Effects of Novel Vasopressin Receptor Antagonists on Renal Function and Cardiac Hypertrophy in Rats with Experimental Congestive Heart Failure


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ABSTRACT

Arginine vasopressin (AVP) plays an important role in renal hemodynamic alterations, water retention, and cardiac remodeling in congestive heart failure (CHF). The present study evaluated the acute and chronic effects of vasopressin V1a receptor subtype (V1a) and vasopressin V2 receptor subtype (V2) antagonists on renal function and cardiac hypertrophy in rats with CHF. The effects of acute administration of SR 49059 [(2S)-1{[2R,3S]-5-chloro-3-(2-chlorophenyl)-1-(3,4-dimethoxybenzene-sulfonyl)-3-hydroxy-2,3-dihydro-1H-indole-2-carbonyl]-pyrrolidine-2-carboxamide}] (0.1 mg/kg) and SR 121463B [1-{[4-(N-tert-butylcarbamoyl)-2-methoxybenzenesulfonyl]-5-ethoxy-3-spiro-[4-(2-morpholinoethoxy)cyclohexane]indol-2-one, fumarate; equatorial isomer} (0.3 mg/kg) and SR 49059, (2R)-3-((N-pyrrolidine-2-carboxamide) (0.1 mg/kg) and SR 121463B, 1-[4-([2R,3S]-5-chloro-3-(2-chlorophenyl)-1-(3,4-dimethoxybenzenesulfonyl)-3-hydroxy-2,3-dihydro-1H-indole-2-carbonyl]-pyrrolidine-2-carboxamide)] (0.1 mg/kg) and SR 121463B, 1-[4-(N-tert-butylcarbamoyl)-2-methoxybenzenesulfonyl]-5-ethoxy-3-spiro-[4-(2-morpholinoethoxy)cyclohexane]indol-2-one, fumarate; equatorial isomer} (0.3 mg/kg), V1a and V2 antagonists, respectively, on renal function, and of chronic treatment (3.0 mg/kg/day for 7 or 28 days, via osmotic minipumps or p.o.), on water excretion and cardiac hypertrophy were studied in rats with aortocaval fistula and control rats. CHF induction increased plasma AVP (12.8 ± 2.5 versus 32.2 ± 8.3 pg/ml, p < 0.05). Intravenous bolus injection of SR 121463B to controls produced dramatic diuretic response (from 5.5 ± 0.8 to 86.3 ± 21.9 μl/min; p < 0.01). In contrast, administration of SR 49059 did not affect urine flow. Likewise, administration of SR 121463B, but not SR 49059, to rats with CHF significantly increased urinary flow rate from 20.8 ± 6.4 to 91.6 ± 26.5 μl/min (p < 0.01). Diuretic effects of SR 121463B were associated with a significant decline in urinary osmolality and insignificant change of Na+ excretion. In line with its acute effects, chronic administration of SR 121463B to CHF rats increased daily urinary volume 2 to 5-fold throughout the treatment period. Both SR 121463B and SR 49059 significantly reduced heart weight in CHF rats when administered for 4 weeks, but not 1 week. These results suggest that V2 and V1a antagonists improve water balance and cardiac hypertrophy in CHF and might be beneficial for the treatment of water retention and cardiac remodeling in CHF.

The importance of the neurohumoral vasoconstrictor systems in the pathogenesis of congestive heart failure (CHF) has been recognized (Francis et al., 1984; Dzau, 1987; Packer, 1992; Shigematsu et al., 2001). In particular, the dominant role of the renin angiotensin aldosterone system (RAAS) and sympathetic nervous system (SNS) in the development of cardiovascular and renal dysfunction in CHF has been established and angiotensin-converting enzyme inhibitors and β-blockers serve as key therapies in clinical CHF (Packer, 1992; Shigematsu et al., 2001; Jessup and Brozena, 2003; Katz, 2003). Although playing an important compensatory role in the early stages of the disease, prolonged activation of these systems has detrimental effects on the kidney and cardiovascular system. For instance, RAAS and SNS are involved in the induction of cardiac hypertrophy in CHF

