Hormonal influence on renal function with particular reference to diabetes mellitus
Hoogenberg, Klaas

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CHAPTER 5

NOREPINEPHRINE-INDUCED BLOOD PRESSURE RISE AND RENAL VASOCONSTRICTION IS NOT ATTENUATED BY ENALAPRIL IN MICROALBUMINURIC IDDM

K. Hoogenberg¹, G. Navis² and R.P.F. Dullaart¹

In non-diabetic subjects an attenuated systemic norepinephrine (NE) responsiveness may contribute to the mechanisms of action of angiotensin-converting enzyme (ACE) inhibitor treatment. We determined whether ACE-inhibitor treatment influences systemic and renal haemodynamic responsiveness to exogenous NE, as well as urinary albumin excretion during NE, in micro-albuminuric IDDM patients, representing a patient category that benefits by strict blood pressure control. In 7 microalbuminuric IDDM patients, systemic and renal responsiveness to NE, infused at individually determined threshold (Δ mean arterial pressure (MAP)=0mmHg), 20% pressor (ΔMAP =4mmHg) and pressor (ΔMAP=20mmHg) doses, were compared before and after 8 weeks treatment with enalapril, 10 mg daily. Blood glucose was clamped at 5 mmol/l and insulin was infused at 30 mU/kg per h. Enalapril decreased MAP (p<0.05) and microalbuminuria (p<0.05), whereas effective renal plasma flow (ERPF) increased (p<0.01) and glomerular filtration rate (GFR) remained unaltered. Filtration fraction tended to decline (p=0.09). The ACE inhibitor-induced fall in MAP disappeared at NE pressor dose, and the overall mean increase in MAP in response to NE was even higher with than without enalapril (p<0.05). After enalapril, ERPF remained higher at all NE doses (p<0.05), but the magnitude of the NE-induced fall in ERPF was not altered by ACE inhibition treatment. Overnight urinary albumin excretion fell by ACE-inhibition (p<0.05), but this effect was not seen during NE infusion. The angiotensin II/active renin ratio and serum aldosterone levels remained lower with enalapril at all NE doses (p<0.05). In conclusion, enalapril does not attenuate systemic and renal vascular responsiveness to exogenous NE in microalbuminuric IDDM despite adequate inhibition of the renin-angiotensin-aldosterone system. These findings suggest that the effect of NE on vasoconstriction is not effectively counteracted by ACE inhibition treatment alone.

Introduction

Blockade of the renin-angiotensin-aldosterone system (RAAS) by angiotensin-converting enzyme (ACE) inhibitors lowers blood pressure, (micro)albuminuria and slows progression of diabetic and non-diabetic renal insufficiency, although such treatment is unable to completely prevent this serious complication [1-3]. The mechanism of action of these agents is still incompletely understood. Both angiotensin II-dependent and independent mechanisms are thought to be involved. The sympathetic nervous system (SNS) and RAAS are closely related [4] and ACE inhibition may influence systemic norepinephrine (NE) reactivity. Experimental studies indeed have shown that systemic

¹Department of Endocrinology and ²Nephrology, Groningen University Hospital, The Netherlands.  
Nephrol Dial Transplant (in press)
vascular NE responsiveness is attenuated by ACE inhibitors [5,6]. A decrease in systemic NE responsiveness after ACE inhibition treatment has been demonstrated in hypertensive subjects [7-9]. Moreover, in healthy subjects the renal vasoconstrictive response has been found to be reduced after ACE inhibition [10]. This would suggests that attenuation of renal NE responsiveness may be involved in the renoprotective effect of ACE inhibitors in non-diabetic subjects.

