Neuroendocrine and renal effects of intravascular volume expansion in compensated heart failure

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Gabrielsen, Anders, Peter Bie, Niels Henrik Holstein-Rathlou, Niels Juel Christensen, Jørgen Warberg, Harriet Dige-Petersen, Erik Frandsen, Søren Galatius, Bettina Pump, Vibeke B. Sørensen, Jens Kastrup, and Peter Norsk. Neuroendocrine and renal effects of intravascular volume expansion in compensated heart failure. Am J Physiol Regulatory Integrative Comp Physiol 281: R459–R467, 2001.—To examine if the neuroendocrine link between volume sensing and renal function is preserved in compensated chronic heart failure (HF), we observed that intravascular and central blood volume expansion by 3 h of water immersion (WI) elicits a natriuresis. In HF, WI suppressed angiotensin II (ANG II) and aldosterone (Aldo) concentrations, increased the release of atrial natriuretic peptide (ANP), and elicited a natriuresis (P < 0.05 for all) compared with seated control. Compared with control subjects (n = 9), ANG II, Aldo, and ANP concentrations were increased (P < 0.05) in HF, whereas absolute and fractional sodium excretion rates were attenuated [47 ± 16 vs. 88 ± 15 μmol/min and 0.42 ± 0.18 vs. 0.68 ± 0.12% (mean ± SE), respectively, both P < 0.05]. When ANG II and Aldo concentrations were further suppressed (P < 0.05) during WI in HF (by sustained angiotensin-converting enzyme inhibitor therapy, n = 9) absolute and fractional sodium excretion increased (P < 0.05) to the level of control subjects (108 ± 34 μmol/min and 0.70 ± 0.23%, respectively). Renal free water clearance increased during WI in control subjects but not in HF, albeit plasma vasopressin concentrations were similar in the two groups. In conclusion, the neuroendocrine link between volume sensing and renal sodium excretion is preserved in compensated HF. The natriuresis of WI is, however, modulated by the prevailing ANG II and Aldo concentrations. In contrast, renal free water clearance is attenuated in response to volume expansion in compensated HF despite normalized plasma AVP concentrations.

renin-angiotensin system; aldosterone; vasopressin; water-electrolyte balance

INFORMATION REGARDING the modulation of renal excretion of sodium and water by changes in extracellular and intravascular volumes in human heart failure (HF) is sparse and primarily obtained in mild degrees of the disease (29–33). It is not clear to what extent intravascular volume expansion promotes renal excretion of sodium and water in HF patients with more severe left ventricular dysfunction. Therefore, it is currently not known if the neuroendocrine link between volume sensing mechanisms and readjustments of renal excretion of sodium and water is preserved in chronic human HF. In such patients, attenuated increases of renal sodium and water excretion in response to intravascular volume expansion might contribute to volume overload and decompensation, a frequent cause of morbidity and hospitalization.

In a recent investigation we observed that intravascular and central blood volume expansion by thermoneutral water immersion suppressed sympathetic nervous activity and renin release in patients with compensated chronic HF (10). The observations demonstrate that volume stimulation suppresses antinatriuretic neuroendocrine activity, an effect that would be expected to promote renal excretion of sodium. On the other hand, plasma renin activity and ANG II concentrations were markedly elevated compared with those of the control subjects. Increased ANG II concentrations inhibit renal sodium excretion through direct effects on the kidney (11, 26) and by causing increased secretion of aldosterone (Aldo) (11, 26). Thus increased activity of the renin-angiotensin-aldosterone axis during volume expansion might attenuate or abolish the natriuretic response to intravascular and central blood volume expansion in compensated HF.

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On the basis of our previous study (10), the aim of the present investigation was to examine whether the neuroendocrine link between volume sensing and control of renal function is preserved in compensated chronic HF. We therefore tested the hypothesis that 1) water immersion elicits a natriuresis in compensated HF and 2) the natriuretic response is modulated by endogenous ANG II and Aldo activity.

METHODS

The experimental protocol was approved by the Ethics Committee of Copenhagen (KF 01–233/98) and in agreement with institutional guidelines and the principles set forth in the declaration of Helsinki. All participants gave written informed consent.

