Chapter 8

Chronic ACE inhibitor therapy differently modulates mediators of endothelium-dependent dilation in small renal and mesenteric arteries. Effect of dietary sodium restriction

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Submitted
Abstract

Background
ACE inhibitor therapy has beneficial effects in a variety of renal and cardiovascular diseases, and this may partly arise from the actions of ACE inhibition on endothelial function. The therapeutic efficacy of ACE inhibition is improved by dietary sodium restriction, although the underlying mechanisms have not yet been clarified. Therefore, we aimed to study the impact of dietary sodium restriction on the effect of ACE inhibition on endothelial function, i.e. on endothelium-dependent dilation and the underlying dilative mediators.

Methods
Male healthy Wistar rats were treated for 3 weeks with the ACE inhibitor lisinopril (75mg/l), while receiving either control diet (CON, 2% NaCl,) or low sodium diet (LS, 0.05% NaCl), respectively, and were compared to non-treated rats (n=8-10 rats for each group). Small mesenteric and renal (interlobar) arteries were studied in a perfusion set-up for pressurized arteries for endothelium-dependent dilation to acetylcholine (ACh, \(10^{-8} - 10^{-4}\) M), and for the contribution of endothelium-derived mediators NO, prostaglandins (PGs), and endothelium-derived hyperpolarizing factor (EDHF), by using specific inhibitors of these pathways, i.e. L-NMMA (\(10^{-4}\) M), indomethacin (\(10^{-5}\) M), and charybdotoxin (\(10^{-7}\) M) + apamin (\(5\times10^{-7}\) M), respectively.

Results
We found that systolic blood pressure (140±4 mmHg and 143±6 mmHg in non-treated CON and LS rats, respectively) was significantly reduced by treatment with lisinopril, the effect being more pronounced in rats receiving LS (102±2 mmHg) as compared to CON diet (125±4 mmHg). In rats receiving CON diet lisinopril did not alter maximal dilation nor sensitivity to ACh in both artery types, but significantly altered the balance between the dilative mediators NO and EDHF, the NO/EDHF ratio being increased in mesenteric arteries (to 0.24) and decreased in renal arteries (to 0.23) as compared to non-treated rats (0.10 and 0.81 for mesenteric and renal arteries, respectively). In contrast, in rats receiving LS diet lisinopril significantly decreased ACh dilation (area under curve in arbitrary units) in mesenteric (-30%) as well as in renal arteries (-62%). This decrease was due to an impairment in EDHF in both artery types, and in renal arteries additionally due to the presence of contractile PGs.

Conclusion
We conclude that chronic ACE inhibitor therapy differently modulates mediators of endothelium-dependent dilation in small mesenteric and renal arteries. Whether the considerable impairment in renal endothelium-dependent dilation after ACE inhibitor therapy specifically under LS diet may be part of its renoprotective action needs to be explored in further studies.


**Introduction**

Originally developed as a vasodilator drug for treatment of hypertension, it has become clear that the beneficial effect of ACE inhibition goes well beyond blood pressure reduction *per se* \(^1\text{-}^4\). ACE inhibitor (ACEi) therapy has been found effective not only in treatment of hypertension, but also in a variety of renal and cardiovascular disorders not directly related to high blood pressure, as evidenced for example by reduced mortality in patients with chronic heart failure (CHF) after ACEi therapy. Such beneficial effects are believed to arise to an important extent from the actions of ACEi's on the vasculature, particularly on endothelial function. Indeed, improvement of endothelial dysfunction after ACEi therapy may explain – at least in part – reduction in peripheral vascular resistance and subsequent improvement of perfusion and exercise capacity in CHF \(^5\).

It has also become clear that the therapeutic efficacy of ACE inhibition is blunted by high dietary sodium intake, occurring irrespective of the underlying disorder. This counts for blood pressure lowering, renal hemodynamic response, and proteinuria \(^6\text{-}^8\). *Vice versa*, in patients not adequately responding to ACEi’s therapy-resistance may be overcome by a moderate dietary sodium restriction \(^9\). For that matter, induction of a negative sodium balance – using diuretics and/or dietary sodium restriction – is a generally employed strategy to improve the therapeutic benefit of ACEi therapy in human \(^7\) and experimental renal dysfunction and hypertension \(^10\). Yet, whether or not and to what extent this involves enhanced improvement in endothelial dysfunction under these pathophysiological conditions remains uncertain. In fact, very little is known about the impact of dietary sodium restriction on the effect of ACEi therapy on mediators of endothelium-dependent dilation in different vascular beds, neither in pathophysiological states nor under normal conditions.