In insulin-dependent and non-insulin-dependent diabetes mellitus (IDDM and NIDDM), the systemic vasopressor response to exogenous NE has been repeatedly found to be enhanced [11-13]. The effect of ACE inhibition on systemic and renal NE responsiveness is less well characterised in diabetes mellitus. In patients with NIDDM and in a heterogeneous group of NIDDM and IDDM patients, treatment with enalapril or captopril did not normalise the exaggerated systemic NE responsiveness [14,15], but this has not been studied in IDDM complicated by microalbuminuria. It is of particular relevance to evaluate the NE responsiveness after ACE-inhibition treatment in microalbuminuric IDDM patients, because control of factors that contribute to a blood pressure rise in such patients appears to be indicated to prevent progression to overt nephropathy. The purpose of our study was, therefore, to evaluate the effect of enalapril treatment on systemic and renal haemodynamic responsiveness to exogenous NE in IDDM patients with microalbuminuria.

**Subjects and methods**

**Subjects**

The study was approved by the local medical ethics committee and all participants gave written informed consent. The patients were considered insulin-dependent, and glucagon stimulated C-peptide levels were less than 0.2 nmol/l. Seven patients (6 men, 1 women) participated. They had a mean age of 48±9 (mean±SD) years and a diabetes duration of 29±5 years. None had severe obesity (body mass index ranging from 23.5 to 27.9 kg/m²). All of the patients had persistent microalbuminuria (defined as an urinary albumin excretion rate (Ualb.V) between 20 to 200 µg/min in at least two out of three overnight urine collections for a 1-year period). All patients were treated with enalapril for elevated blood pressure related to the presence of incipient nephropathy (systolic/ diastolic blood pressure >140/90 mmHg), but none had essential hypertension before development of microalbuminuria. ACE inhibition treatment was stopped for 6 weeks before the start of the study. Without enalapril, 6 patients had a systolic blood pressure > 140 mmHg and 1 patient had a diastolic blood pressure >90 mmHg. None of the patients had untreated proliferative retinopathy, symptomatic coronary heart disease, peripheral vascular disease, or clinical autonomic neuropathy as evaluated by standard tests (beat-to-beat variation during deep breathing, Valsalva manoeuvre and systolic blood pressure response to standing). All participants were instructed by dietician to adhere to a diet containing 100 mmol sodium and 1 gram protein/kilogram body weight per day throughout the studies. Three timed overnight urine collections were obtained directly prior to the studies.
Table 1. Baseline characteristics before and after 8 weeks treatment with enalapril.

<table>
<thead>
<tr>
<th></th>
<th>before ACEi</th>
<th>with ACEi</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c (%)</td>
<td>8.4 ±0.2</td>
<td>8.5 ± 0.2</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>83.2 ± 2.7</td>
<td>83.3 ± 2.7</td>
</tr>
<tr>
<td>Urinary urea excretion (mmol/day)</td>
<td>279 ± 26</td>
<td>282 ± 16</td>
</tr>
<tr>
<td>Sodium excretion (mmol/day)</td>
<td>110 ± 8</td>
<td>123 ± 10</td>
</tr>
<tr>
<td>Mean of 3-timed overnight urinary albumin excretion rates (µg/min)</td>
<td>71.4 (33.5 - 152.2)</td>
<td>50.9 (36.8 - 97.2) (^a)</td>
</tr>
</tbody>
</table>

Data in mean±SEM, except for urinary albumin excretion rate which is geometric mean (95% confidence interval). ACEi: angiotensin converting enzyme inhibition. \(^a\) denotes \(p<0.05\)

