Role of neutral endopeptidase 24.11 in AV fistular rat model of heart failure

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Abstract

Objective: The aortovenocaval fistular (AVF) rat represents a model of heart failure caused by increased cardiac volume overload and reduced renal function. Both circulating vasoconstrictors like the renin–angiotensin–aldosterone system and vasodilators like atrial and brain natriuretic peptides (ANP and BNP) are activated in this animal model of heart failure. In addition, neutral endopeptidase 24.11 (NEP) in plasma and urine is elevated in AVF rats. In the present investigation we examined the renal and hormonal effects of the NEP inhibitor, ecadotril, in acute and chronic studies in rats with an aortovenocaval fistula (AVF).

Methods: Sprague Dawley rats (350–430 g) were prepared by introducing a shunt between abdominal aorta and the vena cava.

Results: Acute administration of the neutral endopeptidase inhibitor, ecadotril (30 mg/kg p.o.), significantly improved the reduced renal excretion of sodium in AVF rats (83 ± 10 to 145 ± 14 μmol/kg/h, P < 0.01) but had no significant effect in sham-operated rats. However, neutral endopeptidase activity in urine was significantly decreased after ecadotril in both groups. Plasma ANP was increased after ecadotril only in AVF rats (275 ± 83 to 748 ± 187 pg/ml, P < 0.05), whereas the increase in plasma BNP was not statistically significant. After 4 weeks of observation the ANP and BNP plasma levels, renin activity (PRA), angiotensin I, and neutral endopeptidase activity were significantly higher in AVF rats than in sham-operated rats. Four weeks on ecadotril (30 mg/kg p.o., b.i.d.) increased plasma ANP (245 ± 48 as opposed to 450 ± 77 pg/ml, P < 0.05) and decreased PRA (11.3 ± 1.5 as opposed to 6.8 ± 1.2 mg/ml/h, P < 0.005) in AVF rats. Plasma NEP activity was inhibited in both groups. Ventricle weight was significantly higher in AVF rats than in sham-operated controls, and ecadotril treatment over 4 weeks decreased ventricular hypertrophy to a slight extent. Conclusion: These results indicate that in the AVF rat model of heart failure the neutral endopeptidase inhibitor, ecadotril, improves the reduced kidney function in AVF rats by raising natriuretic peptides in plasma and probably in urine. NEP inhibition with ecadotril could therefore offer useful therapeutic possibilities in the treatment of heart failure.

Keywords: Heart failure; ANP; BNP; Ecadotril; Rat, AV fistular model

1. Introduction

Congestive heart failure is a pathophysiological condition characterized by elevated natriuretic peptides (atrial and brain natriuretic peptides: ANP and BNP), by activation of the renin–angiotensin–aldosterone system (RAAS), by an activated adrenergic system, and by reduced renal function. ANP is secreted by the atria in response to increased blood pressure or volume. In congestive heart failure the ventricles also participate in regulation of electrolytes and of volume homeostasis via the secretion of ANP and BNP [1].

ANP is the first described 28-amino-acid peptide in a family of hormones known to play an important role in the regulation of extracellular volume. This peptide has a number of functions in the renal and vascular systems which lead to natriuresis, diuresis, a decrease in blood pressure, modulation of cardiac output and plasma volume, and inhibition of the RAAS [2–6].

Neutral metalloendopeptidase EC 3.4.24.11 (NEP) is a zinc-containing membrane-bound enzyme, widely distributed in the organism, with a high activity in the brush border of renal proximal tubules. It is the primary metabolizing enzyme for ANP [7]. Cleavage of the Cys65–Phe106 and Ser173–Phe174 bonds of ANP by NEP destroys the
essential ring structure, resulting in biological inactivation of ANP [6,8].