The present study was initiated to assess the impact of a moderate dietary sodium restriction on the effect of chronic ACEi therapy on normal endothelial function in two distinct vascular beds. To this end, normal healthy Wistar rats were kept on control diet (CON) containing modestly elevated sodium or on a low sodium diet (LS) while receiving daily treatment with vehicle or lisinopril (LIS), before endothelium-dependent dilation was assessed in isolated vessel preparations of these rats. Small mesenteric arteries were studied given the importance of this artery type in the regulation of total peripheral resistance (i.e., increased resistance in CHF for example). Renal (interlobar) arteries were studied because of their importance in maintaining renal perfusion, and the central role of the kidney in circulating volume and blood pressure regulation; hence, blood pressure reduction is often used as an indicator for therapeutic efficacy of ACEi therapy \(^10\). Arteries were studied for morphological vessel characteristics, endothelium-dependent and –independent dilation to acetylcholine and sodium nitroprusside, respectively, and for the contribution of endothelial vasodilator mediators underlying acetylcholine-induced dilation.

**Methods**

**Rat Studies**

Male Wistar rats (250-300 g, Harlan, Zeist, The Netherlands) were housed under standard conditions at the animal facility of the University of Groningen and were studied in compliance with institutional and legisatory regulations. After an adaptation period of one week, rats were allocated to one of four experimental groups (n=8-10 per group) receiving
different treatments. The ACE inhibitor (ACEi) lisinopril (LIS, 75 mg/l) was given for a period of three weeks via tap water to rats either fed a rat chow (Hope Farms, Woerden, The Netherlands) containing modestly elevated sodium as a control (CON-LIS, 2.0% NaCl) or low sodium diet (LS-LIS, 0.05% NaCl), which were compared to rats treated with vehicle (CON and LS, respectively). Once per week rats were put in metabolic cages for collection of 24h urine samples and routine analyses of urinary sodium content. After prior training sessions to get accustomed with the experimental set-up, systolic blood pressure was determined in awake animals at the end of the treatment period by means of the tail-cuff method using an automated multi-channel system (Life Science, Woodland Hills, California); a mean of three subsequent recordings was taken as the final value.

At sacrifice after three weeks of treatment, rats were anesthetized with 1.5% isoflurane in N2O/O2 and blood samples were taken for determination of plasma ACE activity (i.e. hippuryl-his-leu cleavage method as previously described by Hirsch et al.)11. Intestines and kidneys were removed and put into cold Krebs solution. Third-order branches of the superior mesenteric artery and renal interlobar arteries of the (right) kidney were isolated from surrounding perivascular tissue in cold Krebs solution.

Vascular Studies

In Vitro Perfusion Setup for Small Arteries

Small renal (interlobar) arteries and small mesenteric arteries were transferred to an arteriograph system for pressurized arteries12 (Living System Instrumentation, Burlington, VT, USA). Artery segments were cannulated at both ends on glass micropipettes, secured, and the lumen of the vessel was filled with Krebs solution through the micropipettes. Intraluminal pressure was set to 70 mmHg and held constant (blind sac) by a pressure servo system (Living System Instrumentation, Burlington, VT, USA). The vessel chamber was continuously recirculated with warmed (37°C) and oxygenated (5% CO2 in O2) Krebs solution with a pH of 7.4. The vessel chamber was transferred to the stage of an inverted light microscope with a video camera attached to a viewing tube. The video dimension analyzer (Living System Instrumentation, Burlington, VT, USA) was used to analyze the signal obtained from the video image and to continuously register lumen diameter and wall thickness.

PE-induced Constriction and Endothelium-Dependent Dilation to ACh

Arteries were allowed to equilibrate for one hour in regular Krebs solution before being pre-constricted with phenylephrine (PE). Initially vessels were all stimulated with a fixed dose of PE (3x10^-7 mol/L) and the level of contraction was assessed. Thereafter - because this resulted in different contraction levels – the concentration of PE was slowly increased (varying from 3x10^-7 to 3x10^-6 mol/L) to finally obtain similar levels of preconstriction (diameter reduction by 40±2% in mesenteric arteries, and by 37±1% in renal arteries). Preconstricted vessels were then studied for endothelium-dependent relaxation by giving cumulative doses of acetylcholine (ACh; 10^-8 mol/L - 10^-4 mol/L) to the recirculating bath.