ABBREVIATIONS: CHF, congestive heart failure; RAAS, rennin angiotensin aldosterone system; SNS, sympathetic nervous system; RBF, renal blood flow; GFR, glomerular filtration rate; AVP, arginine vasopressin; CD, collecting duct; AQP2, aquaporin 2; V1a, vasopressin V1a receptor subtype; V2, vasopressin V2 receptor subtype; SR 49059, (2S)-1{[2R,3S]-5-chloro-3-(2-chlorophenyl)-1-(3,4-dimethoxybenzene-sulfonyl)-3-hydroxy-2,3-dihydro-1H-indole-2-carbonyl]-pyrrolidine-2-carboxamide}; SR 121463B, 1-{[4-(N-tert-butylcarbamoyl)-2-methoxybenzenesulfonyl]-5-ethoxy-3-spiro-[4-(2-morpholinoethoxy)cyclohexane]indol-2-one, fumarate; equatorial isomer; ACF, aortocaval fistula; UNaV, urinary sodium excretion; FeNa, fractional sodium excretion; MAP, mean arterial pressure; RVR, renal vascular resistance; V, urinary flow rate; Uosm, urinary osmolality; Ang, angiotensin; ET, endothelin; YM087, conivaptan hydrochloride; ANOVA, analysis of variance.
In particular, the kidney is highly sensitive to the actions of the vasoconstrictor agent, and a decrease in renal blood flow (RBF) is one of the most common alterations in clinical and experimental CHF (Davis, 1965). Impaired renal function and reduced glomerular filtration rate (GFR) are actually considered as strong independent predictors of mortality in patients with CHF (Hillega et al., 2000). Yet, the RAAS and SNS comprise only two of the three major components originally proposed to link neurohormonal activation to CHF (Francis et al., 1984). The third component is the arginine vasopressin (AVP), whose circulating levels are also elevated in clinical CHF (Szlatalewicz et al., 1981; Goldsmith et al., 1983). Increased activity of the vasoconstrictor systems in general and AVP in particular leads to salt and water retention by the kidney, thereby causing a further deterioration in cardiac performance (Dzau, 1987; Schrier et al., 2001). AVP is secreted from the posterior pituitary gland into the circulation in response to increased plasma osmolality or hypovolemia/hypotension (Robertson 1987). AVP exerts its biological actions through at least three different G-protein-coupled receptors (Birnbaumer, 2000). V1a and V2 are abundantly expressed in the cardiovascular system and the kidney and mediate the main biological actions of AVP, namely, vasconstriction and water retention. The V1a receptor operates through the phosphoinositide signaling pathway, causing release of intracellular Ca2+ ions (Burrell et al., 1994). The V2 receptor, found in the renal collecting duct (CD) cells, is linked to the adenylyl cyclase pathway, using cAMP as its second messenger. Activation of this receptor leads to the formation of membrane vesicles containing aquaporin 2 (AQP2) water channels located in the subapical domain of CD principal cells to fuse with the apical membrane, thus increasing water permeability of the CD (Bankir, 2001; Wong and Verbalis, 2001). V1b receptors are involved in the stimulating effect of AVP on adrenocorticotropic hormone secretion.

Although it is well documented that AVP plays an essential role in the development of water retention and subsequently hyponatremia in advanced CHF (Goldsmith, 1999; Lee et al., 2003), its relative contribution to the cardiovascular and renal dysfunction in CHF in earlier stages of the disease is less understood compared with the other vasoconstrictor systems. In part, this was due to the lack of effective pharmacologic blockers of AVP. Recent advancements in the development of highly selective V1a and V2 vasopressin-receptor antagonists provide us with the opportunity to evaluate the role of AVP in the pathogenesis of renal and cardiovascular manifestations of CHF.

Thus, in the present study, we studied the acute and chronic effects of SR 49059 and SR 121463B, V1a and V2 antagonists, respectively, on renal function and cardiac hypertrophy in rats with aortocaval fistula (ACF), an experimental model of CHF. We have previously shown that this experimental model of CHF closely mimics renal and cardiac manifestations of advanced clinical CHF (Winaver et al., 1988; Brodsky et al., 1998). After tracheostomy, polyethylene tubes (PE50) were inserted into the carotid artery, jugular vein, and urinary bladder, for blood pressure monitoring, infusion of various solutions, and urine collections, respectively. A solution of 2% of inulin in 0.9% saline was continuously infused at a rate of 1.0 to 1.5% of body weight/h throughout the experiment. After a 60-min equilibration period, two baseline clearance periods of 20 min each were obtained. Then, one of the following drugs or vehicle (0.2 ml of saline) was administered to control and CHF rats; SR 49059 was administered i.v. at a dose of 0.1 mg/kg, and SR 121463B was administered i.v. at a dose of 0.3 mg/kg, based on the protocol of Huang et al. (2000) and Serradeil-Le Gal et al. (1993, 1996). Two or three additional clearance periods were obtained under the influence of the drug. Urine volume was determined gravimetrically, infusion of various solutions, and urine collections, respectively. A solution of 2% of inulin in 0.9% saline was continuously infused at a rate of 1.0 to 1.5% of body weight/h throughout the experiment. After a 60-min equilibration period, two baseline clearance periods of 20 min each were obtained. Then, one of the following drugs or vehicle (0.2 ml of saline) was administered to control and CHF rats; SR 49059 was administered i.v. at a dose of 0.1 mg/kg, and SR 121463B was administered i.v. at a dose of 0.3 mg/kg, based on the protocol of Huang et al. (2000) and Serradeil-Le Gal et al. (1993, 1996). Two or three additional clearance periods were obtained under the influence of the drug. Urine volume was determined gravimetrically, Blood samples were obtained in the midst of every second clearance period. Plasma samples were separated by centrifugation.