BNP is a 32-amino-acid polypeptide homologous with ANP, originally isolated from porcine brain. It displays ANP-like biological activity by binding to the ANP-A receptors [9]. However, it should be mentioned that BNP activates to a lesser extent the ANP-A receptor than ANP [10]. In contrast to ANP the cardiac hormone BNP is secreted predominantly from vessels of the hypertrophic heart, and its elevation in plasma is used as a marker for various stages of heart failure in humans [11]. NEP is largely responsible for inactivating not only ANP, but also BNP and many other peptides, including angiotensin II, bradykinin, CNP, etc. [12]. NEP-inhibitors accordingly prevent the degradation of ANP and BNP, prolonging their half-lives and promoting the known ANP/BNP-mediated actions [13–18]. In addition, in patients with heart failure administration of NEP inhibitors increases not only the already elevated plasma ANP, but also the elevated BNP levels [16].

In the present study we examined the effects of ecadotril in acute and chronic experiments in rats with an aortoveno-caval fistula (AVF). The renal effects of an ANP infusion are attenuated in AVF rats in comparison with their sham-operated controls [19]. In contrast, infusion of the NEP inhibitor, thiorphan, produced a greater increase in urinary sodium and cyclic GMP excretion in AVF rats than in the controls [19]. In view of these findings, it was of interest to gain further insight into the mechanism of the improved renal response to NEP inhibition, and accordingly we performed acute and long-term trials with the orally active NEP inhibitor, ecadotril, in this animal model of heart failure.

2. Methods

2.1. Substances

Ecadotril (N-((S)-(2-[(acetyltthio)methyl]-1-oxo-3-phenylpropyl)-glycine benzylester; formerly called sinorphan) was received from Bioprojet, France.

2.2. Preparation of the AV fistula

After an overnight fast, Sprague-Dawley rats (350–430 g) were prepared by a modification of a method described by Mercadier et al. [20]. Briefly, surgical procedures were carried out under anaesthesia with inhalation of fluothane (2%) in oxygen/N₂O (30% /70%). The aortocaval pedicle was reached through an abdominal midline incision. Clamps were inserted to expand the operation field. Small vascular bulldog clamps were positioned on the two vessels from right to left, the first directly under the origin of the right renal arteries and the second over the aortic and vena cava bifurcation. By a right-to-left rocking motion of the two clamps through 90° using fine threads at the end of the clamps, the vena cava was raised above the aorta. A longitudinal 2- to 3-mm-long incision was made in the vein with scissors under a microscope, and through this incision a hole was cut into the medial common wall shared with the aorta. The incision was washed with physiological saline (0.9% NaCl) with a small addition of heparin to prevent thrombosis, closed with an overcast seam (Ethicon Vicryl TF-6, 8-0, Norderstedt, Germany), and carefully covered with adhesive (Ethicon Bucrylat, Ethicon GmbH Norderstedt, Germany). The clamp over the bifurcation was removed first, and then the clamp distally to the renal arteries. To check whether the operation had been successful, we clamped the right renal artery with tweezers. Red blood appeared in the vena cava, instead of the normal blue colour. The abdominal incision was closed with two seams and a little novocaine (2%) was injected subcutaneously. The inhalation anaesthesia was stopped and the animals recovered within 5 min. The duration of the operation was about 35 min. Mortality within 2 weeks after the experiment was 44% due to ascites and ischaemia. The same procedure was used to prepare the sham rat: the two vessels were clamped as described above for the same time as was needed for the fistula preparation, and the animals then recovered as described above. No sham-operated animal died within 2 weeks.

2.3. Acute experiments

Three to 7 days after the AVF preparation a diuresis experiment was performed. To rats fasted overnight a single dose of ecadotril (30 mg/kg p.o.) was administered as a suspension in polyethylene glycol 400/carboxymethylcellulose (0.5%) solution (v/v = 10/90) in an administration volume of 2 ml/kg body weight (BW). Controls received the vehicle alone by the oral route. For volume loading the rats were given physiological saline (0.9% NaCl) in a dose of 10 ml/kg body weight p.o. and were placed in metabolic cages. Urine was then collected over 6 h. The excretions of sodium, volume, potassium, and cyclic guanosine-monophosphate (cGMP) were measured, and neutral endopeptidase (NEP) activity in urine was determined. At the end of the experiment blood was withdrawn from the tail vein into tubes coated with EDTA (potassium EDTA, Sarstedt, Germany) and centrifuged for the determination of ANP and BNP.

