The cardiovascular consequences of neutral endopeptidase (NEP) inhibition with the NEP inhibitor ecadotril were evaluated by determining acute and long-term effects of the compound on hemodynamic, hormonal, renal, and structural parameters in hypertensive transgenic rats harboring a mouse renin gene (TGR(m(Ren2)27)) and in normotensive controls (Sprague-Dawley rats, SDR). Acute administration of ecadotril (10 and 30 mg/kg, orally) produced a dose-dependent decrease in systolic blood pressure with a maximal effect of \(-23\) mm Hg between 2 and 4 h after oral administration. The NEP activity in plasma was significantly inhibited and the plasma levels of atrial natriuretic peptide (ANP) and brain natriuretic peptide, and their second messenger, cyclic GMP, were distinctly raised after oral administration. In addition, ecadotril (10 and 30 mg/kg, orally) produced a dose-dependent increase in the urinary excretion of sodium and cyclic GMP. These effects were more pronounced in TGR(m(Ren2)27) than in the normotensive SDR rats.

In the long-term study, the systolic pressure in control TG(m(Ren2)27) rats increased from \(213 \pm 5\) to \(255 \pm 7\) mm Hg, whereas, in animals treated with ecadotril (30 mg/kg, orally twice daily), the blood pressure increased only from \(213 \pm 5\) to \(227 \pm 6\) mm Hg during the observation period of 13 weeks. The increase in heart weight and in kidney weight were also delayed. At the end of the study, cyclic GMP was elevated and ANP tended to be higher, whereas plasma renin activity had decreased. These data indicate a beneficial pharmacological profile of neutral endopeptidase inhibition that could prove useful in the treatment of cardiovascular diseases like hypertension. Am J Hypertens 1996;9:795–802

**KEY WORDS:** Transgenic TGR(m(Ren2)27) rats, neutral endopeptidase (enkephalinase) inhibition, atrial natriuretic peptide, brain natriuretic peptide, cyclic GMP, hypertension, sinorphan, ecadotril.
membrane-bound enzyme widely distributed in the organism, especially in the brush border membranes of the proximal tubules of the kidney, the lung, and the brain. It is the primary metabolizing enzyme for ANP. Cleavage of the Cys162-Phe206 and Ser123-Phe206 bonds of ANP by NEP destroys the essential ring structure and results in biological inactivation of ANP. This membrane peptidase is not only responsible for the inactivation of ANP but also for the inactivation of several other peptides, including brain natriuretic peptide (BNP).^1^-^4

BNP is a 32-amino-acid polypeptide homologous with ANP, originally isolated from porcine brain and subsequently from pig and rat hearts. It displays ANP-like biological activity by binding to ANP A-receptors. In contrast to ANP, the cardiac hormone BNP is secreted predominantly from the ventricle of the hypertrophic heart and is used as a marker for the various stages of heart failure in humans. Neutral endopeptidase inhibitors prevent the degradation of ANP, thereby enhancing its half-life and promoting ANP-mediated actions, such as diuresis, natriuresis, and vasodilation.^6^-^10^ The elevated plasma BNP levels in patients with heart failure are also increased further by administration of an NEP inhibitor.9

In the present study we examined the effects of eca
dotril in acute and long-term experiments with transgenic rats harboring the mouse renin gene (TGR(m(Ren2)27)) and in corresponding controls (Sprague-Dawley rats; SDR). Transgenic rats are characterized by high transcription rates of the transgene especially in the adrenal glands. They exhibit high plasma aldosterone concentrations, reduced renal renin production, and low plasma renin activity.11,12 Early in life TGR(m(Ren2)27) rats develop very early a life-threatening hypertension associated with elevated plasma ANP levels and increased heart weights.13

The aim of this study was to evaluate the cardiovascular consequences of NEP inhibition with the NEP inhibitor eca
dotril by determining the compound's acute and long-term effects on hemodynamic, hormonal, renal, and structural parameters in hypertensive transgenic rats harboring a mouse renin gene (TGR(m(Ren2)27)) and in appropriate normotensive controls (SDR).

METHODS

Substances Ecadotril (formerly called sinorphan) was received from Bioprojet, Paris, France.

