Clinical Investigation

Neurohormonal Inhibition and Hemodynamic Unloading During Prolonged Inhibition of ANF Degradation in Patients With Severe Chronic Heart Failure

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Background. The purpose of this study was to investigate the therapeutic potential of prolonged inhibition of atrial natriuretic factor (ANF) degradation in patients with severe chronic heart failure.

Methods and Results. The effects of repeated doses of the endopeptidase inhibitor candoxatrilat (150 mg i.v.) were examined over a 24-hour period in patients with severe chronic heart failure (New York Heart Association class III–IV). Plasma α-hANF(99-126) was elevated at baseline (235±59 pg/ml), increased 2.5-fold at 2 hours after the first dose, and remained significantly elevated throughout the 24-hour protocol. In contrast, pro-hANF(31-67) decreased from 3,151±616 to 2,072±362 pg/ml (p <0.05). Cardiac index (CI) increased only transiently after the first dose of candoxatrilat (CI, 2.11±0.2 to 2.67±0.28 l/min/m², p <0.05). Sodium excretion increased sixfold (p <0.05) 2 hours after the first dose of candoxatrilat and remained significantly elevated throughout the protocol. Degree of natriuresis and diuresis in response to candoxatrilat was closely related to baseline cardiac output. Glomerular filtration rate and volume excretion did not change significantly. Pulmonary capillary wedge pressure fell from 23±3 to 18±3 mm Hg (p <0.05) and remained below baseline throughout the 24 hours. Arterial pressure, heart rate, and total peripheral resistance did not change significantly during the 24-hour period. Urinary cGMP excretion increased fivefold (p <0.05), whereas urinary ANF immunoreactivity and plasma cGMP levels remained unchanged. Excretion of prostacyclin metabolite 6-keto-PGF₁α increased 3.3-fold (p <0.05). Plasma norepinephrine and epinephrine levels decreased significantly after candoxatrilat and remained suppressed over the 24-hour period. There was also a transient reduction in plasma vasopressin, aldosterone levels, and plasma renin activity. Hematocrit, total protein content, and plasma albumin concentrations did not change, indicating that no fluid shift into the extravascular space had occurred.

Conclusions. 1) The inhibition of ANF degradation causes sustained drop in left and right atrial pressures that appears to be mediated by an inhibition of neurohormonal activity; 2) concomitant inhibition of bradykinin breakdown (which in turn stimulates renal prostacyclin synthesis) contributes to natriuresis; 3) the close correlation between renal response and baseline cardiac index indicates that an inadequate renal perfusion secondary to low cardiac output diminishes the efficacy of this treatment modality. This spectrum of action would be advantageous for a first-line diuretic agent early in the course of disease rather than in patients with advanced chronic heart failure. (Circulation 1992;86:1089–1098)

KEY WORDS • chronic heart failure • endopeptidase inhibition • atrial natriuretic factor • cGMP • 6-keto-PGF₁α.

D iuretic therapy in congestive heart failure patients stimulates the renin–angiotensin system, sympathetic nervous system, and the release of antidiuretic hormone, all of which may compromise the desired hemodynamic and renal effects. In contrast, atrial natriuretic factor (ANF) induces natriuresis, diuresis, and vasodilatation and inhibits the neurohormonal axis. These latter actions are particularly useful in treating patients with sodium- and water-retaining disorders such as congestive heart failure and essential hypertension. Although short-term administration of ANF exerted beneficial effects on hemodynamic, renal, and neurohormonal parameters in patients with severe chronic heart failure, its therapeutic potential has been challenged by our observation that prolonged administration of this peptide in pharmacological doses, associated with a marked increase of plasma ANF levels, causes a shift of plasma constituents (fluid and protein) into the third space. At that time, we proposed that either intermittent administration of ANF or only modest increases in plasma ANF may avoid these side effects while still exerting beneficial effects on hemodynamic, renal, and neurohormonal parameters.