2.4. Long-term experiments

Chronic treatment with ecadotril (30 mg/kg p.o., b.i.d.) over a period of 4 weeks was started immediately after the acute experiment (4–8 days after the preparation of AVF). The rats had free access to water and received a standard rat diet (Snniff Versuchstierdiäten Soest, Germany). Renal parameters were measured in the last week of the experi-
ment. For volume loading the rats were given physiological saline (0.9%) in a dose of 10 ml/kg body weight p.o. and were placed in metabolic cages. Urine was collected over 6 h for the determination of the renal excretion of sodium, volume, potassium, and cGMP and of NEP activity. In addition, creatinine clearance was determined. After decapitation blood was collected in tubes prechilled with EDTA and centrifuged, the separated plasma being stored at -70°C until the assay of cGMP, ANP, BNP, and renin activity. For the determination of NEP activity plasma was separated in heparinized tubes (Sarstedt, Germany) and stored at 4°C until assays on the same day. After thoracotomy the kidneys and the hearts were removed and the ventricles isolated by cutting off the pulmonary arteries, atria, and aortas. The ventricles were opened, washed, blotted dry with filter paper, and weighed.

2.4.1. Endopeptidase activity in plasma and urine
Plasma was collected in heparinized tubes and stored on ice until the assay. A fluorimetric method was used for the determination of endopeptidase activity by a two-step fluorimetric assay, as described previously in detail [21]. Succinyl-Ala-Ala-Phe-amidomethyl-coumarin (Bachem Pharma, Germany) served as the substrate. Blanks were obtained by adding 1 μM thiorphan (Sigma, Germany) to substrate solution of parallel incubations. The solution was incubated for half an hour at 37°C and the reaction was then stopped by boiling at 56°C. Fluorescence of the samples was measured with emission at 440 nm and excitation at 367 nm. For the determination of endopeptidase activity in urine the same method was used as described above.

2.4.2. Plasma ANP
The ANP in plasma was determined after extraction using C18-cartridges (Bond Elut®, Varian, Harbor City, USA) and a specific and sensitive radioimmunoassay kit (Biotrend, Cologne, Germany). The antibody did not show any cross-reactivity with ANP.

2.4.3. Plasma BNP
BNP in plasma was similarly determined after extraction using C18-cartridges. After lyophilization, the BNP was reconstituted in assay buffer and the concentration of BNP-like immunoreactivity was measured with a commercial radioimmunoassay kit (Biotrend, Cologne, Germany). The antibody did not show any cross-reactivity with ANP.

2.4.4. Plasma cGMP
For this determination 300 μl of plasma was added to an equal volume of 10% trichloracetic acid. After incubation for 30 min the samples were centrifuged (10 min, 5000 rpm, 4°C) and the supernatant was washed 4 times with water-saturated ether and lyophilized. The cGMP was determined using a commercial radioimmunoassay kit (IBL, Hamburg, Germany).

2.4.5. Plasma renin activity (PRA)
Plasma was incubated with phenylmethylsulfonyl fluoride and the accumulation of angiotensin I was measured by radioimmunoassay (Sorin Biomedia, Saluggia, Italy).

2.4.6. Electrolyte excretion
Urine volume was determined gravimetrically and urinary flow was expressed in ml/kg/h. The electrolyte concentrations were determined with an electrolyte analyser (Type 982-S, AVL, Bad Homburg, Germany). The excretion rates for sodium and potassium were calculated and expressed in pmol/kg/h. Creatinine in plasma and urine was determined by a spectrometric method [22] and the creatinine clearance was expressed in ml/min.