Animals Study I Thirty male Sprague-Dawley rats and 30 male transgenic rats (TGR(m(Ren2)27)) (TGR) aged 10 weeks were randomized by body weight into two groups, a control group and a eca
dotril-treated group. The rats had free access to water and received a standard rat diet (Sniff, Soest, Germany). The SDR were obtained from Moellegaard (Copenhagen, Denmark) and the TGR from the Zentralstelle für Versuchs
	
tiere (Zentra, Germany).

The ecadotril was given as a suspension in polyethylene glycol 400/carboxymethylcellulose (0.5%) solution (v/v ~ 10/90) in a dose of 30 mg/kg orally to rats fasted overnight in an administration volume of 2 mL/kg body weight. Control animals received the vehicle alone by the oral route. The rats received a volume loading (20 mL physiological saline/kg) and were placed in metabolic cages. Urine was collected over an interval of 6 h and the excretions of sodium, volume, potassium, and cyclic guanosine monophosphate (cGMP) were determined. On the next day, the rats received the vehicle solution or ecadotril and were decapitated 1 h later. Blood was collected in EDTA tubes (Sarstedt, Germany) and centrifuged, the separated plasma being stored at −70°C to await the determination of cGMP, ANP, BNP, renin activity (PRA), and aldosterone. For determination of sodium, neutral endopeptidase activity, and the hematocrit, plasma was collected in heparinized tubes (Sarstedt, Germany).

Study II The blood pressure of TGR was determined by the tail cuff method after restraining the rats in tubes prevarmed to 36°C. After the determination of the baseline value, ecadotril (10 or 30 mg/kg, orally) or vehicle was administered and the blood pressure was measured again 1, 2, 4, and 6 h later.

Study III At the age of 10 weeks, transgenic rats were treated daily with ecadotril (30 mg/kg, orally twice daily) or vehicle alone. Systolic blood pressure was measured weekly by the tail-cuff method in conscious animals, kept in thermostated tubes at 36°C. In the last week of the study the animals received ecadotril or vehicle and a volume loading of 20 mL physiological saline/kg and then placed in metabolic cages. Urine was collected for 6 h and the urinary excretions of volume, sodium, potassium, cGMP, urea, creatinine, N-acetyl-β-glucosaminidase (NAG), and lactate dehydrogenase (LDH) were determined. After 13 weeks of treatment the animals were killed by decapitation. Blood samples were collected in prechilled potassium EDTA or heparinized tubes (Sarstedt, Germany) and stored in aliquots at −70°C for determination of the plasma parameters. After thoracotomy, the kidneys and the hearts were removed and the ventricles isolated by cutting off the atria, pulmonary arteries, and aortas. The ventricles were finally opened, washed, dried with filter paper, and weighed.

Determinations in Plasma Enkephalinase Activity For the determination of enkephalinase activity, plasma was collected in ice-cold tubes coated with heparin (Sarstedt, Germany) and stored until the assays at 4°C.
A two step fluorometric assay described previously was used for the determination of enkephalinase activity. Succinyl-Ala-Ala-Phe-amidomethyl-coumarine (Bachem Pharma, Heidelberg, Germany) served as the substrate. Blanks were obtained by adding 1 µmol/L thiorphan (Sigma, Deisenhofen, Germany) to the substrate solution in parallel incubations. The solution was incubated for half an hour at 37°C and the reaction was stopped by boiling for 30 min at 56°C. Fluorescence of the samples was determined with emission at 440 nm and excitation at 367 nm.

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

BNP BNP was similarly extracted from EDTA-plasma using C18-cartridges. After lyophilization, the BNP was reconstituted in assay buffer and the concentration of BNP-like immunoreactivity was measured with a commercially available radioimmunoassay kit according to the instructions (Biotrend, Köln, Germany). The antibody did not show any cross-reactivity with ANP.

cGMP For determination of cyclic GMP an equal volume of 10% trichloroacetic acid was added to the samples, as described previously. After an incubation period of 30 min the samples were centrifuged (10 min, 5000 rpm, 4°C) and the supernatant was washed four times with water-saturated ether and lyophilized. The volume of 10% trichloroacetic acid was added to the samples as described previously. After incubation for half an hour at 37°C and the reaction was stopped by boiling for 30 min at 56°C. Fluorescence of the samples was determined with emission at 440 nm and excitation at 367 nm.

