Cardiorenal and Neurohumoral Effects of Endogenous Atrial Natriuretic Peptide in Dogs With Severe Congestive Heart Failure Using a Specific Antagonist for Guanylate Cyclase–Coupled Receptors

Atsuyuki Wada, MD; Takayoshi Tsutamoto, MD; Yuzuru Matsuda, PhD; Masahiko Kinoshita, MD

Background To elucidate the extent of the compensatory role of endogenous atrial natriuretic peptide (ANP) in severe congestive heart failure (CHF), we examined the changes in hemodynamics and neuroendocrine and renal functions after incremental administration of an ANP antagonist, HS-142-1 (HS), in dogs with CHF.

Methods and Results We assessed the effects of HS on the suppression of plasma and urinary cGMP levels as a marker of endogenous ANP activity in dogs without CHF. Bolus injections of 0.3 and 1.0 mg/kg HS reduced plasma cGMP levels to 77% and 60% and urinary cGMP excretion to 79% and 61% of the relevant control levels, respectively. Then the study was performed in dogs with CHF induced by chronic rapid ventricular pacing, and the plasma ANP level was sixfold higher than that in the controls. Hemodynamic, hormonal, and renal variables were determined both before and after subsequent incremental administration (0.3, 1.0, and 3.0 mg/kg every 30 minutes) of HS. HS lowered the plasma and urinary cGMP levels dose dependently to 32% and 37% of the control levels, respectively. Mean arterial, pulmonary capillary wedge, and right atrial pressures and cardiac output did not change significantly. However, plasma renin activity, aldosterone level, and norepinephrine level increased rapidly to 226%, 179%, and 252% of the control values, respectively. Urine flow rate and urinary sodium excretion were significantly inhibited, with no concomitant change in glomerular filtration rate or renal plasma flow.

Conclusions These findings suggest that endogenous ANP contributes to the suppression of the activation of the renin-aldosterone system and sympathetic nervous activity and body fluid retention but that the vasodilative action of this peptide is attenuated in advanced CHF. (Circulation. 1994;89:2232–2240.)

Key Words • atrial natriuretic factor • guanosine • congestive heart failure

Previous studies have shown that atrial natriuretic peptide (ANP) has potent vasorelaxant, diuretic, and natriuretic actions and shows neurohumoral effects that inhibit renin and aldosterone secretion. In congestive heart failure (CHF), the increase in ANP in the plasma may contribute to the compensatory regulation of hemodynamics, body fluid balance, and interaction of neurohumoral factors. Although the plasma ANP level remains elevated above basal values, the cardiac pressure increases, volume overload develops, and the renin–angiotensin–aldosterone system and sympathetic nervous activity are significantly elevated during the progression of chronic CHF. In CHF, the response to ANP infusion is less sensitive with regard to the renal, hormonal, and hemodynamic changes compared with healthy control subjects. The compensatory effects of ANP appear to be attenuated in advanced CHF. We have shown previously that plasma levels of ANP and cGMP, an intracellular second messenger of ANP, become elevated with the progression of mild CHF. However, the plasma cGMP level reached a plateau despite the elevated level of ANP in chronic severe CHF. Therefore, we suggested that this is due to downregulation of guanylate cyclase (GC)–coupled ANP receptors in vascular beds. Several previous studies using antibodies against ANP have been performed to clarify the role of endogenous ANP activity in CHF. However, the exact role of endogenous ANP in CHF has not yet been determined, and whether its compensatory mechanism is actually attenuated in CHF remains unknown because no specific antagonist for ANP is available. We used a specific antagonist of GC-coupled ANP receptors, HS-142-1 (HS), to determine whether elevated endogenous ANP plays a compensatory role in advanced CHF. Hirata et al.11 used this ANP antagonist, HS, in DOC acetate (DOCA)–salt hypertensive rats and suggested that endogenous ANP is involved in the regulation of blood pressure and body fluid volume. In the present study, we examined the extent of the pathophysiological role of endogenous ANP in the regulation of the systemic hemodynamics, body fluid volume balance, and neurohumoral factors in the pacing-induced canine severe heart failure model system using HS in a dose-response study.
Methods

