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Research Article

Asymptomatic or early left ventricular dysfunction in humans is characterized by increases in circulating atrial natriuretic peptide (ANP) without activation of the renin-angiotensin-aldosterone system (RAAS). We previously reported a canine model of early left ventricular dysfunction (ELVD) produced by rapid ventricular pacing and characterized by an identical neurohumoral profile and maintenance of the natriuretic response to volume expansion (VE). To test the hypothesis that elevated endogenous ANP suppresses the RAAS and maintains sodium excretion in ELVD, we assessed the effects of antagonism of ANP on cardiorenal and neurohumoral function in ELVD. Chronic ANP suppression was produced by bilateral atrial appendectomies before the production of ELVD by rapid ventricular pacing (ELVD-APPX, n = 5). This group was compared with a separate group with ELVD and intact atrial appendages (ELVD-INTACT, n = 8). ELVD-APPX was characterized by lower circulating ANP (50 +/- 11 vs. 158 +/- 37 pg/ml, P < 0.05), activation of plasma renin activity (PRA) (9.4 +/- 2.4 vs. 0.6 +/- 0.4 ng/ml per h, P < 0.05) and aldosterone (36.4 +/- 12.5 vs. 2.5 +/- 0.0 ng/dl, P < 0.05) when compared to ELVD-INTACT. In comparison to the ELVD-INTACT group, sodium excretion was decreased before and during VE in the ELVD-APPX group. Acute ANP antagonism was produced by administration of the particulate guanylate cyclase coupled natriuretic peptide receptor antagonist, […]

A Functional Role for Endogenous Atrial Natriuretic Peptide in a Canine Model of Early Left Ventricular Dysfunction

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Abstract

Asymptomatic or early left ventricular dysfunction in humans is characterized by increases in circulating atrial natriuretic peptide (ANP) without activation of the renin-angiotensin-aldosterone system (RAAS). We previously reported a canine model of early left ventricular dysfunction (ELVD) produced by rapid ventricular pacing and characterized by an identical neurohumoral profile and maintenance of the natriuretic response to volume expansion (VE). To test the hypothesis that elevated endogenous ANP suppresses the RAAS and maintains sodium excretion in ELVD, we assessed the effects of antagonism of ANP on cardiorenal and neurohumoral function in ELVD. Chronic ANP suppression was produced by bilateral atrial appendectomies before the production of ELVD by rapid ventricular pacing (ELVD-APPX, $n = 5$). This group was compared with a separate group with ELVD and intact atrial appendages (ELVD-INTACT, $n = 8$). ELVD-APPX was characterized by lower circulating ANP (50±11 vs. 158±37 pg/ml, $P < 0.05$), activation of plasma renin activity (PRA) (9.4±2.4 vs. 0.6±0.4 ng/ml per h, $P < 0.05$) and aldosterone (36.4±12.5 vs. 2.5±0.0 ng/dl, $P < 0.05$) when compared to ELVD-INTACT. In comparison to the ELVD-INTACT group, sodium excretion was decreased before and during VE in the ELVD-APPX group. Acute ANP antagonism was produced by administration of the particulate guanylate cyclase coupled natriuretic peptide receptor antagonist, HS-142-1, to seven conscious dogs with ELVD and intact atrial appendages (ELVD-INTACT). HS-142-1 decreased plasma concentrations and renal generation of the ANP second messenger, cGMP, and was associated with activation of PRA and sodium retention with enhanced tubular sodium reabsorption. These data support a significant role for elevated endogenous ANP in the maintenance of sodium excretion and regulation of the RAAS in experimental ELVD. (*J. Clin. Invest.* 1995. 95:1101–1108.) Key words: atrial natriuretic peptide • ventricular dysfunction • renin-angiotensin system • sodium • antagonism

Introduction

Atrial natriuretic peptide (ANP)[1] is a hormone of cardiac origin which when released in response to atrial stretch functions to increase sodium excretion, inhibit the renin-angiotensin-aldosterone system (RAAS) and modulate arterial pressure. Most recently, studies using a unique natriuretic peptide receptor antagonist have importantly supported a role for endogenous ANP in cardiorenal homeostasis in normal conditions (1). The significance of ANP, however, in the modulation of hemodynamic, hormonal and renal function in heart failure remains controversial. While previous studies in acute and chronic severe congestive heart failure (CHF) have suggested that endogenous ANP may exert important beneficial effects by lowering filling pressures, suppressing the RAAS and maintaining sodium excretion, other studies have reported a blunted renal response to exogenous ANP in severe CHF (2–5).

Recent clinical studies have demonstrated that overt CHF is preceded by a period of early asymptomatic left ventricular dysfunction (ELVD). As reported in the Studies of Left Ventricular Dysfunction (SOLVD), humans with ELVD have elevated endogenous ANP without activation of the RAAS and presumably are without sodium retention as they remain asymptomatic without diuretic therapy (6). To date, the functional role of elevated endogenous ANP in ELVD in the regulation of sodium excretion and the RAAS remains undefined.

We have reported a canine model of ELVD produced by rapid ventricular pacing at 180 beats per minute for 10 d (7). This model of ELVD results in a neurohumoral profile identical to that observed in humans with ELVD as reported in SOLVD (6). Associated with this unique neurohumoral profile in the canine model of ELVD is a maintenance of the natriuretic response to volume expansion despite significant hemodynamic compromise. A preliminary report from Wright et al. (8) demonstrated preservation of the hemodynamic, hormonal, and renal response to exogenous ANP in this model of ELVD (8) which supports the current hypothesis that endogenous ANP importantly contributes to the maintenance of neurohumoral and renal function in ELVD.

