Vasopeptidase Inhibition Restores Renovascular Endothelial Dysfunction in Salt-Induced Hypertension

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Abstract. Renovascular hemodynamics plays a pivotal role in the regulation of BP. The effect of the vasopeptidase inhibitor omapatrilat (O) and the ACE-inhibitor captopril (C) on endothelial function in the renal circulation in salt-induced hypertension were investigated. Dahl salt-sensitive rats (n = 6 per group) on standard or salt-enriched chow were treated for 8 wk with O (36 ± 4 mg/kg per d), C (94 ± 2 mg/kg per d), or placebo. Renal arteries were suspended in organ chambers for isometric tension recording. Vascular hypertrophy was assessed by determination of standardized heart weight and aortic weight, and morphologic analysis of glomerular injury was performed. Systolic BP of salt-fed, placebo-treated animals increased to 196 ± 6 mmHg, which was reduced by O (162 ± 5 mmHg; P < 0.05) and C (164 ± 7 mmHg; P < 0.05) to a comparable degree. In salt-induced hypertension, endothelium-dependent relaxations in renal arteries (56 ± 6 versus 100 ± 6%; P < 0.05) as well as contractions to endothelin-1 (ET-1) (98 ± 5% versus 128 ± 5%; P < 0.05) and big ET-1 (47 ± 6% versus 116 ± 7%; P < 0.05) were markedly reduced as compared with control animals, whereas standardized aortic weight and heart weight (4.9 ± 0.4 versus 3.2 ± 0.3 g/kg; P < 0.05) increased. Treatment with O restored endothelium-dependent relaxations (88 ± 6%; P < 0.05 versus C) and contractions to ET-1 (120 ± 6%) and big ET-1 (98 ± 9%), O prevented vascular hypertrophy (0.23 ± 0.019 mg/mm² versus 0.31 ± 0.018 mg/mm² in high-salt diet; P < 0.05), but, in contrast to C, it only had a modest effect on glomerular injury. In conclusion, O restored renovascular endothelial function and prevented vascular hypertrophy in salt-induced hypertension and therefore may advance as a beneficial approach in the therapy of various forms of hypertension.

Inhibition of the angiotensin-converting enzyme (ACE) is a well-established treatment option for patients with hypertension and improves morbidity and mortality in large clinical studies (1, 2). The mechanisms involved in the vasculoprotecive effects of ACE inhibitors appear—in large part—to be related to their effects on endothelial function. Indeed, in human coronary arteries and saphenous veins, endothelium-dependent relaxations to bradykinin are enhanced after preincubation with an ACE inhibitor (3, 4).

Vasopeptidase inhibition represents a new therapeutic principle in hypertension (5–7) and heart failure (8), which includes inhibition of neutral endopeptidase (NEP) in addition to ACE inhibition. NEP catalyzes the degradation of a number of endogenous vasodilator peptides, including atrial natriuretic peptide, brain natriuretic peptide, C-type natriuretic peptide, substance P, and bradykinin, as well as vasoconstrictor peptides, including endothelin-1 (ET-1) (9) and angiotensin II (10). Hence the overall effect of NEP inhibition on vascular tone will depend on the effects of a compound on the procession of these different vasoactive substances and is—especially in molecules, which inhibit other systems as well—difficult to predict. Nevertheless, omapatrilat (O), a new vasopeptidase inhibitor, effectively lowers BP in salt-dependent and volume-dependent as well as in renin-dependent forms of hypertension (11). The combination of ACE and NEP inhibition may be particularly useful in the treatment of hypertension (5, 12–14) and heart failure (15–19).

