, obese (body weight 593 ± 3 g), ZSF_1 rats (Genetic Models, Indianapolis, IN). Ten rats received BG9928 (10 mg/kg/day; Biogen Idec, Inc., Cambridge, MA) in their food (BG9928 group), and 10 rats did not (control group). Treatments were continued for 24 weeks. One obese ZSF_1 rat in the control group died during the 24-week treatment period; therefore, the control group had $n = 9$.

Metabolism Cage Studies. After 24 weeks of treatment, rats were placed in metabolic cages. Body weight, 24-hour food and water intake, and urine output were determined. Urine samples were collected and assessed for creatinine, glucose, and total protein. Following the metabolic cage studies, animals were fasted overnight, and the BG9928 group received BG9928 orally by gavage. Rats were anesthetized with halothane, and a 32-gauge needle was inserted into an exposed jugular vein for the collection of 2 ml of blood for analysis of creatinine, triglycerides, cholesterol, and glucose.

Plasma samples were analyzed in duplicate for triglycerides and cholesterol (Sigma-Aldrich, St. Louis, MO), creatinine levels (Beckman creatinine analyzer; Beckman Instruments, Fullerton, CA), insulin (Incstar Corporation, Stillwater, MN), and plasma renin activity (radioimmunoassay; New England Nuclear, Waltham, MA). Glucose and total protein levels in urine were determined by Infinity glucose reagent (Sigma-Aldrich) and by modification of the Lowry method, respectively.

Oral Glucose Tolerance Test. Following metabolic cage studies, all rats were denied food overnight, and an oral glucose tolerance test (OGTT) was conducted. Animals were anesthetized with halothane between 8 AM and 10 AM, and a drop of blood was taken from the tail vein for the measurement of fasting glucose levels. Rats were given glucose (2 g/kg/ml) by oral gavage, and blood withdrawals were repeated after 30, 60, 90, and 120 minutes for the measurement of plasma glucose.

Acute Measurements of Blood Pressure, Heart Function, and Renal Hemodynamics. After 24 weeks of treatment, each rat was anesthetized with pentobarbital (45 mg/kg, i.p.) and instrumented for measurements of blood pressure, heart function, renal hemodynamics, and kidney function, as described previously (Tofovic et al., 2007; Bilan et al., 2011). In brief, a PE-240 polyethylene catheter (Becton, Dickinson and Company, Franklin Lakes, NJ) was inserted into the trachea to facilitate breathing. A polyethylene cannula (PE-50) was inserted into the left femoral artery and connected to a Micro-Med digital blood pressure analyzer (BPA 200; Micro-Med, Inc., Louisville, KY) for automatic and continuous monitoring of mean arterial blood pressure (MABP), systolic blood pressure (SBP), and diastolic blood pressure (DBP) and heart rate, which were integrated over 30-minute intervals. The right carotid artery was cannulated with a short section of PE-50 tubing, which was advanced into the left ventricle and connected to a heart-performance analyzer (HPA 400; Micro-Med, Inc.) for continuous measurement of pressure-time parameters of left ventricular function: heart rate, peak systolic pressure, end-diastolic pressure, maximum dP/dt during ventricular contraction, maximum negative dP/dt during ventricular relaxation (−dP/dt), and time constant of ventricular relaxation (Tau). These parameters were recorded electronically and stored in digital format (Excel; Microsoft, Redmond, WA). The HPA 400 heart performance analyzer used a modern high-sensitive ultra-low-volume transducer with displacement of 0.023 nl/mm Hg, thus permitting low-noise measurements even in small animals with an accuracy of ± 0.2 mm Hg in pressure measurements and 1–2% variations in slope measurements.

A PE-50 cannula was placed in the left jugular vein for infusion of ^{14}C-inulin. Next, through a midline abdominal incision, the left kidney was exposed, and a PE-10 catheter was inserted into the left ureter to facilitate the collection of urine. A flow probe (model 1RB; Transonic Systems, Inc., Ithaca, NY) was placed on the left renal artery for the determination of renal blood flow, which was recorded manually, and intravenous infusion of ^{14}C-inulin (0.035 μCi/50 μl saline/min) was initiated. A 60-minute stabilization period was permitted before two 30-minute clearance periods were conducted. SBP, MABP, and DBP were recorded continuously, and other hemodynamic parameters were recorded at 5-minute intervals and averaged during a 30-minute urine collection. A midpoint blood sample (100 μl) for measurement of radioactivity and hematocrit was collected. Urine volume was determined gravimetrically, and plasma and urine ^{14}C-inulin radioactivity were measured (liquid scintillation analyzer, model 2500TR; Packard Instrument Company, Downers Grove, IL). Inulin clearance [an estimate of glomerular filtration rate (GFR)] and renal vascular resistance were calculated. As there were no differences (time effects) between the two clearance periods, the presented data represent the average of the two clearance periods.