Animal Preparation

Conditioned mongrel dogs of both sexes, weighing 11 to 15 kg, were used for all experiments. Approval was obtained from the Animal Research Committee of Shiga University of Medical Science, and all dogs were preconditioned to the study environment for 4 weeks before experiments. The dogs were fasted overnight but allowed water ad libitum until the start of the experiment. Anesthesia was induced with pentobarbital sodium (25 mg/kg), with supplemental doses administered as necessary to maintain anesthesia. Dogs were intubated and ventilated with an Aika R-60 respirator. Through a left thoracotomy incision at the fourth intercostal space, the heart was exposed, the pericardium was opened, and two cardiac unipolar pacemaker leads (Matsuda M-23) were sutured onto the right ventricular apex. After the incision was closed, the leads were tunneled to the back and connected to the external pacemaker (model 540, Seamed). The left femoral vein was then exposed and a 7F Swan-Ganz thermodilution catheter (model T-047-03, Goodee) was advanced into the pulmonary artery for measurement of right atrial pressure (RAP), mean pulmonary arterial pressure (MPAP), pulmonary capillary wedge pressure (PCWP), and cardiac output (CO). CO was calculated by the thermodilution technique in triplicate using 5 mL of ice-cold 0.9% saline injected into the right atrium. The subsequent thermodilution curves were integrated by a Gould CO computer (SP1435, Statham). A Tygon catheter (flexible plastic tubing, Norton) was placed in the descending thoracic aorta via the right carotid artery for measurement of mean arterial pressure (MAP) with a pressure transducer (Baxter MP5100). The right jugular vein was also catheterized and the Tygon catheter advanced into the vena cava for drug administration. The central filling pressure was recorded on a polygraph (RM-6000, Nihon Kohden). The pacemaker and the ends of the catheters and pacing wires were securely fastened in a small bag worn on the back of the animal. The dogs were allowed to recover from surgery for at least 14 days before control study measurements, during which time they were trained to lie quietly, unrestrained, on the experimental table on a heat mat. Control hemodynamic and venous plasma samples for neurohumoral determinations were obtained before programming of the pacemaker to an asynchronous mode at a rate of 270 beats per minute. After 14 to 36 days of rapid ventricular stimulation (average of 22 days), the dogs were judged to be in severe CHF on the basis of elevation of central filling pressures, evidence of fluid retention, apathy, anorexia, and symptomatic signs of dyspnea. All measurements were recorded at the elevated heart rate, and all subsequent studies were performed with animals in the conscious state. Systemic vascular resistance (SVR) and pulmonary vascular resistance (PVR) were calculated as follows: SVR=(MAP−RAP)/CO×80; PVR=(MPAP−PCWP)/CO×80.

Experimental Protocols

Study 1: Effects of HS on Plasma and Urinary cGMP Production in Dogs Without Pacing

The effects of HS on suppression of endogenous ANP activity were evaluated by measuring plasma and urinary cGMP concentrations. Eight healthy conscious dogs were prepared as described without pacing. The urinary bladder was catheterized with a 7F Clincy balloon drainage tube (Create Medic Co) under short thiopental sodium anesthesia (4 mg/kg). After a 60-minute equilibration period, HS (Kyowa Hakko Kogyo Co) dissolved in 20 mL saline was injected as a bolus into the right atrium at a dose of 0.3 (n=4) or 1.0 mg/kg (n=4). Blood and urine samples were drawn every 30 minutes for 60 minutes. An equal volume of saline was injected to replace the volume lost by blood withdrawal, and changes in plasma and urinary cGMP concentrations were measured. At the same time, changes in cardiac pressures were measured every 10 minutes, and those in other neurohumoral factors, including ANP, plasma renin activity (PRA), aldosterone, and norepinephrine, were determined every 30 minutes.

