Low-Dose Atrial Natriuretic Factor and Furosemide in Experimental Acute Congestive Heart Failure

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ABSTRACT

This study was designed to address three objectives in an experimental model of acute congestive heart failure (CHF) in the dog produced by rapid ventricular pacing. The first objective was to characterize cardiological and humoral responses before and during 2 h of acute CHF. The second objective was to determine the modulating action of iv furosemide upon these biologic responses to acute CHF, testing the hypothesis that furosemide-mediated natriuresis is associated with activation of the renin-angiotensin-aldosterone system (RAAS) compared with the control group. The third objective was to determine the modulating action of continuous low-dose atrial natriuretic factor (ANF) administration during acute CHF upon these biologic responses, testing the hypothesis that exogenous low-dose ANF would prevent activation of the RAAS and enhance the natriuretic action of furosemide. In the control group (Group 1; \( N = 6 \)), plasma ANF increased after the onset of CHF; GFR and sodium excretion were maintained without activation of this RAAS despite arterial hypotension. In Group 2 (\( N = 6 \)), furosemide in acute CHF increased sodium excretion but in association with a decrease in GFR and activation of the RAAS. Low-dose exogenous ANF and furosemide (Group 3; \( N = 6 \)) in acute CHF were associated with a maintenance of GFR, no activation of the RAAS, and potentiation of furosemide-induced natriuresis. In summary, these studies demonstrate that furosemide potently increases sodium excretion in acute CHF, but with a decrease in GFR and activation of the RAAS. Low-dose ANF in acute CHF with furosemide maintains GFR, attenuates activation of the RAAS, and potentiates natriuresis.

Key Words: Diuretics, natriuretic peptides, congestive heart failure

Acute congestive heart failure (CHF) is a clinical syndrome characterized by acute decreases in cardiac output (CO) and arterial pressure in association with increases in cardiac filling pressures. Recent studies have demonstrated a functional role for elevated endogenous atrial natriuretic factor (ANF) in acute CHF to maintain sodium excretion and attenuate the full activation of the renin-angiotensin-aldosterone system (RAAS), despite the potent stimulus of arterial hypotension (1,2). Although these and other studies support a homeostatic role for endogenous ANF in CHF (3), the use of additional potent loop diuretics is often required in the prevention and treatment of acute pulmonary congestion.

Furosemide is an efficacious loop diuretic used in the treatment of acute CHF. The efficacy of furosemide is based upon its ability to markedly enhance sodium excretion and decrease intravascular volume despite the presence of decreased renal perfusion pressure. Although the ability of furosemide to increase sodium excretion in acute CHF is well established, the full natriuretic action of furosemide may be limited by activation of the RAAS (4,5). In addition to the activation of the RAAS by furosemide, a decrease in ANF has been reported during furosemide therapy in humans with acutely decompensated chronic CHF (6). Such a decrease in endogenous ANF may also limit the full natriuretic action of furosemide in acute CHF.

This study was designed to address three objectives in an experimental model of acute CHF in the dog produced by rapid ventricular pacing. The first objective was to characterize the cardiological and endocrine responses before and during 2 h of acute CHF. The second objective was to determine the modulating action of iv furosemide upon these biologic responses to acute CHF, testing the hypothesis that the furosemide-mediated natriuresis is associated with activation of the RAAS and decreases in circulating ANF when compared with the control group without furosemide. The third objective was to determine the modulating action of continuous low-dose ANF administration during acute CHF upon cardiological and...
endocrine function, testing the hypothesis that exogenous low-dose ANF to maintain a steady-state pathophysiological level of plasma ANF would prevent the activation of the RAAS and enhance the natriuretic action of furosemide.

METHODS

Experiments were performed in three groups of mongrel dogs of either sex weighing 17 to 25 kg. Group I (Control; N = 6) underwent rapid ventricular pacing for 2 h to induce acute CHF, which, as in humans, is characterized by increased left ventricular filling pressure, decreased CO, and decreased arterial pressure. Group II (furosemide; N = 6) received an iv bolus of furosemide (1.7 mg/kg) 45 min after the onset of acute CHF. Group III (furosemide plus ANF; N = 6) received furosemide as in Group II with continuous, iv, low-dose ANF (20 ng/kg·min; Peninsula Laboratories, Belmont, CA) initiated at the time of furosemide administration.

