Neutral Endopeptidase Inhibition Potentiates the Effects of Natriuretic Peptides in Renin Transgenic Rats

Max Wegner, Detlev Ganten*, and Johannes-Peter Stasch

The influence of neutral endopeptidase (NEP) inhibition with (S)-thiorphan on the hormonal, renal, and blood-pressure-lowering effects of an infusion of atrial (ANP), brain (BNP), and C-type natriuretic peptide (CNP) was evaluated in hypertensive transgenic rats (TGR) harboring an additional mouse renin gene (TGR(m(Ren2)27)). These TGR possess an activated natriuretic peptide system as compared with Sprague-Dawley rats (SDR), used in this study as control. (S)-Thiorphan significantly decreased blood pressure in anesthetized TGR but not in anesthetized SDR during the 60-min infusion period. Exogenously administered ANP decreased blood pressure in SDR with no significant effects in TGR after 60 min. In contrast, BNP infusion significantly decreased blood pressure in TGR, while changes in SDR were not significant. The blood pressure was further decreased after combined infusion of ANP and BNP with (S)-thiorphan in TGR. No effect on blood pressure was registered during infusion of CNP in either experimental group. The plasma levels of ANP, BNP, and cGMP were higher in TGR than in SDR, whereas plasma renin activity was lower. Co-administration of ANP, BNP, or CNP with the NEP inhibitor (S)-thiorphan potentiated the plasma ANP, BNP, and cGMP. Infusion of ANP alone did not affect BNP plasma levels of TGR and vice versa. In contrast, CNP infusion increased ANP plasma levels in both TGR and SDR. Renal excretion of sodium and cGMP increased after infusion of (S)-thiorphan and ANP or BNP in both TGR and SDR. The combination of ANP and (S)-thiorphan had a slightly greater effect on urinary excretion of sodium and cyclic GMP in TGR than either compound alone, but the effects were more pronounced in SDR than in TGR. Finally, infusion of CNP alone and in combination with (S)-thiorphan influenced the excretion of sodium and cyclic GMP only slightly. These results indicate that inhibition of neutral endopeptidase by (S)-thiorphan potentiates the hemodynamic and renal effects of natriuretic peptides ANP and BNP, and to some extent those of CNP, in hypertensive TGR and normotensive SDR. In contrast to ANP and BNP, infusion of CNP had no effect on the blood pressure in anesthetized TGR or SDR. Inhibition of NEP therefore seems to be a promising way to potentiate endogenous levels of natriuretic peptides, which may be of therapeutic benefit in cardiovascular diseases such as hypertension or heart failure. (Hypertens Res 1996; 19: 229–238)

Key Words: neutral endopeptidase 24.11, (S)-thiorphan, ecadotril, TGR(m(Ren2)27), ANP, BNP, CNP

The natriuretic peptides are a family of related polypeptide hormones including atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP). These natriuretic peptides have a common structural motif consisting of a loop of 17 amino acids formed by intramolecular disulfide bonds. Only 6 of the 17 amino acids in this ring differ among the three peptides isolated from the pig, whereas the N- and C-terminals vary both in amino acid composition and length (1, 2).

Neutral metalloendopeptidase (EC 3.4.24.11; NEP) is a zinc-containing membrane-bound enzyme widely distributed in the organism. It has high activity in the brush border membranes of proximal renal tubules. NEP is the primary metabolizing enzyme for ANP, which is secreted into the circulation mainly by the cardiac atria in response to an increase in atrial pressure or atrial stretch (3–5). Cleavage at the Cys105-Phe106 and Ser123-Phe124 bonds of ANP by NEP destroys the essential ring structure and results in biological inactivation of ANP (4, 6). This membrane peptidase seems to be largely responsible for the inactivation of not only endogenous and exogenous ANP but also of many other peptides, including BNP and CNP (7).

BNP is a polypeptide comprising 32 amino acids homologous with ANP, originally isolated from porcine brain. It exerts ANP-like biological activity by binding to the same ANP-A receptors (8, 9). In

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contrast to ANP, cardiac hormone BNP is secreted predominantly from the ventricles of a hypertrophic heart, and it is used as a marker for various stages of heart failure in humans (10). Mukoyama et al. (11) have shown a higher percentage increase in plasma BNP than in plasma ANP in patients with CHF (11) and immediately after myocardial infarction (12). They also found a relationship between the plasma levels of BNP and both the degree of heart failure and that of left-ventricular dysfunction.