The peptide ANF is degraded enzymatically by a neutral metalloendopeptidase E.C.3.4.24.11. (NEP8–10).
This enzyme is located in high concentrations in renal tissue in microvilli of the brush border of the proximal tubules. Inhibition of this endopeptidase causes modest increases in plasma ANF levels (two- to threefold), along with significant diuresis and natriuresis in control subjects and patients with mild chronic heart failure. Importantly, NEP inhibition has been found to produce greater natriuresis and diuresis than that produced by equivalent plasma levels of ANF obtained from exogenously administered ANF.

Despite these encouraging observations, the therapeutic efficacy of NEP inhibition in patients with severe chronic heart failure, particularly after repeated administration, remains to be determined. Therefore, the aim of the present study was to investigate 1) the efficacy of candoxatrilat, a recently developed NEP inhibitor, on renal and hemodynamic parameters, plasma ANF and pro-ANF(31-67) levels, and on neurohormones and 2) whether the effects are sustained after repeated administration.

Methods

Nine patients with chronic congestive heart failure were studied after giving written informed consent. The experimental protocol was approved by the ethical committee of the University of Freiburg. The group consisted of six men and three women (mean age, 63±2 years; range, 56–78 years) with an ejection fraction (determined by left ventricular angiography) ≤30% (Table 1). Seven patients were in New York Heart Association functional class III, and two were in class IV. Five patients had cardiomyopathy caused by coronary artery disease and four patients idiopathic dilated cardiomyopathy. Patients with a history of myocardial infarction, valvular heart disease, or recent acute decompensation were excluded. Four days before the study, angiotensin converting enzyme inhibitors were discontinued and 2 days before the study, short-acting vasodilators and diuretics were stopped (Table 1). Anti-coagulants, antiarrhythmic agents, and digitalis were continued throughout the study. At 8 AM on the day of the study, a 7F Swan-Ganz thermodilution catheter was placed percutaneously via the basilic vein and advanced into the pulmonary artery under fluoroscopy. A urinary catheter was placed for urine sampling. Thereafter, a light breakfast was provided (one cup of tea and one sandwich). Additional light meals were provided immediately after hemodynamic measurements at 1 PM and 5 PM (Table 2). Urine produced in the initial 60-minute equilibration period was discarded. At 9 AM, a 4-hour control urine collection was started. Hemodynamic stability (<10% variation) was ensured by two consecutive measurements performed at 30-minute intervals beginning at 11 AM.

### Table 1. Clinical Characteristics of Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>NYHA class</th>
<th>Concomitant medication</th>
<th>Previous medication</th>
<th>EF (%)</th>
<th>PCP (mm Hg)</th>
<th>α-hANF (pg/ml)</th>
<th>pro-ANF(31-67) (pg/ml)</th>
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<td>M</td>
<td>IDC</td>
<td>IIa</td>
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Mean±SEM: 22±2, 23±3, 235±59, 3,151±591

n=9 patients.

NYHA, New York Heart Association functional class: III, able to walk at a reduced pace on the flat level; a, indefinitely at a reduced rate; b, have to stop; IV, symptomatic at rest; EF, left ventricular ejection fraction; PCP, pulmonary capillary wedge pressure; ANF, plasma levels of atrial natriuretic factor; pro-ANF(31-67), plasma levels of the ANF-pro hormone; IDC, idiopathic dilated cardiomyopathy; CAD, cardiomyopathy caused by coronary artery disease; ISMN, isosorbide mononitrate; ISDN, isosorbide dinitrate.