Dogs were fasted the night before the experiment but were allowed water ad libitum until the time of the study. After the induction of anesthesia with pentobarbital sodium (30 mg/kg iv), dogs were surgically prepared by the selective cannulation of both femoral veins and the left femoral artery for infusions and arterial pressure monitoring. The right external jugular vein was isolated, and a 7.5 French balloon-tipped thermodilution catheter (American Edwards Laboratory, Santa Ana, CA) was advanced into the pulmonary artery. The trachea was intubated with a 9.5-mm endotracheal tube, and the animal was mechanically ventilated (Harvard Apparatus, Millis, MA). The left kidney was exposed by a retroperitoneal flank incision. The ureter was isolated and cannulated for urine collection. An electromagnetic flow probe was placed around the renal artery and connected to a flow meter (Carolina Medical Electronics, King, NC) for the continuous measurement of RBF. The chest was opened in the left fifth intercostal space. An epicardial pacing wire was placed on the ventricular apex and was subsequently connected to a pulse generator (Model 4563A; Medtronic Corporation, Minneapolis, MN) for ventricular pacing. Each dog received a priming dose of inulin (Nutritional Biochemicals, Cleveland, OH) and a continuous infusion at a rate of 1 mL/min through a femoral vein catheter at concentrations to achieve a steady-state plasma inulin level of approximately 50 mg/dl.

One 30-min control clearance period (Baseline) was performed before acute CHF. Ventricular pacing, at 250 beats/min, was then initiated to produce acute CHF. After 45 min, a 15-min clearance (CHF) was obtained. Furosemide in the presence or absence of low-dose ANF was then administered, followed by a final 30-min clearance performed 2 h after the onset of acute CHF (CHF 2 h). In addition, plasma and 15-minute short-time urine collections were obtained throughout the protocol to permit the determination of circulating ANF and urinary sodium excretion.

During each clearance period, cardiovascular, hormonal, and renal parameters were evaluated. Cardiovascular parameters included systemic arterial pressure, left atrial pressure (LAP), CO, and systemic vascular resistance (SVR). For each period, CO was determined by thermodilution in triplicate and was averaged. Extracted arterial plasma concentrations of ANF were measured by an RIA procedure previously described (7). PRA and plasma aldosterone were measured by RIA as previously reported (8,9). GFR was determined by the clearance of inulin with plasma and urine inulin concentrations measured by the anthrone method (10). Plasma and urinary sodium concentrations were quantified by the use of ion-selective electrodes (Beckman System E2A; Beckman Instruments, Brea, CA).

Data from each group are expressed as mean ± SE. Data were analyzed by analysis of variance for repeated measurements and by paired and unpaired t tests. Comparisons within groups were analyzed by analysis of variance for repeated measurements, followed by paired t test when appropriate. Comparisons between groups were performed by unpaired t test. Statistical significance was accepted for P < 0.05.

RESULTS

Table 1 reports cardiovascular data and Table 2 reports renal and hormonal data at Baseline, at 45 min after the onset of acute CHF before furosemide and/or ANF, and at 2 h after CHF. Figures 1 and 2 illustrate circulating ANF and urinary sodium excretion at intervals throughout the experiment in each group.

Group I (Control)

Acute CHF decreased CO and mean arterial pressure (MAP) in association with increased LAP and SVR (Table 1). Two hours after CHF, MAP returned to baseline in association with a further decrease in CO and an increase in SVR. LAP remained elevated and unchanged.

With the onset of acute CHF, GFR was unchanged in association with a decrease in RBF and an increase in renal vascular resistance (RVR) (Table 2). Urinary sodium excretion and the fractional excretion of sodium were maintained at baseline. At 2 h after acute CHF, these renal parameters were maintained, with the exception of a modest but significant increase in urine flow.