Histologic Evaluation. Rats were euthanized with an overdose of pentobarbital. Heart and kidneys were removed and placed in separate vials containing 10% formalin. Tissues were processed into paraffin blocks for light microscopy. Two histologic sections (3–5 μm thick) were cut and stained with H&E (kidney, heart) or methenamine silver–trichrome (kidney). Samples were analyzed in a blinded fashion by a histopathologist (E.M.S.). Histopathology scores were assessed semiquantitatively as 0 (absent), 0.5 (trace), 1 (mild), 2 (moderate), and

3 (severe) for renal histopathology, including focal general glomerular sclerosis, focal segmental glomerular sclerosis, interstitial fibrosis, tubular atrophy/dilation, proteinaceous material, other casts, glomerular and interstitial inflammation, vascular inflammation, and vascular hypertrophy. The same scale was used to assess cardiac histopathology, which included vascular, pericardial and endocardial inflammation, ischemic and necrotic degeneration, and vascular thickening and vasculitis.

Statistical Analysis. Data are expressed as the mean ± S.E.M. The BG9928 group was compared with the control group using a 2-tailed Student's unpaired *t* test. Statistical significance was at the 5% level ($P < 0.05$).

Results

Effects of Chronic BG9928 on Plasma Creatinine, Triglycerides, Cholesterol, and Renin Activity in Obese ZSF_1 Rats. In obese ZSF_1 rats lightly anesthetized with halothane, chronic BG9928 significantly decreased plasma creatinine, suggesting that BG9928 improved GFR (Fig. 1A). As previously reported, and compared with lean ZSF_1 rats (Rafikova et al., 2008), basal plasma triglyceride (4351 ± 550 mg/dl) and cholesterol (1199 ± 172 mg/dl) levels were extremely elevated in control obese ZSF_1 animals (Fig. 1, B and C, respectively). Chronic BG9928 significantly reduced the high triglyceride levels (Fig. 1B) but did not significantly alter plasma cholesterol (Fig. 1C). Importantly, chronic BG9928 did not increase plasma renin activity (Fig. 1D). These findings indicate that, in animals with severe dyslipidemia, chronic BG9928 exerts a beneficial effect on plasma triglycerides but does not chronically activate the renin-angiotensin system.

Effects of Chronic BG9928 on Food and Water Intake, Urine Volume and Urinary Excretion of Glucose, and Total Protein in Conscious Obese ZSF_1 Rats. In obese ZSF_1 rats, chronic BG9928 significantly increased body weight (Fig. 2A). This was not due to increased food intake or water intake since BG9928 tended ($P = 0.063$) to reduce food intake (Fig. 2B) and significantly decreased water intake (Fig. 2C). As previously reported, and compared with lean ZSF_1 rats (Rafikova et al., 2008), control obese ZSF_1 rats exhibited marked polydipsia (water intake of 122 ± 14 ml/kg/day), polyuria (urine output of 103 ± 11 ml/kg/day), glucosuria (823 ± 179 mg/kg/day), and proteinuria (1702 ± 263 mg/kg/day). BG9928 significantly reduced polydipsia, polyuria, urinary glucose excretion, and urinary protein excretion (Fig. 2, C–F), findings that are consistent with an antidiabetic and renoprotective action of BG9928.

Effects of Chronic BG9928 on OGTT in Obese ZSF_1 Rats. To confirm the presumed antidiabetic effects of chronic BG9928, obese ZSF_1 rats were lightly anesthetized with halothane and administered 2 g/kg of glucose orally while plasma glucose was monitored. In control obese ZSF_1 rats,