CNP, the third member of the natriuretic peptide family, was recently identified in porcine brain (1). CNP has been demonstrated to cause only mild diuresis and natriuresis but to elicit stronger stimulation of cGMP synthesis and stronger growth inhibition of vascular smooth muscle cells than ANP and BNP (1, 13).

Neutral endopeptidase inhibitors prevent ANP (and BNP) from degradation, increase the half-life of ANP, and promote ANP-mediated actions, such as diuresis, natriuresis, and vasodilation (14-18). In addition, Lang et al. (17) have reported that the plasma BNP concentration increased in patients with heart failure after acute administration of an NEP inhibitor.

In the present study we examined the influence of NEP on the clearance of ANP, BNP, and CNP in hypertensive rats carrying an additional mouse renin gene (TGR(m(Ren2)27)) and in normotensive Sprague-Dawley rats (SDR). In comparison with SDR, the TGR(m(Ren2)27) have an activated ANP and BNP system, and are therefore an interesting model for the investigation of substances interacting with the natriuretic peptides system (19, 20). In addition, fulminant hypertension develops in TGR(m(Ren2)27) after the age of 5 weeks, with systolic pressure in excess of 250 mmHg. Despite the additional renin gene, the plasma levels of renin are lower than those in normotensive Sprague-Dawley rats. Here we report the results obtained after infusing the NEP inhibitor (S)-thiorphan and the peptides ANP, BNP, and CNP, alone or in combination with the NEP inhibitor, into anesthetized TGR and SDR and evaluate the repercussions on blood pressure and on hormonal and renal variables.

Materials and Methods

Substances
(S)-Thiorphan ((S)-3-mercapto-2-benzylpropanoylglycine) was synthesized by hydrolysis of ecdotril (trivial name, sinorphan). Ecdotril was received from Bioprojet, France, and the peptides ANP, BNP, and CNP from Sigma (Deisenhofen, Germany).

Animals
In vivo experiments
The experiments were performed using TG(m(Ren2)27) rats (Zentralinstitut Bayer AG Wuppertal, Germany) and Sprague-Dawley rats (Möllegard, Denmark) as control. Body weights ranged from 300 to 370 g. On the evening before the study the rats were deprived of food but not of drinking water. On the day of the experiment the rats were anesthetized with thiopental “Lentia”® (Hormonchemie, Munich, Germany) 100 mg/kg i.p. A tracheotomy was performed, and catheters were inserted into the femoral artery for blood pressure measurement (Gould P23 ID pressure transducer and Gould 2008s recorder) and the femoral vein for test drug administration. Urine was collected through a catheter inserted into the bladder through a suprapubic incision. At the completion of the operation the rats received a starting injection of 5 ml physiological saline/kg followed by a continuous infusion of the same solution at 100 µl/kg/min i.v. The rectal temperature of the rats was kept at 37.5 ± 1 °C with heat lamps. The rats were allowed an equilibration period of 1 h under these conditions before starting urine collection in tared vials. The atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP) have been demonstrated to be released in patients with heart failure after acute administration of an NEP inhibitor. ANP in plasma: The plasma ANP levels were measured after extraction in C₁₅ᵬ-cartridges (Bond Elut®, Varian, Harbor City, USA), using a specific and sensitive radio-immunoassay kit (Anawa, Biotrend, Germany).

BNP in plasma: BNP was similarly extracted from plasma using C₁₅ᵬ-cartridges. After lyophilization, BNP was reconstituted in assay buffer, and the concentration of BNP-like immunoreactivity was detected with a commercially available radioimmunoassay kit (Biotrend, Cologne, Germany) that shows no cross-reactivity to ANP.

cGMP in plasma and urine: For the determination of cGMP in plasma, 300 µl of plasma was added to an equal volume of 10% trichloroacetic acid. After an incubation period of 30 min, the probes were centrifuged (10 min, 5,000 rpm, 4 °C), and the supernatant was washed with four portions of water-saturated ether and lyophilized. cGMP was then determined with the use of a commercially available radioimmunoassay kit (IBL, Hamburg, Germany). The concentration of cyclic GMP in urine was determined after dilution with ice-cold sodium acetate buffer (0.05 M; pH 6.2).