**Study design**

NE-infusions were performed on three separate study days. On the first day, systemic MAP-NE dose response curves were made. The individual NE threshold (\(\Delta_{\text{mean arterial pressure (MAP) = 0mmHg}}\)), 20% pressor (\(\Delta_{\text{MAP = 4mmHg}}\)) and pressor doses (\(\Delta_{\text{MAP = 20mmHg}}\)) were calculated from MAP-NE dose response curves. On a separate day, the stepwise NE infusions were given with renal function measurements during 10 consecutive 45 min clearance periods. Two baseline periods were followed by 6 periods of NE (2 periods for threshold, 20% pressor and pressor doses, respectively), after which 2 determinations were obtained after cessation of the infusion (recovery period). Blood pressure was recorded every 5 min (Dinamap). These systemic and renal haemodynamic studies were repeated after 8 weeks of treatment with the ACE inhibitor enalapril (10 mg/day), given at 0800 h. The same NE doses were administered during this second evaluation. The patients also participated in another study that aimed to compare the renal haemodynamic and microproteinuric NE responses in micro- and normoalbuminuric IDDM patients and control subjects, and the pre-enalapril data were also used for that study [13]. One initially studied patient declined to participate in the second evaluation.

The experiments were carried out in a temperature-controlled room kept at 22°C. The subjects were studied after an overnight fast and remained so during the experiments. Smoking was prohibited and no liquid other than water was allowed to drink. The subjects remained in supine position and were only allowed to stand on voiding. Two hours before the start of the renal haemodynamic measurements, each subject drank 600 ml water to promote diuresis. Thereafter, urinary volume losses were suppleted by water drinking. An euglycaemic insulin clamp was used to minimise effects of differences in actual glycaemia on renal haemodynamics [16] and to avoid that changes in insulin levels during the experiments would potentially influence NE sensitivity. Insulin was infused (Velosulin H.M., Novo-Nordisk, Bagsvaerd, Denmark) at a rate of 30 mU/kg per h. Blood glucose was kept at 5 mmol/l by varying the infusion rate of a 20% glucose solution. Potassium chloride (10 mmol per 100 g of glucose) was added to the glucose vials.

GFR and ERPF were measured simultaneously using primed infusions of \(^{125}\text{I}-\text{iothalamate}\) and \(^{131}\text{I}-\text{hippurate}\), respectively [16]. The clearances were calculated using the formula \(U.V/P\) and \(I.V/P\), respectively. \(U.V\) represents the urinary excretion rate of the
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tracer, I.V represents the infusion rate of the tracer, and P represents the tracer value of plasma samples taken at the end of each clearance period. Errors in the estimation of GFR due to incomplete bladder emptying and dead space were corrected by multiplying the clearance of $^{125}$I-iothalamate with the formula: clearance of $^{125}$I-hippurate (I.V/P) / clearance of $^{131}$I-hippurate (U.V/P). The coefficients of variation for GFR and ERPF are 2.2% and 5.0%, respectively [16]. The GFR and ERPF were corrected to 1.73$\text{m}^2$ of body-surface area. Filtration fraction (FF) was calculated as the quotient of $^{125}$I-iothalamate and $^{131}$I-hippurate clearances.

Analytical methods

Blood samples were taken from an intravenous catheter at the end of each clearance period with the subjects in supine position. Blood was immediately centrifuged at 4°C and samples were stored at -20°C before assay. Aliquots of urine for measurement of urinary proteins were stored at -20°C and analysed within 2 weeks. Plasma insulin was determined by RIA. Plasma NE was analysed by HPLC [17]. Active plasma renin was determined with a commercially available double-antibody RIA (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA) with a cross-reactivity with prorenin of 0.2%. Serum ACE activity was measured with an HPLC technique. Angiotensin II was determined by RIA (Dept. of Pharmacology, State University Maastricht, The Netherlands). Cross-reactivity of the angiotensin II antibody with angiotensin I is <0.1%. Serum aldosterone was determined by RIA. Blood glucose was measured using a Yellow Springs glucose Analyser (Model 23A, Yellow Springs Inc, Yellow Springs, Ohio, USA). Urinary albumin was measured with ELISA using rabbit anti-human albumin antibody (DAKO). The detection limit is 1 µg/l. Sodium, potassium, creatinine and serum albumin in serum and urine were measured on SMA(C) autoanalysers (Technicon Instruments Inc. Tarrytown, N.Y., USA).