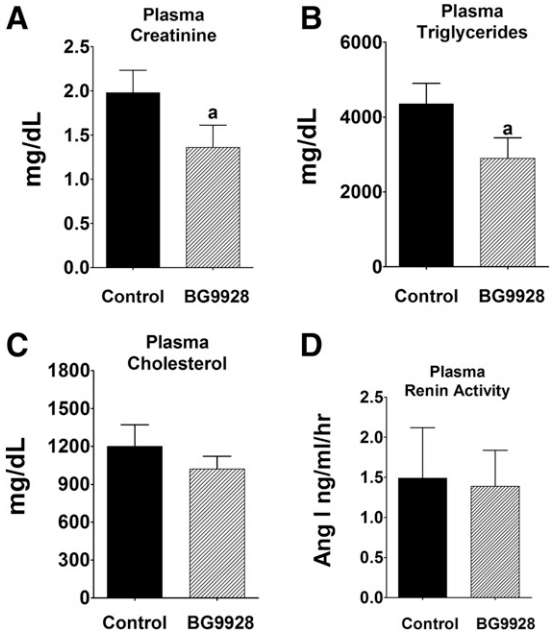

Fig. 1. Bar graphs show the effects of BG9928 on plasma creatinine (A), plasma triglycerides (B), total cholesterol (C), and plasma renin activity (D) in obese ZSF_1 rats under light halothane anesthesia. Values represent the mean ± S.E.M. (n = 9–10). a, $P < 0.05$ versus control. Reference values for age-matched lean nondiabetic ZSF_1 rats (n = 10): plasma creatinine, 0.6 ± 0.1 mg/dl; plasma triglycerides, 81 ± 13 mg/dl; and total cholesterol, 65 ± 3 mg/dl.

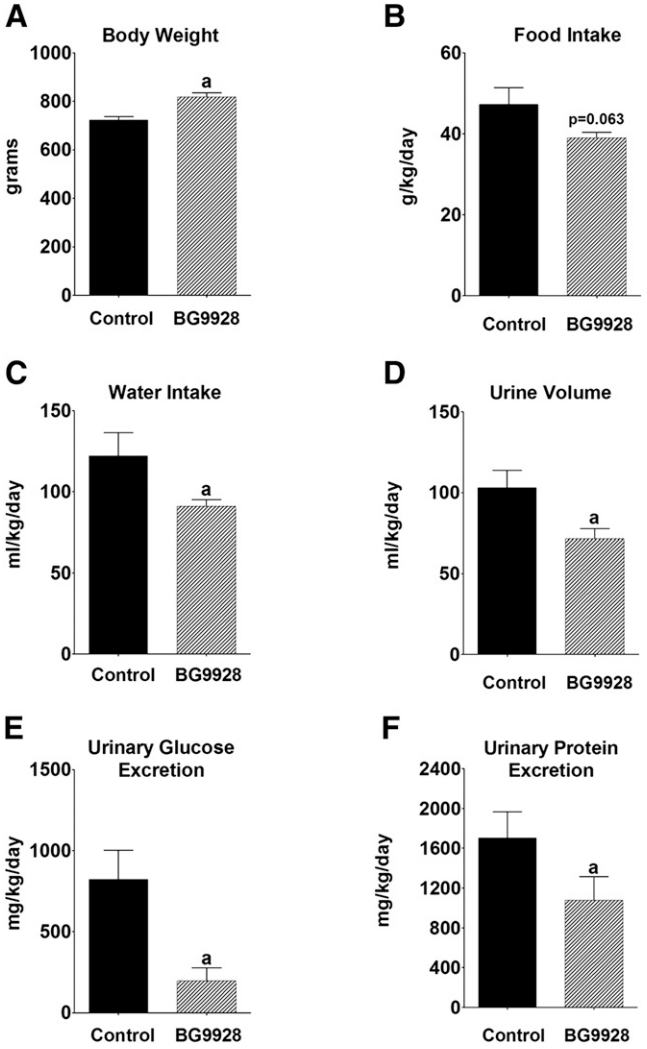

Fig. 2. Bar graphs show the effects of BG9928 on body weight (A), food intake (B), water intake (C), urine volume (D), urinary glucose excretion (E), and urinary protein excretion (F) in conscious obese ZSF_1 rats in metabolic cages. Values represent the mean ± S.E.M. (n = 9–10). a, $P < 0.05$ versus control. Reference values for age-matched lean nondiabetic ZSF_1 rats (n = 10): body weight, 530 ± 7 g; food intake, 41.7 ± 0.6 g/kg/day; water intake, 54.9 ± 2.1 ml/kg/day; urine volume, 30.2 ± 1.3 ml/kg/day; urinary glucose excretion, 0.006 ± 0.001 g/kg/day; and urinary protein excretion, 55 ± 3 mg/kg/day.

fasting plasma glucose levels were in the severely diabetic range (270 ± 14 mg/dl), and chronic BG9928 significantly reduced fasting plasma glucose levels (Fig. 3A). Although not statistically significant (P = 0.1438), BG9928 numerically increased plasma insulin levels (Fig. 3B). Chronic BG9928 also significantly improved glucose tolerance as indicated by a more rapid return to basal plasma glucose levels following a 2-g/kg oral glucose load (Fig. 3C). These studies confirmed the antidiabetic actions of chronic BG9928 in rats with metabolic syndrome.