Study 2: Cardiovascular, Neurohumoral, and Renal Effects of HS in Severe Heart Failure

To evaluate the role of endogenous ANP in severe CHF, we divided the dogs with CHF induced by rapid pacing into two groups; one group (n=6) received HS and the other (n=5) received only saline as time controls. The dogs' bladders were catheterized as in study 1, and 45 minutes later a priming dose of 50 mg/kg creatinine and 8 mg/kg para-aminohippurate (PAH) dissolved in 10 mL saline solution was infused into the pulmonary artery over a 15-minute period according to the method of Riegger et al12 with some modifications. This was followed by a constant infusion (0.75 mL/min) of 1.0 mg·kg⁻¹·min⁻¹ creatinine and 0.3 mg·kg⁻¹·min⁻¹ PAH throughout the experimental period. After a 60-minute equilibration period, when plasma concentrations were at a steady state, the bladder of each dog was completely emptied and flushed five times with sterile distilled water before the first of two clearance periods, each 30 minutes in duration, was begun. After stable basal hemodynamic records were obtained, HS was administered stepwise as a bolus at three doses of 0.3, 1.0, and 3.0 mg/kg dissolved in 20 mL saline or vehicle only (saline 20 mL) to exclude any temporal effects, injected at intervals of 30 minutes into the right atrium. Pressure measurements were repeated every 10 minutes for 90 minutes after injection. The collected pressure data included MAP, RAP, MPAP, and PCWP. CO was determined before and every 30 minutes after HS administration. Blood was drawn for analysis of plasma ANP level, cGMP concentration, PRA, aldosterone, and norepinephrine levels. Blood specimens were centrifuged at 4°C, and the plasma was frozen at −30°C until assay. After the HS injection, three additional 30-minute urine collections were made. Urine for analysis of urinary cGMP excretion was collected at the end of each clearance period, and urinary cGMP specimens were frozen at −30°C until assay.

Analysis of Blood and Urine Samples

Blood for the determination of plasma ANP, cGMP, PRA, aldosterone, and norepinephrine levels was collected from the pulmonary artery. Blood for ANP assay was collected in tubes containing aprotinin (500 kallikrein inhibitory units/mL) and EDTA (1 mg/mL) and immediately placed on ice. After centrifugation at 3000 rpm at 4°C, aliquots of plasma were measured by radioimmunoassay as previously described.13 Cross-reactivity was assumed to be 100% with dog ANP because the amino acid sequences are identical. The intraassay and interassay coefficients of variation were 6.3% and 9.6%, respectively. Plasma and urinary concentrations of cGMP were determined by radioimmunoassay with a commercial kit (Yamasa Syoyu Co Ltd),14 and intra-assay and interassay coefficients of variation were 2.4% and 8%, respectively. PRA was measured by radioimmunoassay (Gamma Coat Renin Kit, Clinical Assay). Plasma aldosterone was measured by radioimmunoassay with a SPA-C Aldosterone Kit (Dai-ichi Isotope Laboratories). Estimation of plasma norepinephrine concentration was made by high-performance liquid chromatography using an autoanalyzer (model HLC-8030, Tosoh Co). Serum and urine creatinine and sodium concentrations were measured by an autoanalyzer (model 723, Hitachi). Serum and urine PAH concentrations were determined by the dimethylaminocinnamaldehyde method. Creatinine clearance and PAH clearance were calculated from standard formulas and were equated with glomerular filtration rate (GFR) and renal plasma flow (RPF), respectively. Filtration fraction (FF) was calculated as GFR/RPF.
TABLE 1. Hormonal and Hemodynamic Response to the Injection of HS-142-1 In Dogs Without Heart Failure

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Dose of HS-142-1, mg/kg</th>
<th>Time, min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>ANP, pg/mL</td>
<td>0.3</td>
<td>61±7</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>53±16</td>
</tr>
<tr>
<td>cGMP, pmol/mL</td>
<td>0.3</td>
<td>10.8±2.8</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>11.6±2.5</td>
</tr>
<tr>
<td>PRA, ng·mL⁻¹·h⁻¹</td>
<td>0.3</td>
<td>1.3±0.4</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>1.8±0.2</td>
</tr>
<tr>
<td>Ald, pg/mL</td>
<td>0.3</td>
<td>120.5±51.3</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>58.8±19.4</td>
</tr>
<tr>
<td>NE, pg/mL</td>
<td>0.3</td>
<td>235±47</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>247±27</td>
</tr>
<tr>
<td>U-cGMP, pmol/mL</td>
<td>0.3</td>
<td>1688±226</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>1919±480</td>
</tr>
<tr>
<td>PCWP, mm Hg</td>
<td>0.3</td>
<td>4.9±1.4</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>5.1±0.7</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>0.3</td>
<td>103.8±1.8</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>104.5±1.7</td>
</tr>
</tbody>
</table>

ANP indicates atrial natriuretic peptide; PRA, plasma renin activity; Ald, aldosterone; NE, norepinephrine; U-cGMP, urinary cGMP excretion; PCWP, pulmonary capillary wedge pressure; and MAP, mean arterial pressure. Values are mean±SEM (n=4). *P<0.05 vs value at time 0.