2.4.7. Statistical analyses
All data are presented as mean ± s.e.m. Comparisons between untreated and ecadotril-treated sham-operated or AVF rats were analyzed by one way ANOVA followed by Dunnett's t-test when appropriate. To check differences between baseline parameters in untreated sham-operated

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham controls</th>
<th>Sham ecadotril</th>
<th>AVF controls</th>
<th>AVF ecadotril</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diuresis (ml/kg/h)</td>
<td>1.4 ± 0.1 (10)</td>
<td>1.6 ± 0.2 (10)</td>
<td>1.4 ± 0.2 (10)</td>
<td>1.8 ± 0.2 (9)</td>
</tr>
<tr>
<td>Natriuresis (μmol/kg/h)</td>
<td>137 ± 17 (10)</td>
<td>195 ± 24 (10)</td>
<td>83 ± 10 (10) *</td>
<td>145 ± 14 (9) **</td>
</tr>
<tr>
<td>Kaliuresis (μmol/kg/h)</td>
<td>78 ± 10 (10)</td>
<td>79 ± 9 (10)</td>
<td>77 ± 10 (10)</td>
<td>89 ± 10 (9)</td>
</tr>
<tr>
<td>Sodium/potassium ratio</td>
<td>1.9 ± 0.2 (10)</td>
<td>2.6 ± 0.3 (10)</td>
<td>1.2 ± 0.2 (10) a</td>
<td>1.7 ± 0.2 (9) *</td>
</tr>
<tr>
<td>cGMP excretion (fmol/kg/h)</td>
<td>2.5 ± 0.4 (10)</td>
<td>5.9 ± 0.8 (10) * * *</td>
<td>3.7 ± 0.6 (10)</td>
<td>15.3 ± 4.7 (9) *</td>
</tr>
<tr>
<td>NEP activity (nmol/ml/min)</td>
<td>1.2 ± 0.2 (10)</td>
<td>0.0 ± 0.0 (9) * * *</td>
<td>1.8 ± 0.2 (10) a</td>
<td>0.0 ± 0.0 (7) * * *</td>
</tr>
</tbody>
</table>

Values are presented as means ± s.e.m. (n).
* P < 0.05, ** P < 0.005 compared with the values in untreated sham and AVF rats, a p < 0.05 compared with the values in sham-operated control rats.
rats, Student's *t*-test was performed. The significant threshold was set at 0.05.

3. Results

3.1. Acute experiment

Fig. 1 and Table 1 show the effects of a single dose of the orally active NEP inhibitor, ecadotril (30 mg/kg), on the renal and plasma parameters 3–7 days after AVF preparation. The renal excretion of sodium was originally significantly lower in AVF rats than in sham-operated rats, whereas diuresis was comparable. Ecadotril increased the excretion of sodium in both groups of animals, but the change was significant only in the AVF rats. The potassium excretion did not change after a single dose of ecadotril, and the sodium/potassium ratio was significantly elevated in AVF rats but not in the sham-operated controls. The urinary excretion of cGMP tended to be higher in AVF rats than in the controls. After oral administration of ecadotril the urinary cGMP increased in both groups, but more so in AVF rats. The NEP activity measured in urine was significantly higher in AVF rats than in controls, and was nearly completely inhibited in both groups treated acutely with ecadotril (Table 1). Fig. 1 shows the plasma levels of ANP and BNP after a single dose of ecadotril. 3 to 7 days after the production of the AV fistula, the plasma levels of ANP and BNP were doubled in AVF rats, while the plasma BNP only tended to be higher. NEP inhibition with ecadotril significantly increased the plasma levels of ANP and tended to increase plasma BNP in rats with experimentally induced CHF but not in sham-operated rats.

3.2. Long-term experiment

Table 2 shows the results of NEP inhibition with ecadotril at the end of a 4-week treatment period with