The objective of the current study was to establish the significance of endogenous ANP in regulating cardiorenal and neurohumoral function in ELVD by investigating the effects of chronic ANP suppression in conscious dogs with ELVD. Specifically, dogs underwent bilateral atrial appendectomy before the induction of ELVD in order to attenuate the chronic elevation of ANP observed in ELVD (ELVD-APPX) while in a separate group, the atrial appendages were left intact (ELVD-

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1. *Abbreviations used in this paper:* ANP, atrial natriuretic peptide; ANP_{corr}, ANP corrected for plasma hematocrit; cGMP, 3′,5′ cyclic guanosine monophosphate; CHF, congestive heart failure; CO, cardiac output; ELVD, early left ventricular dysfunction; MAP, mean arterial pressure; PCWP, pulmonary capillary wedge pressure; PRA, plasma renin activity; PV, plasma volume; RAAS, renin-angiotensin-aldosterone system; RAP, right atrial pressure; RBF, renal blood flow; SVR, systemic vascular resistance; VE, volume expansion.

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INTACT). Hemodynamic, hormonal and renal function were then compared at baseline in ELVD-APPX and ELVD-INTACT and the responses to acute saline volume expansion were compared between the two groups. To further investigate the role of elevated endogenous ANP in ELVD, an antagonist to the biologically active natriuretic peptide receptors, HS-142-1 (9, 10), was administered to a third group of dogs with ELVD and the hemodynamic, hormonal and renal responses were evaluated.

Methods

General methods. 20 male mongrel dogs, weighing between 18 and 21 kg, underwent implantation of a programmable cardiac pacemaker (model 8426; Medtronic, Minneapolis, MN). Under pentobarbital sodium anesthesia (30 mg/kg) and via a left thoracotomy and 1–2 cm pericardiotomy, the heart was exposed and a screw-in epicardial pacemaker lead was implanted into the right ventricle. The pacemaker lead was connected to a pulse generator which was implanted subcutaneously in the chest wall.

Five dogs underwent removal of the left and right atrial appendages at the time the permanent pacemaker was implanted to chronically blunt the increase in plasma ANP associated with ELVD (ELVD-APPX). A separate incision was made in the pericardium over the right and left atrial appendage. The appendage was ligated at the appendage base, with excision of the excess tissue. The pericardium was sutured closed with caution to not distort the anatomy of the pericardium.

In the other fifteen dogs the appendages were left intact (ELVD-INTACT). Eight of these dogs served as controls for the ELVD-APPX group. Five of these eight were chosen at random from the original group of ELVD dogs (7). The surgical preparation in these dogs was identical to that in the appendectomy dogs with the exception that no incision in the pericardium over the atrial appendages was made. Three additional ELVD-INTACT dogs were studied where the surgical preparation was identical to the ELVD-APPX dogs in that bilateral pericardial incisions were made over the atrial appendages, however no atrial appendectomy was performed. Hemodynamic, neurohumoral, and renal data in the three ELVD-INTACT dogs was not significantly different from the group of five ELVD-INTACT dogs from the original study (7). The remaining seven ELVD-INTACT dogs comprised the acute antagonism group which received HS-142-1.

A subcutaneously placed chronic femoral artery catheter (Access Technologies, Skokie, IL) was implanted in all dogs during the sterile surgical period. This was flushed biweekly with heparinized saline. Dogs received antibiotics for the first three post operative days and allowed to recover for an average of 10 d. Dogs were acclimated to the sling in which the conscious studies were performed, allowing standing in a minimally restrained fashion. All dogs were fed normal dog chow (Lab Canine Diet 5506; Purina Mills, St. Louis, MO) and were allowed free access to tap water. All studies were conducted in a manner consistent with the guiding principles of the American Physiological Society. The experimental protocol was approved by the Institutional Animal Care and Use Committee.

At the end of the recovery period, dogs were programmed for rapid right ventricular pacing. The rapid ventricular pacing model (at 240–250 beats per minute) has been used extensively by our laboratory and others to produce a model of severe CHF with marked depression of cardiac function and activation of the RAAS (11–15). The current study uses a modification of this model in the conscious state with a lower pacing rate, 180 beats per minute for 10 d, which produces a milder form of tachycardia-related cardiomyopathy without activation of the RAAS.

A two-dimensional guided M-mode echocardiogram was performed at baseline and after the induction of ELVD on the 11th day of pacing. Echocardiograms were performed in the conscious state with the dog unrestrained, standing quietly and in normal sinus rhythm. Ejection fraction was determined as previously described (7, 16). Although sinus tachycardia in the dog may reach 180 beats per minute intermittently, the animals were paced for the majority of beats when an ECG was monitored to confirm pacemaker function or at the time of echocardiography.

The night before the acute experiments the animals were fasted, given 300 mg of lithium carbonate and allowed access to water ad lib. The day of the acute experiment, 10 d after pacing at 180 beats per minute, the animals were briefly anesthetized with thiopental sodium (15 mg/kg) to allow percutaneous placement of a flow-directed balloon tip pulmonary artery catheter (model 93131-7F; American Edwards Laboratories, AHS del Caribe, Anasco, P.R.) via an external jugular vein for measurement of cardiac output and atrial pressures. A second balloon-tip catheter was inserted in the urinary bladder to allow urine collection. The femoral artery catheter was connected to a pressure monitor for on-line measurement of aortic pressure. After animals regained consciousness, an equilibration period of 90 min was allowed before initiating the acute study.