Subjects. Twelve male out-clinic patients with chronic HF and dilated cardiomyopathy (idiopathic, n = 9; hypertensive, n = 2; ischemic, n = 1) in a stable compensated phase (New York Heart Association functional class II, n = 7; III, n = 5) and nine age- and gender-matched control subjects were included in the investigation (Table 1). The patients were on standard medical treatment with angiotensin-converting enzyme inhibitor (ACE-I; n = 11), ANG II receptor blocker (losartan, n = 1), diuretics (n = 11), and digoxin (n = 8). In addition, four patients received additional nitrate vasodilator therapy, two patients received α-adrenoceptor blocker (cardenilol), and four patients received β-adrenoceptor blocker (metoprolol or atenolol). Medications were unchanged during the period of investigation. All patients were without signs of decompensation (pulmonary rales, peripheral edema) on the days of investigation. One HF patient and one control subject had short duration type II diabetes mellitus (<3 mo) treated by dietary measures. The rest of the control subjects were healthy as indicated by medical history, clinical examination, blood pressure (<140/95), hematocrit, and urine dipstick tests.

Table 1. Characteristics of control subjects and heart failure patients

<table>
<thead>
<tr>
<th>Control Subjects</th>
<th>P Value Control Subjects vs. Heart Failure</th>
<th>Heart Failure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 9)</td>
<td>(n = 11)</td>
</tr>
<tr>
<td>Age, yr</td>
<td>50 ± 5</td>
<td>0.99</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>67.6 ± 0.02</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>53.6 ± 1.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LAD, mm</td>
<td>40.1 ± 1.3</td>
<td>&lt;0.0001</td>
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</table>

Measurements and calculations. Measurements of cardiovascular variables and blood sampling were performed at hourly intervals, and the results of all measurements were averaged over the 3-h study period. Arterial pressures in the

Intravascular and central blood volume expansion. Thermoneutral (34.5–35.0°C) water immersion (8) (upright seated) to the fourth intercostal space was used as a means to induce intravascular and central blood volume expansion. This model increases cardiac preload (10) and induces an increase in plasma volume (15) and a decrease in plasma colloid osmotic pressure (15) (hemodilution) without initial changes in plasma osmolality (10), plasma sodium concentration (10), or total body sodium content.

Experimental protocol. Eleven patients and nine healthy control subjects underwent a 3-h water-immersion study and a 3-h seated control study conducted in a randomized balanced order ~6 wk apart. For 3 days before each of the study days, the participants ingested a standardized diet containing 80 mmol of sodium/day. ACE-I (n = 10) and ANG II- receptor blocker (n = 1) medications were discontinued 24 h before the study, and all other medications were omitted on the day of the study. Pharmacological testing of the degree of ACE activity was not performed.

Nine of the HF patients participated in an additional 3-h water-immersion study during sustained ACE-I treatment 7 wk after the previous immersion or seated control study. The responses were compared with those obtained during withdrawal of ACE-I therapy in the same nine subjects. During the days before this study the participants were allowed to ingest their normal diets and continued to take their ACE-I (n = 8) and ANG II-receptor blocker (n = 1) medications but withheld all other medications on the study day. These measures were undertaken to investigate the patients during their habitual activity of the renin-angiotensin-aldosterone axis. Otherwise, identical procedures and hydration protocols were applied during each of the 3 study days.

Values presented are means ± SE of control subjects and heart failure patients investigated during withdrawal of angiotensin-converting enzyme inhibitor (ACE-I) therapy for 24 h (−ACE-I) and during sustained ACE-I treatment (+ACE-I). LVEF, left ventricular ejection fraction; LVEDD, left ventricular end-diastolic diameter; LAD, left atrial diameter; 24-h UNaV/UkV, 24-h urinary sodium/potassium excretion before the seated control study (seated), water immersion study (WI), and WI with (WI +ACE-I) and without (WI −ACE-I) sustained ACE-I treatment; Hct, hematocrit. *P = 0.02 for WI +ACE-I vs. WI −ACE-I. LVEF, LVEDD and LAD were measured by conventional echocardiography in conjunction with the investigation.
brachial artery were measured by an automatic oscillometric method (Propath 102, Protocol Systems, Beaverton, OR) with the cuff kept at heart level during all measurements. Heart rate was determined from a single-lead electrocardiogram. Cardiac output was derived from measurements of pulmonary blood flow using a noninvasive inert gas rebreathing method as previously described (10). From these measurements, cardiac index, stroke volume index, and systemic vascular resistance were calculated using standard formulas.