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham control</th>
<th>Sham ecadotril</th>
<th>AVF controls</th>
<th>AVF ecadotril</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diuresis (mg/kg/h)</td>
<td>1.7±0.2(10)</td>
<td>1.8±0.2(9)</td>
<td>1.2±0.1(9)</td>
<td>1.6±0.2(7)</td>
</tr>
<tr>
<td>Natriuresis (μmol/kg/h)</td>
<td>134±11(10)</td>
<td>156±20(9)</td>
<td>95±16(9)</td>
<td>133±15(7)</td>
</tr>
<tr>
<td>Kaliuresis (μmol/kg/h)</td>
<td>86±7(10)</td>
<td>84±5(9)</td>
<td>89±9(9)</td>
<td>83±10(7)</td>
</tr>
<tr>
<td>Sodium/potassium ratio</td>
<td>1.7±0.2(10)</td>
<td>1.9±0.2(9)</td>
<td>1.9±0.6(9)</td>
<td>1.8±0.3(7)</td>
</tr>
<tr>
<td>cGMP excretion (nmol/kg/h)</td>
<td>3.0±0.2(10)</td>
<td>7.6±0.7(9)</td>
<td>4.5±0.9(6)</td>
<td>15.2±3.9(7)</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td>1.9±0.1(9)</td>
<td>1.7±0.1(8)</td>
<td>1.3±0.1(9)</td>
<td>1.7±0.1(7)</td>
</tr>
</tbody>
</table>

Values are presented as means ± s.e.m. (n).

* P < 0.05, ** * P < 0.005 compared with the values in untreated sham and AVF rats, # P < 0.05 compared with the values in sham-operated control rats.
respect to renal function. After 4 weeks of ecadotril treatment the excretion of potassium and the sodium/potassium ratio were effectively the same as in untreated sham-operated rats, while the volume excretion was significantly lower in AVF rats. The reduced natriuresis and diuresis in AVF rats was improved after ecadotril, but the effect was not statistically significant. The urinary excretion of cGMP was significantly higher in both groups treated with ecadotril. The creatinine clearance in AVF rats was lower than in sham-operated controls, and was restored to normal values by chronic neutral endopeptidase inhibition.

The plasma levels of ANP, BNP, and NEP activity are shown in Fig. 2 whereas PRA and angiotensin I levels are depicted in Fig. 3. Plasma of ANP was significantly higher in AVF than in sham-operated rats (245 ± 48 as opposed to 140 ± 12 pg/ml, \( P < 0.05 \)). Chronic treatment with ecadotril significantly increased plasma ANP in AVF rats. In addition, the plasma levels of BNP were elevated in AVF rats (54 ± 5 as opposed to 32 ± 3 pg/ml, \( P < 0.05 \)) and ecadotril treatment slightly increased the plasma BNP levels, though the effect was not significant (Fig. 2). According to the levels of neutral endopeptidase activity in urine the NEP activity in plasma was significantly higher in AVF rats than in the controls (46 ± 3 vs. 32 ± 3 pmol/ml/min, \( P < 0.05 \)), and decreased significantly under ecadotril.

Angiotensin I and plasma renin activity were doubled in AVF rats compared with the sham-operated controls. Chronic treatment with ecadotril significantly reduced these parameters, to the levels in untreated controls (Fig. 3).

At the end of the experiment, ventricle weight was significantly higher in AVF rats than in sham-operated rats (328 ± 30 compared with 241 ± 5 mg/100 g body weight, \( P < 0.05 \)). The increase in lung weight in AVF rats was not statistically significant. Chronic ecadotril treatment tended to reduce ventricular hypertrophy and the lung weight in AVF rats.

4. Discussion

The results of the present study provide a number of insights into the regulation of the natriuretic peptide system by neutral endopeptidase in the aortovenocaval fistular rat model of congestive heart failure. We were also able to demonstrate that not only plasma ANP, but also plasma BNP was significantly elevated in AVF rats. The increase in ANP by a factor of 2-4 observed in this study was not as large as reported by other authors using AVF heart failure rats [23,24], probably reflecting only a modest CHF in our study. The AVF rat model of heart failure is characterized not only by high plasma levels of natriuretic peptides, but also by an increase in ANP mRNA in the atria and ventricles [25]. Acute treatment with the NEP inhibitor, ecadotril, significantly increased plasma ANP levels in AVF animals and tended to increase plasma BNP without having an effect on corresponding controls. However, after 4 weeks of observation both plasma ANP and plasma BNP was significantly higher in untreated AVF rats, and chronic NEP inhibition led to an increase in ANP and BNP in AVF rats without affecting these natriuretic peptides in the sham-operated animals.
Neutral endopeptidase activity in plasma and urine is significantly elevated in AVF rats with congestive heart failure in comparison with sham-operated controls. The increase in NEP activity might be one of the reasons for the blunted renal response to ANF infusion in this model of heart failure and for the resistance to the action of increasing doses of ANP [24,26–31]. In addition, enzymatic degradation by increased NEP limits local renal responses to increases in endogenous and exogenous ANP in dogs with congestive heart failure independent of changes in systemic hemodynamics or augmented plasma levels of ANP [32]. Treatment with ecadotril significantly inhibited the increased NEP activity in plasma and urine. A plausible explanation for the higher natriuretic response in AVF than in sham-operated rats after NEP inhibition [33] could be that ecadotril, which is rapidly cleaved into the active metabolite (S)-thiorphan, exerts a local action in the kidneys. More ANP and BNP can therefore reach tubular sites in the kidneys, normally inaccessible to the peptides, with consequent elevation of natriuretic peptide concentration in the nephrons to the level required to elicit a renal response [24,34,35].