Cardiac hemodynamic parameters measured during each acute study clearance period included mean arterial pressure (MAP), right atrial pressure (RAP), pulmonary capillary wedge pressure (PCWP) and cardiac output (CO). CO was determined by thermodilution (CO model 9510-A computer; American Edwards Laboratories, Irvine, CA) and was measured five times during each clearance period and averaged. Systemic vascular resistance (SVR) was calculated by the formula:

$$\text{SVR (dyne} \cdot \text{s} \cdot \text{min}^{-5}) = \frac{\text{MAP} - \text{RAP}}{\text{CO}} \times 80$$

Voided urine was allocated for measurements of sodium, creatinine, lithium, inulin and PAH, and refrigerated until analysis. Urine collected for cGMP analysis was heated to more than 90°C before storage. Arterial blood for sodium, creatinine, lithium, inulin, and PAH determinations was collected in heparinized tubes, placed on ice, and centrifuged at 2,500 rpm at 4°C. Plasma was decanted and refrigerated until analysis. Urinary and plasma sodium concentrations were measured using ion-selective electrodes (Beckman Instruments, Brea, LA). Urinary and plasma creatinine concentrations were measured by the Jaffe reaction (Beckman Instruments). Urinary and plasma lithium levels were determined by flame emission spectrophotometry (model 357; Instrumentation Laboratory, Wilmington, MA). Lithium clearances (ClLi) were used as an indirect method to calculate proximal (PFNaR = [1 − (ClLi/GFR)] · 100) and distal fractional tubular sodium reabsorption (DFNaR = (ClLi − ClNa)/ClLi · 100) (17–18). Urinary and plasma inulin concentrations were measured by the anthrone method (19). In the first study, glomerular filtration rate was estimated by creatinine clearance. In the second study, glomerular filtration rate was estimated by both creatinine clearance and inulin clearance. Renal blood flow was calculated from estimated renal plasma flow (PAH clearance) and hematocrit. A calculated inulin and PAH bolus was followed by a continuous infusion at a rate of 1 cc/min.

Arterial blood samples for hormonal analysis were placed in EDTA tubes on ice, centrifuged at 2,500 rpm at 4°C and stored. After extraction, plasma levels of ANP were measured by radioimmunoassay to α-human ANP as previously described (20). Plasma samples for cGMP were extracted with ethanol and plasma and urinary cGMP were measured by radioimmunoassay using the method of Steiner et al. (21). Renal cGMP generation was calculated as:

$$\text{Renal cGMP gen.} = (\text{Urinary Flow} \times [\text{cGMP}]_{\text{urine}}) - (\text{GFR} \times [\text{cGMP}]_{\text{plasma}}).$$

Plasma renin activity and aldosterone were determined by radioimmunoassay using the methods of Haber et al. (22, 23).

As hematocrit (H) decreased secondary to rapid intravascular volume expansion in the first study, plasma ANP was corrected for the increase in plasma volume (PV). Percent change of plasma volume was calculated using the formula: $\%PV = (100/100\text{-}H_1) \cdot [100([H_1 - H_2/H_2)]$, where H_1 = baseline hematocrit and H_2 = VE hematocrit (24). Corrected ANP (ANP_{corr}) was calculated using the formula: $ANP_{corr} = ANP\ (1 + \%PV)$.

Study no. 1: bilateral atrial appendectomy in chronic ELVD. Five

dogs with ELVD-APPX and eight dogs with ELVD-INTACT were prepared as described above. At the conclusion of the equilibration period, two 30-min baseline urinary clearances were performed. Midway through each urinary clearance period, cardiac hemodynamics were measured and arterial blood was drawn for electrolyte and hormonal analysis. After the two 30-min baseline urinary clearances, volume expansion was initiated. Normal saline was infused through the cordis sheath placed in the superior vena cava at 2 $ml \cdot kg^{-1} \cdot min^{-1}$. This was equivalent to ~ 5% body weight volume expansion over 30 min. A 15-min urinary clearance was performed during the second half of volume expansion, along with hemodynamic and arterial blood collection.

Study no. 2: acute antagonism of ANP in ELVD. Seven ELVD-INTACT dogs were prepared as described above. At the conclusion of the equilibration period, two 30-min baseline urinary clearances were collected. The natriuretic peptide antagonist, HS-142-1 (3 mg/kg in 20 cc normal saline), was then administered as an intravenous bolus through the right atrial port of the Swan Ganz catheter. Two additional 30-min clearances were then collected after the bolus of HS-142-1. Midway through each of the four urinary clearance periods, cardiac hemodynamics were measured and arterial blood was drawn for electrolyte and hormonal analysis.

Statistical methods. All data are presented as mean±SEM. Comparison between ejection fraction before and after pacing in each group was made using the paired Student's *t* test.

In the first study, comparisons between the ALVD-APPX and ELVD-INTACT groups were made using unpaired Student's *t* tests. The two baseline clearance periods were averaged and comparisons between the baseline period and volume expansion in each group were analyzed by the paired Student's *t* test. Statistical significance was defined as $P < 0.05$.

In the second study, comparisons between the three clearance periods (the first clearance representing the average of the two baseline clearances and the next two clearances representing post-HS-142-1 administration) were made using ANOVA for repeated measures and Fisher's Least Significant Difference test for individual comparisons. Statistical significance was defined as $P < 0.05$.

Results

Study no. 1: bilateral atrial appendectomy in chronic ELVD. Bilateral atrial appendectomy resulted in removal of 2.12±0.29 gm of atrial tissue (0.90±0.13 gm on the right and 1.22±0.19 gm on the left). This likely underestimates the actual amount of functional tissue removed as it does not account for tissue isolated and destroyed by the ligatures around the appendage stumps.

Left ventricular ejection fraction decreased similarly in each group, from 52±2 to 33±2% in the ELVD-INTACT group ($P < 0.05$) and from 54±2 to 35±1% in the ELVD-APPX group ($P < 0.05$). No dog in this or the second study exhibited the profound lethargy, muscle wasting and ascites routinely observed in dogs undergoing more rapid ventricular pacing at 250 beats per minute.

Hemodynamic, hormonal and renal data at baseline in the ELVD-INTACT and ELVD-APPX groups are depicted in Figs. 1, 2, and 3, respectively. Cardiac output, pulmonary capillary wedge pressure, and systemic vascular resistance were similar in both groups. Mean arterial pressure was lower and right atrial pressure was higher in the ELVD-APPX group (Fig. 1).