Plasma ANF increased with the onset of acute CHF and decreased thereafter, although remaining at con-
concentrations above baseline (Table 2; Figure 1). PRA was unchanged with the onset of acute CHF and decreased at 2 h compared with the acute CHF period. Plasma aldosterone remained unchanged throughout acute CHF.

Group II (Furosemide)

Cardiac and systemic hemodynamic responses to acute CHF were similar in the furosemide group as compared with the control group (Table 1). MAP and CO decreased, whereas LAP and SVR increased. At 2 h after CHF, MAP increased to baseline in association with a progressive decrease in CO and an increase in SVR.

Renal responses to acute CHF at 45 min were similar to those observed in Group I (Table 2). In response to furosemide, however, GFR decreased 2 h after CHF. Urinary sodium excretion and the fraction excretion of sodium increased with a peak effect 30 min after furosemide (Figure 2) and remained elevated 2 h after CHF.

Plasma ANF increased with the onset of acute CHF (Table 2; Figure 1) and decreased thereafter, although remaining above baseline. PRA did not change with the onset of acute CHF but was increased at 2 h. Aldosterone was unchanged with the onset of CHF but increased 2 h after CHF.

Group III (Furosemide and Low-Dose ANF)

With the onset of acute CHF (Table 1), MAP and CO decreased in association with an increase in LAP and SVR. At 2 h after CHF in the presence of both furosemide and continuous low-dose ANF, MAP remained decreased in association with a further decrease in CO and an increase in SVR. LAP remained elevated.

With the onset of CHF, renal responses were similar to those of the other groups with a maintained GFR, decreased RBF, and increased RVR (Table 2). In contrast to Group II with furosemide, low-dose ANF was associated with a maintenance of GFR. RBF decreased and RVR increased 2 h after CHF. As illustrated in Figure 2, the peak increase in sodium excretion was greatest in Group III.

Plasma ANF increased with the onset of acute CHF and remained elevated with the continuous low-dose ANF infusion (Table 2; Figure 1). With the onset of CHF, PRA remained unchanged and was maintained at baseline concentrations for the duration of the protocol. Plasma aldosterone was also unchanged with the onset of CHF and at 2 h.

**DISCUSSION**

This study demonstrates that experimental acute CHF is characterized by a decrease in CO and arterial pressure with an increase in LAP and plasma ANF. Despite the decrease in renal perfusion pressure, GFR and sodium excretion were maintained without activation of the RAAS. In acute CHF, iv furosemide increased sodium excretion, but in association with a decrease in GFR and activation of the RAAS. The continuous administration of low-dose ANF, which maintained steady-state concentrations of elevated circulating ANF, maintained GFR and prevented the activation of the RAAS, despite sustained arterial hypotension and augmented furosemide-mediated natriuresis.

This study confirms recent investigations of Lee and colleagues that demonstrated that endogenous ANF serves an important functional role in acute CHF (3). In a low-ANF model of acute CHF produced by thoracic inferior vena cava constriction, those investigators observed RAAS activation and marked sodium retention that were not observed in a high-ANF model of acute CHF, despite comparable decreases in CO and arterial pressure. When exogenous ANF was infused in dogs with the low-ANF model produced by acute caval constriction to mimic circulating concentrations achieved in high-ANF heart failure produced by rapid ventricular pacing, sodium
TABLE 2. Renal and endocrine function