Vasopeptidase inhibitors lower BP in a broader range of conditions than inhibition of ACE or NEP alone, and their effectiveness seems to be independent of the activity of the renin-angiotensin system or the degree of salt retention (20). O is a new vasopeptidase inhibitor that induces long-lasting antihypertensive effects in experimental hypertension (12), greater than those elicited by selective inhibition of either enzyme alone (11). Furthermore, O lowers BP and attenuates cardiac hypertrophy in diabetic hypertensive rats (21). Meanwhile, first clinical data are available, demonstrating hemodynamic benefits of treatment with O in patients with hypertension (14, 22–24) and heart failure (8, 25–27). The first large-scale clinical study on heart failure indicates reduced morbidity and mortality on treatment with O as compared with ACE inhibitor treatment (28).
Despite obvious clinical benefit of vasopeptidase inhibitors in heart failure and hypertension, their mechanism of action is still poorly understood. A positive influence of O on vessel stiffness (19) and vascular remodeling (29, 30) has been shown before. Also, long-term vasopeptidase inhibition exerts beneficial effects in the renal circulation (26). Influences of vasopeptidase inhibitors on endothelial function in the renal artery may substantially improve renal hemodynamics and therefore contribute to their beneficial systemic effects.

Therefore, this study was designed to investigate the effects of long-term treatment with the vasopeptidase inhibitor O on renovascular endothelial function as well as its effects on vascular hypertrophy in a model of salt-induced hypertension.

### Materials and Methods

#### Animals

Male Dahl salt-sensitive rats 12 wk of age were obtained from Charles River WIGA GmbH (Sulzfeld, Germany) and randomly assigned to one of four treatment regimens: (1) standard chow (control); (2) salt-enriched (4% NaCl) chow (Harlan Teklad, Madison, WI), which was given alone (salt diet); (3) together with O (salt + O); or (4) with captopril (C) (salt + C). O and C were provided by Bristol-Myers Squibb Pharmaceutical Research Institute (Princeton, NJ). The rats were treated for 8 wk, and chow and drug intakes were monitored throughout the entire study. Systolic arterial BP and heart rate (HR) were measured by the tail-cuff method with a pulse transducer (model LE 5000; Letica, Barcelona, Spain) (31). The study design and the experimental protocols were approved by the institutional animal care committee (Kommission für Tierversuche des Kantons Zürich, Switzerland).

#### Tissue Harvesting

Animals were anesthetized with pentobarbital (50 mg/kg intraperitoneally) after 8 wk treatment, and blood samples were collected through puncture of the right ventricle. The renal arteries were removed, dissected free from adherent connective tissue, and placed immediately into cold (4°C) modified Krebs-Ringer bicarbonate solution: 118.6 mmol/L NaCl, 4.7 mmol/L KCl, 2.5 mmol/L CaCl₂, 1.2 mmol/L MgSO₄, 1.2 mmol/L KH₂PO₄, 25.1 mmol/L NaHCO₃, 0.026 mmol/L ethylenediaminetetraacetic acid, and 10.1 mmol/L glucose. Under a microscope (Leica Wild M3C, Heerbrugg, Switzerland), vessels were cleaned of adherent tissue and cut into 3-mm-long rings.

#### Organ Chamber Experiments

Renal artery rings were suspended to fine tungsten stirrups (diameter, 50 μm), placed in an organ bath filled with 25 ml Krebs solution, and connected to force transducers (UTC 2, Gould Statham, CA) for isometric tension recording as described (32). After an equilibration period of 60 min, renal artery rings were progressively stretched to their optimal passive tension (2.0 ± 0.2 g) as assessed by the response to 100 mmol/L KCl in modified Krebs solution (33). Rings were preconstricted with norepinephrine (approximately 70% of 100 mmol/L KCl) and relaxations to acetylcholine (ACH; 10⁻¹⁰ to 10⁻⁵ mol/L) or sodium nitroprusside (SNP; 10⁻¹¹ to 10⁻⁵ mol/L) were obtained. Relaxations to ACH were assessed with and without preincubation of indomethacin (30 min, 10⁻⁷ mol/L) and in the presence or absence of the nitric oxide synthase (NOS) inhibitor NG-nitro-L-arginine methyl ester (L-NAME) (preincubation for 30 min, 3 × 10⁻⁴ mol/L). In additional experiments, cumulative-concentration-response curves to ET-1 (10⁻¹⁰ to 10⁻⁷ mol/L) and big ET-1 (10⁻⁹ to 10⁻⁷ mol/L) were obtained in quiescent preparations. To avoid the development of tachyphylaxis, only single concentrations of angiotensin I and angiotensin II were used (10⁻⁷ mol/L). All drugs used in the organ bath were obtained from Sigma Chemical Co (Buchs, Switzerland) apart from ET-1 and big ET-1, which were purchased from Novabiochem/Calbiochem AG (La Jolla, CA).