Bilateral atrial appendectomy effectively blunted the increase in ANP associated with ELVD when compared to the ELVD-INTACT group (Fig. 2) and when compared to preventricular failure concentrations of ANP in the ELVD-APPX group (46±15 pg/ml preventricular failure to 50±13 pg/ml, NS). Plasma concentrations of cGMP tended to be lower in the ELVD-APPX group ($P = 0.10$) when compared with the

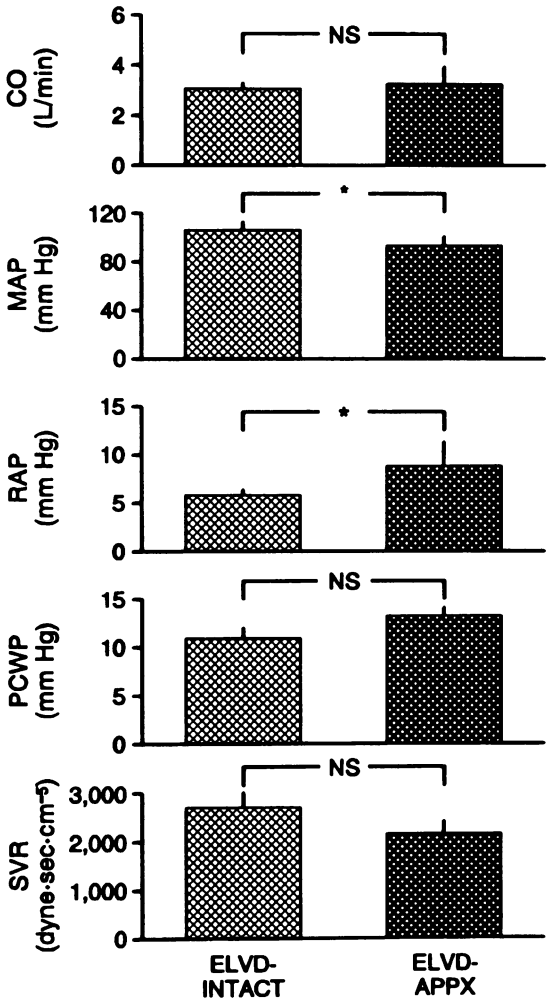

Figure 1. Chronic suppression of ANP in ELVD. Cardiac output (*CO*), mean arterial pressure (*MAP*), right atrial pressure (*RAP*), pulmonary capillary wedge pressure (*PCWP*), and systemic vascular resistance (*SVR*) in ELVD-INTACT ($n = 8$) and ELVD-APPX ($n = 5$) at baseline, prior to volume expansion. Values are mean±SEM. $*P < 0.05$ ELVD-INTACT vs. ELVD-APPX. *NS*, not significant.

ELVD-INTACT group, and were not different from pre-ventricular failure concentrations in the ELVD-APPX group (5.6±0.5 pmol/ml preventricular failure to 5.7±1.4 pmol/ml, NS). This is in contrast to the ELVD-INTACT group in which both plasma ANP (30.0±4.0 pg/ml preventricular failure to 140.6±24.0 pg/ml, $P < 0.05$) and cGMP (4.0±0.4 pmol/ml preventricular failure to 10.3±1.8 pmol/ml, $P < 0.05$) were significantly increased when compared with preventricular failure concentrations. Plasma renin activity and plasma aldosterone were higher in the ELVD-APPX group than in the ELVD-INTACT group. Plasma renin activity (1.77±0.65 ng/ml/h preventricular failure to 9.40±2.40 ng/ml/h, $P < 0.05$) and plasma aldosterone (6.0±0.7 ng/dl preventricular failure to 36.4±12.5 ng/dl, $P < 0.05$) were activated by ventricular dysfunction in the ELVD-APPX group when compared with preventricular failure concentrations.

Sodium retention was present in the ELVD-APPX group at baseline when compared to the ELVD-INTACT group as demonstrated by lower urinary volume and lower fractional and absolute excretion of sodium (Fig. 3). Creatinine clearance tended to be lower and proximal fractional sodium reabsorption

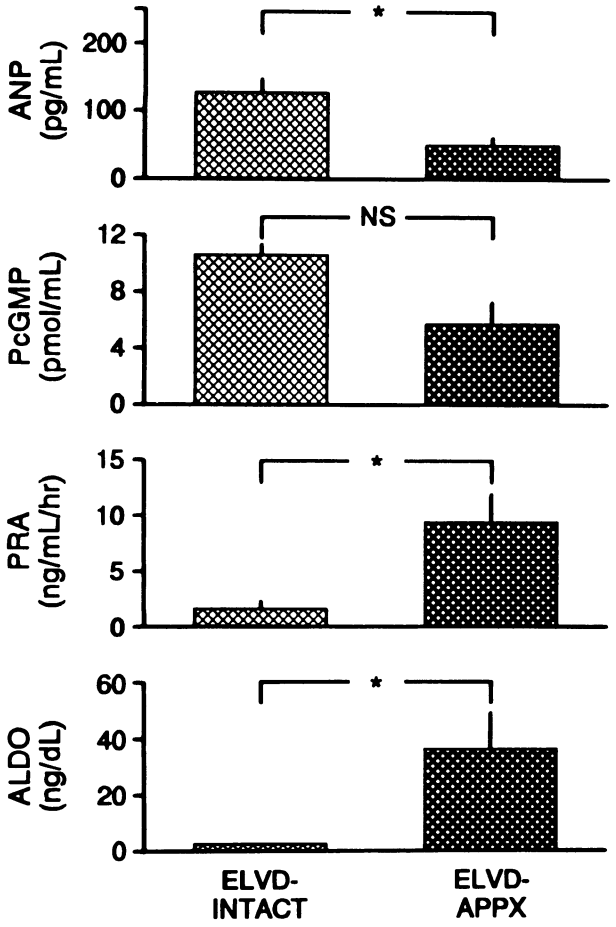

Figure 2. Chronic suppression of ANP in ELVD. Atrial natriuretic peptide (*ANP*), plasma cGMP (*PcGMP*), plasma renin activity (*PRA*), and plasma aldosterone (*ALDO*) in ELVD-INTACT (n = 8) and ELVD-APPX (n = 5) at baseline, before volume expansion. Values are mean±SEM. *$P < 0.05$ ELVD-INTACT vs. ELVD-APPX. *NS*, not significant.