Statistical Analysis

All values are given as mean±SEM. ANOVA for repeated measurements was used to determine the significance of changes during time-dependent multiple observations. Comparisons with control values were analyzed by Dunnett's test after ANOVA for repeated measurements. Student's t test was used for analysis of the significance of single comparisons. Differences at a value of P<0.05 were considered to be statistically significant.

Results

Study 1: Effects of HS Alone on Plasma and Urinary cGMP Levels in Dogs Without Pacing

As shown in Table 1, at doses of 0.3 and 1.0 mg/kg, HS significantly reduced plasma and urinary cGMP concentrations in dogs without pacing. However, the plasma ANP level, levels of other neurohumoral factors, and hemodynamic parameters were not significantly changed. That is, HS at 0.3 and 1.0 mg/kg IV alone did not influence basal hemodynamics or endocrine secretion, with the exception of cGMP level.

Study 2: Response to Rapid Ventricular Pacing

Rapid right ventricular pacing induced CHF in both experimental groups (HS versus vehicle only). In the HS group, MAP was significantly reduced below the pre-pacing value of 105.9±4.1 to 87.6±4.4 mm Hg in CHF (P<0.05) and CO from 2.78±0.23 to 1.52±0.13 L/min (P<0.001). However, PCWP and RAP rose progressively to peak levels of 19.0±3.1 mm Hg (P<0.01) and 7.3±0.9 mm Hg (P<0.001), respectively. Plasma ANP concentrations increased to about sixfold higher than that at basal level (from 77±9 to 395±67 pg/mL, P<0.001). The plasma level of cGMP was also significantly elevated above the pre-pacing value of 11.1±2.3 to 33.7±6.5 pmol/mL in CHF (P<0.01). The neurohormone levels increased during the development of CHF: PRA increased from the prepping value of 1.2±0.3 to 5.2±2.0 ng·mL⁻¹·h⁻¹ in CHF (P<0.05), aldosterone level from 51.7±21.1 to 364.5±121.1 pg/mL (P<0.01), and norepinephrine level from 162±37 to 539±100 pg/mL (P<0.01). When the basal hemodynamic and hormonal data of all experimental animals were pooled, the differences between the HS and vehicle groups were not significant.

cGMP Studies in CHF

We investigated the changes in plasma and urinary cGMP concentrations as a marker of endogenous ANP activity. HS reduced plasma cGMP concentration dose dependently from 33.7±6.5 pmol/mL before injection to 102±1.4 pmol/mL 90 minutes after injection (P<0.001). After injection of HS at 0.3 and 1.0 mg/kg, the plasma cGMP value was reduced to 67.6% and 44.5% of the basal value, respectively, and at the maximal dose, HS lowered the cGMP concentration to 32.0% (Fig 1). Urinary cGMP excretion was also inhibited significantly, from 5129±1542 to 1950±665 pmol/mL, dose dependently (P<0.01). Percent changes in urinary cGMP excretion were reduced to 81.1% of the average basal value by 0.3 mg/kg HS, to 48.4% by 1.0 mg/kg HS, and to 37.3% by 3.0 mg/kg HS (Fig 2).

Hemodynamic Changes in CHF

Fig 3 shows the hemodynamic changes associated with stepwise administration of HS at three different
dose levels in dogs with severe CHF. We observed no significant changes in the systemic hemodynamic response to HS. MAP, PCWP, and RAP throughout the experimental period ranged from 87.2 ± 5.1 to 93.0 ± 4.7 mm Hg, from 19.0 ± 3.1 to 21.7 ± 3.7 mm Hg, and from 7.3 ± 0.9 to 7.8 ± 1.3 mm Hg, respectively. CO ranged from 1.52 ± 0.13 to 1.49 ± 0.15 L/min. PVR and SVR ranged from 275.2 ± 43.0 to 365.3 ± 72.7 dynes ⋅ s⁻¹ ⋅ cm⁻⁵ and from 4342.9 ± 394.0 to 4655.3 ± 403.5 dynes ⋅ s⁻¹ ⋅ cm⁻⁵, respectively. These values did not change significantly during any experimental procedure. The control saline injection also did not affect these hemodynamic parameters.