Renal Hemodynamic Effects of Chronic BG9928 in Pentobarbital-Anesthetized Obese ZSF_1 Rats. Obese ZSF_1 rats were hypertensive as indicated by the high levels of SBP (190 ± 3 mm Hg), MABP (139 ± 2 mm Hg), and DBP (113 ± 2 mm Hg); however, chronic BG9928 did not affect SBP, MABP, or DBP (Fig. 4, A–C). Likewise, chronic BG9928 did not significantly alter inulin clearance (GFR), renal blood flow, or renal vascular resistance in pentobarbital-anesthetized obese ZSF_1 rats.

Cardiac Hemodynamic Effects of Chronic BG9928 in Pentobarbital-Anesthetized Obese ZSF_1 Rats. In obese ZSF_1 rats, chronic BG9928 did not affect left ventricular peak systolic pressure (Fig. 5A) or left ventricular maximum dP/dt during ventricular contraction (Fig. 5B), but did significantly improve left ventricular –dP/dt (Fig. 5C). Chronic BG9928 also reduced left ventricular end-diastolic pressure (Fig. 5D) and shortened the time constant (Tau) for left ventricular relaxation (Fig. 5E). These findings suggest that BG9928 exerts a beneficial effect on left ventricular diastolic function.

Effects of Chronic BG9928 on Kidney and Heart Histopathology. Importantly, chronic BG9928 significantly improved renal and cardiac histopathology. In the kidneys, chronic BG9928 significantly reduced focal segmental glomerular sclerosis (Fig. 6A), tubular atrophy and dilation (Fig. 6B), as well as proteinaceous casts (Fig. 6C). In the heart, chronic BG9928 significantly reduced histologic signs of cardiac ischemia (Fig. 6D) and markedly decreased cardiac necrosis (Fig. 6E) and cardiac vasculitis (Fig. 6F). Figure 7 illustrates H&E stains of the myocardium showing severe myocardial necrosis (Fig. 7A) and ischemia changes (Fig. 7B) in hearts from control ZSF_1 rats compared with moderate myocardial necrosis (Fig. 7C) and ischemic changes (Fig. 7D) in the myocardium from BG9928-treated ZSF_1 rats. Neither kidney weights nor heart weights were significantly altered by chronic BG9928 in ZSF_1 rats (kidney: 3.88 ± 0.17 mg/g body weight in controls vs. 3.62 ± 0.15 mg/g body weight in BG9928-treated animals; heart: 2.35 ± 0.06 mg/g body weight in controls vs. 2.29 ± 0.04 mg/g body weight in BG9928-treated animals).

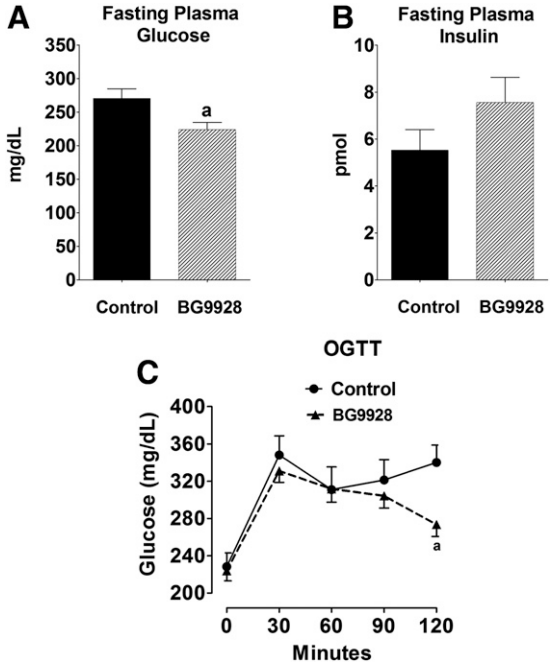

Fig. 3. Graphs show the effects of BG9928 on fasting plasma glucose (A), fasting plasma insulin (B), and time versus plasma glucose relationship following a 2-g/kg oral glucose load (C) in obese ZSF_1 rats under light halothane anesthesia. Values represent the mean ± S.E.M. (n = 9–10). a, $P < 0.05$ versus control. Reference values for age-matched lean nondiabetic ZSF_1 rats (n = 10): fasting plasma glucose, 134.2 ± 5.9 mg/dl and fasting plasma insulin, 0.79 ± 0.14 ng/ml.

