Effects of novel vasopressin receptor antagonists on renal function and cardiac hypertrophy in rats with experimental congestive heart failure


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List of abbreviations

ACF: Aorto-caval fistula; ACTH: Adrenocorticotropic hormone; Ang II: Angiotensin II; AQP2: Aquaporin 2; AVP: Arginine vasopressin; CD: Collecting duct; CHF: Congestive heart failure; ET: Endothelin; FE_Na: Fractional sodium excretion; GFR: Glomerular filtration rate; LV: Left ventricle; MAP: Mean arterial pressure; NO: Nitric oxide; RAAS: Rennin angiotensin aldosterone system
RBF: Renal blood flow; SNS: Sympathetic nervous system; V: Urinary flow rate
V_{1a}: Vasopressin V_{1a} receptor subtype; V_{2}: Vasopressin V_{2} receptor subtype
U_NaV: Urinary sodium excretion; U_osm: Urinary osmolality

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Abstract

Objective: 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 V₁ₐ and V₂ antagonists on renal function and cardiac hypertrophy in rats with CHF.

Methods: The effects of acute administration of SR49059 (0.1mg/kg) and SR 121463B (0.3mg/kg), V₁ₐ and V₂ antagonists, respectively on renal function, and of chronic treatment (3.0mg/kg/day for 7 or 28 days, via osmotic minipumps or P.O), on water excretion and cardiac hypertrophy were studied in rats with aorto-caval fistula and control rats. Results: CHF induction increased plasma AVP (12.8±2.5 vs. 32.2±8.3 pg/ml, P<0.05). Intravenous bolus injection of SR121463B 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 SR49059 did not affect urine flow. Similarly, administration of SR121463B, but not SR49059, to rats with CHF significantly increased V from 20.8±6.4 to 91.6±26.5 µl/min (p<0.01). The diuretic effects of SR 121463B were associated with significant decline in urinary osmolality and insignificant change of Na⁺ excretion. In line with its acute effects, chronic administration of SR121463B to CHF rats increased daily urinary volume 2-5 fold throughout the treatment period. Both SR121463B and SR49059 significantly reduced heart weight in CHF rats when administered for 4, but not 1 week.

Conclusions: 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.
Introduction

The importance of the neuro-humoral vasoconstrictor systems in the pathogenesis of congestive heart failure (CHF) has been recognized (Dzau, 1987; Francis et al., 1984; 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 ACE inhibitors and β-blockers serve as key therapies in clinical CHF (Katz, 2003; Jessup & Brozena, 2003; Packer, 1992; Shigematsu et al, 2001). Although playing an important compensatory role in the early stages of the disease, prolonged activation of these systems have detrimental effects on the kidney and cardiovascular system. For instance, RAAS and SNS are involved in the induction of cardiac hypertrophy in CHF (Goldsmith, 1999; Yamazaki & Yazaki, 2000). In particular, the kidney is highly sensitive to the vasoconstrictor agents actions, and a decrease in renal blood flow (RBF) is one of the most common alterations in clinical and experimental CHF (Davis, 1965). Actually, impaired renal function and reduced glomerular filtration rate (GFR) are considered a strong independent predictor of mortality in patients with CHF (Hillege et al., 2000). Yet, the RAAS and SNS comprise only two of the three major components originally proposed to link neuro-hormonal activation to CHF (Francis et al., 1984). The third component is the arginine vasopressin (AVP), whose circulating levels are also elevated in clinical CHF (Goldsmith et al., 1983; Szatalowicz et al., 1981). Increased activity of the vasoconstrictor systems in general and AVP in particular leads to salt and water retention by the kidney, and thereby causes 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 3 different G-protein-coupled receptors (Birnbaumer 2000). V\textsubscript{1a} and V\textsubscript{2}, are abundantly expressed in the cardiovascular system and the kidney, and mediate the main biological actions of AVP, namely, vasoconstriction and water retention. The V\textsubscript{1a} receptor operates through the phosphoinositide signaling pathway, causing release of intracellular Ca\textsuperscript{2+} ions (Burrell et al 1994). The V\textsubscript{2} receptor, found in the renal collecting duct (CD) cells, is linked to the adenylate cyclase pathway, utilizing cAMP as its 2\textsuperscript{nd} messenger. Activation of this receptor leads to performed 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 (Wong & Verbalis, 2001, Bankir 2001). V\textsubscript{1b} receptors are involved in the stimulating effect of AVP on ACTH 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 V\textsubscript{1a} and V\textsubscript{2} 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 & SR 121463B, V\textsubscript{1a} and V\textsubscript{2} antagonists, respectively on renal function and cardiac hypertrophy in rats with aorto-caval fistula (ACF), an experimental model of CHF.
Previously, we have shown that this experimental model of CHF closely mimics renal and cardiac manifestations of advanced clinical CHF (Abassi et al., 2001a; Brodsky et al., 1998; Winaver et al., 1988). These include neuro-humoral activation, including RAAS and SNS, and elevated plasma AVP levels (Abassi et al., 2001a; Winaver et al., 1988). In addition, this model is characterized by a marked decrease in RBF and GFR, with a tendency to sodium retention, edema formation, and initial increase in cardiac output followed by low cardiac output heart failure (Abassi et al., 2001a; Winaver et al., 1988). Finally, rats with ACF develop a marked degree of cardiac hypertrophy (Brodsky et al., 1998). The selection of SR 49059 and SR 121463B was made since they are the most potent and selective, orally active V₂ and V₁ₐ antagonists described so far (Serradeil-Le Gal et al, 1993 and 1996), and since their renal and cardiac effects were not examined thoroughly in heart failure.