Plasma renin activity (PRA): For the determination of PRA, edetated rat plasma was incubated with phenylmethylsulfonyl fluoride, and the accumulated angiotensin I was measured with a commercial radioimmunoassay kit (Sorin Biomeda, Saluggia, Italy).

Electrolyte excretion: The urine volume was determined gravimetrically, and urinary flow was expressed in ml/kg/h. Electrolyte concentrations were measured by flame photometry (Autocal Flammenphotometer 743, Instrumentation Laboratory, Hersel, Germany). The excretion rates of sodium and potassium were calculated and expressed in
Table 1. Effects of (S)-thiorphan (100 μg/kg/min i.v.) and of the Peptides ANP, BNP, and CNP (100 ng/kg/min i.v.) Alone or in Combination on Plasma Levels of ANP, BNP, and cGMP in Hypertensive TG(m(Ren2)27) Rats and in Normotensive Sprague-Dawley Rats

<table>
<thead>
<tr>
<th>Substance and dose</th>
<th>ANP (pg/ml)</th>
<th>BNP (pg/ml)</th>
<th>cGMP (pmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>248 ± 97 (6)</td>
<td>43 ± 6 (6)*</td>
<td>8.6 ± 0.7 (6)*</td>
</tr>
<tr>
<td>(S)-Thiorphan (100 μg/kg/min i.v.)</td>
<td>296 ± 109 (6)</td>
<td>41 ± 6 (6)</td>
<td>12.5 ± 1.1 (6)*</td>
</tr>
<tr>
<td>ANP (100 ng/kg/min i.v.)</td>
<td>433 ± 16 (6)*</td>
<td>44 ± 5 (6)</td>
<td>16.4 ± 1.2 (6)‡</td>
</tr>
<tr>
<td>ANP+STH</td>
<td>1133 ± 189 (6)***</td>
<td>57 ± 7 (6)</td>
<td>62.0 ± 2.9 (6)§</td>
</tr>
<tr>
<td>BNP (100 ng/kg/min i.v.)</td>
<td>273 ± 100 (6)</td>
<td>139 ± 39 (7)**</td>
<td>22.6 ± 4.7 (7)*</td>
</tr>
<tr>
<td>BNP+STH</td>
<td>411 ± 113 (5)</td>
<td>472 ± 102 (6)***</td>
<td>70.4 ± 10.9 (6)§</td>
</tr>
<tr>
<td>CNP (100 ng/kg/min i.v.)</td>
<td>339 ± 52 (5)</td>
<td>—</td>
<td>21.0 ± 1.9 (6)§</td>
</tr>
<tr>
<td>CNP+STH</td>
<td>354 ± 47 (6)</td>
<td>—</td>
<td>34.0 ± 5.0 (6)§</td>
</tr>
</tbody>
</table>

Sprague-Dawley rats

<table>
<thead>
<tr>
<th>Substance and dose</th>
<th>ANP (pg/ml)</th>
<th>BNP (pg/ml)</th>
<th>cGMP (pmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>78 ± 6 (7)</td>
<td>10 ± 2 (5)</td>
<td>4.0 ± 0.2 (7)</td>
</tr>
<tr>
<td>(S)-Thiorphan (100 μg/kg/min i.v.)</td>
<td>104 ± 13 (6)</td>
<td>13 ± 3 (5)</td>
<td>6.6 ± 0.6 (6)***</td>
</tr>
<tr>
<td>ANP (100 ng/kg/min i.v.)</td>
<td>107 ± 9 (4)*</td>
<td>15 ± 3 (4)</td>
<td>17.0 ± 3.0 (4)***</td>
</tr>
<tr>
<td>ANP+STH</td>
<td>198 ± 30 (4)***</td>
<td>34 ± 4 (3)***</td>
<td>33.0 ± 3.4 (4)§</td>
</tr>
<tr>
<td>BNP (100 ng/kg/min i.v.)</td>
<td>76 ± 5 (7)</td>
<td>24 ± 5 (5)*</td>
<td>10.0 ± 3.8 (7)</td>
</tr>
<tr>
<td>BNP+STH</td>
<td>141 ± 18 (6)**</td>
<td>185 ± 25 (5)§</td>
<td>67.8 ± 8.1 (6)§</td>
</tr>
<tr>
<td>CNP (100 ng/kg/min i.v.)</td>
<td>163 ± 37 (5)**</td>
<td>—</td>
<td>8.2 ± 0.9 (6)§</td>
</tr>
<tr>
<td>CNP+STH</td>
<td>168 ± 34 (6)**</td>
<td>—</td>
<td>32.5 ± 4.9 (6)§</td>
</tr>
</tbody>
</table>