Contribution of PGs, NO and EDHF to ACh-induced Dilation

To determine the contribution of vasoactive prostaglandins (PGs), nitric oxide (NO), and endothelium-derived hyperpolarizing factor (EDHF) to endothelium-dependent ACh-induced dilation, the response to ACh was additionally studied as in the above but now in presence of various inhibitors added to the bath 20 minutes prior to addition of ACh. To this end, the cyclooxygenase (COX)-inhibitor indomethacin (10^-5 mol/L), given to the superfusion
ACE inhibition modulates endothelium-derived mediators

medium, was used to inhibit prostaglandin production. Nω-monomethyl-L-arginine (L-NMMA, 10^{-4} mol/L) - given to the superfusion medium in presence of indomethacin - was used to further inhibit NO production. A combination of charybdotoxin (chtx, 10^{-7} mol/L) and apamin (apa, 5x10^{-7} mol/L), applied into the lumen of the artery as well as to the superfusion medium in presence of indomethacin and L-NMMA, was used to inhibit EDHF^{13,14}. Note that the exact nature of EDHF has not yet been established, meaning that specific inhibitors are not yet available. Nevertheless, the inhibition of calcium-dependent potassium channels with the combination of chtx/apa has consistently been shown to inhibit the L-NMMA- and indomethacin-resistant relaxation and hyperpolarization believed to be mediated by EDHF^{14}. It is important to further mention that the way in which the endothelial mediators are determined may be critical as they may not be independent but may interact. In this context NO has been described to attenuate EDHF(-release)^{15,16}, and thus EDHF may be fully active only when NO is inhibited or decreased. Furthermore, NO itself may in part mediate its vasodilatory effect via opening of potassium channels and hyperpolarization (similar to the mechanism of EDHF)^{17}. Therefore, if potassium channel blockers are used alone to determine EDHF, not only EDHF-mediated relaxation but also a part of the NO-mediated relaxation may be measured at the same time. Because of that we determined the contribution of EDHF always in presence of NO inhibition.

**Endothelium-Independent Dilation**

In a limited number of the arteries (n=4 for each group) - after endothelial function measurements - additional concentration-response curves to sodium nitroprusside (SNP, 10^{-9} – 3x10^{-4} mol/L) were obtained in preconstricted arteries to account for dilative ability of arterial smooth muscle to NO.

**Solutions and Drugs**

Vessel segments were superfused with Krebs solution containing (in mM): 120.4 NaCl, 5.9 KCl, 2.5 CaCl₂, 1.2 MgSO₄, 25.0 NaHCO₃, 1.2 NaH₂PO₄, 11.5 glucose (Merck, Darmstadt, Germany). Acetylcholine, apamin, charybdotoxin, indomethacin, L-NMMA, phenylephrine and sodium nitroprusside, were obtained from Sigma-Aldrich Chemie B.V., The Netherlands. They were dissolved in de-ionized water and diluted with Krebs solution. Stock solution (10^{-2} mol/L) for indomethacin was prepared in 96% ethanol.

**Data Analysis**

Myogenic constriction was expressed as a percent constriction = 100 x [(D_{base} – D_{myo})/D_{base}], where D is the diameter before the development of myogenic tone (D_{base}) or the diameter after the development of myogenic tone (D_{myo}). Concentration-response curves to acetylcholine (ACh) and maximal relaxation (Emax) were expressed in percentage of preconstriction to phenylephrine (PE). The Area Under each individual Curve (AUC) was determined (Sigma Plot, Jandell Scientific) and expressed in arbitrary units. The AUC was used to present total (individual) ACh dilation, and for subsequent analysis of differences in ACh dilation with and without indomethacin, L-NMMA and chtx/apa to estimate the contribution of PG, NO and EDHF, respectively^{18}. Data are expressed as mean ± standard error of the mean (SEM). Group-comparison was performed using one-way ANOVA, or repeated measures ANOVA in case of full concentration-response curves to ACh and SNP, and when appropriate corrected for multiple comparison by Duncan's multiple range test. Statistical differences were determined using student’s paired or unpaired t-test, where appropriate. Significance was accepted at P<0.05.
Results

Rat Characteristics
In conjunction with their diets, urinary sodium excretion was significantly higher in control rats (CON) as compared to those fed chow containing low sodium (LS), and this was most pronounced in control rats treated with lisinopril (CON-LIS) (Table 1). There were no significant differences between untreated LS- and CON-rats regarding body weight (BW), systolic blood pressure (SBP) and plasma ACE activity. Treatment with LIS significantly inhibited plasma ACE activity to a similar extent in both sodium groups. BW was significantly reduced after treatment with LIS specifically in the LS-LIS, but not in the CON-LIS group. Although treatment with LIS reduced SBP in both sodium groups, this effect was more pronounced in LS-LIS compared to CON-LIS (approximately 30%- and 10%-reduction, respectively). These findings may be taken indicative for the enhanced therapeutic efficacy of ACEi therapy during dietary sodium restriction.