Methods

Studies were conducted on a local strain of male Sprague Dawley rats (Harlan Laboratories, Ltd., Jerusalem), weighing 290-380 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, IIT.

Induction of congestive heart failure

Heart failure was induced by surgical creation of an arterio-venous fistula between the abdominal aorta and the inferior vena cava by the method originally described by Stumpe et al (1973). In short, the abdominal aorta and inferior vena-cava were
exposed through a mid-abdominal incision under pentobarbital anesthesia (60 mg/kg, i.p.), and an arterio-venous shunt was surgically created in the common wall of the two vessels (side to side, 0.9- 1.2 mm O.D.), as previously described from our laboratory (Abassi et al., 2001a; Abassi et al., 1997; Brodsky et al., 1998; Winaver et al., 1988, Francis et al, 2004).

**Acute studies**

These studies were designed to evaluate the acute effects of SR121463B (1-[4-(Ntert-butylcarbamoyl)-2-methoxybenzenesulfonyl]-5-ethoxy-3-spiro-[4-(2-morpholinoethoxy)cyclohexane]indol-2-one, fumarate; equatorial isomer), and SR49059 ((2S) 1-[(2R 3S)-5-chloro-3-(2-chlorophenyl)-1-(3,4-dimethoxybenzenesulfonyl)-3-hydroxy-2,3-dihydro-1 H-indole-2-carbonyl]-pyrrolidine-2-carboxamide), (kindly supplied by Dr C. Serradeil-Le Gal, Sanofi-Aventis, Toulouse Cedex- France) on urine flow, urinary sodium excretion (UNaV), fractional sodium excretion (FENa), glomerular filtration rate (GFR), renal blood flow (RBF), and Mean arterial blood pressure (MAP) in rats with CHF compared with sham-operated controls.

**Clearance studies**

**Acute Effects of SR49059 and SR121463B 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 thermo-regulated (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-
1.5% of body weight per hour throughout the experiment. After a 60 min.
equilibration period, two baseline clearance periods of 20 minutes each were
obtained. Then, one of the following drugs or vehicle (0.2 ml 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
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 2\textsuperscript{nd} clearance period. Plasma samples were separated by
centrifugation.

\textit{Measurements of total RBF}

\textbf{Acute Effects of SR49059 and SR121463B on renal hemodynamics in control and
CHF rats:} On the 7\textsuperscript{th} postoperative day, the acute effects of SR121463B and
SR49059 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 carotid artery were
canulated and their abdominal cavity 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
Hemodynamic recordings were obtained for an additional 60 minutes after
administration of the drugs. Measurement of RBF was performed by ultrasonic flowmeter (Transonic Corp, Ithaca, N.Y.) using a flow-probe placed around the left renal artery of control and CHF rats (Brodsky et al 1998; Abassi et al, 1997, 1998). MAP was continuously monitored through a pressure transducer and RVR was calculated on-line 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 (K$_3$EDTA) 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 via 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. Prior to 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, 2ML4 Alzet Pharmaceuticals.), 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, additional group of rats with ACF was subjected to treatment with SR 49059 or SR 121463B given orally (gavage at 10 o'clock AM) at similar doses for 7 days beginning on the same day of the creation of ACF.