#### Vascular and Cardiac Hypertrophy

For assessment of vascular hypertrophy, aortic rings were blotted dry and weighed, and the arterial surface area of opened rings was measured as described (33). Aortic surface area was calculated using formulas for diameter and radius of a cylinder for each individual ring, and values were averaged. After exsanguination of the animals, hearts were removed, isolated, and snap-frozen in liquid nitrogen. Wet weight of hearts was measured, standardized for body weight, and reported as mg heart weight/kg body weight.

#### Morphologic Analysis of Glomerular Injury

Renal injury was assessed as described previously (34). Briefly, paraffin-embedded sections of whole kidneys (5 to 7 μm) stained with periodic acid-Schiff reagent were viewed by light microscopy at a magnification of X40 using a Zeiss microscope (Carl Zeiss GmbH, Jena, Germany). One hundred glomeruli per slide were evaluated. Morphologic evaluation of glomerular injury was performed using semi-quantitative scoring methods. Lesions were graded by glomerulosclerosis (grade 1 to 4: 1 to 25%, 26 to 50%, 51 to 76%, and 76 to 100% sclerosis, respectively). The glomerular injury score was calculated by summarizing the products of severity grade times the percentage of glomeruli displaying the same degree of severity.

#### Calculations and Statistical Analyses

Relaxations to agonists in isolated arteries are reported as percent preconstriction in rings precontracted with norepinephrine to about 70% of contraction induced by KCl (100 mmol/L). The contractions were expressed as a percentage of 100 mmol/L KCl–induced contractions, which were obtained at the beginning of each experiment. Results are presented as mean ± SEM. Functional endothelin-converting-enzyme (ECE) activity was calculated as the ratio of the contraction to big ET-1 (10⁻⁷ mol/L) divided by the contraction to ET-1 (10⁻⁷ mol/L). In all experiments, n equals the number of rats per experiment. For statistical analyses, the sensitivity of the vessels to the drugs was expressed as the negative logarithm of the concentration that caused half-maximal relaxation or contraction (pD₂). Maximal relaxation (expressed as a percentage of precontraction) or contraction was determined for each individual concentration-response curve by nonlinear regression analysis with MatLab software (Math Works Inc., Natick, MA). For comparison between two values, the unpaired t test or the nonparametric Mann-Whitney test was used when appropriate. For multiple comparisons, results were analyzed by ANOVA followed by Bonferroni’s correction (35). Pearson correlation coefficients were calculated by linear regression. Significance was defined as P < 0.05.

#### Results

### Characteristics of Animals

Systolic BP increased after chronic administration of a high-salt diet (4% NaCl) in salt-sensitive Dahl rats as compared with rats on a standard chow at days 14, 28, and 56 after introduction of the diet (Table 1). Treatment with either O or C prevented the salt-induced BP increase (P < 0.05 versus rats
on high-salt diet alone). O, at a mean daily dose of 36.2 mg/kg, was equipotent in lowering BP as 94.1 mg/kg of C.

Changes in heart rate during treatment and differences in heart rate among the treatment groups did not reach statistical significance.

**Vascular Relaxations**

In hypertensive animals, maximal endothelium-dependent relaxations to ACH and sensitivity (pD2 value) in renal arteries were markedly impaired when compared with control rats (Figure 1A; \( P < 0.05 \)). Both O and C improved endothelium-dependent relaxations, but the maximal relaxation achieved by O was significantly higher than by C (Figure 1A; \( P < 0.05 \) versus C) and was comparable to the control animals. Preincubation with the NOS inhibitor L-NAME blunted relaxations to ACH in all groups completely (Figure 2).