### Table 2. Study Protocol

<table>
<thead>
<tr>
<th>Protocol time (hours)</th>
<th>Candoxatrilat (150 mg) (1 PM)</th>
<th>Candoxatrilat (150 mg) (9 PM)</th>
<th>Candoxatrilat (150 mg) (5 AM)</th>
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<td>Protocol time (hours)</td>
<td>~2 ~1 0 1 2 4 8 12 16 17 18 20 24</td>
<td>Hemodynamic</td>
<td>Blood samples</td>
<td>Urine samples</td>
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<td></td>
<td>x x x x x x x x x x x x</td>
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<td>x x x x x x</td>
<td>x x x x x x</td>
</tr>
</tbody>
</table>
After baseline measurements, the atriopeptidase inhibitor candoxatrilat (Pfizer Central Research, Sandwich, Kent, England) was given intravenously as a bolus at a dose of 150 mg. This dose was repeated at 8 and 16 hours. Hemodynamic measurements and blood and urine sampling were performed as indicated by the protocol (x, Table 2). Blood samples were drawn from the right atrial port of the Swan-Ganz catheter for plasma α-hANF(99-126) and pro-hANF(31-67) (N-terminal prohormone cleavage peptide as a marker for the endogenous ANF secretion\(^1\)\(^2\)\(^3\)). Plasma cGMP concentrations, electrolytes, aldosterone, catecholamines, plasma renin activity, plasma vasopresin levels, and creatinine were also determined from the right atrium sample. Urine samples were analyzed with respect to volume, sodium, potassium, creatinine, α-hANF immunoreactivity, and c-GMP concentrations. To assess relative changes within fluid compartments, we determined hematocrit, total plasma protein, and albumin concentrations. Fluid intake was restricted to a maximum of 1.0 l within 28 hours. Body weight was recorded before and at the completion of the protocol.

Systolic and diastolic blood pressures were determined by sphygmomanometry with an automated oscillographic technique (Dinamap, model 845 XT, Criticon Corp., Tampa, Fla.). The triple-lumen Swan-Ganz catheter allowed the measurement of right atrial pressure (RAP), mean pulmonary arterial pressure, and pulmonary capillary wedge pressure (PCP). Cardiac output was determined by the thermodilution technique. Measurements were performed in triplicate using iced normal saline. Heart rate was derived from a continuously recorded ECG. Mean arterial pressure (MAP), cardiac index (CI), stroke volume, and systemic vascular resistance were calculated using standard formulas.\(^1\)\(^7\)

Urine volume was measured using graduated cylinders. Urine potassium and sodium excretion (milliequivalents per liter) were determined by flame photometry and osmolality by freezing-point depression. Urine volume was expressed as urinary flow rate (milliliters per minute). The glomerular filtration rate (GFR) was calculated as endogenous creatinine clearance. Osmolar clearance and free water clearance were determined for each collection period and expressed as milliliters per minute. Free water clearance was calculated as the difference between urinary volume and clearance of osmoles per minute. Sodium and potassium excretion rates were calculated using the standard formula. Plasma α-hANF and pro-hANF(31-67) levels, plasma catecholamines, plasma and urine c-GMP, plasma renin activity, aldosterone and arginine vasopressin (AVP) levels, and the urinary excretion rate of the stable prostacyclin metabolite 6-keto-PGF\(_1\alpha\) were determined as described previously.\(^7\)\(^8\)\(^9\) Plasma vasopresin levels of age-matched normal individuals averaged 0.47±0.1 pg/ml. The excretion rate of the stable prostacyclin (PGI\(_2\)) metabolite 6-keto-PGF\(_1\alpha\) was analyzed to determine whether the concomitant inhibition of bradykinin breakdown, and therefore enhanced renal PGI\(_2\) production, contributes to natriuresis.

**Statistical Analysis**

Values are given as mean±SEM. Intraindividual comparisons within the 24-hour protocol (n=7 patients) were evaluated by ANOVA. The ANOVA for hormonal and renal data were performed after logarithmic transformation, but absolute values are given in the results. When the F test indicated significant differences, individual comparisons were made by the Student-Newman-Keuls test. Single comparisons were made by paired t test. A value of p<0.05 was considered significant.

**Results**

**Atriopeptidase Inhibition: Initial Hemodynamic, Hormonal, and Renal Response (n=9)**

Atriopeptidase inhibition by candoxatrilat caused a significant increase in plasma ANF with a peak enhancement (2.5-fold) 2 hours after the first dose (from 235±59 to 591±148 pg/ml). Plasma cGMP did not change significantly (baseline, 3.57±0.52 pmol/ml). Plasma pro-hANF(31-67) levels declined from 3.151±599 to 2.103±461 pg/ml 4 hours after candoxatrilat dose (p<0.05).