Following the operation, the rats were returned into their metabolic cages for additional 6 days, during which measurements of daily and cumulative water and sodium excretions were performed. On day 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 \( C_{in} \). Sodium concentrations in plasma and urine were determined by flame photometry (model IL 943, Instrumentation Laboratories). The osmolality of plasma and urine samples was determined by vapor pressure osmometer (Wescor Inc, Model 5500, Logan, Utah). Plasma levels of AVP were measured by a radioimmunoassay method (Peninsula Laboratories).

**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 paired t-test to compare each experimental value to its own baseline (see figure legends and result section for more details). 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 SR49059 and SR121463B on urine flow rate (V; Fig. 1A) and urine 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, t-test) and 86.3±21.9 µl/min (p<0.01, t-test and one way ANOVA) 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, 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=NS). Similarly, 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 µl/min, P=NS) (Fig. 1A). The diuretic effects of SR 121463B were associated with 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, 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 H$_2$O, p<0.01, t-test and one way ANOVA) (Fig. 1B). As expected, SR 49059 did not affect the urine osmolality neither in normal rats nor in rats with CHF (Fig. 1B).

The effects of SR 49059 and SR 121463B on U$_{Na}V$, GFR, FENa% and MAP in sham operated controls and rats with CHF are summarized in Figure 2. Bolus injection of SR 121463B to sham controls caused a non-significant increase in U$_{Na}V$ from basal values of 0.63±0.37 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 non-significantly enhanced U$_{Na}V$ from 0.34±0.21 to 0.65±0.49, 0.55±0.42 and 0.644±0.53 µEq/min after 20, 40, 60 min, respectively. SR 49059 had no significant effect on U$_{Na}V$ in sham controls throughout the experiment. In contrast to sham controls, injection of SR 49059 to animals with CHF produced non significant increases in U$_{Na}V$ (from 0.16±0.07 to 0.48±0.21, 0.30±0.10, and 0.39±0.14 µEq/min, P=NS). The lack of natriuretic effect of SR 121463B in controls and rats with CHF and of SR 49059 in rats with CHF persists also when the values were expressed as FE$_{Na}$ (Fig. 2C). The diuretic effect of SR 121463B in control rats were accompanied by a significant increase in GFR from 1.57±0.22 to 2.99±0.27 and 1.93±0.28 ml/min after 20 (p<0.05, t-test) and 40 min, respectively, (Fig. 2B). Administration of SR 121463B to CHF rats did not affect GFR as compared with its stimulatory effect on GFR in sham controls. SR 49059 did not significantly affect GFR in control rats, However, when administered to CHF animals, it enhanced GFR from 1.79±0.29 to 2.23±0.66 after 60 min (p=NS). These findings suggest that V$_{1a}$ dependent mechanisms contribute to renal vasoconstriction in rats with experimental CHF, more than in control rats. As
shown in Fig. 2D, baseline MAP was significantly lower in CHF rats compared with control rats. Bolus injection of SR 121463B caused a transient, yet not significant decrease in MAP in CHF rats (from 90.4±2.9 to 82.5±2.5 mmHg, P=NS) but not in control animals. SR 49059 caused a more pronounced and prolonged systemic vasodilatory effect in CHF rats compared with controls: MAP decreased in CHF animals from 102±8.7 to 85.7±4.9 mmHg, P=NS, and in sham controls from 114.8±1.6 to 108.8±4.4 mmHg, P=NS). These findings suggest that V1a 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:** Fig. 3 depicts the acute effects of SR 49059 and SR 121463B on MAP and 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, t-test), decrease in RBF, without affecting MAP or RVR (Fig. 3). In contrast, SR 121463B did not influence neither one of these parameters in CHF animals. Administration SR 49059 did not cause neither renal nor systemic vasodilatory effect in sham controls but slightly decreased MAP from 81.7±1.2 to 77.2±2.9 mmHg after 60 min (P=NS), without affecting RBF and RVR in rats with CHF (Fig. 3). These findings suggest that V2 dependent mechanisms contribute to renal vasodilation 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 vs. 32.2±8.3 pg/ml, P<0.05, 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

Fig. 4 summarizes the data on the effects of chronic administration of SR 49059 and SR 121463B (via osmotic minipumps, for 7 days) on daily (upper panel) and cumulative (lower panel) water and sodium excretion, in CHF rats. The basal daily urinary volume, U$_{NaV}$, and Uosm were: 10±2 ml/day, 1120.5±96.8 µEq/day and 2302.1±85.8 mosm/kg H2O, respectively. In agreement with our previous reports, absolute and cumulative sodium excretions were lower in CHF rats compared with sham-operated control rats (Fig. 4C,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 as compared to baseline values (Fig. 4A). This was noticed on the first day, disappeared on the 2$^{nd}$, 3$^{rd}$, 4$^{th}$ day, enhanced again on the 5$^{th}$ day of treatment and lasted for 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 5$^{th}$ 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. 4A, B).