<table>
<thead>
<tr>
<th></th>
<th>Group I, Control</th>
<th>Group II, Furosemide</th>
<th>Group III, ANF + Furosemide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>CHF</td>
<td>CHF 2 h</td>
</tr>
<tr>
<td>GFR (mL/min)</td>
<td>47 ± 8</td>
<td>47 ± 5</td>
<td>45 ± 5</td>
</tr>
<tr>
<td>RBF (mL/min)</td>
<td>223 ± 17</td>
<td>168 ± 8(^b)</td>
<td>154 ± 16(^b)</td>
</tr>
<tr>
<td>RVR (R.U.)</td>
<td>0.44 ± 0.04</td>
<td>0.50 ± 0.02</td>
<td>0.66 ± 0.07</td>
</tr>
<tr>
<td>V (mL/min)</td>
<td>0.16 ± 0.04</td>
<td>0.13 ± 0.04</td>
<td>0.17 ± 0.05(^a)</td>
</tr>
<tr>
<td>UNaV (μEq/min)</td>
<td>41.9 ± 15.0</td>
<td>21.4 ± 10.3</td>
<td>26.0 ± 13.4</td>
</tr>
<tr>
<td>FENa (%)</td>
<td>0.55 ± 0.23</td>
<td>0.27 ± 0.12</td>
<td>0.37 ± 0.21</td>
</tr>
<tr>
<td>ANF (pg/mL)</td>
<td>39 ± 9</td>
<td>364 ± 56(^b)</td>
<td>204 ± 27(^c)</td>
</tr>
<tr>
<td>PRA (ng/mL)</td>
<td>2.3 ± 0.7</td>
<td>4.3 ± 1.1</td>
<td>1.8 ± 0.3(^c)</td>
</tr>
<tr>
<td>Aldo (ng/mL)</td>
<td>17.0 ± 0.7</td>
<td>15.1 ± 3.5</td>
<td>18.0 ± 4.2</td>
</tr>
</tbody>
</table>

\(^a\) Abbreviations: V, urine flow; UNaV, urinary sodium excretion; FENa, fractional excretion of sodium; Aldo, aldosterone; R.U., resistance units.

\(^b\) \(P < 0.05\) CHF versus baseline.

\(^c\) \(P < 0.05\) CHF 2 h versus baseline.

In the presence of furosemide, increases in PRA and plasma aldosterone were observed. Increases in PRA and aldosterone were observed in normal humans or animals and speculated that atrial natriuretic factor and RAAS activation did not occur. This study is consistent with such a role for ANF in acute CHF.

Figure 2. Urinary sodium excretion (UNaV) before and during experimental acute CHF in anesthetized dogs in control (Group I), open (Group II, furosemide), and closed (Group III, furosemide plus ANF) (control; open; closed). Group II (furosemide, 7 mg/kg; open circle); Group III (furosemide, 7 mg/kg, closed circle) with endogenous ANF. Group I (control, closed circle) with endogenous ANF.

Figure 3. Plasma ANF, pg/ml, in anesthetized dogs before and during experimental acute CHF in anesthetized dogs in control (Group I), open (Group II, furosemide), and closed (Group III, furosemide plus ANF) (control; open; closed). Group II (furosemide, 7 mg/kg; open circle); Group III (furosemide, 7 mg/kg, closed circle) with endogenous ANF. Group I (control, closed circle) with endogenous ANF. 20 ng/kg-min, IV, closed circle.
changes in the RAAS. This study confirms and extends this speculation and suggests that such an attenuating action upon the RAAS in acute CHF may be secondary to increases in endogenous ANF activated by atrial stretch. The observation that the low-dose administration of ANF, resulting in higher pathophysiologic concentrations of circulating ANF, reversed furosemide-mediated changes in the RAAS supports this speculation. Moreover, such a biologic action may explain in part the report of Bennett and coworkers that acute CHF in humans with myocardial infarction may be associated with a transient diuresis, despite ventricular dysfunction (11).

This study also importantly extends previous reports and describes the temporal alterations in endogenous ANF with acute CHF. Despite continued acute CHF and elevation of LAP, the peak elevation of endogenous circulating ANF was not maintained. In this study, plasma ANF decreased by approximately 40% at 2 h after the onset of acute CHF. The mechanism of this decrease is unclear but may be secondary to the depletion of stored atrial ANF. Indeed, Perrella et al. reported that acute CHF for 3 hours is unassociated with detectable increases in atrial ANF mRNA, suggesting that increased ANF synthesis may not be augmented in acute CHF, resulting in a relative deficiency state during the initial hours after the onset of ventricular dysfunction (12).