Materials and Methods

Studies were conducted on a local strain of male Sprague-Dawley rats (Harlan Laboratories, Ltd., Jerusalem, Israel), weighing 290 to 380 g. The animals were kept in individual metabolic cages in a temperature-controlled room and were fed standard rat chow containing 0.5% NaCl and tap water ad libitum. All experiments were performed according to the guidelines of the committee for the supervision of animal experiments, Technion, Israel Institute of Technology.

Acute Studies

These studies were designed to evaluate the acute effects of SR 121463B and SR 49059 (kindly supplied by Dr. C. Serradeil-Le Gal, Sanofi-Aventis, Toulouse cedex, France) on urine flow, urinary sodium excretion (UNaV), fractional sodium excretion (FENa), GFR, RBF, and mean arterial blood pressure (MAP) in rats with CHF compared with sham-operated controls.

Clearance Studies

Acute Effects of SR 49059 and SR 121463B on Clearance Parameters in Control and CHF Rats. On the 7th postoperative day, sham controls (n = 5) and CHF rats (n = 5) were anesthetized with Inactin (100 mg/kg i.p.), placed on a thermoregulated (37°C) surgical table, and prepared for hemodynamic and clearance studies (Brodsky et al., 1998). After tracheostomy, polyethylene tubes (PE50) were inserted into the carotid artery, jugular vein, and urinary bladder, for blood pressure monitoring, infusion of various solutions, and urine collections, respectively. A solution of 2% of inulin in 0.9% saline was continuously infused at a rate of 1.0 to 1.5% of body weight/h throughout the experiment. After a 60-min equilibration period, two baseline clearance periods of 20 min each were obtained. Then, one of the following drugs or vehicle (0.2 ml of saline) was administered to control and CHF rats; SR 49059 was administered i.v. at a dose of 0.1 mg/kg, and SR 121463B was administered i.v. at a dose of 0.3 mg/kg, based on the protocol of Huang et al. (2000) and Serradeil-Le Gal et al. (1993, 1996). Two or three additional clearance periods were obtained under the influence of the drug. Urine volume was determined gravimetrically. Blood samples were obtained in the midst of every second clearance period. Plasma samples were separated by centrifugation.

Measurements of Total RBF

Acute Effects of SR 49059 and SR 121463B on Renal Hemodynamics in Control and CHF Rats. On the 7th postoperative day, the acute effects of SR 121463B and SR 49059 on RBF, MAP, and renal vascular resistance (RVR) were evaluated in additional groups of rats with CHF (n = 5) and sham-operated controls (n = 5). For this purpose, rats were anesthetized, the jugular vein and ca-
rotid artery were cannulated, and their abdominal cavities were opened. After a 60-min equilibration period, baseline parameters were obtained. One of the following drugs was then administered to these rats: SR 49059 was administered i.v. at a dose of 0.1 mg/kg, and SR 121463B was administered i.v. at a dose of 0.3 mg/kg (based on the protocol of Serradeil-Le Gal C et al., 1993, 1996; Huang et al., 2000). Hemodynamic recordings were obtained for an additional 60 min after administration of the drugs. Measurement of RBF was performed by ultrasonic flowmeter (Transonic Systems Inc., Ithaca, NY) using a flow probe placed around the left renal artery of control and CHF rats (Abassi et al., 1997, 1998; Brodsky et al., 1998). MAP was continuously monitored through a pressure transducer, and RVR was calculated online by a computerized data acquisition system using the formula RVR = MAP/RBF.

### Chronic Studies

#### Plasma AVP Levels in Control and CHF Rats

Plasma levels of AVP were determined in sham-operated controls (n = 8) and rats with CHF (n = 12). Seven days after surgery, the animals were decapitated, and blood samples were collected into precooled tubes (K3EDTA) and immediately centrifuged at 4°C for 10 min at 3000 rpm. Plasma samples were stored at −70°C until analysis.

**Effects of Chronic Administration of SR 49059 and SR 121463B on Sodium Balance and Cardiac Hypertrophy in Rats with CHF.** This protocol is designed to examine the effects of chronic administration (7 and 28 days) of SR 49059 and SR 121463B on the development of sodium and water retention and cardiac hypertrophy in rats with CHF. Rats with CHF (n = 6–10) and sham-operated controls (n = 5) were prepared as described in the previous protocol. Before the operation, the rats were placed in individual metabolic cages for 2 days (days 1 and 0), for baseline measurements of urine volume and sodium excretion. Two experimental approaches were used. In the first, rats with CHF were treated with either SR 49059 or SR 121463B via osmotic minipumps beginning on the day of fistula placement. During the operation an osmotic minipumps (models 2001 and 2ML4; Alzet, Cupertino, CA), releasing either SR 49059 or SR 121463B, at a dose of 3.0 mg/kg/day for 7 and 28 days, or the vehicle, were inserted into the peritoneal cavity of the rat. In the second approach, an additional group of rats with ACF was subjected to treatment with SR 49059 or SR 121463B given orally (gavage at 10:00 AM) at similar doses for 7 days beginning on the same day of the creation of ACF.