In this context, Wilkins et al. [24] and Hoffman et al. [31] have previously demonstrated an attenuated renal response to exogenous ANP in AVF rats in comparison with the action of ANP in sham-operated controls. This finding is consistent with the attenuated renal response to ANP in humans with congestive heart failure [26,27]. In contrast, infusion of the NEP inhibitor, thiorphan, produced a greater effect on sodium and cGMP excretion in AVF rats than in the controls. This strong renal response was associated with only a modest increase in plasma ANP but a large increase in urinary ANP secretion. The effects of thiorphan, therefore, could not be explained simply in terms of an increase in plasma ANP, and it was proposed that thiorphan has a local action within the kidneys and protects ANP from degradation by NEP in the proximal tubules.

With the onset of acute CIIF a decrease occurred in the urinary clearance of ANP, despite a marked increase in plasma ANP. Very little ANP was detected in the urine of sham-operated or AVF rats under baseline conditions [24]. Abassi et al. [19] have reported that the urinary excretion of ANP is reduced in comparison with sham-operated controls, probably due to the increased activity of neutral endopeptidase located primarily in the brush border of renal proximal tubules. Many peptides seem to be involved in the renal responses to NEP inhibition, but, on the other hand, it is clear that ANP is a prime factor in initiating the response of these drugs. In the first place, the renal effects are accompanied by a significant rise in cGMP excretion, and secondly, the effect of NEP inhibition in AVF rats can be markedly attenuated by pretreatment with a monoclonal antibody directed against ANP [24].

In the light of the findings that plasma ANP levels and NEP activity are significantly higher in AVF than in sham-operated rats [33,36,37] it can be proposed that, on the one hand, the clearance receptor-mediated mechanism is largely involved in clearance of ANP under normal conditions, and that, on the other hand, NEP is upregulated and plays an additional significant role in the clearance when the natriuretic peptides are elevated, as is the case under conditions of heart failure. However, so far nothing is known about the distribution, regulation and quantitative importance of ANP clearance receptors in this animal model of heart failure.

In various animal hypertension models acute administration of various NEP inhibitors has been shown to increase plasma ANP even when the latter is already elevated as in NaCl-sensitive spontaneously hypertensive rats [38] and in DOCA/salt hypertensive rats [39]. In contrast, NEP inhibitors fail to elevate plasma ANP in normotensive rats [5] and in dogs [40]. Furthermore, we have demonstrated in an earlier work that in hypertensive transgenic rats with an extra renin gene (TGR(mRen2)27) a single administration of ecadotril significantly increased plasma ANP and BNP levels [18]. Plasma BNP was higher in TGR than in normotensive Sprague-Dawley rats [18], but despite the longer half-life of BNP [41] its levels were lower than the plasma levels of ANP, probably due to the only moderate cardiac hypertrophy in these young TGR. In addition, we had demonstrated that plasma NEP activity was significantly higher in hypertensive transgenic rats carrying an additional renin gene (TGR(mRen2)27) than in normotensive Sprague-Dawley rats [18]. For DOCA/salt hypertensive rats, also, it has been reported that their urinary NEP activity was 7 times higher than in controls [17]. The reason for the increased NEP activity in animals with hypertension or heart failure is not yet fully understood. There is some evidence that cGMP elevates NEP activity in smooth vascular muscle cells [42]. On the other hand, it could be that neutral endopeptidase substrates which are elevated under conditions of hypertension and heart failure, like ANP and BNP, are responsible for the upregulation of NEP. Taken together, these findings support the concept that NEP is elevated under conditions of hypertension and heart failure, and that NEP inhibitors have a stronger effect in animals with elevated NEP activity.