Renin Activity Plasma renin activity (PRA) was determined by incubation of rat EDTA plasma with phenylmethylsulfonyl fluoride. Angiotensin I accumulated in the plasma samples during incubation for 1 h at 37°C at pH 6.0 and was measured using a commercial radioimmunoassay kit (Sorin Biomedica, Saluggia, Italy).

Plasma Aldosterone Plasma aldosterone was also determined with a radioimmunoassay kit from Sorin Biomedica.

Plasma Creatinine, Plasma Urea, and Plasma Uric Acid These were determined by methods described previously.

Renal Parameters In the last week of the study the rats were placed in metabolic cages for the determination of renal excretion. The collection period was 3 h. The urine volume was determined gravimetrically and the urinary flow rate was expressed in microliters/minute. Urinary sodium and potassium were determined flame-photometrically (Instrumentation Laboratories, Hersel, Germany), and creatinine was determined spectrophotometrically with picric acid. The color produced during the reaction was measured at 492 nm. Urea was determined by an enzymatic UV test.

Statistical Analyses All results are given in the form of mean ± SEM. Intraindividual comparisons within the blood pressure measurements of the acute experiment and the long-term treatment were evaluated by analysis of variance for repeated measurements (ANOVA). To check differences for significance of hormonal and renal data, ANOVA was also performed. When an F test indicated significant differences, individual comparisons were made by the Student-Bonferroni test. A P < 0.05 was considered to be significant.

RESULTS

Study I Table 1 summarizes the results of acute oral doses of 10 and 30 mg of ecedotril/kg with respect to the renal parameters: after a collection period of 6 h in hypertensive TGR(mRen2:27) rats and in normotensive Sprague-Dawley controls. Ecedotril induced a dose-dependent increase in natriuresis, which reached statistical significance after 10 mg/kg, orally, in the TGR and after 30 mg/kg, orally, in the SDR. In contrast, there was little effect on the potassium excretion. The sodium/potassium ratio was accordingly significantly increased. A significant increase in diuresis was only observed after the administration of 30 mg/kg, orally, in the TGR. Ecedotril doubled the urinary cGMP excretion in the SDR and quadrupled the cGMP excretion in the TGR. The effects of ecedotril (30 mg/kg, orally) on plasma parameters 1 h after administration, are shown in Table 2. The baseline levels of ANP, BNP, cGMP, plasma aldosterone, and NEP activity are significantly higher in TGR than in SDR, whereas PRA is significantly lower in the TGR than in SDR. After ecedotril administration the NEP activity in plasma was distinctly reduced by about 75%. The ANP and cGMP plasma levels were consequently significantly elevated both in SDR and TGR, the effect being more pronounced in TGR than in SDR. Plasma BNP levels were also significantly increased in the TGR. A slight decrease in plasma aldosterone that did not reach statistical significance was observed. Hemococoncentration occurred in both groups.

Study II Figure 1 shows a dose-dependent effect of ecedotril on the systolic blood pressure in TGR. No significant change in systolic pressure was observed in the controls during the observation period of 6 h. After administration of 10 mg of ecedotril/kg, orally, a decrease in systolic pressure of 14 mm Hg was observed and after the administration of 30 mg/kg, orally, a decrease of 23 mm Hg was seen. The reduction of blood pressure by a dose of 30 mg/kg, orally, was maximal 2 h after administration and was still nearly maximal after 6 h.

Study III In the 13 week study the systolic blood pressure in the TGR controls increased from 213 ± 5 to 255
TABLE 1. EFFECTS OF SINGLE DOSES OF ECADOTRIL (10 AND 30 mg/kg, ORALLY) ON THE URINARY EXCRETION OF SODIUM, POTASSIUM, VOLUME, cGMP, AND ON THE SODIUM/POTASSIUM RATIO IN SPRAGUE-DAWLEY RATS AND TGR(m(Ren2)27) RATS (N = 10 PER GROUP). COLLECTION PERIOD 6 h.