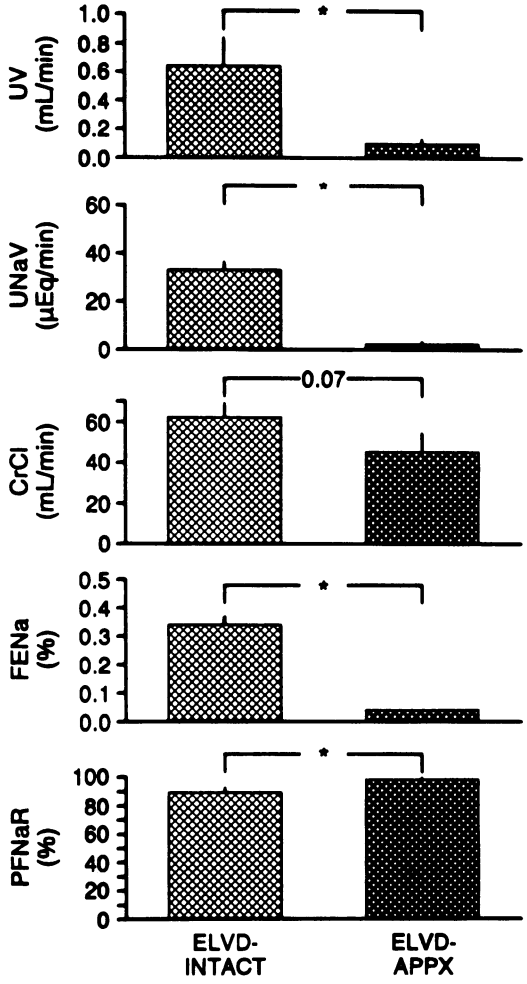

Figure 3. Chronic suppression of ANP in ELVD. Urinary flow rate (*UV*), urinary sodium excretion (*UNaV*), creatinine clearance (*CrCl*), fractional excretion of sodium (*FENa*), and proximal fractional sodium reabsorption (*PFNaR*) in ELVD-INTACT (n = 8) and ELVD-APPX (n = 5) at baseline, prior to volume expansion. Values are mean±SEM. *$P < 0.05$ ELVD-INTACT vs. ELVD-APPX.

was higher in the ELVD-APPX group at baseline. No significant difference between the two groups was demonstrated in distal fractional sodium reabsorption.

Intravenous saline volume expansion (VE) produced similar increases in cardiac output (Δ CO = 1.8±0.5 vs. 2.3±0.8 liters/min, NS), mean arterial pressure (Δ MAP = 9.3±2.0 vs. 13.0±3.0 mm Hg, NS), right atrial pressure (Δ RAP = 5.2±1.0 vs. 4.6±0.6 mm Hg, NS) and pulmonary capillary wedge pressure (ΔPCWP = 9.6±1.4 vs. 7.4±0.6 mm Hg, NS) in the ELVD-INTACT and ELVD-APPX groups, respectively. Systemic vascular resistance similarly decreased in the INTACT and APPX ELVD groups (ΔSVR = 845±317 vs. 852±288 dyne·sec·cm^{-5}, NS). While the chronic elevation in ANP associated with ELVD was blunted by bilateral atrial appendectomy in all 5 dogs, acute saline VE produced increases in ANP_{corr} in 2 of the 5 ELVD-APPX dogs and there was no significant difference in the change in ANP_{corr} with VE in the INTACT and APPX ELVD groups (Δ ANP = 88±38 vs. 164±106 pg/ml, NS). Plasma renin activity decreased by 6.3±1.5 ng/ml per h and plasma aldosterone levels decreased by 13.2±2.9 ng/dL with VE in the ELVD-APPX group. While the mean values for plasma renin activity and aldosterone decreased with VE in the ELVD-INTACT group, these changes were not analyzed statistically because many of the VE values were below the limits of detection of the assay. The renal responses to VE in the two groups are compared in Fig. 4. The diuretic and natriuretic response to VE was markedly blunted in the ELVD-APPX group with reduced increases in urinary flow and urinary sodium excretion. The change in creatinine clearance with VE in the ELVD-APPX group was higher than that observed in the ELVD-INTACT group. No significant change occurred in distal tubule sodium reabsorption. The blunted natriuretic response was thus mediated by a blunted decrease in proximal tubule fractional sodium reabsorption in the ELVD-APPX group.

Study no. 2: acute antagonism of ANP in ELVD. ELVD-INTACT was characterized by decreases in left ventricular ejection fraction (54±2 to 30±3%, $P < 0.05$), increases in plasma ANP (22±4 to 187±64 pg/ml, $P < 0.05$) and its second messenger cGMP (3.9±1.4 to 6.4±1.3 pmol/ml, $P < 0.05$) and no significant change in plasma renin activity (1.8±0.7 to 3.5±1.3 ng/ml per h, NS) or plasma aldosterone concentration (4±3 to 5±3 ng/dl, NS).