The increase in plasma ANF concentrations was associated with a reduction in plasma norepinephrine (from 533±131 to 332±86 pg/ml), epinephrine (from 115±24 to 62±15 pg/ml), aldosterone levels (from 187±51 to 65±10 pg/ml), plasma renin activity (from 4.01±1.7 to 1.91±0.7 ng/ml/hr), and plasma vasopresin (from 2.70±0.7 to 1.51±0.43 pg/ml) for all p<0.05 2 hours after the first candoxatrilat dose.

Candoxatrilat increased CI from 2.11±0.2 to 2.67±0.28 l/min/m\(^2\) and decreased central filling pressures (PCP from 23±2.5 to 18.0±2.8 mm Hg, RAP from 11.8±2.15 to 9.1±1.9 mm Hg, and PAM from 37±4 to 33±4 mm Hg). The maximal increase in CI occurred 2 hours after candoxatrilat coincident with peak plasma ANF levels. Heart rate, total peripheral resistance, and arterial pressure did not change significantly with candoxatrilat.

Renal baseline status was on average abnormal compared with healthy controls,\(^12\) consistent with severe heart failure. Baseline values were: sodium excretion, 58±23 μEq/min; fractional sodium excretion, 0.29±0.34%; potassium excretion, 57±10 μEq/min; fractional potassium excretion, 14.4±2%; volume excretion, 0.93±0.19 ml/min; GFR, 57±10 ml/min; free water clearance, 0.59±0.17 ml/min; and osmolar clearance, 1.49±0.28 mosm/min. After the first candoxatrilat dose, potassium excretion, fractional potassium excretion, GFR, and free water clearance did not change significantly. Volume excretion tended to increase, but the response was heterogeneous, on average, minimal, and not significant. Only patient 5 (see Table 1), with the lowest baseline ANF levels and a CI >2.5 l/min/m\(^2\), responded to candoxatrilat with a good diuresis (from 0.73 to maximally 6.33 ml/min). In contrast, patients 2, 3, and 6, with the highest plasma α-hANF levels (>300 pg/ml) and CI <1.7 l/min/m\(^2\) did not respond at all. To determine which hemodynamic or neurohumoral parameter most accurately predicts diuretic responders, linear regression analysis was performed. The maximal diuretic response within the first 4 hours after candoxatrilat dose correlated best with basal CI (r=0.78, p=0.012). No significant correlation was observed with basal renin activity (r=−0.61, p=0.73), basal plasma norepinephrine levels (r=−0.23), and PCP (r=0.002).

Atriopeptidase inhibition caused a significant enhancement of sodium and fractional sodium excretion.
and an increase in osmolar clearance with a maximum 2 hours after the first candoxatrilat dose (to 234±80 \(\mu\)Eq/min, to 1.23±0.45%, and to 2.77±0.42 mosm/min, respectively). There was also a close correlation between baseline CI and the maximal natriuretic effect within 4 hours after the first candoxatrilat dose \((r=0.77, p=0.01)\). Urinary cGMP excretion rate increased about fivefold (from 1.318±130 to 7.080±1,281 pmol/min, \(p<0.05\)) 2 hours after the first candoxatrilat dose coincident with peak plasma ANF levels. The excretion rate of ANF immunoreactivity remained unchanged (266±108 pg/min). The inhibition of ANF degradation caused a significant 3.3-fold increase \((p=0.01)\) in the excretion rate of 6-keto-PGF-1\(\alpha\) (see Figure 1), which suggests that coincident inhibition of bradykinin breakdown and therefore enhanced prostaglandin formation contributes to the observed increase in sodium excretion.