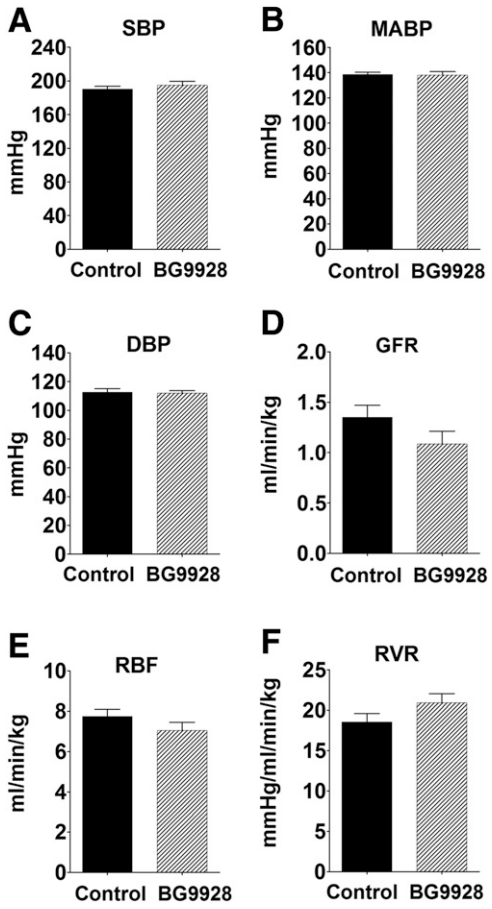

Fig. 4. Bar graphs show the effects of BG9928 on SBP (A), MABP (B), DBP (C), GFR (D), renal blood flow (RBF) (E), and renal vascular resistance (RVR) (F) in ZSF_1 rats under pentobarbital anesthesia. Values represent the mean ± S.E.M. (n = 9–10).

Discussion

In this study, we show that chronic (24 weeks) administration of an orally active and potent A_1/A_{2B} receptor antagonist (BG9928) improved meaningful outcomes in obese ZSF_1 rats, an animal model of HFpEF with diabetes and metabolic syndrome. This study is unique in that it examined the effects of combined A_1/A_{2B} receptor antagonism on clinically meaningful outcomes utilizing a model that best mimics the human

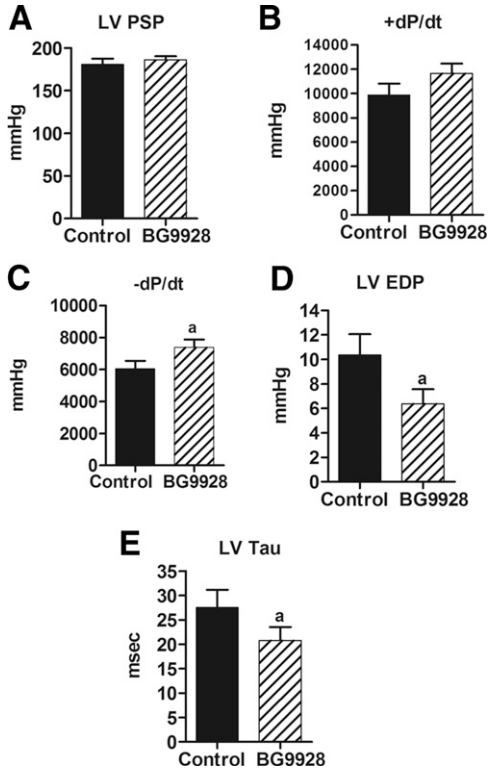

Fig. 5. Bar graphs show the effects of BG9928 on left ventricular peak systolic pressure, (A; LV PSP), +dP/dt (B), -dP/dt (C), left ventricular end diastolic pressure (D; LV EDP), and the constant of isovolumetric left ventricular relaxation (E; LV Tau) in obese ZSF_1 rats under pentobarbital anesthesia. Values represent the mean ± S.E.M. (n = 9–10). a, $P < 0.05$ versus control.