<table>
<thead>
<tr>
<th>Renal Parameters</th>
<th>SDR</th>
<th>TGR(m(Ren2)27)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
<td>Ecadotril 10 mg/kg</td>
</tr>
<tr>
<td>Natriuresis (µmol/kg/h)</td>
<td>51.9 ± 8.3</td>
<td>69.4 ± 14.2</td>
</tr>
<tr>
<td>Kaliuresis (µmol/kg/h)</td>
<td>74.4 ± 7.9</td>
<td>78.6 ± 5.5</td>
</tr>
<tr>
<td>Diuresis (mL/kg/h)</td>
<td>2.0 ± 0.1</td>
<td>2.0 ± 0.1</td>
</tr>
<tr>
<td>cGMP excretion (nmol/kg/h)</td>
<td>0.9 ± 0.4</td>
<td>0.9 ± 1.4**</td>
</tr>
<tr>
<td>Sodium/potassium ratio</td>
<td>0.7 ± 0.1</td>
<td>0.9 ± 0.1</td>
</tr>
</tbody>
</table>

The values are means ± SEM. †P < .05, ‡P < .01, §P < .005, ††P < .001 compared with the values in untreated controls. **P < .05 compared with the value in Sprague-Dawley rats.

± 7 mm Hg (n = 11), whereas in animals treated with ecdotril (30 mg/kg, orally twice daily) it increased only from 213 ± 5 to 227 ± 6 mm Hg (n = 10, P < .01). Body weight was lower in controls than in ecdotril treated TGR at the end of the study (255 ± 3.2 vs 270 ± 4.4 g, P < .05). The increases in heart weight and in kidney weight relative to body weight (BW) were also delayed in ecdotril treated transgenic rats (393 ± 10.9 vs 36 ± 15.2 mg/100 g BW, P < .01 and 728 ± 12.0 vs 696 ± 6.7 mg/100 g BW, P < .05, respectively).

Table 3 summarizes the basic plasma parameters in the controls and in ecdotril-treated rats at the end of the study. In TGR(m(Ren2)27) rats, the plasma ANP levels were not significantly elevated in the ecdotril-treated group, while the plasma cGMP levels in this group were significantly increased. Renin activity, urea, uric acid, and creatinine were significantly lower in the plasma of ecdotril-treated TGR than in the plasma of untreated TGR.

The urinary excretions of volume, sodium, potassium, cGMP, and creatinine and also the creatinine clearance, established in the last week of the study, are shown in Table 4. The natriuresis and the urinary excretion of cGMP were significantly higher in the ecdotril group than in the vehicle-treated controls (Table 4). Ecdotril caused a slight but statistically significant improvement of plasma urea and plasma creatinine. In addition, creatinine clearance tended to be higher in ecdotril-treated animals.

DISCUSSION

Transgenic rats carrying an additional mouse renin gene (TGR(m(Ren2)27)) provide a new model of hypertension with a well-defined genetic background. TGR are characterized by high transcription rates of the transgene in the adrenal glands, high plasma aldosterone concentrations, suppression of renal renin production, and low plasma renin activity. The finding of exaggerated natriuresis and diuresis both in hypertensive patients with low plasma renin activity but high aldosterone concentrations 18 and in TGR13 suggest a clinical relevance for this new hypertension model. We have demonstrated that ANP, BNP, and cGMP plasma levels are elevated in the TGR in comparison with normotensive controls (Sprague-Dawley rats).

TABLE 2. EFFECTS OF ECADOTRIL (30 mg/kg, ORALLY) ON ATRIAL NATRIURETIC PEPTIDE (ANP), BRAIN NATRIURETIC PEPTIDE (BNP), CYCLIC GMP, RENIN ACTIVITY (PRA), ALDOSTERONE, HEMATOCRIT, AND NEUTRAL ENDOPEPTIDASE (NEP) ACTIVITY IN PLASMA IN SPRAGUE-DAWLEY RATS (SDR) AND TGR(m(Ren2)27) RATS 1 h AFTER ADMINISTRATION