In contrast to endothelium-dependent relaxations, maximal endothelium-independent relaxations to the NO donor SNP (Figure 1B) in renal arteries were comparable in all groups. Preincubation with indomethacin (10^-7 mol/L) did not alter maximal relaxations or sensitivity (pD2 value) to either ACH or SNP.

**Vascular Contractions**

Contractions of renal arteries to ET-1 were reduced in Dahl rats on a high-salt diet (Figure 3A; \( P < 0.05 \)) and were normalized by long-term administration of O or C, respectively (\( P < 0.05 \) versus placebo-treated, salt-fed Dahl rats for maximal response; Figure 3A). In addition, renal artery contractions to big ET-1 were markedly reduced in salt-sensitive hypertension (Figure 3B; \( P < 0.05 \)). Treatment with O but not with C (\( P < 0.05 \) for maximal contractions versus O) normalized contractions to big ET-1 (Figure 3B). Therefore, functional ECE activity, expressed as the ratio of the contraction to 10^-7 mol/L big ET-1 divided by the contraction to 10^-7 mol/L ET-1, was significantly lowered in salt-sensitive hypertension (Figure 4; \( P < 0.05 \) versus controls). ECE activity was normalized by O (Figure 3; \( P < 0.05 \) versus placebo treatment) but was not significantly affected by C. In addition, ECE activity was blunted by incubation with O (10^-7 mol/L) in vitro (data not shown).

The effectiveness of ACE inhibition, as assessed by determination of functional ACE activity, did not differ between O and C (0.28 ± 0.04 versus 0.33 ± 0.06, respectively; NS). Also, ACE activity, as determined by the ratio of the contraction to angiotensin I (10^-7 mol/L) divided by the contraction to angiotensin II (10^-7 mol/L), was significantly reduced by either C or O compared with the control group (0.74 ± 0.08; \( P < 0.01 \)).

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**Table 1.** Systolic BP (mmHg) of salt-sensitive Dahl rats during 56 d of treatment with different regimens

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 0</th>
<th>Day 14</th>
<th>Day 28</th>
<th>Day 56</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>143 ± 5</td>
<td>146 ± 5</td>
<td>148 ± 6</td>
<td>148 ± 9</td>
</tr>
<tr>
<td>Salt diet (4%)</td>
<td>143 ± 4</td>
<td>177 ± 6b</td>
<td>197 ± 6b</td>
<td>196 ± 8b</td>
</tr>
<tr>
<td>Salt + omapatrilat</td>
<td>140 ± 7</td>
<td>151 ± 6c</td>
<td>156 ± 6c</td>
<td>162 ± 8c</td>
</tr>
<tr>
<td>Salt + captopril</td>
<td>144 ± 5</td>
<td>149 ± 5c</td>
<td>157 ± 6c</td>
<td>164 ± 7c</td>
</tr>
</tbody>
</table>

a Data are given as mean ± SEM of six rats in each group. Day 0 indicates BP before treatment.

b \( P < 0.01 \) versus control rats (ANOVA and Bonferroni’s correction).

c \( P < 0.05 \) versus rats on salt diet.
Vascular and Cardiac Hypertrophy

After 8 wk of salt feeding, standardized heart weight of salt-sensitive rats was significantly elevated (4.9 ± 0.4 g/kg versus 3.2 ± 0.3 g/kg in control rats; \( P < 0.05 \); Figure 5A), indicating cardiac hypertrophy. Increase in heart weight was prevented by \( \text{O} \) (3.7 ± 0.4 g/kg; \( P < 0.05 \) versus high-salt diet; Figure 5A) but not by \( \text{C} \) (4.3 ± 0.3 g/kg, NS). In parallel, aortic weight increased in salt-induced hypertension (0.31 ± 0.018 mg/mm² versus 0.22 ± 0.02 mg/mm² in control rats; \( P < 0.05 \), Figure 5B). Vascular hypertrophy was prevented by \( \text{O} \) (0.23 ± 0.019 mg/mm²; \( P < 0.05 \) versus high-salt diet; Figure 5B) but not by \( \text{C} \) (0.28 ± 0.02 mg/mm², NS). The difference in aortic hypertrophy between \( \text{C} \)-treated and \( \text{O} \)-treated animals reached statistical significance (\( P < 0.05 \)).