Statistical analysis

Results are expressed as mean±SEM for parametrically distributed data and as geometric mean (95% confidence intervals) for non-parametrically distributed data unless stated otherwise. The 2 clearance periods obtained at baseline, threshold, 20% pressor, pressor and recovery were averaged for analysis. Where appropriate, repeated measurements ANOVA for parametrically or non-parametrically distributed data was used to analyse the effects of the NE-infusions before and after ACE inhibition. Paired $t$-tests or Wilcoxon tests were then used to establish differences between corresponding observation periods. Differences in NE effects on MAP and ERPF before and after ACE inhibition were evaluated by comparing the overall mean changes of each individual. $p$-Values less than 0.05 were considered to be significant.
Enalapril and norepinephrine reactivity in IDDM

Results

Metabolic control, body weight, urinary urea and sodium excretion were similar before and after treatment with enalapril (Table 1). Enalapril lowered baseline MAP by 5%, but did not change pulse rate (Table 2). GFR was unaltered, whereas ERPF increased by 9% (Table 2). FF tended to fall \((p=0.09\)). Overnight microalbuminuria fell by 28% (Table 1). Enalapril increased plasma active renin and decreased serum ACE activity and serum aldosterone levels (Table 3). Baseline plasma AII did not significantly change, but the AII/active renin ratio profoundly decreased, indicating adequate RAAS inhibition.

Plasma NE concentrations during NE infusions were similar before and after ACE inhibition (Table 2). Before enalapril, NE increased MAP at 20% pressor and pressor doses to target levels (Table 2). With enalapril, NE infusion also increased MAP. At NE pressor dose there was no difference in MAP compared to before enalapril. The overall mean increment in MAP was greater during than before enalapril \((p<0.05\), Figure 1A). At recovery, MAP was again lower with than without enalapril. ACE inhibition treatment did not influence the fall in pulse rate at NE-pressor dose and its rise at recovery (Table 2).

GFR remained essentially unaltered during NE infusions before and after enalapril, but declined at recovery (Table 2). ERPF progressively decreased at 20% pressor and at pressor dose both before and during enalapril, and corresponding rises in FF were seen. During all observation periods, ERPF remained significantly higher with than before enalapril, and the magnitude of the NE-induced decrease in ERPF was not influenced by enalapril treatment (Figure 1B). The overall urinary albumin excretion rate during NE infusion was 51.5 (24.3-109.1) µg/min before and 57.6 (26.3-126.3) µg/min after enalapril \((p>0.20\)).

Before enalapril, plasma active renin, angiotensin II and serum aldosterone levels increased at NE pressor dose, whereas the angiotensin II/active renin ratio remained unaltered (Table 3). With enalapril, plasma active renin and serum aldosterone levels rose at NE-pressor dose, but plasma angiotensin II did not significantly increase. At NE-pressor dose a slight fall was seen in the angiotensin II/active renin ratio. The overall mean levels of plasma active renin, serum ACE activity, angiotensin II, the angiotensin II/active renin ratio and serum aldosterone were lower after enalapril.

Mean blood glucose and plasma insulin concentrations were 5.2±0.1 and 5.1±0.1 mmol/l and 230±22 and 251±19 pmol/l, before and after enalapril, respectively (NS).

Discussion

As expected, enalapril lowered blood pressure and overnight urinary albumin excretion, and induced renal vasodilatation in microalbuminuric IDDM patients. The systemic haemodynamic and the renal responsiveness to exogenous NE, as well as urinary albumin excretion during NE, were however not altered by ACE inhibition treatment.
### Table 3. Parameters of the renin-angiotensin II-aldosterone system during stepwise norepinephrine infusions without and with enalapril