<table>
<thead>
<tr>
<th>Plasma Parameters</th>
<th>SDR</th>
<th>TGR(m(Ren2)27)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
<td>Ecdotril</td>
</tr>
<tr>
<td>ANP (pg/mL)</td>
<td>38.9 ± 4.3 (14)</td>
<td>58.7 ± 4.9 (15†)</td>
</tr>
<tr>
<td>BNP (pg/mL)</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>cGMP (pmol/mL)</td>
<td>5.0 ± 0.3 (15)</td>
<td>7.6 ± 0.7 (15†)</td>
</tr>
<tr>
<td>PRA (ng/mL/h)</td>
<td>7.5 ± 1.0 (15)</td>
<td>10.0 ± 1.8 (14)</td>
</tr>
<tr>
<td>Aldosterone (pg/mL)</td>
<td>164 ± 47 (15)</td>
<td>118 ± 72 (14)</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>42.6 ± 0.6 (15)</td>
<td>44.7 ± 0.8 (15)†*</td>
</tr>
<tr>
<td>NEP activity (pmol/mL/min)</td>
<td>24 ± 2 (11)</td>
<td>6 ± 1 (11)‡‡</td>
</tr>
</tbody>
</table>

The values are means ± SEM (n). †P < .05, ‡P < .005, §P < .001 compared with the values in untreated controls. **P < .05 and ††P < .001 compared with the values in Sprague-Dawley controls. n.d. = not detectable, below the detection limit of 10 pg/mL.
The purpose of the present study was to evaluate the cardiovascular consequences of neutral endopeptidase inhibition by determining the acute and the long-term effects of ecadotril on hemodynamic, hormonal, renal, and structural parameters in TCX and SDR.

### TABLE 3. EFFECTS OF LONG-TERM TREATMENT (13 WEEKS) WITH ECADOTRIL (30 mg/kg ORALLY TWICE DAILY) ON ATRIAL NATRIURETIC PEPTIDES (ANP), RENIN ACTIVITY (PRA), cGMP, UREA, URIC ACID, AND CREATININE IN PLASMA AND ON THE HEMATOCRIT IN TGR(m(Ren227) RATS 1 h AFTER ADMINISTRATION

<table>
<thead>
<tr>
<th>Plasma Parameters</th>
<th>Controls</th>
<th>Ecadotril</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANP (pg/mL)</td>
<td>220 ± 16.2 (11)</td>
<td>242 ± 39 (10)</td>
</tr>
<tr>
<td>cGMP (pmol/mL)</td>
<td>38.2 ± 3.1 (11)</td>
<td>55.9 ± 5.0 (10)*</td>
</tr>
<tr>
<td>PRA (ng/mL/h)</td>
<td>12.6 ± 1.0 (11)</td>
<td>9.3 ± 1.1 (10)*</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>7.7 ± 0.4 (11)</td>
<td>6.2 ± 0.3 (10)**</td>
</tr>
<tr>
<td>Uric acid (μmol/L)</td>
<td>65.9 ± 5.8 (11)</td>
<td>56.6 ± 3.6 (10)**</td>
</tr>
<tr>
<td>Creatinine (μmol/L)</td>
<td>56.4 ± 1.6 (11)</td>
<td>50.4 ± 0.9 (10)**</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>47 ± 0.6 (11)</td>
<td>47 ± 0.7 (10)</td>
</tr>
</tbody>
</table>

The values are means ± SEM (n). *P < .05, **P < .01, compared with the values in untreated controls.

Oral administration of a single dose of ecadotril significantly increased the plasma levels of ANP and cGMP both in hypertensive TGR and in normotensive SDR, but the increase was more pronounced in the former. In view of the greater increase in the ANP and cGMP in plasma, it seems likely that high baseline plasma ANP levels are an essential prerequisite for the efficacy of NEP inhibitors. Moreover, acute administration of various NEP inhibitors has been shown to increase plasma ANP in various animal models even when the latter is already elevated, eg, in NaCl-sensitive spontaneously hypertensive rats and in DOCA/salt hypertensive rats, whereas NEP inhibitors failed to elevate plasma ANP in intact normotensive rats and dogs. Furthermore, Koepke et al and Seymour et al have reported that NEP inhibitors like thiorphan and SQ 28,603 given intravenously were ineffective in elevating plasma ANP in spontaneously hypertensive rats (SHR) and in normotensive Wistar-Kyoto rats.