Blood samples were obtained from a catheter inserted into a cubital vein. Plasma protein concentrations, plasma sodium ([PNa]), and potassium concentrations and plasma osmolality were measured on fresh plasma samples. Plasma concentrations of arginine vasopressin (AVP; 7) (detection limit = 0.15 pg/ml, average recovery 73%), ANG II (16) (detection limit = 1.0 pg/ml, average recovery 103%), norepinephrine (17), epinephrine (17), and plasma renin activity (23) were measured as previously described. Aldo was measured by a commercially available kit (Coat-A-Count; Diagnostic Products, Los Angeles, CA). NH2 terminal pro-atrial natriuretic peptide-(1–30) (ANP) was measured radioimmunologically using antiserum and calibration material from Peninsula Laboratories (Belmont, CA) and in-house prepared tracer. Plasma samples were diluted 1:15 with RIA buffer before assay.

At hourly intervals, the subject voided, and urine volume, osmolality, and concentrations of sodium and potassium were measured. At each void, complete emptying of the bladder was verified by an ultrasound scan. In the few instances where residual urine was observed, the volume was estimated (25) and the void volume corrected. Subsequently, urine flow rates and urinary excretion rates of sodium, potassium, and solute free water were calculated from standard formulas and averaged over the 3-h study period. Urine concentrations of cGMP were measured radioimmunologically using antiserum from Calbiochem-Novabiochem (San Diego, CA), calibrator material from Sigma-Aldrich (St. Louis, MO), and in-house prepared tracer. Urine samples were diluted 90-fold with RIA buffer before assay. Urinary excretion rates of cGMP were calculated to provide an index of the activity of mechanisms mediating cGMP-dependent sodium excretion.

Glomerular filtration rate (GFR) was estimated from the renal clearance of 51Cr-EDTA (2), and the renal clearance of endogenous lithium was used to estimate changes in fluid delivery out of the proximal tubules for the whole kidney (22, 28). The renal clearances of 51Cr-EDTA, endogenous lithium (CLi), and sodium (CNa) were calculated as the ratio between the urinary excretion rate and the mean plasma concentrations during the 3-h study periods. Filtered load of sodium ([FNa] = GFR × [PNa]) and whole kidney fractional excretion of sodium ([FENa] = CNa/GFR) were calculated, and endogenous CLi was used for the calculation of absolute proximal tubular reabsorption rate of sodium ([APRNa] = (GFR × CLi) × [PNa]), fractional proximal tubular sodium reabsorption ([FPRNa] = 1 – (CNa/CLi)), absolute distal tubular reabsorption rate of sodium ([ADRNa] = (C14 – CNa)/[PNa]), and fractional distal tubular sodium reabsorption ([FDRNa] = 1 – (CNa/C14)].

Statistical analysis. Changes in variables in response to water immersion within the control and HF groups were analyzed by a paired two-sided t-test. An unpaired t-test was used to detect differences in variables between the control and HF (withdrawn ACE-I treatment) groups during seated control and water immersion, respectively. Data are presented as means ± SE, and the level of statistical significance was chosen at 0.05.

RESULTS

Effects of water immersion in control subjects and HF during withdrawal of ACE-I treatment. Intravascular volume expansion by water immersion increased cardiac index (Fig. 1) and decreased systemic vascular resistance (Table 2) similarly in both groups of subjects without changes in mean arterial pressure (Fig. 1).

Fig. 1. Cardiac index (CI), mean arterial pressure (MAP), glomerular filtration rate (GFR), and filtered load of sodium ([FNa]) during seated control (SEAT) and intravascular volume expansion by water immersion (WI) in control subjects and heart failure subjects investigated after withdrawal of angiotensin converting enzyme inhibitor (ACE-I) treatment for 24 h (~ACE-I). The 2 columns to the right illustrate effects of WI in heart failure subjects ~ACE-I (WI) compared with those obtained in the same subjects during WI on sustained ACE-I treatment (WI+ACE-I). Results are presented as means ± SE. *P < 0.05 vs. SEAT; ‡P < 0.05 vs. WI in heart failure subjects ~ACE-I.