**Plasma Hormonal Changes in CHF**

Fig 4 shows the hormonal changes with administration of HS in dogs with CHF. Administration of HS caused a dose-dependent reduction in plasma cGMP concentration, with a significant increase in plasma ANP level from the preinjection value of 395 ± 67 to 550 ± 58 pg/mL, 90 minutes after injection (P < .01). PRA and plasma aldosterone and plasma norepinephrine levels also increased from 5.2 ± 2.0 to 12.2 ± 3.3 ng ⋅ mL⁻¹ ⋅ h⁻¹ (P < .01), from 364.5 ± 121.1 to 651.7 ± 184.1 pg/mL (P < .01), and from 538.8 ± 99.7 to 1358.8 ± 322.8 pg/mL (P < .01), respectively, with injection of HS. There were no significant changes in these values throughout the experimental period in the vehicle-only group.

**Changes in Renal Function in CHF**

Table 2 and Fig 5 show the changes in renal variables after HS injection. Urinary flow rate was significantly reduced, to 0.32 ± 0.11 mL/min, from the average basal value of 0.55 ± 0.18 mL/min (P < .01), and absolute urinary sodium excretion was reduced to 7.6 ± 2.8 μEq/min from the average basal value of 41.5 ± 17.2 μEq/min (P < .05), although there were no significant changes in GFR, RPF, or FF. None of these variables changed significantly in the vehicle group.

**Discussion**

To elucidate the pathophysiological roles of endogenous ANP in severe CHF, we administered the antagonist of GC-coupled ANP receptors, HS, to dogs with heart failure induced by rapid ventricular pacing. We investigated the extent to which endogenous ANP actually contributes to the regulation of the hemodynamic, neurohumoral function, and body fluid balance in severe CHF by HS administration. HS dose dependently lowered both plasma and urinary cGMP concentrations while increasing PRA and aldosterone and norepinephrine levels. Renal excretion of sodium and water was reduced, with no associated changes in GFR or RPF, although cardiac hemodynamics remained unchanged throughout the experimental period. Plasma and urinary cGMP levels have been demonstrated to serve as biological markers of endogenous ANP activity.15-18 Although cGMP is produced by the activation of two different isoenzyme forms of GC, soluble and particulate GC, ANP is well known to interact specifically only with particulate GC. Conversely, nitric oxide (NO) activates soluble GC and in turn increases the cGMP levels in endothelial and vascular smooth muscle cells. The NO system may alter the renin-angiotensin-aldosterone system and sodium excretion.19,20 HS specifically blocks the actions of ANP without inhibiting other cGMP-linked substances such as sodium nitroprusside and acetylcholine, which are known to activate soluble GC and stimulate NO production. HS does not inhibit sodium nitroprusside-induced accumulation of cGMP observed in porcine kidney epithelial LLC-PK₁ cells and rabbit aortic rings10,21 or prevent the vasodilative response to acetylcholine observed in isolated rat renal artery.11 Thus, HS specifically blocks ANP-induced cGMP production through specific blockade of the particulate GC-linked receptor. The present study showed that HS blocks the biological activities of endogenous ANP in CHF.

**Hemodynamic Changes**

ANP is considered to play a significant role in vascular tone regulation via its vasodilative activity.22,23 However, the extent of the role of endogenous ANP in the
ANP-induced ta21 central experiment. 