The present study shows that the plasma levels of brain natriuretic peptide are markedly increased in heterozygous transgenic hypertensive rats compared with normotensive SDR. Despite the longer half-life of brain natriuretic peptide, the plasma levels of BNP were lower than the plasma levels of ANP. It is interesting to note that BNP can be degraded by neutral endopeptidase. Following this idea, Hirata et al have reported that the NEP inhibitor candesartan elevates plasma BNP to a greater extent in DOCA-salt rats.
hypertensive rats than in control rats. In addition, Lang et al.2 have found that the same NEP inhibitor increased the plasma concentration of BNP in patients with heart failure. In the present work, we found that ecadotril increased not only the plasma levels of ANP but also the plasma levels of BNP in TGR. The plasma BNP levels stayed below the detection limit in ecadotril treated SDR.

In agreement with published data, the plasma renin activity in TGR was significantly lower than the plasma renin activity in SDR, and conversely, plasma aldosterone was significantly higher in TGR than in SDR. In this acute study, ecadotril caused a slight increase in the plasma renin activity, which reached statistical significance in the TGR, probably as a result of the acute excretion of sodium. On the other hand, ANP-mediated natriuresis should be accompanied by a reduction in plasma renin activity. At least in clinical studies, the plasma renin activity remained unchanged after ecadotril in healthy normotensive subjects and in patients with chronic renal failure and decreased slightly in patients with congestive heart failure. The reason for this difference in the acute effect of ecadotril is still unclear. A slight but significant hemoconcentration was observed both in TGR and in SDR after administration of ecadotril, consistent with the idea that ANP itself promotes extravasation and increases the hematocrit in animals and in humans.

In our acute study, we found exaggerated natriuresis, kaliuresis, diuresis, and cGMP excretion in the TGR. Ecadotril significantly elevated the natriuresis, cGMP excretion, and the sodium/potassium ratio in SDR, and in addition produced a dose-dependent increase in kaliuresis and diuresis in TGR. This response to ecadotril is more pronounced in transgenic rats than in the corresponding normotensive controls, suggesting that NEP inhibitors are more active under conditions of elevated plasma ANP levels. Similar results have been obtained after infusion of S-thiorphan, the active metabolite of ecadotril, into anaesthetized dogs. The effects of S-thiorphan on natriuresis, diuresis, and urinary ANP are more pronounced under the conditions of elevated plasma ANP levels induced by blocking the ANP clearance receptors than under normal conditions. In healthy volunteers, ecadotril inhibited 90% of the NEP activity and increased ANP plasma and urinary cGMP by 70% and 100%, respectively. Ecadotril also increased the urinary sodium output by 50%, decreased fractional distal reabsorption, and increased the glomerular filtration rate. Also, in patients with chronic heart failure, natriuresis, but not kaliuresis, increased after a single dose of ecadotril.

In the present study, the neutral endopeptidase activity in plasma was significantly higher in transgenic rats than in the SDR, and a distinct inhibition was observed after ecadotril treatment in both animal models. Hirata et al. have reported that the urinary NEP activity was about seven times higher in DOCA/salt hypertensive rats than in control rats. The reason for the increased NEP activity in animal hypertension models is not yet fully understood. There is some evidence that cGMP is involved because it has been shown in in vitro experiments that cGMP elevates the activity of NEP in smooth vascular muscle cells. On the other hand, it could be that substrates of neutral endopeptidase, which are elevated under conditions of hypertension, such as ANP, BNP, and kinins, are responsible for the up-regulation of NEP. Taken together, these findings support the concept that NEP inhibitors have a stronger effect in animal models with elevated NEP activity. Ecadotril exhibits a dose-dependent and long-lasting antihypertensive activity in conscious TGR by up to 22 mm Hg after 120 min, but is devoid of any antihypertensive activity in normotensive controls (data not shown). The NEP inhibitor candoxatri significantly lowered blood pressure in hypertensive Dahl salt-sensitive rats with elevated plasma ANP levels, but did not have this effect in normotensive Sprague-Dawley rats. In DOCA/salt hypertensive rats, Seymour et al. and Hirata et al. have observed an effect on blood pressure, and they suggest that DOCA/salt hypertensive rats appear to be more sensitive to the depressor effects of NEP inhibitors than normotensive Sprague-Dawley rats. These results are similar to the earlier findings according to which NEP inhibitors, such as thiorphan, SCH 39370, and SQ 28.603, do not decrease blood pressure in normotensive rats and dogs. In addition, sustained low-dose infusions of ANP were natriuretic but were unable to lower the blood pressure significantly in normotensive subjects.