The hemodynamic responses to acute administration of HS-142-1 in ELVD-INTACT are displayed in Table I. Both mean

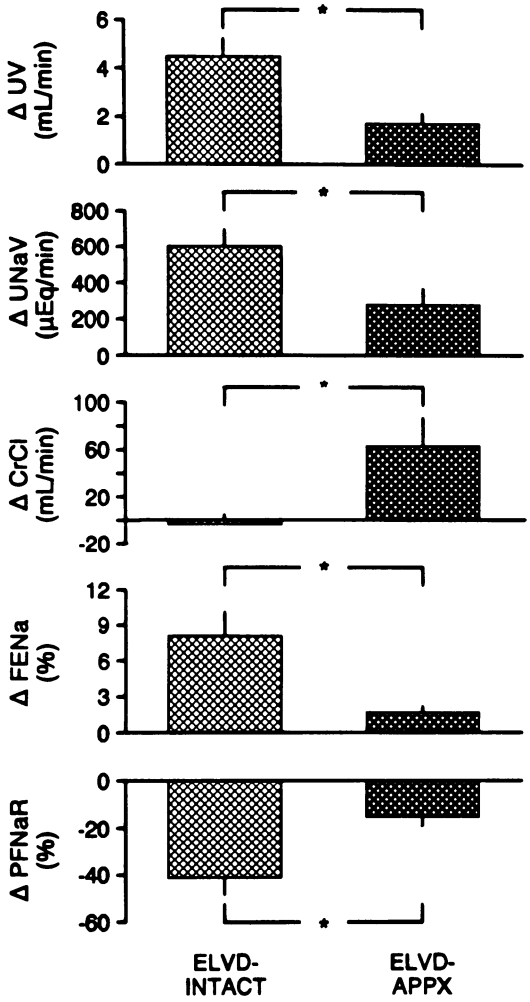

Figure 4. Chronic suppression of ANP in ELVD. Changes in urinary flow rate (Δ *UV*), urinary sodium excretion (Δ *UNaV*), creatinine clearance (Δ*CrCl*), fractional excretion of sodium (Δ*FENa*), and proximal fractional sodium reabsorption (Δ*PFNaR*) with volume expansion in ELVD-INTACT (n = 8) and ELVD-APPX (n = 5). Values are mean±SEM. *P < 0.05 ELVD-INTACT vs. ELVD-APPX.

arterial pressure and pulmonary capillary wedge pressure increased and remained elevated after administration of HS-142-1. There was a trend towards an increase in systemic vascular resistance following HS-142-1. No significant change was noted in cardiac output, right atrial pressure or renal blood flow.

Table I. Hemodynamic Responses to Acute Administration of HS-142-1 in ELVD

n = 7	ELVD	ELVD + 30′ post-HS-142-1	ELVD + 60′ post-HS-142-1
MAP (mm Hg)	110±4	124±6*	124±6*
RAP (mm Hg)	8.5±0.76	9.1±0.74	9.7±1.2
PCWP (mm Hg)	14.0±0.82	15.0±0.99*	17.0±1.2*
CO (L/min)	3.56±0.58	3.56±0.49	3.53±0.7
SVR (dyne·s·min^{-5})	2640±400	2880±480 (P = 0.06)	3520±880
RBF (ml/min)	432±43	464±63	556±85

Data are mean±SEM. * P < 0.05 vs. ELVD.

Table II. Hormonal Responses to Acute Administration of HS-142-1 in ELVD

n = 7	ELVD	ELVD + 30′ post-HS-142-1	ELVD + 60′ post-HS-142-1
ANP (pg/ml)	187±64	209±55	187±63
cGMP (pmol/ml)	6.4±1.3	3.6±0.97*	2.4±0.55*
PRA (ng/ml per h)	3.5±1.3	6.3±1.5*	3.7±1.2
Aldo (ng/dl)	5.0±3.1	11.0±5.2 (P = 0.06)	12.0±5.3 (P = 0.07)

Data are mean±SEM. * P < 0.05 vs. ELVD. *aldo*, aldosterone.

The hormonal responses to acute administration of HS-142-1 in ELVD-INTACT are displayed in Table II. Plasma ANP did not change after HS-142-1. Plasma cGMP was decreased and was associated with a significant increase in plasma renin activity. There was a trend towards an increase in plasma aldosterone concentrations. Of note, the cGMP and aldosterone effects were sustained while PRA returned to pre-HS-142-1 concentrations after HS-142-1.

The renal responses to acute administration of HS-142-1 in ELVD-INTACT are displayed in Table III. HS-142-1 produced decreases in renal cGMP generation and this was associated with decreases in urinary sodium excretion and fractional excretion of sodium. Fractional reabsorption of sodium at both the proximal and distal tubular level increased. These renal responses returned toward pre-HS-142-1 levels 60 min following the HS-142-1 bolus. There was a significant increase in creatinine clearance in the second urinary clearance following HS-142-1 administration. There was a similar trend in GFR as estimated by inulin clearance. While the values for GFR as estimated by creatinine clearance were lower than those obtained by inulin clearances the changes in these values over time were similar.

Fig. 5 summarizes the cardiorenal and neurohumoral responses to acute natriuretic peptide receptor antagonism in ELVD-INTACT dogs. Attenuation of plasma and renal cGMP

Table III. Renal Responses to Acute Administration of HS-142-1 in ELVD

n = 7	ELVD	ELVD + 30′ post-HS-142-1	ELVD + 60′ post-HS-142-1
UV (ml/min)	0.52±0.12	0.42±0.09	0.4±0.11
UNaV (μEq/min)	79±26	32±11*	55±17
FENa (%)	0.49±0.15	0.24±0.11*	0.29±.08
GFR (ml/min)	96±12	97±17	107±18
CrCl (ml/min)	70±5	72±7	83±7*
PFNaR (%)	81.0±2.2	87.0±1.3*	83.0±1.3
DFNaR (%)	98.0±0.6	99.0±0.3*	98.0±0.4
cGMP gen (pmol/min)	1326±357	560±128*	873±198*

Data are mean±SEM. * P < 0.05 vs. ELVD. *UV*, urine volume; *UNaV*, urinary sodium excretion; *FENa*, fractional excretion of sodium; *GFR*, glomerular filtration rate; *CrCl*, creatinine clearance; *PFNaR*, proximal fraction of sodium reabsorption; *DNFaR*, distal fraction of sodium reabsorption; cGMP gen, renal cGMP generation.