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Thus systemic vasodilatation was preserved in HF despite elevated ANG II concentrations. In contrast to the preserved systemic vasodilatation, the suppression of heart rate in response to volume expansion was blunted in HF patients (Table 2). In addition to these hemodynamic responses, the decrease in plasma protein concentrations (Table 2) indicates that volume expansion by water immersion induced the expected increase in plasma volume and decrease in plasma colloid osmotic pressure in controls and HF patients, respectively.

During seated control, plasma concentrations of ANP and Aldo were higher in HF patients than in control subjects and ANG II concentrations also tended to be elevated \( (P = 0.10, \text{Fig. 2}). \) Although these differences persisted during volume expansion, a significant increase in plasma ANP concentrations and a decrease in plasma concentrations of Aldo and ANG II was initiated in both groups of subjects. Consistent with the hydrated state of the subjects, plasma AVP concentrations were low (Fig. 2). Changes in response to volume expansion or differences between the groups were not observed. Plasma norepinephrine concentrations (Table 2) were suppressed in response to volume expansion in both groups of subjects, but differences between groups were not detected.

During seated control, absolute sodium excretion (Fig. 3) tended to be lower in HF patients than in controls \( (P = 0.06). \) Volume expansion increased \( F_{E_{\text{Na}}}, \text{(Fig. 3) and produced a significant natriuresis in both groups of subjects, but the natriuretic response was attenuated in HF patients compared with that of controls. Because arterial pressure, GFR, and } F_{\text{Li}} \text{(Fig. 1) were similar in controls and HF patients during volume expansion, differences in intrarenal sodium handling most likely accounted for the differences in sodium excretion. In this regard, } C_{\text{Li}} \text{ (Table 3) increased more in control subjects than in HF patients in response to volume expansion, indicating an attenuated increase in delivery of sodium and water to the distal tubular segments in HF patients. Calculation of } APR_{\text{Na}} \text{ (Table 3) from } C_{\text{Li}} \text{ also indicated that } APR_{\text{Na}} \text{ was unchanged in controls but increased in HF patients in response to volume expansion.}

In both groups of subjects, the increased distal delivery of sodium and water in response to volume expansion elicited an increase in \( ADR_{\text{Na}} \text{(Table 3), which did not totally compensate for the increased load. Hence, unchanged or slightly lowered } FDR_{\text{Na}} \text{(Table 3) at a higher load increased the output of sodium from the distal tubular segments and participated in the natriuresis. Although statistically signific-

### Table 2. Cardiovascular and plasma variables during seated control and WI in control subjects and heart failure patients