One might conclude that more severe heart failure could have increased circulating ANF to higher levels, which may have attenuated furosemide-induced renal and endocrine responses. Studies by Redfield et al., however, have suggested a maximal ceiling for plasma ANF in acute CHF that limits circulating ANF to levels observed in this study (13). Under such circumstances, a relative deficiency could occur, augmenting the reductions in GFR and the increases in the RAAS in association with furosemide in acute CHF. Such an occurrence would underscore the rationale for low-dose iv ANF as adjunct therapy to furosemide in acute left ventricular failure.

Intravenous furosemide resulted in an immediate natriuretic response despite reduced renal perfusion pressure. Although the natriuretic action of furosemide has been well characterized, investigations conflict with regard to furosemide-mediated renal hemodynamic responses. Such a conflict may be related to whether investigations were performed under normal physiologic conditions or in pathophysiologic states associated with reduced renal perfusion pressure. Our findings are consistent with the reports of Dikshit et al., which described the renal hemodynamic effects of furosemide in the humans after acute myocardial infarction (14). In these previous reports, furosemide resulted in an initial increase in GFR 15 min after diuretic administration. However, 2 h after furosemide therapy, in association with reduced arterial pressure and elevated cardiac filling pressures, GFR was below baseline.

The physiologic mechanism(s) that mediate the decrease in GFR in this and previous studies in response to furosemide may be multifactorial. Because RBF decreased in all three groups but GFR decreased only in the furosemide group, a direct or indirect action of furosemide upon the ultrafiltration coefficient, $K_f$, could be speculated. Because the decrease in PRA observed in Group I without furosemide did not occur in the furosemide group, a role for increased activity of the intrarenal renin-angiotensin system could mediate the reduction in GFR (15), although angiotensin may alternatively serve to maintain GFR via efferent arteriolar constriction (16). Further, although not measured in this investigation, furosemide may have activated the sympathetic nervous system, as reported in human studies by Francis et al., resulting in decreases in the filtration process (17). Indeed, increases in plasma norepinephrine, together with decreases in CO, could importantly contribute to reductions in GFR.

The low-dose administration of ANF to maintain steady-state pathophysiologic concentrations after furosemide resulted in a maintenance of GFR. The mechanism of this preservation is consistent with the known ability of ANF to increase GFR (18–20). This action is mediated by a decrease in afferent arteriolar resistance, an increase in efferent arteriolar resistance, an increase in $K_f$, and an inhibition of tubuloglomerular feedback (18–24). The action of ANF in this study is also remarkable because it occurred despite sustained renal hypoperfusion.

Furosemide-mediated natriuresis was increased by ANF, despite a persistently lower renal perfusion pressure. The mechanism of this enhanced natriuresis may also be multifactorial. The maintenance of GFR with low-dose ANF may have resulted in a greater delivery of sodium to the loop of Henle, resulting in a greater increase in sodium excretion. Although not determined in this study, low-dose ANF could have resulted in decreases in both the proximal and distal reabsorption of sodium, which are tubular sites of ANF action, thus augmenting the decrease in the loop of Henle reabsorption secondary to furosemide (1,25). Further, the lack of activation of the RAAS observed with low-dose ANF may have prevented an antinatriuretic action of angiotensin II upon the proximal tubule and an antinatriuretic action of aldosterone on the distal tubule (26).

In summary, this study provides continuing insight into the role of endogenous ANF in acute CHF in which increases in this cardiac hormone may serve a functional role to maintain sodium excretion and prevent activation of the RAAS, despite arterial hypotension. These studies also demonstrate that furosemide-induced natriuresis is associated with de-
creases in GFR with activation of the RAAS. Such responses could limit the full natriuretic action of furosemide in acute CHF. Last, these investigations also demonstrate that low-dose continuous ANF infusion potentiates furosemide-induced natriuresis and that this modulating action may occur by the preservation of GFR and the prevention of the activation of the RAAS.

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REFERENCES