Fig 3. Graphs showing the time course of changes in hemodynamics with administration of vehicle (o, n=5) or HS-142-1 (HS; c, n=6) in dogs with heart failure. HS 0.3, 1.0, and 3.0 mg/kg or the saline vehicle was administered stepwise at 30-minute intervals. Pressure measurements were repeated every 10 minutes from 0 to 90 minutes after administration, and cardiac output (QO) was determined before and every 30 minutes after administration. Systemic vascular resistance was calculated at these times. PCWP indicates pulmonary capillary wedge pressure; MAP, mean arterial pressure; and SVR, systemic vascular resistance. Values are mean±SEM.

control of cardiac hemodynamics in advanced CHF has not been determined. The stepwise administration of HS in the present study demonstrated that the regulation of blood pressure by endogenous ANP was attenuated in severe CHF. Since administration of HS in stepwise doses reduced the plasma cGMP concentration to 32% of the basal level, we expected that the central filling pressure would be elevated during the experiment. However, no statistically significant changes were observed. HS has been shown to prevent ANP-induced relaxation of isolated rabbit thoracic aorta21 and inhibit the depressor response to human ANP infusion in rats.24 When ANP antiserum was used in rats with CHF, different results were reported concerning the hemodynamic regulation by endogenous ANP. However, plasma cGMP levels were not estimated in these previous studies.7,8 Recently, Hirata et al11 also administered HS to DOCA-salt hypertensive rats and showed that a higher dose of HS (8.0 mg/kg) induced an increase in MAP associated with a 77% reduction in plasma cGMP levels. The differences in the hemodynamic responses in these various studies could be due to differences in the level and duration of elevation of endogenous ANP concentration and/or differences in

Fig 4. Bar graphs showing changes in plasma hormone levels with administration of vehicle (open bars, n=5) or HS-142-1 (HS; hatched bars, n=6) in dogs with heart failure. HS 0.3, 1.0, and 3.0 mg/kg or the saline vehicle was administered stepwise at 30-minute intervals. ANP indicates atrial natriuretic peptide; Ald, aldosterone; PRA, plasma renin activity; and NE, norepinephrine. Values are mean±SEM. P<.05, oP<.01 compared with basal values; fP<.05 compared with values with vehicle.
experimental animals used and basal diseases studied. Previously, we reported that the effect of attenuation of endogenous ANP activity on hemodynamics observed in patients with severe chronic CHF may be due to the downregulation of ANP receptors in the vascular beds.\textsuperscript{6,14} In addition, the mechanism of the reduction in vasodilative action of endogenous ANP in this study may be related to a defect in intracellular mechanisms other than those involved in the production of cGMP, because the hemodynamics were not altered significantly when the plasma cGMP levels were reduced below the basal level.\textsuperscript{3,25,26} However, there is an alternative possibility that the reduced level of plasma cGMP does not originate mainly from the smooth muscle but rather from the vascular endothelium. Porter et al\textsuperscript{25} recently reported that the potency of ANP in the induction of cGMP production is even higher in endothelial than in smooth muscle cells. Therefore, inhibition of the ANP receptor in smooth muscle may not be sufficient to elicit significant hemodynamic changes.

**Plasma Hormonal Changes**

In study 2, the plasma ANP concentrations were significantly elevated after HS administration. Although HS very selectively blocks the biologically active receptor (B-receptor) that is coupled to GC, HS does not affect the binding of ANP to the metabolic clearance receptor, which lacks GC activity.\textsuperscript{10,28,39} The attenuation of ANP metabolism may be due to impaired internalization of ANP-receptor complexes in CHF.\textsuperscript{30-33} However, the increase in plasma ANP levels observed in the present study may be a consequence of HS displacing ANP from the B-receptor or blocking its binding to the B-receptor, since we found that HS also tended to induce an increase in plasma ANP levels in dogs without CHF in study 1.

We observed rapid and marked elevation of PRA, aldosterone, and norepinephrine levels despite the lack of overall deterioration in systemic hemodynamics. This is, to the best of our knowledge, the first demonstration of a significant role of endogenous ANP in countering these vasoconstrictive/antidiuretic hormones in CHF. In the present study, PRA increased to 226% of the control level when the cGMP level had decreased to 32%. Several previous studies have shown that exogenous ANP, administered intravenously, suppresses renin secretion, despite a sustained decrease in arterial pressure.\textsuperscript{3,5,33,34} However, whether endogenous ANP has an inhibitory effect on renin release remains controversial. After administration of ANP antiserum in a normal rat model, Naruse et al\textsuperscript{35} observed an elevation in PRA, whereas Rudd et al\textsuperscript{36} reported no change in PRA. In the present study, we observed a significant elevation of the already activated PRA compared with the prepping values when endogenous ANP activity was suppressed by HS. Although ANP-mediated renin inhibition may involve a macula densa mechanism, the results of the present study do not allow us to distinguish between this and a direct receptor-mediated mechanism.\textsuperscript{3,37}