The cardiovascular consequences of NEP inhibition with ecadotril were evaluated by determining the long-term effects of the drug on hemodynamic, renal, hormonal, and structural parameters in the TGR. During the observation period of 13 weeks, these animals develop malignant hypertension. The plasma levels of ANP and BNP in TGR are elevated, and may reflect a compensatory response to maintain normal blood pressure and plasma volume. We surmised that ecadotril, by preventing degradation of natriuretic peptides, would potentiate the activity of the endogenous hormone and thereby prevent the development of hypertension and the associated cardiac hypertrophy. The rise in systolic blood pressure could be delayed significantly by ecadotril treatment. Although by the end of the study plasma ANP levels were not significantly elevated, the elevated plasma cGMP and urinary cGMP excretions, which are used as markers for the involvement of ANP, suggest an activation of the
ANP system was still achieved under chronic ecdadotril therapy.

Long-term treatment with ecdadotril in young transgenic rats not only delayed the development of hypertension but also the associated cardiac and renal hypertrophy. There is some evidence that the reductions in cardiac and kidney mass are independent of the hemodynamic changes in these rats. It was previously reported that chronic treatment for 1 month with the NEP inhibitor's SCH 34826 and SCH 42495 in adult SHR with manifest hypertension reduced cardiac mass and the amount of fibrotic tissue present in the left ventricle, despite the lack of antihypertensive activity. It has been suggested that ANP acts as a physiologi-
cal antagonist of the renin-angiotensin system. The left ventricular myocytes and cells within the kidney contain detectable amounts of angiotensin II, which may act as a trophic growth promoting factor. Local interaction of angiotensin II by enhanced ANP activity in ventricles and in kidneys could therefore exert a protectoic and antihypertrophic influence on cardiac myocytes and on kidney cells. This interpretation does not rule out other unexplored mechanisms that may play a part in determining changes in cardiac hypertrophy. NEP hydrolyzes numerous substances, including brain natriuretic peptide, bradykinin, substance P, etc. The role of these peptides in ventricular and kidney hypertrophy remains to be elucidated.

At the end of the treatment, the renin activity and angiotensin I in the plasma compartment are distinctly higher in comparison with the young rats in the acute study, but significantly lower in ecdadotril-treated animals than in the controls. The delayed increase in plasma renin activity is probably a consequence of the activated ANP system under treatment with ecdadotril. On the other hand, it could reflect a slight kidney protective action of ecdadotril because plasma urea, uric acid, and creatinine are also slightly lower in ecdadotril-treated animals than in the controls. In the last week of the experiment, the sodium and cGMP excretions under ecdadotril were still elevated, whereas kaliuresis and diuresis were not significantly different between the controls and the ecdadotril group. In addition, creatinine clearance was increased under ecdadotril, although the difference did not reach the level of statistical significance.

In conclusion, our results demonstrate that TGR can be regarded as an animal model with an activated natriuretic peptide system. These rats show significantly higher ANF, BNP, cGMP, and NEP activities in plasma, as well as an exaggerated natriuresis and cGMP excretion in comparison with normotensive Sprague-Dawley rats serving as controls. TGR are, therefore, a suitable model for the investigation of NEP inhibitors, as exemplified by ecdadotril. The effect of ecdadotril is more pronounced in TGR than in SDR. The latter has an antihypertensive effect in TGR and an inhibitory effect on malignant hypertension and the associated cardiac hypertrophy, and it may offer the possibility of a rational and therapeutically effective approach to cardiovascular diseases.

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