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects ((n = 9))</th>
<th>Heart Failure ((-ACE-I, n = 11))</th>
<th>Heart Failure ((-ACE-I, n = 9))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WI + ACE-I</td>
<td>WI + ACE-I</td>
<td>WI + ACE-I</td>
</tr>
<tr>
<td>SVI, ml·beat(^{-1})·m(^{-2})</td>
<td>Seated control 31 ± 2</td>
<td>27 ± 2</td>
<td>41 ± 3</td>
</tr>
<tr>
<td></td>
<td>WI 49 ± 3(^{+})†</td>
<td>38 ± 3(^{a})</td>
<td>42 ± 3</td>
</tr>
<tr>
<td>SVR, dyn·s/cm(^{-5})</td>
<td>Seated control 2,020 ± 104</td>
<td>1,921 ± 216</td>
<td>1,352 ± 140</td>
</tr>
<tr>
<td></td>
<td>WI 1,373 ± 91(^a)</td>
<td>1,480 ± 171(^a)</td>
<td>1,248 ± 99</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>Seated control 63 ± 2(^{+})</td>
<td>73 ± 4</td>
<td>71 ± 4</td>
</tr>
<tr>
<td></td>
<td>WI 58 ± 2(^{+})</td>
<td>70 ± 4</td>
<td>68 ± 4</td>
</tr>
<tr>
<td>PRA, ng·ml(^{-1})·h(^{-1})</td>
<td>Seated control 2.7 ± 0.5(^{+})</td>
<td>30.1 ± 11.3</td>
<td>18.1 ± 7.4</td>
</tr>
<tr>
<td></td>
<td>WI 1.2 ± 0.2(^{+})</td>
<td>17.8 ± 5.9</td>
<td>172 ± 22</td>
</tr>
<tr>
<td>NE, pg/ml</td>
<td>Seated control 305 ± 55</td>
<td>293 ± 20</td>
<td>208 ± 22</td>
</tr>
<tr>
<td></td>
<td>WI 145 ± 25(^{+})</td>
<td>187 ± 21(^{a})</td>
<td>172 ± 22</td>
</tr>
<tr>
<td>Epi, pg/ml</td>
<td>Seated control 16 ± 3</td>
<td>25 ± 6</td>
<td>20 ± 5</td>
</tr>
<tr>
<td></td>
<td>WI 7 ± 3(^{+})</td>
<td>13 ± 4(^a)</td>
<td>14 ± 4</td>
</tr>
<tr>
<td>( P_{\text{Na}} ), mmol/l</td>
<td>Seated control 138 ± 1</td>
<td>135 ± 1</td>
<td>134 ± 2</td>
</tr>
<tr>
<td></td>
<td>WI 138 ± 1</td>
<td>135 ± 1</td>
<td>137 ± 1</td>
</tr>
<tr>
<td>( P_{\text{K}} ), mmol/l</td>
<td>Seated control 4.2 ± 0.04</td>
<td>4.1 ± 0.1</td>
<td>3.9 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>WI 4.1 ± 0.05(^a)</td>
<td>4.0 ± 0.1</td>
<td>3.9 ± 0.1</td>
</tr>
<tr>
<td>( P_{\text{osm}} ), mosmol/kgH(_{2})O</td>
<td>Seated control 283 ± 2</td>
<td>281 ± 2</td>
<td>280 ± 2</td>
</tr>
<tr>
<td></td>
<td>WI 283 ± 1</td>
<td>281 ± 2</td>
<td>284 ± 2(^{+})</td>
</tr>
<tr>
<td>( P_{\text{prot}} ), g/l</td>
<td>Seated control 69.6 ± 1.6</td>
<td>72.0 ± 1.7</td>
<td>70.5 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>WI 63.9 ± 1.4(^{+})</td>
<td>68.9 ± 2.4(^{a})</td>
<td>65.1 ± 1.0(^{+})</td>
</tr>
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</table>

Values presented are means ± SE. SVI, stroke volume index; SVR, systemic vascular resistance; HR, heart rate; PRA, plasma renin activity; NE, plasma norepinephrine concentration; Epi, plasma epinephrine concentration; \( P_{\text{Na}} \), plasma sodium concentration; \( P_{\text{K}} \), plasma potassium concentration; \( P_{\text{osm}} \), plasma osmolality; \( P_{\text{prot}} \), plasma protein concentration; \(^{+}\)P < 0.05 vs. seated control; \(^{†}\)P < 0.05 vs. corresponding experimental intervention in heart failure; \(^{‡}\)P < 0.05 vs. water immersion – ACE-I.
In HF patients, urine flow rate increased in response to volume expansion; however, free water clearance did not. In addition, the urine remained isosmotic (Table 3) during volume stimulation in HF patients in contrast to the hyposmotic urine of the control subjects. Thus HF patients exhibited an attenuated renal diluting capacity during volume stimulation.

**Effects of water immersion in HF during sustained ACE-I treatment.** Volume expansion by water immersion during sustained ACE-I therapy compared with brief withdrawal of ACE-I medications induced a minor reduction of mean arterial pressure without detectable changes in cardiac index or systemic vascular resistance (Fig. 1 and Table 2). Neither systemic vaso-

![Graphs showing plasma concentrations of various hormones](image)

**Fig. 2.** Plasma concentrations of vasopressin (AVP), NH₂ terminal-(1–30) fragment of atrial natriuretic peptide [N-ANP(1–30)], ANG II, and aldosterone (Aldo). *P < 0.05 vs. SEAT; †P < 0.05 vs. corresponding experimental intervention in heart failure subjects; ‡P < 0.05 vs. WI in heart failure subjects – ACE-I. Plasma ANG II concentrations of the 1 subject receiving ANG II receptor blocker treatment have not been included in the ANG II data presented or the statistical analysis because they represent single outliers. Plasma ANG II concentrations for this subject averaged 275 pg/ml during SEAT, 195 pg/ml during WI, and 374 pg/ml during WI+ACE-I.

significant differences in FDR₉Na were not observed in response to immersion or between groups, the HF patients exhibited a numerically smaller decrease in FDR₉Na in response to volume expansion than did the control subjects.