Table 1 Rat characteristics

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>CON-LIS</th>
<th>LS</th>
<th>LS-LIS</th>
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<tbody>
<tr>
<td>Urinary Sodium (mg/d)</td>
<td>3.3±0.3#</td>
<td>4.9±0.4*#</td>
<td>0.66±0.1</td>
<td>0.54±0.1</td>
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<tr>
<td>Body Weight (g)</td>
<td>398±10</td>
<td>398±11</td>
<td>398±12</td>
<td>312±5*</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mmHg)</td>
<td>140±4</td>
<td>125±4*#</td>
<td>143±6</td>
<td>102±2*</td>
</tr>
<tr>
<td>Plasma ACE-activity (His-Leu nM/ml/min)</td>
<td>75±7</td>
<td>30±2*</td>
<td>68±6</td>
<td>23±4*</td>
</tr>
</tbody>
</table>

Data are mean±SEM of n=8-10 observations in all cases. Rats received either control diet (2.0% NaCl) or low sodium diet (0.05% NaCl), and were treated with vehicle (CON and LS, respectively) or the ACE inhibitor lisinopril (75 mg/l drinking water, CON-LIS and LS-LIS, respectively). * indicates P<0.05 for treated versus non-treated rats in the same sodium group. # indicates P<0.05 for control diet versus low sodium diet in the same treatment group.

Note that the LS diet per se, as compared to the CON diet, had no significant effect on either of the parameters investigated in the following sections. Therefore, for reasons of clarity, we will not present data obtained from the LS-group in the following Tables and Figures.
Morphological and Functional Vessel Characteristics

*Mesenteric Arteries* - In mesenteric arteries at 70 mmHg lumen diameter and wall thickness did not significantly differ among the experimental groups (P=NS) (*Table 2*). LIS increased the development of myogenic tone as well PE induced tone, specifically in the LS-LIS group but not in the CON-LIS group (*Table 2, Figure 1A*). Interestingly, presence of indomethacin strongly reduced the increased PE induced tone in LS-LIS, indicating the marked involvement of contractile PGs (*Figure 1A*).

*Renal Arteries* – In renal arteries at 70 mmHg, LIS increased lumen diameter and decreased PE induced tone, the effect being similar in the CON-LIS and LS-LIS group, i.e. independent of sodium diet (*Table 2, Figure 1B*). Inhibition of vasoactive prostaglandins with indomethacin reduced PE induced tone in renal arteries in all groups (*Figure 1B*). Nevertheless, the effect of LIS on PE induced tone also persisted in presence of indomethacin, i.e was not mediated by vasoactive prostaglandins (*Figure 1B*).

*Table 2* Vessel characteristics

<table>
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<th>CON</th>
<th>CON-LIS</th>
<th>LS-LIS</th>
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<tr>
<td><strong>Mesenteric Arteries</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diameter (µm)</td>
<td>339±6</td>
<td>343±6</td>
<td>336±12</td>
</tr>
<tr>
<td>Wall thickness (µm)</td>
<td>41±2</td>
<td>43±3</td>
<td>36±2</td>
</tr>
<tr>
<td>Wall-to-lumen ratio</td>
<td>0.12±0.01</td>
<td>0.13±0.01</td>
<td>0.11±0.01</td>
</tr>
<tr>
<td>Myogenic tone (%)</td>
<td>0.2±0.2</td>
<td>0.5±0.3</td>
<td>24±1*</td>
</tr>
<tr>
<td>PE induced tone (%)</td>
<td>4±2</td>
<td>5±4</td>
<td>46±3*</td>
</tr>
<tr>
<td><strong>Renal Arteries</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diameter (µm)</td>
<td>296±8</td>
<td>331±12#</td>
<td>325±15#</td>
</tr>
<tr>
<td>Wall thickness (µm)</td>
<td>35±3</td>
<td>42±3</td>
<td>42±4</td>
</tr>
<tr>
<td>Wall-to-lumen ratio</td>
<td>0.12±0.01</td>
<td>0.13±0.01</td>
<td>0.13±0.02</td>
</tr>
<tr>
<td>Myogenic tone (%)</td>
<td>2±2</td>
<td>0.4±0.4</td>
<td>2±2</td>
</tr>
<tr>
<td>PE induced tone (%)</td>
<td>35±4</td>
<td>24±3#</td>
<td>26±4#</td>
</tr>
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</table>