After the operation, the rats were returned into their metabolic cages for an additional 6 days, during which measurements of urine volume and sodium excretion were performed. On days 7 or 28, the rats were anesthetized with Inactin (100 mg/kg i.p.), blood samples were collected via carotid artery for chemical analysis, and hearts were immediately removed and weighed for determination of heart/body weight ratio, an index of cardiac hypertrophy.

### Chemical Analysis

Concentrations of inulin in plasma and urine samples were measured by the colorimetric anthrone method (Abassi et al., 2001a,b). GFR was equated with the renal clearance of inulin. Sodium concentrations in plasma and urine were determined by flame photometry (model IL 943; Instrumentation Laboratories, Milano, Italy). The osmolality of plasma and urine samples was determined by a vapor pressure osmometer (model 5500; Wescor Inc., Logan, UT). Plasma levels of AVP were measured by a radiiodinnoassay method (Peninsula Laboratories, San Carlos, CA).

### Statistical Analysis

One-way analysis of variance (ANOVA) for repeated measures followed by Dunnett test was used for comparison of treatment values with baseline value in each group in the acute and chronic experiments. In addition, in some cases, we used the paired Student’s t test to compare each experimental value with its own baseline; for comparison of the graphs representing control and experimental groups in chronic experiments, two-way ANOVA was used. A value of p < 0.05 was considered statistically significant. Data are presented as mean ± S.E.M.

### Results

#### Acute Studies

**Clearance Studies.** Acute effects of SR 49059 and SR 121463B on renal function in control and CHF rats. Figure 1 summarizes the acute effects of SR 49059 and SR 121463B on urinary flow rate (V; Fig. 1A) and urinary osmolality (Uosm; Fig. 1B) in sham-operated controls and rats with CHF. Administration of SR 121463B to sham-operated rats significantly increased V from 5.5 ± 0.8 to 25.7 ± 8.7 (p < 0.05, Student’s t test) and 86.3 ± 21.9 (p < 0.01, Student’s t test and one-way ANOVA) μl/min after 20 and 40 min, respectively. Similar to control rats, administration of SR 121463B to rats with CHF significantly increased V from baseline levels of 20.8 ± 6.4 to 54.3 ± 19.0, 91.6 ± 26.5, and 99.6 ± 25.3 μl/min, after 20, 40, and 60 min, respectively (p < 0.05, Student’s t test) (Fig. 1A). Administration of SR 49059 to sham controls did not significantly affect V (from 4.7 ± 1.0 to 5.4 ± 1.2 and 5.4 ± 1.0 μl/min, p = N.S.). Likewise, administration of SR 49059 to CHF rats did not significantly influence V (from 10.4 ± 1.9 to 21.9 ± 7.0, 17.0 ± 7.1, 12.5 ± 3.0

**Fig. 1.** Acute effects of i.v. administered SR 49059 at a dose of 0.1 mg/kg and SR 121463B at a dose of 0.3 mg/kg on V (A) and Uosm (B) in sham-operated rats (n = 5) and rats with CHF (n = 5). *p < 0.05 versus baseline; #, p < 0.05 versus sham controls according to Student’s t test or one-way ANOVA followed by Dunnett test.
μl/min, p = N.S.) (Fig. 1A). The diuretic effects of SR 121463B were associated with a significant decline in the osmolality of the urine both in sham controls (from 1408 ± 209.9 to 1100 ± 200.7 and 271 ± 78.9 mOsm/kg H2O, p < 0.01, Student’s t test and one-way ANOVA) and in animals with CHF (from 967 ± 118.9 to 718 ± 155.5, 342 ± 127.4 and 277.4 ± 123.6 mOsm/kg H2O, p < 0.01, Student’s t test and one-way ANOVA) (Fig. 1B). As expected, SR 49059 did not affect the urinary osmolality either in normal rats or in rats with CHF (Fig. 1B).