Secretion of aldosterone is known to be inhibited by exogenous ANP, both in vivo and in vitro.\textsuperscript{38,39} In the present study, plasma aldosterone level increased to 179% of the control level; plasma angiotensin II level also increased, but not significantly (data not shown). We consider the increase in aldosterone secretion not to be stimulated by angiotensin II but rather to be a result of the release from direct ANP-mediated inhibition. Oda et al\textsuperscript{40} demonstrated a positive correlation between the restoration of ANP-induced inhibition of aldosterone production and the reduction of intracellular cGMP level by administration of HS to adrenal glomerulosa cells. Our findings in the present study indi-
from basal levels after HS treatment. Urinary cGMP originates from renal cells, and its level is correlated with the natriuresis induced by ANP. Thus, excretion of urinary cGMP is assumed to be a biological marker for renal ANP activity in vivo. Therefore, HS may have blocked the renal endogenous ANP activity in the present study, and endogenous ANP may have actually contributed to the excretion of sodium and water in severe CHF without causing significant changes in GFR or RPF. Pharmacological doses of ANP have been shown to induce an elevation in the excretion of water and sodium associated with a significant increase in GFR. Marin-Grez et al showed that ANP induces a preglomerular vasodilatation and a postglomerular vasoconstriction with enhanced natriuresis. Since no significant changes in FF were observed in the present study, the vasodilative effect of endogenous ANP on the afferent arteriole seemed to be diminished, as it was on the systemic vasculature. Low doses of ANP infusion in dogs have been reported to induce natriuresis without changes in GFR or blood pressure. A very high density of ANP receptor binding sites in the kidney was reported over the inner medulla and the increase in cGMP content in response to ANP is largest in the inner medullary collecting ducts (IMCD) among the renal tubular segments. The threshold ANP concentration for an increase in cGMP accumulation in the IMCD has been reported to be about 100 times higher than that in the glomeruli. The IMCD appears to be a major site of endogenous ANP action, and ANP-induced natriuresis and diuresis are mainly the result of the effect of ANP in the inner medulla associated with a reduction in reabsorption of sodium and water in the IMCD. In addition, ANP acts to reduce proximal tubular reabsorption at physiological concentrations by inhibition of angiotensin-stimulated sodium and water transport. Since HS significantly elevated the plasma aldosterone level and tended to increase that of angiotensin II, the inhibition of these hormones by endogenous ANP may also contribute to natriuresis and diuresis.

**Limitations of the Study**

Recent studies have shown that the plasma brain natriuretic peptide (BNP) concentration is elevated in CHF. BNP, in addition to ANP, may play a pathophysiological role in CHF. Although two similar membrane-bound GCs (GC-A and GC-B) are known to be natriuretic peptide receptors, GC-A is an ANP and BNP receptor, whereas GC-B is a specific receptor for the C-type natriuretic peptide (CNP). HS was equally effective in inhibiting the increase in cGMP production stimulated by BNP, CNP, or ANP. HS shows almost equal antagonistic activity on GC-A and GC-B. In the present study, we could not measure the plasma concentrations of either BNP or CNP, and therefore, we cannot exclude the possibility that our findings are due to inhibition of these peptides. Further investigation is required to determine to what extent HS inhibits the effects of BNP or CNP in CHF.

In summary, the increment in endogenous ANP observed in severe CHF actually suppresses the activation of the renin-aldosterone system and sympathetic nervous activity and inhibits the reabsorption of water and sodium without inducing changes in GFR or RPF.
However, endogenous ANP exerts no significant hemodynamic effect through its vasodilative activity.

Acknowledgments
We wish to thank Mikio Nakagawa and Chika Yamamoto for excellent technical assistance. We also express thanks to Daniel Mrozek for his assistance in preparing the manuscript.

References