Rats were treated with the ACE inhibitor lisinopril (75 mg/l drinking water) while receiving either control diet (2.0% NaCl, CON-LIS) or low sodium diet (0.05% NaCl, LS-LIS), and were compared to non-treated rats under control diet (CON). Data are mean±SEM of n=8-10 observations in all cases. Myogenic and PE (phenylephrine, 3x10^-7 mol/L)-induced tone are expressed as % constriction from baseline diameter. * indicates P<0.05 versus rats receiving control diet (CON and CON-LIS). # indicates P<0.05 versus non-treated rats (CON).
Figure 1 Constriction to a single dose of $3 \times 10^{-7}$ M phenylephrine (PE) in small mesenteric (A; top panel), and renal arteries (B; bottom panel) from normal rats chronically treated with lisinopril while on a control diet (CON-LIS, 2.0% NaCl), or on a low-sodium diet (LS-LIS, 0.05% NaCl), as compared to non-treated rats on control diet (CON). Constrictions were generally reduced in presence (+) of indomethacin (right-side) as compared to absence (-) of indomethacin (left side). Data are mean±SEM of n=8-10 observations in all cases. * indicates $P<0.05$ for LS-LIS versus CON-LIS and CON. # indicates $P<0.05$ versus CON.
ACh induced Dilation and the Contribution of PGs, NO and EDHF

Full concentration-response (CR-) curves to ACh and SNP for individual groups in absence of any inhibitor (i.e. total dilation) are given in Figure 2. Endothelium-independent dilation to SNP did not differ between the groups - neither for mesenteric nor for renal arteries – implying that potential alterations at the level of vascular smooth muscle cell reactivity may not account for possible group-differences in ACh induced dilation. Using the area under curve (AUC) to represent ACh induced dilation in absence and presence of various inhibitors, the absolute and relative contributions of PGs, NO and EDHF to total ACh induced dilation was calculated for individual groups, as presented in Table 3 and Figure 3.

Mesenteric Arteries – In general, pretreatment of mesenteric arteries with indomethacin modestly shifted the CR-curve to ACh to the right and/or decreased the maximal dilation (CR-curve not shown), implying the contribution of dilative PGs (approximately 12-16% to total dilation in CON rats). Additional treatment with L-NMMA further reduced ACh induced dilation (CR-curve not shown), indicating a significant – albeit small - contribution of NO (approximately 8-9% to total dilation in CON rats). ACh induced relaxation resistant to indomethacin plus L-NMMA was fully abolished after additional treatment with the combination of chtx/apa (CR-curve not shown), demonstrating the major contribution of EDHF (approximately 75-80% to total dilation in CON rats). Absolute and relative contributions of PGs, NO and EDHF to total ACh induced mesenteric artery dilation were not altered by differences in sodium-intake per se (i.e. untreated LS- vs CON-rats, data not shown). When combined with LIS, however, total dilation to ACh was significantly decreased specifically during LS-LIS, but not during CON-LIS (Figure 2A). As can be derived from Table 3, the decrease in total dilation to ACh in LS-LIS was due to a marked absolute decrease in contribution of EDHF, and despite an apparent compensatory (hence, though insufficient) absolute increase in dilative PGs, because ACh-induced dilation was significantly decreased in presence of indomethacin in this group (Figure 3A). Also of interest is that although LIS did not alter total mesenteric dilation to ACh during CON-LIS, the absolute contribution of NO was increased while that of EDHF was decreased, resulting in a higher NO-to-EDHF ratio, i.e. compared to the NO-to-EDHF ratio in CON-rats (Figure 4, Table 3).

Renal Arteries – In general, pretreatment of renal arteries with indomethacin was with negligible/minor effects on dilation to ACh (CR-curve not shown). Treatment with L-NMMA, however, markedly reduced ACh induced dilation (CR-curve not shown), indicating the important contribution of NO (approximately 40-50% to total dilation in CON rats). Relaxation to ACh persisting in presence of both indomethacin and L-NMMA was fully abolished after additional treatment with the combination of chtx/apa (CR-curve not shown), confirming that the remainder dilation was mediated by EDHF (approximately 60% to total dilation in CON rats). Absolute and relative contributions of PGs, NO and EDHF to total renal artery dilation to ACh were not altered by differences in sodium-intake per se (i.e. untreated LS- vs CON-rats, data not shown). When combined with LIS, however, total dilation to ACh was profoundly decreased specifically during LS-LIS, but not during CON-LIS (Figure 2, Table 3). To some extent, this seems to be due to the marked presence of contractile PGs because ACh induced dilation in LS-LIS was significantly enhanced in presence of indomethacin (Figure 3B). Nevertheless, also when corrected for contractile PGs, ACh induced dilation in LS-LIS was still significantly smaller compared to CON-rats (see Figure 3B), apparently due to absolute
decreases in contribution of EDHF as well as NO (Table 3). Despite these absolute decreases the NO-to-EDHF ratio was more or less maintained in LS-LIS (Figure 4). In contrast, in CON-LIS the NO-to-EDHF ratio was dramatically decreased - due to increased EDHF and decreased NO but without change in total dilation. Note that the NO-to-EDHF ration in this group was similar in the two different artery types (Figure 4).