Fig. 5 summarizes the data on the effects of chronic administration of SR 49059 and SR 121463B (given P.O. for 7 days) on daily (upper panel) and cumulative (lower 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 basal value of 10.2±4.4 to 46.1±5.0 ml/day (P<0.05), on the first 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 neither daily urinary volume nor cumulative urine volume. Taken together with the results of the acute studies, these findings indicate that SR 121463B affect water, but not sodium excretion. This notion is further supported by the measurements of the urine osmolality as depicted in Fig 6. As shown in Fig. 6, P.O, but not intraperitonial administration of SR 121463B to rats with CHF caused a remarkable decrease in urinary osmolality of 68% below baseline value, beginning on the first 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 (Figure 6). Despite its dramatic diuretic effect, SR 121463B caused a minor and non significant increase in blood osmolality.
when administered via osmotic minipumps (295.7±3.3 vs. 293.6±2.1 mosm/kg H₂O, P=NS), or when administered P.O (297.2±1.9 vs. 293.6±2.1 mosm/kg H₂O, P=NS) (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 V1 or V2 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. Since chronic treatment for 7 days did not affect cardiac hypertrophy in rats with CHF (n=5-6), we performed additional experimental protocol where either SR 49059 or SR 121463B were given for 28 days via osmotic minipump as described in 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 weigh 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 to statistical significance only with the later. 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 neither MAP nor 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 U NaV, 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 V 2 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). While the urinary osmolality in untreated sham controls and CHF animals ranged between 976-2491 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 V 2 antagonists may be beneficial in the correction of hyponatremia in CHF (Gheorghiade et al., 2003; Gheorghiade et al., 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 V$_{1a}$ and V$_2$ 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, orally administration of this compound at similar doses was more efficient as diuretic agent than when given intra-peritonealy 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 24h 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 utilizing various non-peptide orally active AVP antagonists in normal conditions and clinical diseases such as CHF (Naitoh et al., 1994; Wada et al., 2002; Yatsu et al., 1999; Yatsu et al., 2002). However, in light of the high levels of AVP in rats with CHF rats as compared with sham controls, and enhanced renal expression of AQP2 in the formers (Ishikawa & 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 V\textsubscript{1a} in CHF is responsible for increased peripheral and renal vascular resistance (Bankir, 2001; Birnbaumer, 2000). In addition, AVP may adversely influence renal hemodynamics (Cowley, 2000). This effect is mediated by the V\textsubscript{1a} and may be modulated by local release of nitric oxide (NO) and prostaglandins. In pathophysiological concentrations, AVP may also decrease total RBF and glomerular filtration rate (GFR) as a part of the generalized vasoconstriction (Christiansen et al, 2001). These effects are further supported by our findings that SR 49059 improved GFR in CHF rats but not in sham controls rats 60min after the drug administration. Moreover, by acting through V\textsubscript{1a}, 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 V\textsubscript{1a} may attenuate the diuretic action of SR 121463B in CHF by diverting the binding of the hormone selectively toward V\textsubscript{1a} receptors. It is of interest that rats with CHF display enhanced systemic vasodilatory effect to SR 49059 compared with control rats. Similarly, 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 V\textsubscript{1a} 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 V\textsubscript{1a}-mediated vasoconstriction in rats with chronic myocardial infarction, an
experimental CHF. These findings are compatible with the assumption that V_{1a} receptors on peripheral arterial and renal vasculature mediate the vasoconstrictive action of AVP (Loichot et al., 2000, Birnbaumer, 2000), whose levels are elevated in CHF (Goldsmith et al., 1983; Szatalowicz et al., 1981). The vasoconstrictive action of AVP is mediated by the V_{1a}-receptor and may be modulated by local release of nitric oxide (NO) and prostaglandins (Loichot et al, 2000). In pathological concentrations AVP also decreases total RBF and glomerular filtration rate (GFR) as a part of the generalized vasoconstriction induced by the peptide (Christiansen et al, 2001). Taken together, these findings provide new insights into the involvement of AVP via V_{1a} receptor in the elevated renal and systemic vascular resistance characterizing CHF (Davis, 1965). The marked increase in GFR obtained at 20 min in the sham controls treated with SR 121463B group, is most likely an artifact and may stem from the sudden increase in urine flow following the drug administration.