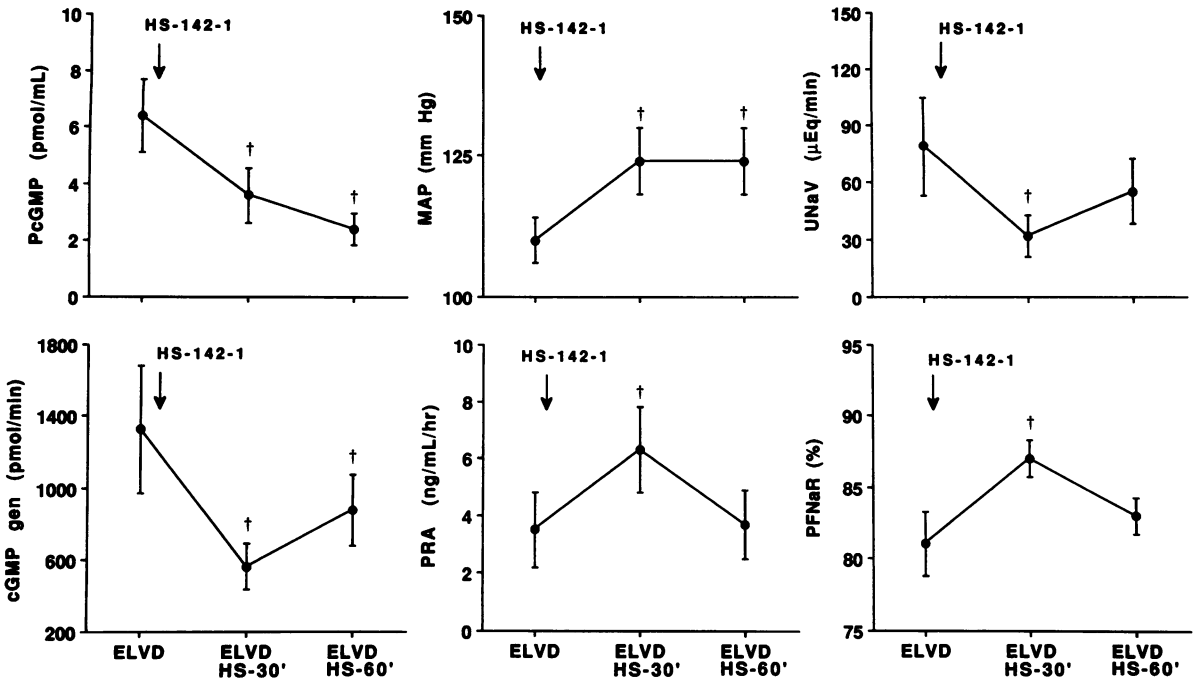

Figure 5. Acute antagonism of ANP in ELVD. Responses of plasma cGMP (*PcGMP*), renal cGMP generation (*cGMP gen*), mean arterial pressure (*MAP*), plasma renin activity (*PRA*), urinary sodium excretion (*UNaV*), and proximal fractional sodium reabsorption (*PFNaR*) at baseline and 30 and 60 min after HS-142-1 (3 mg/kg intravenous bolus, $n = 7$). Values are mean±SEM. $^{\dagger}P < 0.05$ vs. ELVD.

generation was associated with increases in MAP, activation of plasma renin activity and sodium retention which was mediated in part by enhanced reabsorption of sodium at the proximal tubule.

Discussion

The current studies demonstrate that attenuation of the increase in endogenous ANP produced by bilateral atrial appendectomy in ELVD results in elevation of cardiac filling pressures, activation of the RAAS, and sodium retention with an associated reduction in the natriuretic response to saline volume expansion. Second, acute antagonism of endogenous ANP in experimental ELVD by the natriuretic peptide antagonist, HS-142-1, results in decreases in plasma and renal generation of the natriuretic peptide second messenger, cGMP, and is associated with elevation of filling pressures, activation of plasma renin activity and sodium retention, which is mediated by enhanced reabsorption of sodium by the proximal and distal tubule.

The neurohumoral profile associated with asymptomatic or early left ventricular dysfunction is thought to be essential to the maintenance of the asymptomatic state (25). Studies have speculated that activation of endogenous ANP may be essential in maintaining suppression of the RAAS early in the course of ventricular dysfunction as previously suggested by the studies of Lee et al. in acute heart failure (2). In these previous studies Low ANP Acute Heart Failure produced by acute inferior thoracic vena caval constriction was characterized by activation of PRA and aldosterone with sodium retention, responses not observed with Elevated ANP Acute Heart Failure produced by rapid ventricular pacing. In chronic severe CHF an escape from the RAAS-suppressing actions of ANP may result in sodium retention (26). Studies in humans and animal models of CHF have demonstrated a blunting of the second messenger cGMP response to endogenous and exogenous ANP in severe CHF, in contrast to a preservation of the second messenger response to acute and chronic endogenous elevation of ANP and exogenous administration of ANP in mild CHF (5, 8, 27). Such studies suggest an important role for ANP in early ventricular dysfunction which is lost in the late stage of ventricular dysfunction. While one may speculate that ANP remains biologically active in ELVD, the previous inability to antagonize the effect of ANP has hampered efforts to establish the physiologic importance of ANP as a modulator of hemodynamic, hormonal, and renal function in ELVD.