*p < 0.05, **p < 0.01, ***p < 0.005, ‡p < 0.001 compared with values in untreated controls. *p < 0.05 compared with values in Sprague-Dawley controls.

μmol/kg/h.

Statistical analyses: All results are expressed as mean ± SEM. Intraindividual comparisons of blood pressure measurements and renal data in the acute experiment were evaluated by analysis of variance for repeated measurements (ANOVA). To check the significance of differences in hormonal data, ANOVA was also performed. When F-test indicated significant differences, individual comparisons were made by the Student-Bonferoni-test. A p value less than 0.05 was considered to indicate statistical significance.

Results

Hormonal Variables

Table 1 shows the plasma variables after infusion of the NEP inhibitor (S)-thiorphan and infusions of ANP, BNP, CNP, alone or in combination, into TG(m(Ren2)27) rats (TGR) and Sprague-Dawley rats (SDR). The basal plasma levels of ANP (248 ± 97 vs. 78 ± 6 pg/ml, p < 0.05), BNP (43 ± 6 vs. 10 ± 2 pg/ml, p < 0.01), and cyclic GMP (8.6 ± 0.7 vs. 4.0 ± 0.2 pmol/ml, p < 0.005) were significantly higher in control TGR than in the corresponding control SDR, while plasma renin activity (PRA) was significantly lower in the hypertensive TGR than in SDR (6.4 ± 3.2 vs. 18.7 ± 3.6 ng/ml/h, p < 0.05).

In anesthetized TGR, after infusion of the NEP inhibitor (S)-thiorphan no significant increase in plasma ANP or plasma BNP was observed after the 60-min infusion period. However, plasma cGMP, a specific marker for the involvement of natriuretic peptides, was significantly increased after (S)-thiorphan in the transgenic rats. After infusion of ANP the plasma cGMP rose slightly, while plasma BNP remained unaffected. After infusion of BNP increases in plasma BNP and cGMP, without any influence on plasma ANP, were observed in TGR at the end of the study. CNP slightly increased the plasma ANP and cGMP levels in TGR (Table 1).

Combined infusion of (S)-thiorphan and ANP increased plasma ANP 5-fold and plasma cGMP 7-fold, but had no effect on plasma BNP. Combined
infusion of (S)-thiorphan and BNP led to a 10-fold increase in plasma BNP and an 8-fold increase in plasma cGMP, with only a slight rise in plasma ANP. Combined infusion of (S)-thiorphan and CNP tended to increase the plasma ANP levels and led to a 4-fold increase in plasma cGMP (Table 1).

In anesthetized Sprague-Dawley rats, infusion of (S)-thiorphan increased plasma ANP only slightly, whereas cGMP was significantly increased. Exogenously administered ANP led to a 4-fold increase in plasma cGMP, while plasma BNP was unchanged. Exogenously administered BNP increased the cGMP levels in normotensive Sprague-Dawley rats, but had no effect on plasma ANP. After infusion of CNP the plasma ANP and cGMP levels significantly increased. Infusions of ANP or BNP combined with (S)-thiorphan had a greater effect on plasma ANP, BNP, and cGMP than infusions of each compound alone. In addition, the combination of (S)-thiorphan and CNP doubled plasma ANP and significantly increased plasma cGMP (Table 1).