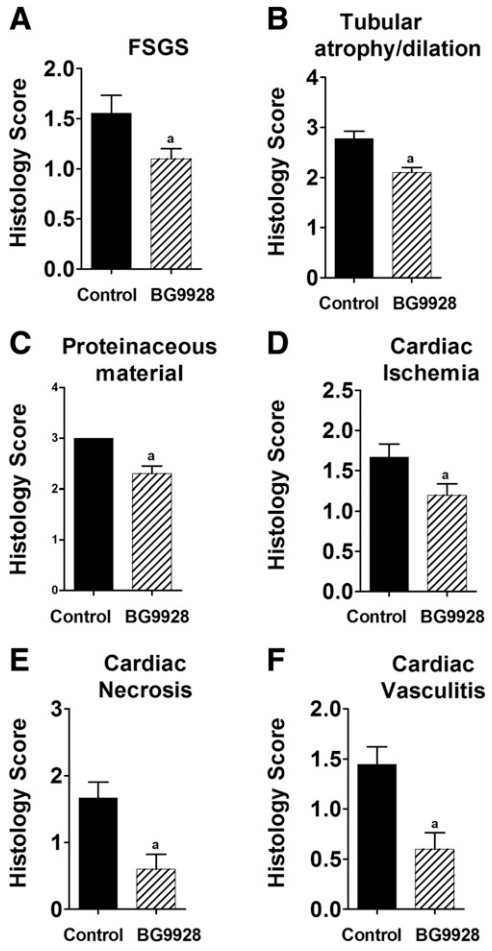

Fig. 6. Bar graphs show the effects of chronic BG9928 treatment on renal and cardiac histopathology scores (0 = absent; 0.5 = trace; 1 = mild; 2 = moderate; 3 = severe) in obese ZSF_1 rats: focal segmental glomerular sclerosis (FSGS) (A), tubular atrophy/dilation (B), proteinaceous casts (C), cardiac ischemia (D), cardiac necrosis (E), and cardiac vasculitis (F). Values represent the mean ± S.E.M. (n = 9–10). a, $P < 0.05$ versus control.

ailment of HFpEF in the setting of metabolic syndrome with diabetic nephropathy (Tofovic et al., 2000, 2007; Tofovic and Jackson, 2003; Hamdani et al., 2013).

An important finding of the present study is that BG9928 exerted significant long-term antidiabetic actions in rats with metabolic syndrome. This conclusion is supported by the observations that chronic BG9928 decreased glucosuria, polydipsia, polyuria, and fasting plasma glucose, and improved the oral glucose tolerance test. Compared with the control group, BG9928 also caused a significant gain in body weight, an effect that was unrelated to food or water intake. This too is consistent with an antidiabetic effect of BG9928, because decreased spillage of glucose into the urine would cause weight gain since the calories in glucose would be conserved. Indeed, long-term treatment of obese ZSF_1 rats with rosiglitazone nearly normalizes blood glucose and HbA1c, attenuates glycosuria, and is associated with a large increase in body weight (Bilan et al., 2011). A greater understanding of the metabolic effects of BG9928 requires future studies with more detailed metabolic measurements.

The antidiabetic effects of BG9928 are likely mediated through inhibition of both adenosine A_1 and adenosine A_{2B} receptors. Studies by Figler et al. (2011) demonstrated that, in mice fed a high-fat diet, treatment with the orally active A_{2B} receptor antagonist ATL-801 for 10 weeks significantly reduced diet-induced elevated fasting blood glucose. Adenosine stimulates hepatic glucose production via activation of A_{2B} receptors (Buxton et al., 1987; Yasuda et al., 2003; Rusing et al., 2006), and selective A_{2B} receptor antagonists produce hypoglycemia in diabetic mice (Harada et al., 2001). However, the role of A_{2B} receptors in glucose regulation is not without controversy. For example, Johnston-Cox et al. (2012) reported that A_{2B} receptor knockout mice fed a high-fat diet actually developed type 2 diabetes. With regard to the A_1 receptor, the oral glucose tolerance test in obese Zucker rats was improved following antagonism of adenosine A_1 receptors (Xu et al., 1998). To our knowledge, the present work represents the first study examining the effects of chronic combined A_1/A_{2B} receptor antagonism in a genetic rat model of metabolic syndrome and confirms that dual blockade of A_1/A_{2B} receptors is antidiabetic in this translational animal model. These findings are consistent with the observation that caffeine, a nonselective adenosine receptor antagonist with a rank order of antagonist potency of $A_1 > A_{2B}$, reduces glycosuria and improves OGTT in obese ZSF_1 rats (Tofovic et al., 2007).

Although combined A_1/A_{2B} receptor antagonism exerts an antidiabetic effect, there is concern that chronic A_{2B} antagonism could worsen the plasma lipid profile. In this regard, in A_{2B} receptor knockout mice fed a high-fat diet, plasma cholesterol and triglycerides are increased (Koupenova et al., 2012). However, we find that in obese ZSF_1 rats, BG9928 significantly lowers plasma triglycerides and tends (not