Bilateral atrial appendectomy effectively blocked the increases in circulating ANP associated with experimental ELVD. The blockade of pacing-induced increases in ANP by atrial appendectomy are consistent with the findings of Shen et al. who demonstrated that removal of the atrial appendages blunts the increase in ANP associated with chronic severe heart failure produced by rapid ventricular pacing (28) and those of Perrella et al. who demonstrated that increases in synthesis and storage of ANP in the atrial free wall occur relatively late in the course of pacing induced heart failure and that pacing induced ventricular dysfunction is unassociated with ventricular hypertrophy and therefore is unassociated with ventricular ANP synthesis (14).

The current study demonstrates that the moderate reduction in ventricular function produced in this model is associated with activation of the RAAS and sodium retention when unaccompanied by chronic increases in plasma ANP. The reduction in sodium excretion and the blunted natriuretic response to volume expansion in the ELVD-APPX group are consistent with the effects of ANP to enhance sodium excretion via known renal tubular effects (29, 30) and by opposing the antinatriuretic effects of the RAAS. The physiological mechanism responsible for activation of the RAAS and sodium retention in the absence of elevation of ANP can only be speculated. One possible mech-

anism suggested by the data is the increased proximal tubule fractional sodium reabsorption observed in the ELVD-APPX group. This may have resulted in enhanced renin release due to decreased sodium delivery to the macula densa (31) and further activation of aldosterone. Indeed, ANP has been demonstrated to reduce proximal tubule sodium reabsorption via a dopamine mediated mechanism (30). In the current study, proximal tubule sodium handling was indirectly determined by lithium clearance, as supported by previous studies which have demonstrated the validity of lithium clearance as an accurate method of estimating whole kidney proximal tubular sodium reabsorption (17, 18). ANP also has a direct suppressive effect on aldosterone release (32) which was lost in the ELVD-APPX group. While not measured in the current study, enhanced sympathetic activation mediated by the RAAS activation and ANP depletion may also contribute to enhanced proximal tubule reabsorption.

The blunted natriuretic response to VE reported in the current study cannot be attributed to an attenuated increase in ANP which increased similarly in the atrial intact animals. The mechanism of the blunted response may more importantly be related to activation of the RAAS. This is consistent with the known actions of angiotensin II to increase proximal tubule reabsorption and aldosterone to increase distal tubular reabsorption. Moreover, the current studies are equally consistent with the known action of angiotensin II and aldosterone to attenuate the natriuretic actions of ANP (33). The effects of atrial appendectomy on atrial innervation and its subsequent effect on renal nerve activity are unknown, though normal cardiac innervation is not reported to traverse the atrial appendages.

HS-142-1, a naturally occurring polysaccharide compound which was initially discovered from the fungal strain of Aureobasidium sp., inhibits binding to the natriuretic peptide particulate guanylyl cyclase receptors (9, 10). These receptors include the natriuretic peptide-A receptor which binds ANP and brain natriuretic peptide (BNP) and the natriuretic peptide-B receptor which binds C-type natriuretic peptide (CNP) (34, 35). This inhibitory action is selective for the particulate guanylyl cyclase receptor (9, 36–40) and has no action on the clearance receptor or soluble guanylyl cyclase (9, 34, 38). As a result, natriuretic peptide-mediated actions are attenuated by HS-142-1 via decreasing the second messenger, cGMP. In the current study, intravenous HS-142-1 decreased plasma concentrations and renal generation of cGMP and this was associated with significant deleterious effects on hemodynamic, neurohumoral and renal function, suggesting that ANP (and conceivably BNP and CNP) plays a significant role in the maintenance of cardiorenal and humoral function in ELVD. The findings demonstrated by acute ANP antagonism thus are consistent with the findings in the studies which used bilateral atrial appendectomy to attenuate the increase in ANP associated with ELVD. Although a natriuretic peptide receptor antagonist now exists and is utilized to assess the response to acute antagonism of ANP in the current study, HS-142-1 is a costly and scarce compound with a short half-life and chronic infusions in large animals such as the dog are impractical and would be prohibitively expensive.

While acute administration of HS-142-1 resulted in a rather marked increase in mean arterial pressure, this was not observed in the ELVD-APPX group despite lower levels of the endogenous vasodilator ANP and chronic activation of endogenous vasoconstrictor systems. Preventricular failure hemodynamics were not assessed in the ELVD-APPX group; thus it is unknown whether pre-ventricular failure mean arterial pressure was different in the two groups. Presumably, atrial appendectomy primarily attenuates ANP release while HS-142-1 also antagonizes the vasodilatory actions of BNP and CNP and thus may account for the observed difference in MAP in chronic vs. acute ANP antagonism.

The findings of the current study support a major role for chronic elevation of ANP in mediating cardiorenal homeostasis in evolving heart failure. These data demonstrate that ANP importantly contributes to the maintenance of hemodynamic, hormonal and renal function in a canine model of ELVD. The similarities between this model and humans with asymptomatic left ventricular dysfunction support speculation that the neurohumoral profile described in patients with asymptomatic left ventricular dysfunction is mechanistically related to the asymptomatic state. Recent studies have demonstrated that ANP is a sensitive and specific marker for asymptomatic ventricular dysfunction (41) which provides prognostic information in patients with asymptomatic and symptomatic ventricular dysfunction (42). Indeed, chronic pharmacologic augmentation of ANP via inhibition of neutral endopeptidase has recently been shown to be of potential therapeutic benefit in patients with symptomatic heart failure, though this type of therapy may have the greatest role in early or mild heart failure and may be of benefit in delaying escape from the beneficial effects of ANP (43). Thus ANP emerges as a cardiac hormone with a major role in the pathophysiology of evolving heart failure.

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