**Table 3** Contribution of endothelial mediators to ACh induced dilation

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>CON-LIS</th>
<th>LS-LIS</th>
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<tbody>
<tr>
<td><strong>Mesenteric Arteries</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total dilation</td>
<td>302±35</td>
<td>309±11</td>
<td>210±7*#</td>
</tr>
<tr>
<td>PGs</td>
<td>35±17 (12%)</td>
<td>51±9 (16%)</td>
<td>93±3* (44%)##</td>
</tr>
<tr>
<td>NO</td>
<td>25±4 (8%)</td>
<td>50±8 (16%)#</td>
<td>21±2 (10%)*</td>
</tr>
<tr>
<td>EDHF</td>
<td>242±14 (80%)</td>
<td>208±7 (68%)#</td>
<td>96±4* (46%)##</td>
</tr>
<tr>
<td><strong>Renal Arteries</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total dilation</td>
<td>178±19</td>
<td>211±13</td>
<td>67±16*#</td>
</tr>
<tr>
<td>PGs</td>
<td>-14±13 (-8%)</td>
<td>2±11 (1%)</td>
<td>-54±11 (-80%)##</td>
</tr>
<tr>
<td>NO</td>
<td>86±12 (49%)</td>
<td>39±11 (19%)#</td>
<td>50±12 (74%)#</td>
</tr>
<tr>
<td>EDHF</td>
<td>106±15 (59%)</td>
<td>170±15 (80%)#</td>
<td>71±30 (106%)##</td>
</tr>
</tbody>
</table>

Rats were treated with the ACE inhibitor lisinopril (75 mg/l drinking water) while receiving either control diet (2.0% NaCl, CON-LIS) or low sodium diet (0.05% NaCl, LS-LIS), and were compared to non-treated rats under control diet (CON). Data are mean±SEM of n=8-10 observations in all cases. * indicates P<0.05 for LS-LIS versus CON-LIS. # indicates P<0.05 versus CON.
Figure 2 Full concentrations-response curves to acetylcholine (ACh, left-side) and sodium nitroprusside (SN, right-side) in small mesenteric arteries (A; top panel) and renal arteries (B; bottom panel) from normal rats chronically treated with lisinopril while on a control diet (CON-LIS, 2% NaCl) or a low-sodium diet (LS-LIS, 0.05% NaCl), as compared to non-treated rats on control diet (CON). Data are mean±SEM of n=8-10 observations in all cases, and * indicates P<0.001 (repeated measures ANOVA) versus the LS-LIS group.
Figure 3 ACh induced dilation represented as area under curve (AUC) in arbitrary units in the absence (left side) and presence (right side) of indomethacin in small mesenteric arteries (A: upper pannel), and renal arteries (B: lower pannel) from normal rats chronically treated with lisinopril while on a control diet (CON-LIS, 2.0% NaCl), or on a low-sodium diet (LS-LIS, 0.05% NaCl), as compared to non-treated rats on control diet (CON). Data are mean±SEM of n=8-10 observations in all cases. * indicates P<0.05 for LS-LIS versus CON-LIS and CON.
ACE inhibition modulates endothelium-derived mediators

Figure 4 Contribution of NO relative to EDHF in ACh induced dilation for normal rats chronically treated with lisinopril while on a control diet (CON-LIS, 2.0% NaCl), or on a low-sodium diet (LS-LIS, 0.05% NaCl), as compared to non-treated rats on control diet (CON).

Discussion

Given the generally enhanced therapeutic efficacy of ACE inhibition under conditions of endogenous RAS-activation, we set out to investigate the effect of ACE inhibitor (ACEi) therapy on (mediators of) endothelial function during dietary sodium restriction in normal rats. The main findings of the present study are that chronic ACE inhibition reduced endothelium-dependent dilation to ACh due to a marked decrease in EDHF contribution in small mesenteric arteries as well as small renal interlobar arteries specifically under conditions of low dietary sodium intake. In renal arteries the decreased dilative response additionally involved ACh induced production of constrictive PGs opposing dilation. Furthermore, ACEi treatment under conditions of mildly elevated sodium intake differentially altered the contribution of NO relative to EDHF in ACh induced dilation in both artery types the ratio being increased in mesenteric and decreased in renal arteries – without affecting total dilation to ACh.
Effect of dietary sodium restriction on rat characteristics and arterial function

*Per se* dietary sodium restriction did not affect rat characteristics, i.e. BW or SBP, in normal healthy Wistar rats in the present study, and this seems consistent with normal functioning of regulatory mechanisms of the renal-body fluid system for arterial pressure control. The lack of significant effects of dietary sodium restriction on morphological and functional vascular properties in small mesenteric resistance arteries and small renal interlobar arteries may be consistent with the data on arterial blood pressure showing no differences.