The effects of SR 49059 and SR 121463B on UNaV, GFR, FENa%, and MAP in sham-operated controls and rats with CHF are summarized in Fig. 2. Bolus injection of SR 121463B to sham controls caused a nonsignificant increase in UNaV from basal values of 0.63 ± 0.07 to 1.42 ± 0.54 and 1.22 ± 0.54 μEq/min after 20 and 40 min, respectively (Fig. 2A). Administration of similar doses of SR 121463B to rats with CHF nonsignificantly enhanced UNaV from 0.34 ± 0.21 to 0.65 ± 0.49, 0.55 ± 0.42, and 0.644 ± 0.53 μEq/min after 20, 40, and 60 min, respectively. SR 49059 had no significant effect on UNaV in sham controls throughout the experiment. In contrast to sham controls, injection of SR 49059 to animals with CHF produced nonsignificant increases in UNaV from 967 to 118.9 mOsm/kg H2O, p = N.S.). The lack of natriuretic effect of SR 49059 at a dose of 0.1 mg/kg on UNaV (from 0.16 ± 0.07 to 0.48 ± 0.21, 0.30 ± 0.10, and 0.39 ± 0.14 μEq/min, p = N.S.). The placement of A-V fistula

The effects of SR 49059 and SR 121463B on renal hemodynamics in control and CHF rats. Figure 3 depicts the acute effects of SR 49059 and SR 121463B on MAP, RBF, and RVR in control rats and CHF animals. As shown in Fig. 3, baseline MAP and RBF were significantly lower, and RVR was higher, in CHF rats compared with control animals. Bolus injection of SR 121463B to sham controls caused a transient (Δ = −17%), yet insignificant (p < 0.05, Student’s t test), decrease in RBF, without affecting MAP or RVR (Fig. 3). In contrast, SR 121463B did not influence any one of these parameters in CHF animals. Administration SR 49059 did not cause either renal or systemic vasoconstrictive effect in sham controls but slightly decreased MAP from 81.7 ± 1.2 to 77.2 ± 2.9 mm Hg after 60 min (p = N.S.), without affecting RBF and RVR in rats with CHF (Fig. 3). These findings suggest that Vα-dependent mechanisms contribute to systemic vasoconstriction in rats with experimental CHF, more than in control rats. Administration of vehicle alone (saline) did not affect renal excretory function or GFR in both CHF and sham controls (data are not shown).

Measurement of Total RBF. Acute effects of SR 49059 and SR 121463B on renal hemodynamics in control and CHF rats. Figure 3 depicts the acute effects of SR 49059 and SR 121463B on MAP, RBF, and RVR in control rats and CHF animals. As shown in Fig. 3, baseline MAP and RBF were significantly lower, and RVR was higher, in CHF rats compared with control animals. Bolus injection of SR 121463B to sham controls caused a transient (Δ = −17%), yet insignificant (p < 0.05, Student’s t test), decrease in RBF, without affecting MAP or RVR (Fig. 3). In contrast, SR 121463B did not influence any one of these parameters in CHF animals. Administration SR 49059 did not cause either renal or systemic vasoconstrictive effect in sham controls but slightly decreased MAP from 81.7 ± 1.2 to 77.2 ± 2.9 mm Hg after 60 min (p = N.S.), without affecting RBF and RVR in rats with CHF (Fig. 3). These findings suggest that Vα-dependent mechanisms contribute to renal vasoconstriction in control rats.

Chronic Studies

Plasma Levels of AVP. The placement of A-V fistula caused a significant increase in AVP plasma levels after 7
days from surgery (from 12.8 ± 2.5 versus 32.2 ± 8.3 pg/ml, $p < 0.05$, Student’s $t$ test). These results demonstrate that the creation of A-V fistula is associated with activation of this antidiuretic system.

Effects of Chronic Administration of SR 49059 and SR 121463B on Daily and Cumulative Water and Sodium Excretion in Rats with CHF. Figure 4 summarizes the data on the effects of chronic administration of SR 49059 and SR 121463B (via osmotic minipumps, for 7 days) on daily (top panel) and cumulative (bottom panel) water and sodium excretion in CHF rats. The basal daily urinary volume, $U_{NaV}$, and $U_\text{osm}$ were: 10 ± 2 ml/day, 1120.5 ± 96.8 μEq/day, and 2302.1 ± 85.8 mOsm/kg H$_2$O, respectively. In agreement with our previous reports, absolute and cumulative sodium excretions were lower in CHF rats compared with sham-operated control rats (Fig. 4, C and D). Treatment with SR 121463B induced a significant ($p < 0.05$, one-way ANOVA) diuretic response (~2-fold increase in urinary volume) in rats with CHF compared with baseline values (Fig. 4A). This was noticed on the 1st day, disappeared on the 2nd, 3rd, and 4th days, was enhanced again on the 5th day of treatment, and lasted for an additional 2 days. The diuretic effect of SR 121463B was significant and impressive when the results were expressed as cumulative urine volume (Fig. 4B). In similarity to the findings in the acute protocols, chronic administration of SR 121463B to CHF rats produced mild natriuresis on the 5th day and was notable in the last day of treatment. In contrast, treatment with SR 49059 did not produce any change in both daily and cumulative urine volume (Fig. 4, A and B).