**Atrial natriuretic factor**

**Effects of Repeated Doses** \((n=7)\)

Figures 2-6 summarize the renal, hormonal, and hemodynamic effects of repeated doses of candoxatrilat during the entire 24-hour protocol. The inhibition of ANF degradation resulted in constantly elevated plasma ANF levels \((p<0.05)\). The endogenous ANF secretion (assessed by the determination of the pro-hANF cleavage peptide(31-67) remained suppressed throughout the entire protocol \((p=0.01)\). The increase in CI was only transient. The second and third administration of candoxatrilat did not elicit significant increases in CI \((p=0.01)\). The increase in CI was only transient. The second and third administration of candoxatrilat did not elicit significant increases in CI \((p=0.01)\). Central filling pressures remained significantly reduced throughout the protocol period. Heart rate, total peripheral resistance, and arterial pressure did not change significantly. Plasma norepinephrine and epinephrine levels remained suppressed \((p<0.05)\), and there was no evidence of activation of the renin–angiotensin–aldosterone system and plasma vasopressin \((p<0.01)\) despite persistent natriuresis and a negative 24-hour fluid balance of 702±194 ml.

**Side Effects**

One patient (patient 1) experienced a vasovagal attack 4 hours after the first candoxatrilat dose. At that time, plasma ANF levels had fallen from 346 to 277 pg/ml. Therefore, it seems difficult to differentiate

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**FIGURE 1.** Bar graph shows excretion rate of 6-keto-PGF-1\(\alpha\) during control and 2 hours after the first candoxatrilat dose in nine patients. Data are presented as mean±SEM, *p=0.01.

**FIGURE 2.** Plots of atrial natriuretic factor (α-hANF) and pro-ANF(31-67) responses to intravenous candoxatrilat (C, 150 mg t.i.d). Values are given as mean±SEM and as individual data. B1 and B2 refer to the two baseline measurements. *p<0.05 vs. baseline.
whether this syncope was due to a direct parasympathomimetic action of ANF\textsuperscript{20} or a consequence of the indwelling Swan-Ganz catheter. Another patient (patient 3) was withdrawn during the study protocol and was put on high-dose furosemide because of left heart decompensation. This patient had the highest PCP, CI <1.6 l/min/m\textsuperscript{2}, the highest pro-ANF(31-67) levels (6,531 pg/ml), and plasma renin activity (16 ng/ml/hr). Both patients had uneventful recoveries.

**Discussion**

The present study was undertaken to study the therapeutic potential of inhibiting ANF degradation in patients with severe chronic heart failure. Inhibition of ANF metabolism enhances the responses to exogenous ANF\textsuperscript{21,22} and leads to significant hemodynamic unloading,\textsuperscript{13} to inhibition of the neurohumoral axis,\textsuperscript{11} and to a striking diuresis and natriuresis in dogs with experimental heart failure\textsuperscript{15} and patients with mild heart failure.\textsuperscript{13,14} Moreover, the renal effects in response to NEP inhibition appear to be superior to exogenously administered ANF,\textsuperscript{15} suggesting different or additional diuretic mechanisms.\textsuperscript{23} Because potassium balance is in general unaffected,\textsuperscript{10-14} this therapeutic principle of NEP inhibition combines several features, which make it particularly useful as a diuretic agent in patients with chronic heart failure.

**Changes in Plasma ANF and Pro-ANF(31-67)**

Candoxatrilat is a selective inhibitor of the metalloendopeptidase E.C.3.4.24.11.,\textsuperscript{24} an enzyme that has a widespread distribution on membranes in many tissues and organs.\textsuperscript{25} Although the kidney has been proposed to be a major site of ANF metabolism,\textsuperscript{26} recent experimental data revealed that the renal NEP plays a relatively minor role in the clearance of ANF from the plasma.\textsuperscript{27} Indeed, the liver, lung,\textsuperscript{28} vascular endothelium,\textsuperscript{29} neutrophils,\textsuperscript{30} and the so-called clearance receptor\textsuperscript{31} have been identified as important participants in the clearance of ANF. NEP inhibition by candoxatrilat in the present study caused a significant increase in plasma ANF concentrations, with a peak enhancement 2 hours after the first dose. The dose of candoxatrilat (150 mg t.i.d.) was sufficient to cause persistently elevated plasma ANF levels for a 24-hour period. Time course and degree of ANF enhancement are consistent with reports from NEP inhibition in animals,\textsuperscript{15} control subjects,\textsuperscript{10-12} and patients with mild congestive heart failure.\textsuperscript{13,14} In contrast to the sustained elevation of plasma ANF, the N-terminal cleavage peptide pro-ANF(31-67) levels declined within 2 hours after the first candoxatrilat dose and remained significantly suppressed during the entire protocol. Recent experimental data demonstrated that in response to atrial stretch, pro-ANF(31-67) and the C-terminus appear to be secreted simultaneously.\textsuperscript{32} This indicates that pro-ANF(31-67) can be used as a marker for the endogenous ANF secretion during exogenous administration of the biological active peptide. Furthermore, the demonstration of a stretch-dependent release suggests that the suppression of
pro-ANF(31-67) levels is secondary to the reduction of left atrial pressure and RAP.