Water immersion induced pronounced increases in urine flow rate and free water clearance (Fig. 3) in control subjects, which exceeded those of HF patients.

![Graphs showing urine flow rate and clearance](image)

**Fig. 3.** Urine flow rate (UV), urinary sodium excretion rate (Unaq/V), fractional excretion of sodium (FENa), and renal free water clearance (CH₂O). *P < 0.05 vs. SEAT; †P < 0.05 vs. corresponding experimental intervention in heart failure subjects; ‡P < 0.05 vs. WI in heart failure subjects – ACE-I.
dilatation nor the attenuated decrease in heart rate in response to volume expansion was affected by ACE-I (Table 2).

Sustained ACE-I treatment decreased plasma concentrations of ANG II and Aldo compared with volume expansion during withdrawal of ACE-I treatment but did not affect plasma ANP and norepinephrine concentrations (Fig. 2 and Table 2). Plasma concentrations of AVP (Fig. 2), however, were slightly decreased during volume expansion with sustained ACE-I treatment compared with during withdrawal of ACE-I. Decreased plasma AVP concentrations were not caused by lower plasma osmolalities (Table 2), because plasma osmolalities were highest during sustained ACE-I therapy.

When volume expansion was performed during sustained ACE-I therapy, absolute and fractional (FE\textsubscript{Na}) sodium excretions were restored to levels similar to those of the control subjects (Fig. 3, P = 0.59 and 0.95, respectively, vs. control subjects). Compared with volume expansion during withdrawal of ACE-I therapy, GFR and FL\textsubscript{Na} (Fig. 1) were increased when ACE-I treatment was sustained, indicating an increase in the proximal tubular load of sodium and water. The increased load to the proximal tubules, however, did not increase delivery of sodium to the distal tubular segments as indicated by the unchanged C\textsubscript{Li} (Table 3). Therefore, the increased proximal tubular load of sodium and water was associated with an increase in APR\textsubscript{Na} (Table 3), which prevented an increase in distal delivery of sodium and water. The pronounced decrease in distal tubular reabsorption (FDR\textsubscript{Na}, Table 3) suggests that the increased natriuresis was primarily caused by increased sodium output from the distal tubular segments.

Urine flow rate and solute free water excretion did not differ when volume expansion was compared with and without ACE-I therapy (Fig. 3). Hence, renal diluting capacity during volume expansion was not restored by sustained ACE-I treatment.

**DISCUSSION**

The results of this investigation demonstrate that intravascular and central blood volume expansion in compensated HF suppresses the activity of the renin-angiotensin-aldosterone system, increases the release of ANP, and elicits a natriuresis. Compared with control subjects, sodium excretion is attenuated during volume expansion in HF patients after brief withdrawal of ACE-I therapy when plasma concentrations of ANG II and Aldo are high. However, volume expansion during sustained ACE-I treatment decreases plasma concentrations of ANG II and Aldo and improves the natriuresis and FE\textsubscript{Na} in HF, probably by decreasing distal tubular sodium reabsorption. Renal free water excretion is attenuated in response to vol-
ume expansion in HF patients despite normal plasma AVP concentrations and is not restored by sustained ACE-I treatment.

We recently demonstrated that short-term (30 min) intravascular volume expansion by water immersion in compensated HF inhibits efferent sympathetic nervous activity and decreases systemic vascular resistance and the release of AVP and renin, whereas forearm vasodilatation and suppression of heart rate are blunted (10). The dissociation of reflex control of heart rate from the mechanisms involved in the control of neuroendocrine activities was confirmed in the present study. In addition, the results of the present study extend our previous findings by showing that increased cardiac output, systemic vasodilatation, suppression of renin-angiotensin-aldosterone axis, and increased ANP secretion are sustained during more prolonged (3 h) intravascular volume expansion in compensated HF and that these responses elicit a natriuresis. Hence, the neuroendocrine link between volume sensing and renal adjustments of sodium excretion is functionally preserved in these patients, albeit neuroendocrine activities may be operating at increased intensity.