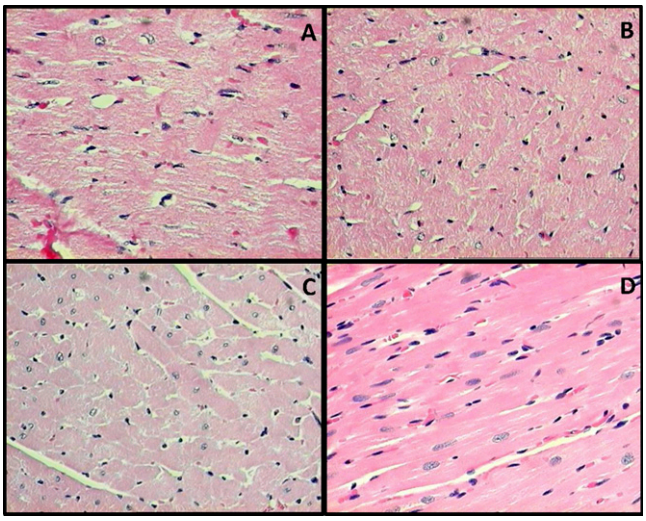

Fig. 7. H&E stains of myocardium illustrate the severe myocardial necrosis (A) and ischemia changes (B) in hearts from control ZSF_1 rats compared with moderate myocardial necrosis (C) and ischemic changes (D) in myocardium from BG9928-treated ZSF_1 rats.

significant) to lower plasma cholesterol. Thus, chronic combined A_1/A_{2B} receptor antagonism exerts a beneficial effect on plasma lipids in a model of metabolic syndrome with severe hyperlipidemia. Since A_1 receptors regulate lipolysis (Johansson et al., 2008; Dhalla et al., 2009; Szkudelski et al., 2009), the ability of BG9928 to antagonize A_1 receptors likely offsets any adverse effects of A_{2B} blockade on plasma lipids. This affords a great advantage of combined A_1/A_{2B} receptor antagonism compared with selective A_{2B} blockade.

The renin-angiotensin-aldosterone system plays a central role in electrolyte homeostasis, regulation of blood pressure, and development/progression of renal and heart disease. The level of activity of this system is determined primarily by the rate at which the kidneys secrete renin into the circulation. Adenosine and adenosine agonists inhibit renin release (Jackson, 2001), and short-term administration of A_1 antagonists increases release of renin (Pfeifer et al., 1995). Importantly, caffeine, a nonselective adenosine receptor antagonist, increases renal renin secretion in obese spontaneously hypertensive heart failure rats, a genetic model of congestive heart failure and metabolic syndrome (Tofovic et al., 1999). In the present study, 24 weeks of treatment with BG9928 did not affect plasma renin activity. This suggests that chronic BG9928 does not activate renin release, a driving force in the development and progression of cardiovascular and renal disease. Again, the unique combination of A_1 and A_{2B} blockade could be responsible for the neutral effects of BG9928 on renin release.

In obese ZSF_1 rats, the kidneys are histologically abnormal. Importantly, BG9928 improved renal histopathology, including reductions in focal segmental glomerulosclerosis, tubular atrophy and dilation, and proteinaceous casts. This is consistent with our observation that BG9928 also decreased urinary protein excretion and reduced plasma creatinine levels. Although BG9928 did not increase inulin clearance in pentobarbital-anesthetized obese ZSF_1 rats, this could be due to high levels of neurohumoral activation in the setting of pentobarbital anesthesia with extensive surgical manipulation.

Theoretically, BG9928 might increase pressure in the glomerular capillaries via inhibition of preglomerular vasoconstrictive A_1 receptors and thereby worsen glomerulosclerosis. The fact that glomerulosclerosis is improved suggests that BG9928 is renoprotective via A_{2B} receptors. A_{2B} receptors are expressed in the kidney (Jackson et al., 2002; Vitzthum et al., 2004), and activation of A_{2B} receptors promotes a higher expression of vascular endothelial growth factor (VEGF) in kidney glomeruli, particularly upon exposure to high glucose concentrations (Valladares et al., 2008). In addition, renal VEGF overproduction induces glomerular hypertrophy that is associated with proteinuria (Liu et al., 2007), and urinary excretion of VEGF correlates to the degree of proteinuria in both humans and rats (Cha et al., 2000, 2004). Likewise, inhibition of expression of renal VEGF is associated with reduced proteinuria and renal injury in aged obese ZSF_1 rats (Zhang et al., 2007). Furthermore, several lines of evidence suggest that, under conditions of increased extracellular adenosine (i.e., hyperglycemia, hypoxia, increased angiotensin II levels), A_{2B} signaling promotes renal fibrosis (Dai et al., 2011; Zhang et al., 2013; Roberts et al., 2014). Finally, although A_1 receptors constrict the preglomerular microcirculation, A_{2B} receptors dilate preglomerular microvessels (Feng and Navar, 2010). Thus, some of the beneficial effects of chronic treatment with BG9928 in the kidney may reflect balanced antagonism of adenosine A_1 and A_{2B} receptors.