Inhibition of Neurohumoral Axis

An important observation of the present study is that the two- to threefold increase in plasma ANF concentrations was associated with a sustained reduction in circulating plasma norepinephrine and epinephrine levels. This observation of an inhibitory action on sympathetic activity by ANF is consistent with studies performing sympathetic nerve recordings, and reflex responses in human subjects. The effects on circulating catecholamines seem to be more pronounced when the increase in plasma ANF concentrations is within a physiological rather than a pharmacological range. This indicates that the sympathetic inhibitory action of ANF may be frequently masked with administration of pharmacological doses, e.g., due to a drop in MAP and subsequent activation of baroreceptor reflexes. Concomitantly, we observed a significant reduction in plasma renin activity and plasma aldosterone levels similar to observations in normal controls. The inhibition of aldosterone release might be explained by a reduction in plasma renin levels or by direct inhibition of aldosterone production in adrenal zona glomerulosa cells. Interestingly, in the adrenal cortex, endopeptidase 24.11 is localized to the glomerulosa cells, where it may serve to inactivate ANF. Therefore, it is tempting to speculate that inhibition of the adrenal endopeptidase contributes to the observed reduction of aldosterone secretion. We also observed a transient reduction in plasma vasopressin levels. This is in line with recent reports demonstrating that ANF inhibits AVP neurons in the paraventricular nucleus and the posturally mediated release in humans.

Hemodynamic Effects

The principal hemodynamic response to repeated NEP inhibition was characterized by a persistent reduction in PCP and RAP similar to control subjects and patients with mild heart failure and by a brief increase in CI. These hemodynamic effects were similar to previous observations using pharmacological doses of intravenous ANF in patients with severe chronic heart failure. This shows that NEP inhibition, associated with only modest increases in plasma ANF concentrations, results in an effective hemodynamic unloading in patients with severe heart failure. The reduction in central filling pressures appear to be mediated by a decrease in preload rather than afterload, because systemic vascular resistance did not change.

In patients with severe chronic heart failure, prolonged infusion of ANF in pharmacological concentrations is accompanied by fluid and protein extravasation, suggesting that part of the observed preload effect might be triggered by extravasation of plasma to interstitial compartments. Moreover, low-dose ANF infusion, associated with a four- to fivefold increase in plasma ANF levels, increased forearm capillary filtrations.

Figure 4. Plots of neurohumoral changes in response to intravenous candesartan (C, 150 mg i.d.). Values are given mean±SEM and individual data. *p<0.05; B1 and B2 refer to the two baseline measurements. AVP, arginine vasopressin; EPI, epinephrine; N-EPI, norepinephrine; ALDO, aldosterone; PRA, plasma renin activity.
Renal Effects
The present study demonstrates that inhibition of NEP results in a significant increase in sodium and fractional sodium excretion and osmolar clearance associated with an increase in urinary cGMP (Figures 5 and 6). In contrast, GFR, water and potassium excretion, and free water clearance remained unchanged, and fractional potassium excretion was significantly suppressed.

Candoxatrilat is less effective in inducing natriuresis and diuresis in patients with severe heart failure when compared with normal controls12 (also see “Results”). The close correlation between diuretic and natriuretic response and baseline CI indicates that a low cardiac output and therefore compromised renal perfusion most accurately predicts natriuretic and diuretic resistance in patients with heart failure, a phenomenon also demonstrated for loop diuretics and thiazides.48

tion, hematocrit, and total plasma protein content.44 This suggests that the concomitant fall in central filling pressure was primarily mediated by fluid extravasation rather than by vasodilating or renal effects. In the presence of unchanged hematocrit, total protein content, and plasma albumin, it seems unlikely that intravascular volume contraction was a significant factor contributing to the significant decrease in preload observed in the present study.