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Plasma active Renin (mU/l)</th>
<th>Angiotensin-converting enzyme (U/l)</th>
<th>Angiotensin II (pmol/l)</th>
<th>Angiotensin II/ plasma active renin ratio</th>
<th>Serum aldosterone (nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>without enalapril</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>61 (33-111)</td>
<td>29 ± 3</td>
<td>15 ± 2</td>
<td>0.26 ± 0.05</td>
<td>360 ± 40</td>
</tr>
<tr>
<td>Threshold</td>
<td>55 (32-94)</td>
<td>28 ± 3</td>
<td>14 ± 1</td>
<td>0.28 ± 0.05</td>
<td>330 ± 40</td>
</tr>
<tr>
<td>20% Pressor</td>
<td>64 (49-187)</td>
<td>28 ± 4</td>
<td>16 ± 1</td>
<td>0.28 ± 0.06</td>
<td>420 ± 40</td>
</tr>
<tr>
<td>Pressor</td>
<td>95 (49-187)</td>
<td>28 ± 4</td>
<td>25 ± 5</td>
<td>0.25 ± 0.05</td>
<td>710 ± 130</td>
</tr>
<tr>
<td>Recovery</td>
<td>91 (51-160)</td>
<td>25 ± 3</td>
<td>21 ± 4</td>
<td>0.24 ± 0.03</td>
<td>460 ± 50</td>
</tr>
<tr>
<td><strong>with enalapril</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>121 (41-359)</td>
<td>6 ± 2b</td>
<td>14 ± 4</td>
<td>0.10 ± 0.02</td>
<td>90 ± 20b</td>
</tr>
<tr>
<td>Threshold</td>
<td>127 (49-335)</td>
<td>6 ± 2b</td>
<td>11 ± 2</td>
<td>0.09 ± 0.02c</td>
<td>100 ± 30b</td>
</tr>
<tr>
<td>20% Pressor</td>
<td>137 (54-485)</td>
<td>7 ± 2b</td>
<td>12 ± 2</td>
<td>0.09 ± 0.02b</td>
<td>110 ± 20b</td>
</tr>
<tr>
<td>Pressor</td>
<td>201 (85-485)</td>
<td>8 ± 2b</td>
<td>18 ± 4</td>
<td>0.08 ± 0.01b, e</td>
<td>220 ± 30b, e</td>
</tr>
<tr>
<td>Recovery</td>
<td>198 (98-402)</td>
<td>9 ± 2b</td>
<td>15 ± 2</td>
<td>0.07 ± 0.01c, e</td>
<td>170 ± 40b</td>
</tr>
</tbody>
</table>

Data in mean±SEM and geometric mean (95% confidence intervals). a p<0.01 overall mean values without vs with enalapril, b p<0.05 and c p<0.01 from corresponding infusion period before enalapril, d p<0.05 and e p<0.01 from baseline values.
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Figure 1(A,B). Plot comparing individual changes in mean arterial pressure (MAP, A) and effective renal plasma flow (ERPF, B) in response to stepwise norepinephrine infusions before and after ACE inhibition. The lines indicate identical changes. Overall mean change in MAP was greater with than without enalapril (n=7, \( p < 0.05 \) by paired Wilcoxon test). No difference in overall ERPF response was observed.
Apart from general factors like basal vasomotor tone and vascular reactivity, sodium and volume homeostasis, as well as baroreceptor feedback control are determinants of the systemic reactivity to exogenous NE, and may thus contribute to an enhanced systemic NE responsiveness in diabetes mellitus [8,11,13,18]. Such an exaggerated responsiveness has been found to be associated with an increased exchangeable sodium and a higher extracellular volume, and this hyperreactivity decreases after diuretic treatment [11]. In addition, microalbuminuric IDDM patients have an attenuated pulse rate decline during NE infusion [13], which suggests that an altered baroreceptor feedback control may also contribute to the systemic NE hyperresponsiveness in IDDM [18].