ACE inhibitor treatment in control rats

Many studies have reported on the effects of chronic ACEi treatment on endothelial function, but the majority of these studies were performed in settings of cardiovascular disorders. Impaired ACh induced dilation - reflecting endothelial dysfunction in the diseased state - may be commonly observed in these studies, as well as improvement of the dilator response after chronic ACEi treatment \(^{19-22}\). Several studies additionally aimed to identify endothelium-derived mediators underlying impaired relaxation (often focussed on NO), and to establish the chronic effect of ACEi’s on these mediators \(^{20,23,24}\). Nevertheless, data in this respect are still far from complete and interpretations often potentially compromised by the haemodynamic (arterial pressure lowering) actions of ACEi’s indirectly affecting endothelial function, particularly in disorders associated with hypertension.

Even less is known about the effect of chronic ACEi treatment on apparently normal endothelial function in un-diseased conditions. Studying normal Wistar rats kept on a regular (sodium) diet, Berkenboom *et al.* found maximal dilation to ACh in isolated aortic rings to be increased from 70% in untreated rats to 90% after chronic ACEi treatment (ramipril for 6 weeks), due to enhancement of NO availability. Moreover, treatment with hydralazine in that study caused a reduction in blood pressure comparable to that with ramipril but without affecting ACh induced dilation, suggesting that the effect was specific to the ACEi and not due to blood pressure reduction *per se* \(^{4}\). However, similarly employing young normotensive Wistar rats and applying comparable treatment duration in the present study, we found no effect of chronic treatment with lisinopril on maximal ACh induced dilation in small mesenteric arteries nor in small renal arteries. Then again, these small arteries already showed near 100% relaxation to ACh in the present study – i.e. unlike the aorta in the above study by Berkenboom *et al.* – implying that there may not be much to be gained by ACEi. For that matter, Atkinson *et al.* previously did find maximal relaxation to ACh to be increased in mesenteric arteries of normal WAG/Rij rats after long-term chronic ACEi treatment with perindopril. However, they also showed that untreated rats in their study developed a time-dependent decrease in maximal ACh induced dilation in mesenteric artery, meaning that long-term ACEi treatment in that study prevented aging-induced mesenteric endothelial dysfunction - hence, which is not comparable to the present study \(^{21,25}\).

Despite the lack of effect of chronic ACEi on maximal dilation to ACh, chronic treatment with lisinopril in the present study did significantly alter the relative contribution of underlying mediators involved. Particularly, the contribution of NO relative to EDHF (NO-to-EDHF ratio) in mesenteric arteries increased from 0.10 in untreated controls to 0.24 after lisinopril treatment. The tendency for increased NO (-availability) after ACEi has been suggested by various other investigators \(^{24}\), and could also account for the above mentioned absolute increase in ACh induced dilation after chronic ramipril treatment in the aorta in the study by Berkenboom *et al.* \(^{4}\) - further note that this would have increased the NO-to-EDHF ratio in the aorta albeit that EDHF was not determined in their study. Because of the alleged inverse relationship between NO and EDHF \(^{15,16,26-30}\), increased NO after chronic lisinopril in
mesenteric arteries already displaying maximal dilation may also account for the decreased contribution of EDHF in the present study. Interestingly, in renal arteries – in which NO contribution to ACh induced dilation is higher than in mesenteric arteries - the NO-to-EDHF ratio was decreased from 0.81 in untreated controls to 0.23 after lisinopril treatment. Thus dependent on the initial contribution of NO in a certain vessel, chronic ACEi either increased or decreased the NO-to-EDHF ratio to a converged ratio of approximately 0.20 – 0.25 without changing total dilation. Whether or not this converged NO-to-EDHF ratio after lisinopril in small arteries of two different vascular beds is coincidental or resulting from specific properties of ACEi (i.e. lisinopril) in relation to an inverse NO-EDHF relationship cannot be determined from this study. Nevertheless, our present data demonstrate differential effects of chronic ACEi on mediators of endothelium-dependent dilation in different vascular beds under normal conditions.