Figure 5 summarizes the data on the effects of chronic administration of SR 49059 and SR 121463B (given p.o. for 7 days) on daily (top panel) and cumulative (bottom panel) water and sodium excretion in CHF rats, compared with untreated CHF animals and sham controls. In similarity to the findings obtained in the protocol of chronic administration of the drugs via osmotic minipumps, administration of SR 121463B p.o. to rats with CHF produced a dramatic diuretic response (Fig. 5A). Daily urinary volume increased from a basal value of 10.2 ± 4.4 to 46.1 ± 5.0 ml/day ($p < 0.05$), on the 1st day of treatment (Fig. 5A). This effect persisted throughout the whole treatment period. The diuretic effect of SR 121463B was obvious and impressive when the results were expressed as cumulative urine volume (Fig. 5B). Despite its diuretic effect and in similarity to acute protocols, SR 121463B did not influence daily sodium excretion as well as the cumulative sodium excretion in the CHF group. In contrast, chronic administration of SR 49059 to rats with CHF did not increase either daily urinary volume or cumulative urine volume. Taken together with the results of the acute studies, these findings indicate that SR 121463B affects water, but not sodium excretion. This notion is further supported by the measurements of the urinary osmolality as depicted in Fig. 6. As shown in Fig. 6, p.o. but not i.p. administration of SR 121463B to rats with CHF caused a remarkable decrease in urinary osmolality of 68% below baseline value, beginning on the 1st day and continued throughout the whole treatment period. Administration of SR 49059 either via osmotic minipumps or p.o. did not change the osmolality of the urine significantly (Fig. 6). Despite its dramatic diuretic effect, SR 121463B caused a minor and nonsignificant increase in blood osmolality when administered via osmotic minipumps (295.7 ± 3.3 versus 293.6 ± 2.1 mOsm/kg H$_2$O, $p = \text{N.S.}$) or when administered p.o. (297.2 ± 1.9 versus 293.6 ± 2.1 mOsm/kg H$_2$O, $p = \text{N.S.}$) (Table 1). SR 49059 did not significantly affect blood osmolality of the various groups (Table 1).

Effects of Chronic Treatment with SR 49059 and SR 121463B on Cardiac/Body Weight Ratio. Table 1 summarizes the effects of chronic treatment (7 and 28 days) with SR 49059 and SR 121463B on body weight, heart weight, and heart/body weight ratio, an index of cardiac hypertrophy. Heart/body weight ratio was significantly higher in CHF rats (1 week) compared with sham-operated controls and was not affected by chronic treatment (1 week) with the drugs. Cardiac hypertrophy was not significantly affected in the CHF group treated with either the V$_1$ or V$_2$ antagonist, compared with the untreated CHF animals (Table 1). Thus, administration of the drug for 7 days did not reduce cardiac hypertrophy in rats with experimental CHF.

Because chronic treatment for 7 days did not affect cardiac hypertrophy in rats with CHF ($n = 5–6$), we performed an additional experimental protocol where either SR 49059 or SR 121463B was given for 28 days via osmotic minipump as described under Materials and Methods. The obtained results are summarized in Table 1. Absolute heart weight was
significantly higher in CHF rats (4 weeks) compared with sham-operated controls and was reduced by chronic treatment with both V₂ and V₁a antagonists. This trend also persisted when the heart weight was normalized to body weight. HW/BW was significantly higher in CHF rats (4 weeks) compared with sham-operated controls and was reduced by chronic treatment with both V₂ and V₁a antagonists but reached statistical significance only with the former. Body weight increased by 9, 22, 8, and 28% in sham, untreated CHF rats, CHF + SR 121463B, and CHF + SR 49059, respectively.