Three to 7 days after the induction of AVF we found a significantly reduced renal excretion of sodium and reduced sodium/potassium ratio in comparison with those in sham-operated controls. The excretion of cGMP as a marker for ANP involvement tended to be higher in AVF rats without reaching statistical significance. Acute treatment with the NEP inhibitor, ecadotril, improved the reduced sodium excretion and the sodium/potassium ratio only in AVF rats, whereas cGMP was increased in both groups. Nearly the same results were observed at the end of the 4-week ecadotril treatment except that the renal excretion of sodium was not significantly increased in AVF rats. In this respect we have already reported earlier
that in hypertensive transgenic rats (TGR(m(Ren2)27)) the renal and endocrine response to ecadotril is also more pronounced than in corresponding normotensive Sprague-Dawley rats [18]. In addition, in patients with chronic renal failure the sodium excretion increased after a single dose of ecadotril without any effect on the potassium excretion [13]. The effects of intravenously administered (S)-thiorphan, the active metabolite of ecadotril, on natriuresis, diuresis, and urinary ANP are also distinctly greater under the conditions of elevated plasma ANP levels induced by a blockade of ANP clearance receptors than under normal conditions [40].

At the end of the 4-week trial the plasma renin activity and plasma angiotensin I were significantly higher in AVF rats than in sham-operated rats. After chronic ecadotril treatment for 4 weeks these plasma parameters were significantly lower in AVF rats. The delayed rise of plasma renin activity and of angiotensin I in ecadotril-treated animals with heart failure is probably a consequence of activation of the natriuretic peptide system. It is interesting to note that treatment with the converting enzyme inhibitor, enalapril, in AVF rats has been demonstrated to provoke dramatic natriuresis, showing that when the influence of the RAAS is removed the natriuretic effects of ANP may be expressed [23].

In the present study, ventricle weights and lung weights were significantly higher in AVF rats and prolonged treatment (4 weeks) with the NEP inhibitor, ecadotril, tended to decrease both. However, in a long-term trial with ecadotril in hypertensive transgenic rats carrying an extra renin gene we found significantly reduced heart weights [18]. It has been suggested that ANP acts as a physiological antagonist of the renin–angiotensin system [5, 7, 43, 44]. Left-ventricular myocytes and cells within the kidneys contain detectable amounts of angiotensin II, which may act as a trophic growth-promoting factor. Local interaction of angiotensin II with enhanced ANP activity in ventricles could therefore exert a protective and antihypertrophic influence on cardiac myocytes. This interpretation does not rule out other still-unexplored mechanisms that play a part in determining changes in cardiac hypertrophy, because NEP hydrolyze numerous substances, including BNP, bradykinin, substance P, etc. [12]. On the other hand, the reason for the only slight reduction in cardiac hypertrophy observed in the present investigation remains to be elucidated.

In conclusion, in the AVF rat model of heart failure, not only the plasma levels of ANP and BNP and the plasma renin activity are significantly higher than in sham-operated animals, but also the NEP activity in plasma and urine. In addition, renal excretion of sodium is reduced and cGMP excretion slightly increased. Inhibition of the increased NEP activity by acute and chronic oral administration of ecadotril led to increased plasma ANP, plasma BNP, and cGMP excretion and to a reduced PRA and angiotensin I in AVF rats, without having any significant effects in corresponding sham-operated controls. We conclude that the increased NEP activity in AVF rats attenuates the effects of endogenous natriuretic peptides in this model of heart failure. The pharmacological profile of the NEP inhibitor, ecadotril, therefore makes it attractive for the treatment of cardiovascular diseases such as heart failure.

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