Importantly, the present study provides evidence that BG9928 improves left ventricular diastolic dysfunction in ZSF_1 rats, a model of HFpEF with metabolic syndrome (Tofovic et al., 2000; Tofovic and Jackson, 2003; Bilan et al., 2011; Hamdani et al., 2013; Leite et al., 2015a,b). In this regard, we observed that 24 weeks of treatment with BG9928 reduced left ventricular end-diastolic pressure and the isovolumetric relaxation time constant (Tau) and –dP/dt. Consistent with these changes, we also found that BG9928 improved cardiac histopathology with significant reductions in cardiac vasculitis, degenerative ischemic changes, and necrosis. Nonetheless, there are limitations regarding the analysis of heart performance in the present study. Specifically, we assessed cardiac function using left ventricular pressure-time variables that reflect alterations in left ventricular performance. The mechanical behavior of the left ventricle during diastole is complex and affected by both active relaxation (Pasipoularides et al., 1986) and viscoelastic properties (Rankin et al., 1977) of the left ventricle. Although –dP/dt and Tau may be affected by changes in compliance (i.e., passive stiffness of the left ventricle), these parameters are mostly a measure of active relaxation, and therefore do not provide a direct measure of left ventricular compliance. Importantly, HFpEF in humans is due to both abnormalities in active relaxation and passive stiffness of the left ventricle (Zile et al., 2004). Therefore, the changes in –dP/dt and Tau observed with BG9928, whether due to improvements in active or passive properties of the left ventricle, would be of benefit in patients with HFpEF. Notably, Leite and coworkers evaluated HFpEF in obese ZSF_1 rats using both invasive pressure-volume analysis and noninvasive echocardiography. These investigators found significant correlations between invasively measured left ventricular end-diastolic pressure and echocardiographic parameters of left ventricular stiffness (Leite et al., 2015a). This suggests that the decreases in left ventricular end-diastolic pressure by BG9928 in the present

study were mediated in part by improvements in left ventricular compliance. More recently, Leite et al. (2015b) reported that, in obese ZSF_1 rats, left ventricular stiffness was accompanied by a prolonged Tau. In fact, Tau was sufficiently prolonged such that the predicted time for complete relaxation exceeded effective filling time and thus contributed to the rise in left ventricular end-diastolic pressure. Therefore, the finding that BG9928 reduces Tau may have important implications for reducing left ventricular end-diastolic pressure and improving symptoms in HFpEF.

Our cardiac findings with BG9928 are consistent with several other studies. Our previous studies showed that 1,3-dipropyl-8-p-sulfophenylxanthine, a nonselective adenosine receptor blocker, reduced infarct size in closed-chest dogs subjected to 90 minutes of left anterior descending coronary artery occlusion and 72 hours of reperfusion (Forman et al., 2000). This effect may be mediated by a reduction in the chemoattractant response of neutrophils (Forman et al., 2000). Studies by Auchampach et al. (2004) in anesthetized dogs revealed that neither BG9719 (selective A_1 antagonist) nor BG9928 (A_1/A_{2B} antagonist) affected infarct size reduction by multiple-cycle ischemic preconditioning. In the absence of preconditioning, pretreatment with BG9928 before occlusion or immediately before reperfusion significantly reduced infarct size, whereas treatment with BG9719 was without effect. Taken together with the present study, it appears that inhibition of A_{2B} signaling provides cardioprotective effects. Indeed, recent studies show that blockade of A_{2B} receptors reduces left ventricular dysfunction and remodeling after myocardial infarction in mice and rats (Toldo et al., 2012; Zhang et al., 2014).

In the PROTECT study, rolofylline (a selective A_1 antagonist similar in structure to BG9928) induced seizures in 0.8% of heart failure patients (Massie et al., 2010), suggesting that blockade of A_1 receptors in the brain decreases seizure threshold. Although this is a limitation of this class of drugs, given the lack of treatments for HFpEF and the poor prognosis in such patients (5-year survival rate of 43%) (Tribouilloy et al., 2008), the risk of central nervous system side effects may be acceptable. Also, the design of A_1/A_{2B} antagonists that do not penetrate the blood-brain barrier could eliminate the potential for adverse effects in the brain.

In conclusion, in heart and renal disease complicated by metabolic syndrome, chronic administration of BG9928 improves type 2 diabetes, lowers plasma triglycerides, attenuates renal and cardiac histopathology, and improves diastolic dysfunction in HFpEF. This study raises the novel concept that dual A_1/A_{2B} receptor antagonists may be useful for treating a range of cardiovascular diseases.

Authorship Contributions

Participated in research design: Tofovic, Jackson, Smits, Whalley, Ticho.

Conducted experiments: Tofovic, Salah, Jackson.

Performed data analysis: Tofovic, Jackson, Salah, Smits, Whalley, Ticho.

Wrote or contributed to the writing of the manuscript: Jackson, Tofovic, Smits, Whalley, Ticho, Deykin.

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