**Effect on Blood Pressure**

The basal values of mean arterial pressure were significantly higher in TGR than in the corresponding SDR controls (146.8 ± 3.2 vs. 114.0 ± 2.9 mmHg, p<0.005). In these hypertensive TGR, infusion of the NEP inhibitor (S)-thiorphan significantly decreased the mean arterial pressure after 40 min of infusion. Infusion of ANP only slightly decreased mean arterial pressure at the end of the infusion period, whereas the effect observed with BNP was statistically significant. No significant blood pressure reduction was seen during infusion of CNP. Combination of (S)-thiorphan with ANP and with BNP produced a stronger blood-pressure-lowering effect than administration of the compounds alone in TGR. No effect was observed after combined infusion of (S)-thiorphan and CNP (Fig. 1).

In normotensive Sprague-Dawley rats, no blood-pressure-lowering response was observed after infusion of (S)-thiorphan. Infusions of ANP and BNP lowered blood pressure, and their combination with (S)-thiorphan resulted in only minor additional reductions in blood pressure. CNP did not decrease blood pressure, either alone or in combination with (S)-thiorphan, in SDR (Fig. 2).

**Effect on Renal Parameters**

(S)-Thiorphan significantly increased the urinary excretion of sodium and cGMP both in hypertensive TGR and in normotensive SDR. Comparable renal effects were observed after the infusion of ANP or BNP alone in both TGR and SDR (Figs. 3–6). Combined infusion of (S)-thiorphan with ANP or with BNP produced greater effects on urinary excretion of sodium and cGMP than the infusion of each compound alone, the effects being more pronounced in the SDR than in TGR. Infusion of CNP influenced the excretion of sodium and cGMP only slightly. However, after infusion of a combination of CNP and (S)-thiorphan an increase in cGMP was observed.

**Discussion**

The results of the present study yield a number of insights into the roles played by neutral endopeptidase in the clearance of various natriuretic peptides in hypertensive renin TGR (TG(m(Ren2)27)) carrying an additional mouse renin gene and having an activated system of natriuretic peptides, and in normotensive control SDR. The NEP inhibitor (S)-thiorphan and the natriuretic peptides ANP, BNP, and CNP were infused alone or in combination, and the consequences on the animals’ blood pressure and on hormonal and renal variables were evaluated in TGR for the first time.

We demonstrated in this study that in anesthetized TGR not only the plasma levels of ANP but also those of brain natriuretic peptide (BNP) were higher than those in the SDR. Infusion of (S)-thiorphan had no effect on plasma ANP or BNP at the end of the infusion period. In contrast, cGMP, the second messenger of natriuretic peptides, was signi-
significantly increased after infusion of (S)-thiorphan. Combined infusion of (S)-thiorphan with ANP or BNP led to a superadditive effect on plasma ANP and BNP, in parallel with a significant increase in second messenger cGMP in plasma both in hypertensive TGR and in SDR. In addition, infusion of CNP alone or in combination with (S)-thiorphan slightly elevated plasma ANP and significantly increased plasma cGMP in both TGR and SDR.

In an earlier study (20) we had demonstrated that in conscious hypertensive transgenic rats with an extra renin gene (TGrn(Ren2)27)) a single administration of the NEP inhibitor ecedotril, the orally active prodrug of (S)-thiorphan, significantly increased plasma ANP levels (11). In rats with experimentally induced congestive heart failure (aortovenocaval fistula rats) acute treatment with ecedotril increased the plasma levels of endogenous ANP without having any effect in their sham-operated controls (21). In addition, acute administration of various NEP inhibitors has been shown to increase plasma ANP in various animal models of hypertension when the ANP was already elevated, e.g., in NaCl-sensitive spontaneously hypertensive rats (22) and in DOCA/salt hypertensive rats (23), whereas NEP inhibitors failed to modify plasma ANP in intact normotensive rats (3), dogs (24), and humans (25). In hypertensive patients, NEP inhibition raised ANP levels (26) and a small decrease in blood pressure was observed (15, 27, 28).