Finally, our findings demonstrate that chronic treatment (7 days) with either SR 49059 or SR 121463B, did not attenuate the development of cardiac hypertrophy in CHF rats. However, when administered for 4 weeks both antagonists significantly reduced the absolute cardiac mass. However, normalization of heart weight to body weight revealed that both antagonists reduced HW/BW, but only SR 121463B produced statistically significant reduction. These differences could be attributed to the weight gain, which was greater in SR 49059 treated CHF rats as compared to SR 121463B treated animals. The antihypertrophic effect of SR 49059 suggest that AVP, via V_{1a} plays a role in cardiac remodeling at least in this model of CHF and for the applied treatment period. This observation is in line with experimental studies evidence that AVP has direct effect on the heart. AVP has been shown to
increase calcium levels in cultured myocytes and stimulate cardiomyocyte hypertrophy and protein synthesis in neonatal rat cardiomyocytes through a V1a-dependent mechanism (Fukuzawa et al., 1999; Nakamura et al., 2000; Xu and Gopalakrishnan, 1991). These effects are very similar to those obtained with exposure of cardiomyocytes to angiotensin II (Ang II) or catecholamines. Actually, 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 (Brodsky et al., 1998; Francis et al., 1984) demonstrated that Ang II and ET systems contribute to cardiac hypertrophy and that blockade of these peptides for one 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 enlargement/remodeling in this model of CHF, as 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, 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 high dose (60 mg/kg, b.i.d) for few days mildly reduced left ventricle (LV) 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 antihypertrophic effect of SR 121463B, it may secondary to improvement of the hemodynamic status in CHF rats by reducing the pre and after
load due its potent diuretic effect. In addition, SR 121463B abolished the increase in BW as compared with untreated CHF and SR 49059 treated animals, thus contributing to the decrease in HW/BW ratio.

In summary, our findings indicate that V₂ is involved in the mechanism of water retention in rats with CHF. Acute or chronic administration of SR 121463B, a highly selective V₂ antagonist may be therefore an optimal treatment modality to improve water balance and cardiac remodeling in CHF.
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Footnotes:

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Legends for Figures

**Figure 1:** Acute effects of intravenously administered SR 49059 at a dose of 0.1 mg/kg, and SR 121463B at a dose of 0.3 mg/kg, on A) urine flow rate (V) and B) urine osmolality (Uosm) in sham-operated rats (n=5) and rats with CHF (n=5). *, P<0.05 vs. baseline; #, P<0.05 vs. sham controls according to t-test or one way ANOVA followed by Dunnett test.

**Figure 2:** Acute effects of intravenously administered SR 49059 at a dose of 0.1 mg/kg, and SR 121463B at a dose of 0.3 mg/kg, on A) Absolute urinary sodium excretion (U$_{Na}$V), B) Glomerular filtration rate (GFR), C) Fractional sodium excretion (FE$_{Na}$), and D) mean arterial blood pressure (MAP) in sham-operated rats (n=5) and rats with CHF (n=5). *, P<0.05 vs. baseline according to t-test or one way ANOVA followed by Dunnett test.

**Figure 3:** Acute effects of intravenously administered SR 49059 at a dose of 0.1 mg/kg, and SR 121463B at a dose of 0.3 mg/kg, on mean arterial pressure (MAP), renal blood flow (RBF) and renal vascular resistance (RVR) in control (n=5) and CHF rats (n=5). There were no statistical differences between the values obtained following the drugs administrations compared with basal values. *, P<0.05 vs. baseline, according to t-test.

**Figure 4:** Daily urine volume (A) and sodium excretion (B) (upper panel), and cumulative urine volume (C) and sodium excretion (D) (lower panel) in control rats (n=5) and in CHF rats (n=6-10) chronically treated or untreated with SR 49059 or SR 121463B at a dose of 3.0 mg/kg/day via osmotic minipumps for 7 days.
Baseline values refer to 2 days collection periods (day -1 and 0) that were averaged and combined. *, P<0.05 compared to baseline, #, P<0.05 compared to untreated CHF, $, P<0.05 compared to sham controls, according to one way ANOVA.