Morphologic Analysis of Glomerular Injury

High-salt diet induced marked glomerulosclerosis in salt-sensitive Dahl rats (glomerulosclerosis index, 22.0 ± 4.6 versus 9.4 ± 3.2 in control rats on standard chow). Both \( \text{O} \) and \( \text{C} \) reduced glomerular alterations (glomerulosclerosis index, 16.6 ± 2.6 and 9.6 ± 2.0, respectively), but only the reduction of glomerular damage by \( \text{C} \) reached statistical significance (\( P < 0.05 \) versus salt diet). Both compounds tended to lower the percentage of sclerotic glomeruli (Figure 6), but a significant reduction of affected glomeruli was only achieved by \( \text{C} \) in grade 1 and grade 4 sclerotic glomeruli (Figure 6; \( P < 0.05 \) versus salt diet).

Discussion

In this study, we demonstrated normalization of endothelial function in renal arteries of salt-sensitive Dahl rats by the vasopeptidase inhibitor \( \text{O} \). \( \text{O} \) not only improved endothelium-dependent relaxations and renovascular reactivity to ET-1, but it also restored renovascular ECE activity. Furthermore, \( \text{O} \) prevented cardiac and vascular hypertrophy but only tended to improve glomerular injury.

Salt-sensitive hypertension is associated with impaired endothelial function in the aorta (33, 36). Here we extend this observation to renal arteries in which we documented impaired endothelium-dependent relaxations toACH as well as reduced contractile responses to ET-1 and big ET-1. Long-term treatment with the ACE inhibitor \( \text{C} \) improved vascular responsiveness but did not restore endothelial function to a degree comparable to \( \text{O} \)—even though achieved BP and inhibition of functional and biochemical ACE activity in renal arteries was comparable in the two treatment groups (21). In vitro inhibitory constants of \( \text{O} \) against ACE and NEP are in the nanomolar range (11); therefore, sufficient inhibition of both enzymes can be assumed. Thus, with equipotent ACE inhibition, the different effects of the two drugs on renovascular endothelial function must be related to properties of \( \text{O} \) other than ACE inhibition.

Vasopeptidase inhibitors simultaneously block ACE and
NEP (10), therefore, the metabolism of several vasoactive peptides, such as angiotensin, natriuretic peptides, bradykinin, and ET-1, and their clearance is altered. In accordance with recent findings (33), plasma ET levels were elevated in placebo-treated animals with salt-induced hypertension (37), although functional ECE activity was decreased. Thus, clearance of ET must be reduced in this model. As pure NEP inhibitors cause vasoconstriction due to decreased breakdown of ET-1 (38), selective blockade of this enzyme may not be appropriate under these conditions. In contrast, the combined ACE and NEP inhibitor O normalized both plasma ET-1 levels (37) as well as functional ECE activity. It has to be remembered, however, that the predictive value of ET-1 plasma levels on local, and in particular renal, ET tissue levels is rather limited (39). However, these findings certainly reflect the complex influence of vasopeptidase inhibition on the endothelin system, including inhibition of ET-1 degradation (38) as well as inhibition of ET-1 generation from big ET-1 (40). In any case, lowering of plasma ET-1 (37) and elevation of ECE activity demonstrate normalization of this altered paracrine system by O in this model of hypertension, and this may be one constituent that contributes to renovascular protection of vasopeptidase inhibitors.

In this study, we only investigated large renal arteries. Whether or not these alterations also occur in renal arterioles is uncertain, particularly because the endothelin system exhibits tissue specificity. Distribution of endothelin isoforms and their receptors differs in the renal cortex and medulla (39). Also, alterations in vascular structure and function in salt-induced hypertension are heterogenous, depending on the size and vascular bed involved (41,42). As in large renal arteries, however, endothelium-dependent relaxations of renal resistance vessels are impaired in salt-sensitive Dahl rats (43). Correspondingly, improvement of vasorelaxation in large renal arteries may contribute to normalization of renal plasma flow and therefore may be beneficial for both renal function and lowering of BP.