Discussion

Administration of SR 121463B to sham controls and CHF rats induced significant diuresis without significantly affecting either MAP or GFR (Fig. 2). Of interest is the finding that $U_{NaV}$ was not significantly influenced by either short-term or chronic administration of SR 121463B in rats with CHF and controls. In fact, both daily and cumulative $U_{NaV}$ were comparable in chronically SR 121463B-treated and untreated CHF rats despite the remarkable diuretic response of the
drug. Likewise, acute administration of SR 121463B to CHF rats and to sham controls did not cause significant change in UNaV, although there was a mild tendency to increase these parameters in the treated CHF rats and sham controls (Fig. 2). AVP exerts its antidiuretic response through the V₂ receptor located on renal CD principal cells, where its activation promotes AQP2 transport to the apical membrane, thus permitting free water reabsorption (Nielsen et al., 1999). Although the urinary osmolality in untreated sham controls and CHF animals ranged between 976 and 2491 mOsm/kg H₂O, rats that received SR 121463B either acutely or chronically exhibited significant reduction of urinary osmolality to ~271 mOsm/kg H₂O. Several recent clinical studies have demonstrated that chronic treatment with selective V₂ antagonists may be beneficial in the correction of hyponatremia in CHF (Gheorghiade et al., 2003, 2004; Schrier et al., 2006). Moreover, in patients hospitalized with CHF, oral tolvaptan (selective V₂-receptor antagonist), in addition to standard therapy including diuretics, improved many heart failure signs and symptoms (Gheorghiade et al., 2007). However, these studies examined primarily the acute effects of the drugs, and only limited and incomplete data are available at present on their long-term effects in experimental CHF (Burrell et al., 1998; Van Kerckhoven et al., 2002). Our study extends these reports by investigating both the acute and chronic renal and cardiac effects of selective V₁a and V₂ antagonists in controls and animals with CHF. We demonstrated that SR 121463B elicited a pronounced diuretic response in CHF animals either when given acutely or chronically. However, oral administration of this compound at similar doses was more efficient as a diuretic agent than when given i.p. via osmotic minipump. These differences could be attributed to that administration of SR 121463B (p.o., a daily dose at once) resulted in a high plasma level of the antagonist and thus in an abrupt and intense blockade of V₂-mediated action of endogenous AVP. This effect could have induced a complete loss of the urine-concentrating ability and high urinary flow, which washed out the medullary osmotic gradient. Thereafter, a slow recovery of this gradient will result in a relative inability to concentrate urine for a large fraction of the whole 24-h urine collection. In contrast, the osmotic minipumps deliver the antagonist much more slowly and regularly, resulting in a moderate decline in the urinary concentrating ability throughout the day.

Our findings that SR 121463B has potent comparable diuretic action in both normal and CHF animals are in agreement with other studies using various nonpeptide orally active AVP antagonists in normal conditions and clinical diseases such as CHF (Naitoh et al., 1994; Yatsu et al., 1999, 2002; Wada et al., 2002). However, in light of the high levels of AVP in rats with CHF rats compared with sham controls and enhanced renal expression of AQP2 in the former (Ishikawa and Schrier, 2003), one may expect an exaggerated diuretic response in the CHF animals. The lack of preferential diuretic action of SR 121463B in rats with CHF can be attributed to several factors. It is well established that activation of V₁a in CHF is responsible for increased peripheral and renal vascular resistance (Birnbaumer, 2000; Bankir, 2001). In addition, AVP may adversely influence renal hemodynamics (Cowley, 2000). This effect is mediated by the V₁a and may be modulated by local release of nitric oxide and prostaglandins. In pathophysiological concentrations, AVP may also decrease total RBF and GFR as a part of the generalized vasoconstriction (Christiansen et al., 2001). These effects are further supported by our findings that SR 49059 slightly improved GFR in CHF rats but not in sham controls rats 60 min after the drug administration. Moreover, by acting through V₁a, AVP causes contraction, proliferation, and hypertrophy of the mesangial cells, leading to decrease in GFR and ultrafiltration coefficient (Ganz et al., 1988; Roald et al., 2004). Taken together, the adverse renal and systemic effects of AVP mediated via V₁a may attenuate the diuretic action of SR 121463B in CHF by diverting the binding of the hormone selectively toward V₁a receptors. It is of interest that rats with CHF display enhanced systemic vasodilatory effect to SR 49059 compared with control rats. Likewise, administration of this compound to CHF animals produced a +24% increase in GFR but did not affect this parameter in sham animals. The higher sensitivity of CHF animals to systemic and renal vasodilatory action of SR 49059 compared with control animals suggests that V₁a-dependent mechanisms contribute to systemic and renal vasoconstriction in rats with experimental CHF, more than in controls. This notion is supported by the report of Lankhuizen et al. (2001), who demonstrated an enhanced renal and systemic V₁a-mediated vasoconstriction in rats with chronic myocardial infarction, an experimental CHF. These findings are compatible with the assumption that V₁a receptors on peripheral arterial and renal vasculature mediate the vasoconstrictive action of AVP (Birnbaumer, 2000; Loichot et al.,...
through a V1a-dependent mechanism (Xu and Gopalakrishnan, 1991; Fukuzawa et al., 1999; Nakamura et al., 2000). The vasoconstrictive action of AVP is mediated by the V1a-receptor and may be modulated by local release of nitric oxide and prostaglandins (Loichot et al., 1981; Goldsmith et al., 1983). The vasoconstrictive action of AVP augments the synthesis of endothelin (ET) and contributes to the adverse effects of Ang II in CHF. Previous studies by Ruzicka et al. (1993) and from our laboratory (Francis et al., 1984; Brodsky et al., 1998) demonstrated that Ang II and ET systems contribute to cardiac hypertrophy and that blockade of these peptides for 1 week largely attenuated the increase in cardiac mass in rats with ACF. These findings may underscore the dominant role of the RAAS and ET systems in the development of myocardial remodeling in this model of CHF, compared with the moderate contribution of AVP to this phenomenon. Therefore, it took 4 weeks of treatment to achieve beneficial effect of SR 49059 on cardiac enlargement. Similar findings were reported by Wada et al. (2002), who demonstrated that administration of a single dose of YM087, a V1a and V2 antagonist, significantly reduced cardiac hypertrophy after 8 but not 5 weeks from CHF induction in rats. Likewise, administration of SR 49059 at a high dose (60 mg/kg b.i.d.) for a few days mildly reduced left ventricle loading conditions (Clair et al., 2000). Nevertheless, administrations of either SR 49059 or SR 121463B to rats with CHF induced by coronary artery ligation for 21 days failed to show any reduction in cardiac hypertrophy, although an improvement in cardiac function was observed in rats receiving the V1a antagonist, SR 49059 (Van Kerckhoven et al., 2002). Concerning the obtained antihypertrophic effect of SR 121463B, it may be secondary to improvement of the hemodynamic status in CHF rats by reducing the pre- and afterload due to its potent diuretic effect. In addition, SR 121463B abolished the increase in BW compared with untreated CHF and SR 49059-treated animals, thus contributing to the decrease in the HW/BW ratio.