In our study BNP was as potent as ANP with respect to the increase in plasma cGMP and the renal excretion. According to Kohno et al. (29), the plasma BNP concentrations were significantly higher in hypertensive patients than in borderline hypertensive patients or in normotensive controls, and this was most pronounced in the presence of left-ventricular hypertrophy. These reports are very similar to our findings, because plasma BNP was significantly higher in TGR than in normotensive SDR. Our results show that NEP inhibitors can potentiate
the effects of BNP infusion (30, 31), suggesting that, similar to ANP, BNP is degraded by endopeptidase 24.11 (32, 33). In addition, NEP inhibition with oral ecadotril in conscious TGR increased not only ANP but also BNP in the plasma (20), and NEP inhibition with candoxatril increased plasma BNP in patients with chronic heart failure (17). Therefore, an increase in the plasma BNP concentration may play a substantial role in the function of NEP inhibitors, and this may partly explain why NEP inhibitors-induced natriuresis is not completely suppressed by ANP antiserum (34). The reason why we did not observe an increase in BNP in our studies is not yet fully understood.

The BNP plasma levels did not change during infusion of ANP. This is understandable, because when ANP clearance receptors are occupied by exogenous ANP, the endogenous BNP levels do not change (17). The authors suggested that ANP clearance receptors are of little or no importance in the clearance of BNP, at least in patients with chronic heart failure. In this context, in-vitro studies have demonstrated that the binding affinity of human BNP for human ANP clearance receptors is only 7% of the binding affinity of human ANP (11, 35).

In the present study, infusion of CNP increased plasma ANP and plasma cGMP in both hypertensive TGR and normotensive SDR, with a supplementary increase when combined with the NEP inhibitor (S)-thiorphan. Koller et al. (36) have shown that CNP activates the ANP-B receptor selectively, and that this receptor is also linked to guanylate cyclase. The distribution of ANP-B receptors varies among tissues and among species. Interestingly enough, in hamster and rat glomeruli no cGMP response was seen with CNP (37, 38), and no ANP-B receptors have been found in the kidneys of monkeys or rats (39, 40). Furuya et al. (38) have reported that CNP potently stimulates cGMP production in cultured rat vascular smooth muscle...
In the present work we observed a significant blood-pressure-lowering effect of (S)-thiorphan infusion in hypertensive anesthetized TGR, but not in normotensive SDR. This is in accord with the anti-hypertensive activity of ecadotril in TGR, which has no such activity in SDR (20). The increased NEP activity in TGR is evidently responsible for the anti-hypertensive effect after infusion of (S)-thiorphan, because NEP activity was increased in the hypertensive TGR as compared with that in normotensive SDR (20). For DOCA/salt hypertensive rats too it has been reported that their urinary NEP activity was 7 times higher than that in corresponding controls (18). In addition, the NEP activity in the urine and plasma of rats with congestive heart failure (AVF) was significantly higher than that in sham-operated rats (21). The reason for the increased NEP activity in animals with hypertension or heart failure is not yet fully understood, but there is some evidence that cGMP elevates NEP activity in vascular smooth muscle cells (45). On the other hand, it could be that substrates of neutral endopeptidase which are elevated under conditions of hypertension and heart failure, such as ANP and BNP, are responsible for the upregulation of NEP.

Infusion of ANP and BNP decreased blood pressure in both animal models to more or less the same extent. Combination of the NEP inhibitor (S)-thiorphan with ANP or with BNP exerted a supplemental blood-pressure-lowering effect only in the hypertensive TGR. In addition, we observed no effect on blood pressure in these anesthetized rats either after CNP or after a combined infusion of CNP with (S)-thiorphan. Our findings do not agree with the results of Stingo et al. (46), who reported for CNP a significant decrease in blood pressure associated with a significant fall in renal sodium excretion in mongrel dogs.

It has been suggested that CNP circulates in low picomolar concentrations. It has also been found that CNP has a much stronger effect than ANP in lowering blood pressure in anesthetized dogs, whereas ANP is more potent in increasing plasma cGMP and the urinary excretion of sodium and cGMP (46). The reason why CNP did not decrease the blood pressure in hypertensive or normotensive anesthetized rats in this study is so far unclear, but a possible explanation could be the absence of ANP-B receptors in rat kidney (39, 40) as mentioned above.