As in the aorta of salt-sensitive Dahl rats (33), ACH-induced relaxations in renal arteries were blunted in the presence of the NOS inhibitor l-NAME and therefore are mediated by NO. In renal arteries of hypertensive salt-sensitive Dahl rats, endothelium-dependent relaxations to ACH, but not the response to

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**Figure 4.** Functional endothelin-converting-enzyme (ECE) activity (given as contraction to $10^{-7}$ mol/L big ET-1 divided by contraction to $10^{-7}$ mol/L ET-1) in renal artery rings of salt-sensitive Dahl rats after 8 wk of treatment with different regimens. Results are shown as mean ± SEM (n = 6 per group). * P < 0.01 versus control rats; ** P < 0.01 versus rats on salt diet.

**Figure 5.** Standardized heart weight (A) and standardized aortic weight (B) in salt-sensitive Dahl rats after 8 wk of treatment with different regimens. Results are shown as mean ± SEM (n = 6 per group). * P < 0.05 versus rats on salt diet. ** P < 0.05 versus rats on omapatrilat (O) treatment. C, captopril.
sclerosis, grade 2 denotes 26 to 50% sclerosis, grade 3 denotes 51 to 75% sclerosis, and grade 4 denotes 76 to 100% sclerosis.

SNP, were impaired; therefore, reduced bioavailability of NO must be involved, which is in accordance with recent findings of reduced endothelial NOS protein expression in this model (44). Therefore, restoration of NO bioavailability may contribute to the normalization of endothelium-dependent relaxations in both treatment groups, but it cannot account for the greater endothelial protection by O as compared with C. Hence, other properties of O, such as the reduced breakdown of natriuretic peptides, are most likely involved.

Besides functional changes, structural vascular and parenchymal alterations, such as cardiac and vascular hypertrophy (33), occur in salt-induced hypertension. O prevented vascular hypertrophy to a greater extent than C did. This is in line with the effect of O in resistance arteries of stroke-prone spontaneously hypertensive rats (30) and salt-sensitive Dahl rats (29).

Surprisingly, assessment of glomerular morphology revealed only moderate improvement of glomerulosclerosis by O but a significant reduction of the glomerulosclerosis index by C. The lack of renal tissue protection by O contrasts with the marked improvement of vascular remodelling. Although the morphologic assessment may be prone to greater variation than that of larger vessels, this finding requires further investigation of the mechanisms by which vasopeptidase inhibition influences glomerular scarring. Differential, tissue-specific effects of O reflect its complex interaction with local regulatory systems. Possibly the beneficial effect of O on ET plasma levels reflects its complex interaction with local regulatory systems.

In conclusion, this study demonstrates marked improvement of renovascular endothelial function by long-term treatment of salt-sensitive hypertension with equipotent dosages of O or C. In contrast to C, O completely restored renovascular NO-mediated relaxation and ECE activity and prevented vascular hypertrophy. Therefore, vasopeptidase inhibition may represent an interesting, new approach in the treatment of hypertension and renovascular disease. In addition to recently published data on vasopeptidase inhibition in hypertension (14, 22–24, 46) and heart failure (8, 26–28), a number of large clinical studies—in part already under way (25, 47)—will be necessary to further evaluate the future clinical role of vasopeptidase inhibitors in the treatment of cardiovascular and renovascular disease and to better understand their beneficial effects.

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### References


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**Figure 6.** Morphologic assessment of glomerular injury in salt-sensitive Dahl rats. Results are shown as mean ± SEM (n = 5 or 6 per group). *P < 0.05 versus salt diet. Grade 1 denotes 1 to 25% sclerosis, grade 2 denotes 26 to 50% sclerosis, grade 3 denotes 51 to 75% sclerosis, and grade 4 denotes 76 to 100% sclerosis.