**Figure 5:** Daily urine volume (A) and sodium excretion (B) (upper panel), and cumulative urine volume (C) and sodium excretion (D) (lower panel) in control rats (n=5) and in CHF rats (n=6-10) chronically treated or untreated with SR 49059 or SR 121463B at a dose of 3.0 mg/kg/day given P.O. for 7 days. Baseline values refer to 2 days collection periods (day -1 and 0) that were averaged and combined. The lines representing untreated CHF animals and SR 121463B-treated CHF rats differed significantly between the control and CHF rats (by two-way ANOVA). *, P<0.05 compared to baseline, #, P<0.05 compared to untreated CHF, $, P<0.05 compared to sham controls, according to one way ANOVA.

**Figure 6:** Effects of chronic treatment with SR 49059 or SR 121463B (3.0 mg/kg/day) given via osmotic minipumps (A) or P.O. (B) for 7 days on urinary osmolality of sham operated controls (n=5) and CHF rats (n=6-10). Baseline values refer to 2 days collection periods (day -1 and 0) that were averaged and combined. #, P<0.05 compared to untreated CHF, $, P<0.05 compared to sham controls, according to one way ANOVA.
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 &amp; Duration</th>
<th>Experimental Groups</th>
<th>BW (gram)</th>
<th>HW (gram)</th>
<th>HW/BW (%)</th>
<th>Blood Osmolality (mosm/kg H2O)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>Before Treatment</td>
<td>297±9</td>
<td></td>
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<td></td>
<td></td>
<td>After Treatment</td>
<td>322±6</td>
<td>1.04±0.02</td>
<td>0.320±0.004</td>
</tr>
<tr>
<td>7 Days (P.O)</td>
<td>CHF</td>
<td>Before Treatment</td>
<td>318±21</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>After Treatment</td>
<td>324±13</td>
<td>1.26±0.08</td>
<td>0.39±0.02^6</td>
</tr>
<tr>
<td></td>
<td>CHF+ SR 121463B</td>
<td>Before Treatment</td>
<td>286±5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>After Treatment</td>
<td>293±6</td>
<td>1.11±0.03</td>
<td>0.37±0.01</td>
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<td></td>
<td>CHF+ SR 49059</td>
<td>Before Treatment</td>
<td>282±4</td>
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<td></td>
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<td>After Treatment</td>
<td>289±2</td>
<td>1.15±0.04</td>
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<td>Sham</td>
<td>Before Treatment</td>
<td>309±10</td>
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<td></td>
<td>After Treatment</td>
<td>322±6</td>
<td>1.04±0.02</td>
<td>0.320±0.004</td>
</tr>
<tr>
<td>7 Days Mini pump</td>
<td>CHF</td>
<td>Before Treatment</td>
<td>318±21</td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>After Treatment</td>
<td>324±14</td>
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<td>0.39±0.02</td>
</tr>
<tr>
<td></td>
<td>CHF+ SR 121463B</td>
<td>Before Treatment</td>
<td>292±7</td>
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<td>After Treatment</td>
<td>312±6</td>
<td>1.35±0.06^6</td>
<td>0.43±0.02^5</td>
</tr>
<tr>
<td></td>
<td>CHF+ SR 49059</td>
<td>Before Treatment</td>
<td>307±13</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>After Treatment</td>
<td>312±6</td>
<td>1.30±0.07</td>
<td>0.41±0.03^6</td>
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<tr>
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<td>Sham</td>
<td>Before Treatment</td>
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<td></td>
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<td>After Treatment</td>
<td>391±12</td>
<td>1.19±0.04</td>
<td>0.320±0.003</td>
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<td>28 Days Mini pump</td>
<td>CHF</td>
<td>Before Treatment</td>
<td>340±17</td>
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<td>After Treatment</td>
<td>415±21</td>
<td>1.95±0.08</td>
<td>0.47±0.01^3</td>
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<tr>
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<td>Before Treatment</td>
<td>380±16</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>After Treatment</td>
<td>419±14</td>
<td>1.65±0.13^5</td>
<td>0.39±0.03^5,^6</td>
</tr>
<tr>
<td></td>
<td>CHF+ SR 49059</td>
<td>Before Treatment</td>
<td>338±15</td>
<td></td>
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<td></td>
<td>After Treatment</td>
<td>433±25^6</td>
<td>1.78±0.13^5</td>
<td>0.41±0.01^5</td>
</tr>
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</table>

$, P<0.05 vs Sham; #, P<0.05 vs untreated CHF; &, P<0.05 vs its own before treatment.
Figure 1

(A) Urine Flow Rate (µl/min)

(B) Urine Osmolality (mosm/kg H₂O)

* indicates statistically significant differences from baseline.

Drug Administration arrow indicates time of drug administration.
Figure 6