The observation that enalapril treatment failed to diminish systemic vascular NE reactivity in microalbuminuric IDDM is in keeping with previous findings in diabetic patients [11,12]. By contrast, in essential hypertension, the systemic responsiveness to NE appears to be attenuated after ACE inhibition treatment [7-9]. Several factors could be involved in the lack of attenuation of the systemic NE responsiveness after ACE inhibition in diabetes mellitus. First, ACE inhibition therapy is unlikely to have influenced sodium and volume status in the present study since body weight remained unchanged. Indeed, captopril does not significantly decrease exchangeable sodium in diabetes mellitus [14]. In this respect, it is noteworthy that ACE inhibition treatment induces a negative sodium balance in essential hypertension [19]. Second, a similar heart rate decline during NE before and after enalapril was observed, suggesting no important alterations in baroreceptor sensitivity, in agreement with findings in hypertensive diabetic patients [14]. Third, although enalapril induced adequate RAAS suppression, it cannot be excluded that a more complete inhibition of angiotensin II formation is required to blunt systemic NE reactivity. Finally, we consider a type II error very unlikely to explain the lack of attenuation of enalapril on systemic NE reactivity, since the mean increment in MAP in response to NE was even higher after ACE inhibition.

This study is the first to document the effect of ACE-inhibition treatment on NE-induced renal vasoconstriction in microalbuminuric IDDM patients. In IDDM, ACE inhibitors increase renal plasma flow without having much effect on glomerular filtration rate. As a consequence, filtration fraction tends to decline [20]. These changes are ascribed to a predominantly postglomerular dilatation which is in part due to decreased angiotensin II formation. In our study, the renal vasodilatory effects of enalapril remained present during NE infusion, a finding thus far only documented in healthy volunteers [10]. Enalapril prevented the rise in angiotensin II levels during NE pressor infusion. It may thus be inferred that this partial RAAS blockade accounted for the ongoing renal vasodilatation of the ACE inhibitor during NE infusion as compared to pretreatment. Although difficult to demonstrate in humans, animal studies have unequivocally documented bidirectional interactions between the RAAS and the SNS in a way such that each system is able to potentiate the other [4]. ACE inhibition treatment could, therefore, induce a decrease in SNS activation. In the isolated kidney, ACE inhibitors indeed have been demonstrated to diminish renal vascular NE responsiveness [5,6], and neuronally-induced renal vasoconstriction [21], suggesting interference in the RAAS-SNS interaction. The present in vivo study does, however, not support the assumption of an intrarenal sympatholytic effect of enalapril in microalbuminuric IDDM patients, since the
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Exogenous NE-induced fall in ERPF was not altered by ACE inhibition treatment.

Our findings may have clinical implications for the future design of renoprotective strategies in IDDM patients. Currently, ACE inhibition treatment is considered to be the main tool to retard the decline in renal function loss in IDDM, both by lowering systemic blood pressure and by decreasing intraglomerular pressure [1-3]. Despite this proven renoprotective potential, many patients still progress to end stage renal failure. Albeit in a small number of patients, the present study is consistent with the possibility that microalbuminuric IDDM patients are not sufficiently protected by ACE inhibition treatment against rises in systemic blood pressure due to bouts of SNS activation during daily life activities. Although the effects of exogenous NE cannot be directly extrapolated to physiological SNS stimulation, it is noteworthy that increases in urinary albumin excretion are well related to rises in blood pressure and plasma NE levels during exercise, which represents a strong endogenous SNS stimulus [17].

In conclusion, we found that enalapril does not attenuate systemic and renal vascular responsiveness to exogenous NE in microalbuminuric IDDM patients. These findings suggest that a potentially adverse effect of NE on systemic and renal vascular tone is not effectively counteracted by ACE inhibition treatment alone.

References

1 Viberti GC, Mogensen CE, Groop LC, Pauls JF. Effect of captopril on progression to clinical proteinuria in patients with insulin-dependent diabetes mellitus and microalbuminuria. JAMA 1994; 271: 275-279

Acknowledgments

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