It is notable and in agreement with our previous investigation (10) that elevated plasma concentrations of the potent vasoconstrictor ANG II in HF did not significantly attenuate systemic vasodilatation in response to volume expansion compared with control subjects. Furthermore, inhibition of the renin-angiotensin-aldosterone axis by ACE-I did not cause any additional systemic vasodilatation during volume expansion in HF. The findings suggest that reflex systemic vasodilatation in response to volume expansion in HF is not importantly influenced by brief (24 h) increases in ANG II and Aldo concentrations.

On the other hand, our findings suggest that increased activity of the renin-angiotensin-aldosterone axis modulates the renal response to volume expansion in HF. First, elevated ANG II and Aldo concentrations in HF compared with control subjects were associated with a blunted natriuretic response to volume expansion despite higher ANP concentrations. Second, sustained ACE-I treatment lowered plasma concentrations of ANG II and Aldo without changes in ANP and increased absolute and fractional sodium excretion to a level similar to that of control subjects. Normalization of the natriuresis in HF during ACE-I compared with that of control subjects is in accordance with observations obtained in patients with mild HF, where ACE-I also restored sodium excretion during volume loading (29, 32). Thus ACE-I improves renal sodium excretion during volume expansion not only in mild stages of HF but also in the later compensated phase of the syndrome.

As indicated above, the beneficial effect of sustained ACE-I therapy in HF on renal sodium excretion was apparently not related to effects on plasma ANP concentrations or renal cGMP generation. In fact, the very similar levels of plasma ANP concentrations and urinary cGMP excretion during volume expansion in HF patients with and without sustained ACE-I treatment indicate that differences in sodium excretion are not explained by differences in the natriuretic actions of ANP or cGMP-mediated natriuretic mechanisms.

In contrast, plasma ANG II and Aldo concentrations were significantly further suppressed during volume expansion in HF patients on sustained ACE-I treatment compared with during withdrawal of ACE-I therapy. The lower ANG II concentrations might have contributed to the increased sodium excretion by direct effects on tubular function or by modulating renal plasma flow and glomerular filtration dynamics during volume expansion (11). Furthermore, decreased ANG II concentrations probably inhibited Aldo release (11, 26), leading to lower plasma Aldo concentrations and withdrawal of Aldo-mediated effects on distal tubular reabsorption (11, 26). It is therefore conceivable that decreased distal tubular sodium reabsorption also contributed to the increased natriuresis during volume expansion in HF patients on sustained ACE-I therapy. Indeed, this was also suggested by estimation of segmental renal tubular sodium handling derived from Cli.

Compared with control subjects, an attenuated increase in distal delivery of sodium and water (Cl,i) in combination with an attenuated decrease in distal tubular sodium reabsorption (FDRNa) apparently contributed to the attenuated natriuretic response in HF during volume expansion after withdrawal of ACE-I treatment (high ANG II and Aldo concentrations). However, the improved renal sodium excretion during volume expansion in HF on sustained ACE-I treatment (low ANG II and Aldo concentrations) occurred without further increase in delivery of sodium and water to the distal tubular sites (Cl,i), suggesting that decreased distal tubular sodium reabsorption contributed importantly to the increased natriuresis. Thus the decreased Aldo concentrations and the Cl,i measurements consistently indicate that decreased distal tubular sodium reabsorption participated in the increased sodium excretion in HF during sustained ACE-I therapy.

Although it is likely that direct renal tubular effects of differences in ANG II and Aldo concentrations contributed to differences in sodium excretion, other factors might also be involved. It is possible that differences in renal plasma flow, intrarenal blood flow distribution, and intrarenal peritubular factors (e.g., capillary hydrostatic/oncotic pressure) contributed to the differences in renal sodium excretion during volume expansion in controls and in HF with and without sustained ACE-I treatment (11). In addition, ACE-I might have increased bradykinin and prostaglandin E2 concentrations (21). Therefore, it cannot be excluded that enhanced renal effects of these substances also contributed to the increased natriuresis (6, 19).