In the present study we observed increased renal excretion of sodium and cGMP after infusing the NEP inhibitor (S)-thiorphan, ANP, or BNP. These two peptides, and to a still greater extent the i.v. combinations with (S)-thiorphan, increased urinary excretion of cGMP and sodium in both TGR and SDR, the percentage increase being greater in the normotensive SDR. In contrast, infusion of CNP influenced the excretion of sodium and cGMP only slightly. Renal excretion of sodium was unaffected by exogenous CNP alone or in combination with (S)-thiorphan both in the TGR and in SDR. In agreement with our findings, early studies of the biological activity of CNP in rats showed it to be natriuretic, diuretic, and a vasodepressor when injected intravenously, but the potency of CNP was

![Graph showing effects of natriuretic peptides on sodium excretion](image-url)
about 1% of the related hormones ANP and BNP (38). CNP, given to anesthetized dogs had greater hemodynamic activity than ANP, but it was not natriuretic even when administered directly into the renal artery (46, 48). The observation of no change in sodium excretion is unique to this peptide when compared with ANP or BNP, which in previous studies produced marked natriuresis despite similar systemic hemodynamic changes (49). This is consistent with the report of Currie et al. (49), who suggested that the renal hemodynamic and natriuretic actions of ANP are affected by the COOH-terminal part of the peptide, which is absent in CNP. In contrast, in the presence of NEP inhibition, CNP has a natriuretic action in dogs, which is due to inhibition of distal sodium reabsorption without increasing urinary cGMP excretion (50). The reason for our failure to observe an increase in the renal excretion of sodium after infusions of CNP into anesthetized normotensive or hypertensive rats in the present study may be the absence of ANP-B receptors in rat kidneys (39, 40), as already mentioned.

In contrast to CNP, ANP and BNP have been reported to produce natriuresis, diuresis, and depressor activity in anesthetized normotensive (51) and hypertensive (52–54) rats. Seymour et al. (30) have also reported that homologous BNP produced significantly greater renal activity than ANP in conscious SDR. In addition, previous investigations (52–54) have shown that NEP inhibition potentiates the reduction in mean arterial pressure stimulated by exogenous ANP. In our study the strong renal response to the BNP infusion observed in hypertensive as well as in normotensive rats was comparable to the effects documented after infusion ANP. Interestingly enough, the percentage increases after peptide infusion, especially after the combination, were greater in the normotensive SDR than in TGR. In this context, it was demonstrated by Stasch et al. (20) that the renal responses to endoCP are more pronounced in hypertensive TGR than in corresponding normotensive Sprague-Dawley rats, although those studies were done in conscious animals. It is still not clear why our results differ from these previous findings. One explanation might be that the basal excretion values in Sprague-Dawley rats are lower than in the hypertensive TGR, so that any renal changes lead to a greater percentage increase in these variables. Moreover, it is difficult to compare conscious with anesthetized rats, where many factors might be involved, e.g., an activated renin-angiotensin-aldosterone-system (R-AAS). Furthermore, many peptides such as bradykinin or substance P might be involved in the renal responses to NEP inhibition but, on the other hand, it is clear that the natriuretic peptides ANP and BNP act as prime factors in initiating the response of these drugs. In the first place the renal effects are accompanied by significant rises in cGMP excretion, and secondly, the effect of NEP inhibition in rats can be markedly attenuated by pretreatment with a monoclonal antibody directed against ANP (56). We therefore suggest, on the basis of our study, that especially increased endogenous ANP and BNP, and to a minor extent CNP, are involved in the acute renal effects of these peptides both in hypertensive TGR and in normotensive SDR.

It is well known that the plasma renin activity is lower in conscious TGR than in conscious SDR despite the additional renin gene (19, 20). Although anesthesia activates the renin-angiotensin-aldosterone system, this difference in PRA between TGR and SDR is still evident in our studies.

In conclusion, in hypertensive renin TGR (TGR(m(Ren)27)) the plasma levels of ANP, BNP, and cGMP are significantly higher than in corresponding SDR, whereas PRA is lower. In this study NEP inhibition potentiated the renal and hemodynamic effects of exogenously administered ANP and BNP, but only slightly increased those of CNP, both in hypertensive TGR and in normotensive SDR. Enhancement of plasma levels of natriuretic peptides by the NEP inhibitor (S)-thiorphan therefore opens up interesting possibilities for the treatment of cardiovascular diseases such as hypertension and heart failure.

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References