**Influence of sodium restriction on the effect of ACE inhibitor therapy**

Lisinopril significantly reduced the dilative ACh response specifically during low sodium (LS) conditions, both in mesenteric and renal arteries. As arteries were preconstricted to similar levels before ACh induced dilation was tested differences in preconstriction levels may not have contributed to the different dilative response in this group. Furthermore, endothelium-independent dilation to SNP was not altered in this group suggesting that endothelial alterations rather than general differences in smooth muscle dilative ability accounted for the impaired ACh induced dilation. This finding seems unexpected because ACEi therapy has rather consistently been reported to either improve endothelial function or perhaps not to exert any significant effect, although there is indeed one study performed in healthy Sprague-Dawley rats describing that chronic (8 week) captopril treatment decreased ACh induced dilation in skeletal muscle arterioles. The authors of this study concluded that this decrease in the dilative response may be due to structural remodeling of the arteriolar wall, as the response to other (endothelium-independent) dilators was also impaired. In the present study, however, it seems unlikely that the decrease in ACh induced dilation after ACEi treatment under LS was due to remodeling processes as wall thickness as well as wall-to-lumen ratio were not significantly different in this group in both artery types. To the surprise of the authors in another study by Barton et al., intact relaxation of renal arteries to ACh in salt-treated salt-resistant Dahl rats was reduced after chronic treatment with the ET\(_A\) receptor antagonist LU135252, but could be normalized by acute COX-inhibition with indomethacin. In the present study, indomethacin also partially restored ACh induced dilation but did not fully normalize the response in renal arteries of lisinopril treated rats during LS. In contrast, moreover, relaxation to ACh in mesenteric arteries of lisinopril treated rats during LS was decreased despite the enhanced contribution of dilative PGs. Hence, the effect of ACEi therapy under LS on endothelium-derived mediators seems to be differentially pronounced in the two artery types concerning vasoactive PGs, demonstrating an increase in constrictive PGs in renal, and an increase in dilative PGs in mesenteric arteries. In contrast, however, in both artery types the absolute EDHF contribution was significantly decreased under this condition.

The underlying mechanisms of the impairment in ACh induced dilation specifically under LS conditions cannot be derived from the present study. It seems very unlikely that *per se* lisinopril as a drug caused this inhibitory effect because the effect of lisinopril on endothelium-dependent dilation was seen only during LS and not in our control rats in the present study. Note, however, that lisinopril treatment under LS conditions was accompanied by a significantly more pronounced blood pressure reduction and decrease in body weight,
suggesting a general decrease in body fluid volume and the possible treat for under-filling of the renal-body fluid system for arterial pressure control. Therefore, to us a plausible explanation for the reduced response to ACh after lisinopril under LS may be that it is part of an adaptation-response of the vessels occurring secondary to the augmented effect of ACEi on the renal-body fluid system for maintaining arterial pressure. Also, the profound myogenic as well as PE induced tone development in mesenteric arteries after lisinopril during LS may act to increase peripheral vascular resistance. This may partly compensate blood pressure decrease under conditions of low body fluid volume / cardiac output, and may redistribute blood flow to essential organ, as is the case in CHF. In this respect, an important cooperative interplay between the COX pathway and the renin-angiotensin-system has been described to maintain blood pressure during sodium depletion, supported by the finding of substantial blood pressure fall in COX-deficient mice under LS conditions, and an even more pronounced (deleterious) blood pressure fall after additional ACEi treatment. In line with this, an important role of the COX pathway in this response may also be supported by our current findings showing that the increased PE induced tone in mesenteric arteries after lisinopril under LS was strongly attenuated by acute COX inhibition with indomethacin suggesting a role for constrictive PGs in mediating the increased PE induced constriction. Furthermore, as described above, in renal arteries constrictive PGs seem to (partly) account for the impaired ACh induced dilation in this condition. The important regulatory function of a balanced release of dilative and constrictive PGs for renal hemodynamics especially in disease states has already been described. However, we can only speculate on the physiological role of the decreased dilative response of renal arteries under LS-LIS, as this seems to be an unexpected finding. It may be considered in this respect that the LS condition is characterized by adaptational mechanisms to retain sodium and water via the RAS. In the case of RAS blockade, i.e. ACEi, however, other mechanisms may become necessary to overcome excessive water and sodium excretion. It may be hypothesized that, in contrast to the well-described "PG-dependent state", in which dilative PGs counteract Ang II mediated (constrictive) effects in the kidney, the LS-LIS condition could be a different form of "PG-dependent state", in which (in absence of Ang II) the production of constrictive PGs may act to attenuate dilation in renal arteries may be to prevent excretion of (limited) sodium and water under this (extreme) condition.

In conclusion, we found that ACEi treatment under conditions of mildly elevated sodium intake differentially altered the contribution of NO relative to EDHF in ACh induced dilation in both artery types - the ratio being increased in mesenteric and decreased in renal arteries – without affecting total dilation to ACh. Furthermore, under conditions of low dietary sodium intake, chronic ACEi reduced endothelium-dependent dilation to ACh due to a marked decrease in EDHF contribution in small mesenteric arteries as well as small renal interlobar arteries. In renal arteries the decreased dilative response additionally involved ACh induced production of constrictive PGs opposing dilation. Whether these alterations may be part of a renoprotective action of ACEi therapy under LS conditions needs to be explored in further studies.
ACE inhibition modulates endothelium-derived mediators

References