It is noteworthy, that increases in GFR and FLNa and decreases in ANG II concentrations induced by volume expansion in HF patients with and without sustained ACE-I therapy failed to increase distal delivery of sodium and water (Cl,i) to the same extent as in controls. Rather, increased proximal tubular sodium reabsorption (APRNa) attenuated the increase in distal
delivery of sodium. This is in contrast to the unchanged or decreased APRNa usually observed in response to volume expansion (3, 24) with decreased ANG II concentrations or administration of ACE-I in healthy humans (12). The observations suggest that sodium-retaining mechanisms acting at the proximal tubule are active during volume expansion in HF. In this regard, sympathetic nerve activity to the kidney is increased in HF (5, 13), and sustained high levels of renal sympathetic nerve activity and/or a blunted decrease during volume expansion (4, 5) in HF may have contributed to the increase in APRNa.

Although the increase in APRNa limited the distal delivery of sodium during volume expansion in HF patients, this could be compensated for by pharmacological suppression (ACE-I) of distal tubular sodium reabsorption. Hence, the increase in APRNa was not preventing the normalization of the natriuretic response. Therefore, during volume expansion in compensated HF, an attenuation of the normal decrease in distal tubular sodium reabsorption seems to be the more important determinant of sodium retention and thus might contribute to volume overload under circumstances when pharmacological suppression is insufficient.

Despite similar plasma osmolalities and AVP concentrations in HF patients and controls, excretion of free water was attenuated in HF patients in response to volume expansion. In hydrated subjects, AVP concentrations are low, and further suppression is difficult to detect by standard methods. Therefore, undetectable suppression of AVP concentrations in response to volume expansion might have contributed to the increased free water excretion in control subjects. Undetectable differences in plasma AVP concentrations between HF and controls, however, does not seem to explain the impaired water excretion in HF patients, because volume expansion during ACE-I in HF significantly suppressed AVP concentrations but did not improve water excretion. Thus it is more likely that intrarenal factors accounted for the impaired water handling in HF patients. In this regard, delivery of fluid to the diluting segments of the nephron (as indicated by Cli) increased less during volume expansion in HF patients than in control subjects and might have contributed (14). Furthermore, increased intrarenal osmotic gradients and/or enhanced chronic expression of aquaporin 2 water channels in collecting duct cells in HF might be involved (20, 34).

**Limitations.** We used Cli, which is currently the best available indirect method to estimate segmental renal sodium handling (12, 28). The endogenous Cli method was chosen because administration of exogenous lithium per se may increase sodium excretion and thus may confound the results (27). The endogenous method provides lower absolute values for Cli than the exogenous method, but the ability to detect changes is similar (9, 22). Therefore, interpretation should be based on the absolute changes in endogenous Cli. In addition, it is unlikely that Aldo-mediated distal tubular sodium reabsorption (18) confounded our measurements, because decreased Aldo concentrations in HF during sustained ACE-I treatment did not alter Cli. Furthermore, we investigated HF patients on medical treatment in a compensated stable phase. Thus the results cannot be extrapolated to patients with decompensated or untreated heart disease.

In conclusion, intravascular and central blood volume expansion in compensated HF suppresses the activity of the renin-angiotensin-aldosterone system, increases the release of ANP, and elicits a natriuresis, which is enhanced when ANG II and Aldo concentrations are suppressed by sustained ACE-I therapy. Hence, the neuroendocrine link between volume sensing and renal adjustments of sodium excretion is functionally preserved in compensated HF, but the natriuresis induced by volume stimulation is modulated by the prevailing activity of the renin-angiotensin-aldosterone axis. In contrast, renal free water excretion is attenuated in response to volume expansion in HF patients despite normalized plasma AVP concentrations and is not restored by sustained ACE-I treatment.

**Perspectives**

The ACE-I-induced normalization of renal sodium excretion during intravascular and central blood volume expansion occurred in the absence of significant systemic hemodynamic effects. These observations demonstrate that ACE-I might not be regarded only as a drug with favorable “cardiovascular” effects in compensated chronic HF but that favorable direct effects of ACE-I on the control of renal sodium excretion during volume expansion and hence control of extracellular volume might also be beneficial.

It has previously been demonstrated that nonosmotic (baroreflex)-mediated release of vasopressin is increased in HF (1) and contributes to renal water retention and hyponatremia. The results of this investigation suggest that the renal diluting capacity is attenuated in HF even at lower or normalized vasopressin concentrations. Future investigations should address the underlying mechanisms, as a further understanding could improve treatment of water balance abnormalities in HF. In particular, it would be useful to know if renal excretion of aquaporin 2 is increased in human HF, and whether vasopressin receptor antagonism can improve the impaired water handling.

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