Northridge and coworkers13 proposed that an ANF-induced increase in venous capacitance may be responsible for the hemodynamic unloading in their patients with mild chronic heart failure. Although ANF per se has virtually no venodilator capacities,45 it is conceivable that the beneficial hemodynamic effects might be mediated by a reduction of sympathetic and angiotensin II–induced constriction of venous capacitance vessels.46,47
The mode of natriuretic action induced by ANF is still debated. Proposed mechanisms include renal hemodynamic changes or a direct inhibition of sodium absorption at the proximal tubules or the inner medullary collecting duct. The demonstration that the inhibition of the neutral endopeptidase causes natriuresis in the absence of changes in GFR and systemic hemodynamics suggests a renal tubular action of ANF. This assumption is supported by experimental data demonstrating an increased fractional excretion of lithium (marker for proximal tubule sodium reabsorption) and an inhibition of angiotensin II–stimulated proximal tubule sodium reabsorption in response to ANF degradation, inhibition, or administration. However, a drug that inhibits proximal sodium transport should stimulate the potassium secretory pathway in the distal nephron. The observed unchanged potassium excretion rate in the present study (see Figure 5) might therefore be compatible with a more distal effect, e.g., in the inner medullary collecting duct. An additional site of action would also explain why atriopeptidase inhibitors produce a greater and more persistent natriuresis when compared with exogenously administered ANF in physiological and pharmacological concentrations.

The inhibition of ANF degradation at the proximal tubule has been reported to be linked to an increased excretion of ANF immunoreactivity. This led several investigators to speculate that high, intraluminal concentrations of undegraded ANF may act intraluminally or on distal ANF receptors, even when they are located on basolateral membranes of the centro-luminal side. In the present study, however, we observed a dissociation between urinary ANF and sodium excretion. Whereas the sodium excretion rate increased almost sixfold, the urinary ANF did not change.

In this context, it should be stressed that NEP is known to cleave a variety of other vasoactive peptides such as bradykinin, encephalin, and substance P in addition to ANF, which might contribute to the observed hemodynamic, renal, and hormonal changes induced by candoxatrilat. Bradykinin stimulates prostaglandin production together with natriuresis in the kidney, and recent experimental data demonstrate that NEP inhibition is associated with a substantial increase in the excretion rate of kinins and sodium. Furthermore, antibradykinin antibodies blocked quantitatively similar to ANF antibodies, natriuresis induced by NEP inhibitor thiorphan in volume-expanded rats. Therefore, the observed increase in 6-keto-PGF-1α excretion rate (secondary to inhibition of bradykinin breakdown) might indicate that the endogenous kinin-generating system of the kidney may also participate in the observed natriuretic responses to candoxatrilat in patients with congestive heart failure.

Summary

The present study demonstrates that repeated NEP inhibition with candoxatrilat (150 mg i.v. t.i.d) causes persistent elevation of plasma ANF levels, hemodynamic unloading associated with a reduction of endogenous ANF secretion (pro-ANF[31–67]), and suppression of neurohumoral activity. However, candoxatrilat failed to elicit a significant diuresis in patients with low resting cardiac output. Nevertheless, in the presence of moderate renal effects, the inhibitory actions of candoxatrilat on the neurohumoral axis, together with hemodynamic unloading, suggests that the inhibition of ANF degradation may be a useful adjunct in moderate heart failure. This spectrum of action would be advantageous for a first-line diuretic agent early in the course of the disease rather than in patients with advanced chronic heart failure. Further studies are warranted to elucidate whether combination therapy with a vasodilator or another diuretic agent may be more effective in inducing a diuretic response in the severely ill patient population than candoxatrilat alone.

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Endopeptidase Inhibition in Chronic Heart Failure


Neurohormonal inhibition and hemodynamic unloading during prolonged inhibition of ANF degradation in patients with severe chronic heart failure.
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