### TABLE 1
Effects of chronic treatment with SR 49059 or SR 121463B on body weight, cardiac hypertrophy, and blood osmolality

<table>
<thead>
<tr>
<th>Treatment Mode and Duration</th>
<th>Experimental Groups</th>
<th>BW, g</th>
<th>HW, g</th>
<th>HW/BW, %</th>
<th>Blood Osmolality, mOsm/kg H2O</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 Days (p.o.)</td>
<td>Sham Before treatment</td>
<td>297 ± 9</td>
<td>321 ± 6</td>
<td>1.04 ± 0.02</td>
<td>0.320 ± 0.004</td>
</tr>
<tr>
<td></td>
<td>CHF + Vehicle Before treatment</td>
<td>322 ± 6</td>
<td>318 ± 21</td>
<td>1.26 ± 0.08</td>
<td>0.39 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>CHF + SR 121463B Before treatment</td>
<td>324 ± 13</td>
<td>286 ± 5</td>
<td>1.11 ± 0.03</td>
<td>0.37 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>CHF + SR 49059 Before treatment</td>
<td>289 ± 2</td>
<td>282 ± 4</td>
<td>1.15 ± 0.04</td>
<td>0.39 ± 0.02</td>
</tr>
<tr>
<td>7 Days minipump</td>
<td>Sham Before treatment</td>
<td>309 ± 10</td>
<td>322 ± 6</td>
<td>1.04 ± 0.02</td>
<td>0.320 ± 0.004</td>
</tr>
<tr>
<td></td>
<td>CHF + Vehicle Before treatment</td>
<td>318 ± 21</td>
<td>324 ± 4</td>
<td>1.26 ± 0.08</td>
<td>0.39 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>CHF + SR 121463B Before treatment</td>
<td>307 ± 13</td>
<td>312 ± 6</td>
<td>1.35 ± 0.06</td>
<td>0.43 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>CHF + SR 49059 Before treatment</td>
<td>307 ± 13</td>
<td>312 ± 6</td>
<td>1.30 ± 0.07</td>
<td>0.41 ± 0.03</td>
</tr>
<tr>
<td>28 Days minipump</td>
<td>Sham Before treatment</td>
<td>358 ± 19</td>
<td>391 ± 12</td>
<td>1.19 ± 0.04</td>
<td>0.320 ± 0.003</td>
</tr>
<tr>
<td></td>
<td>CHF + Vehicle Before treatment</td>
<td>358 ± 19</td>
<td>415 ± 21</td>
<td>1.95 ± 0.08</td>
<td>0.47 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>CHF + SR 121463B Before treatment</td>
<td>380 ± 16</td>
<td>419 ± 14</td>
<td>1.65 ± 0.13</td>
<td>0.39 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>CHF + SR 49059 Before treatment</td>
<td>338 ± 15</td>
<td>433 ± 25</td>
<td>1.78 ± 0.13</td>
<td>0.41 ± 0.01</td>
</tr>
</tbody>
</table>

*p < 0.05 vs. sham.

*p < 0.05 vs. untreated CHF.

*p < 0.05 vs. its own before treatment.

These effects are very similar to those obtained with exposure of cardiomyocytes to angiotensin (Ang) II or catecholamines. Actually, AVP augments the synthesis of endothelin (ET) and contributes to the adverse effects of Ang II in CHF. Redrawn from ASPET Journals on July 5, 2017 jpet.aspetjournals.org Downloaded from
In summary, our findings indicate that V2 is involved in the mechanism of water retention in rats with CHF. Therefore, acute or chronic administration of SR 124163B, a highly selective V2 antagonist may be an optimal therapeutic modality to improve water balance and cardiac remodeling in CHF.

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References


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