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Sodium intake and therapy resistance to ACE inhibition

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Chapter 3
The role of renal ACE activity in sodium induced resistance to ACE inhibition.
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Introduction

Angiotensin-converting enzyme inhibition (ACE inhibition) reduces proteinuria and retards the rate of progressive renal function loss in man and experimental models (1-3). A high sodium intake, however, consistently induces therapy resistance to ACEi (4-7). This therapy resistance is likely to be due to sodium status per se, as it is reversible upon re-institution of low sodium diet or by co-treatment with a diuretic (8). The mechanism of the sodium-induced resistance to ACEi has not been well explored. Usually it is attributed to the suppression of the renin angiotensin aldosterone system by high sodium intake, inferring that blockade of a biological cascade will exert less effect under conditions where activity of the cascade is already suppressed by endogenous mechanisms.

Interestingly, several studies provided evidence that high sodium intake can affect tissue ACE activity, as shown by increased vascular ACE expression, and increased conversion of angiotensin I during high sodium intake (9-11). This raises the possibility that, effects of high sodium intake on tissue ACE might be involved in the blunted efficacy of ACE inhibition during high sodium. In support of this hypothesis, we found that the inhibition of functional conversion of angiotensin I to angiotensin II in the vessel wall by ACE inhibition was blunted by high sodium intake in healthy rats (12). It has been shown that ACE activity is not regulated similarly in all tissues (13;14). Whether high sodium intake also can modify renal ACE activity (rACE) during ACE inhibition and whether this might be involved in renal resistance to therapy during ACE inhibition is unknown.

Therefore, in the present study we investigated the effects of sodium intake on rACE, and its relationship to renal therapy response to ACE inhibition in rats. First, we studied the effect of high as compared to low sodium intake on rACE in healthy rats, in the untreated condition and during ACE inhibition.

Secondly, in proteinuric rats on maintenance treatment with ACE inhibition we studied the effect of sodium intake on rACE in relation to the responses of blood pressure and proteinuria. This study was conducted in adriamycin-induced proteinuria, a rat model of proteinuria-induced renal damage that is well established to respond to ACE inhibition during low sodium intake, with blunting of the response during normal or high sodium intake (3;15).

Methods

Experimental procedures

Male Wistar rats (Hsd:Wu Harlan, Zeist, The Netherlands) were housed in groups of 5 to 6 in a light and temperature controlled environment at the animal research facility of the University of Groningen. The animals had free access to fresh tap water and a semi-synthetic diet containing 0.3% (normal sodium, NS), 2.0% (high sodium, HS) or 0.05% (low sodium, LS) NaCl and 20 % protein (Hope Farms Inc. Woerden, The Netherlands). Urine was collected weekly during a 24-hour stay in metabolic cages with free access to food and water in all groups. Tail -cuff systolic blood pressure (SBP) was measured weekly after appropriate training as described previously (16). Two experimental protocols were performed.

Experimental protocols

A: Effects of sodium intake on renal ACE activity in healthy rats, untreated and during ACE inhibition.
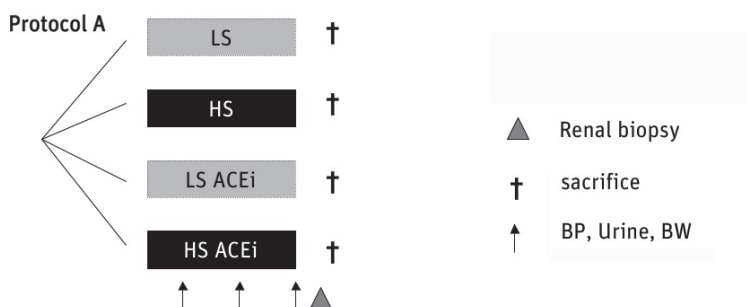

Figure 1 Four parallel groups sacrificed after renal biopsy procedure at the end of three weeks of therapy

Rats (BW range: 280-350) fed with LS or HS rat chow, were randomized to receive either vehicle (LS and HS, each n = 10) or lisinopril 75 mg/l in the drinking water (LS and HS, each n=10) for three weeks. The dose of lisinopril is well on top of the dose-response in this model, as established in prior experiments (3;17). After three weeks, kidneys were harvested and the rats were sacrificed.

B: Effects of a shift in sodium intake on renal ACE activity and therapy response in ACEi treated rats.

In 30 rats (BW range: 280-320) fed NS, Adriamycin nephrosis was inducted by a single injection in the tail vein under light anesthesia with Isoflurane/O2/N2O as described in detail elsewhere (3;16). After development of stable proteinuria (week 6) rats were randomized to start with LS (group I) or HS (group II) diet and all rats started on lisinopril (75 mg/l). After 3 weeks of therapy (week 9), a renal biopsy was performed by lower pole resection, and

Protocol B
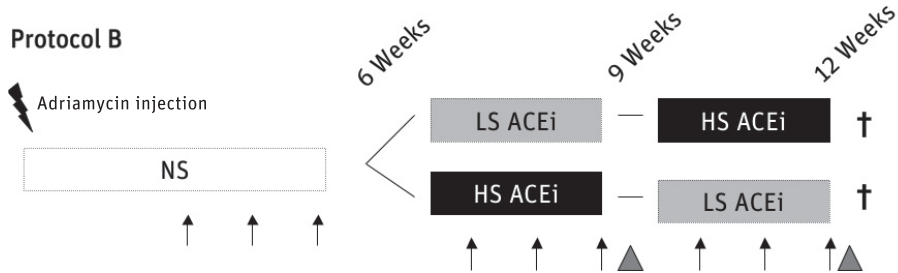
Figure 2 Two parallel groups after proteinuria induction with a switch in dietary sodium

after recovery, the rats crossed over to the other sodium intake, while ACE inhibition was continued unchanged. After another three weeks (week 12), the rats were sacrificed and kidneys harvested. For determination of plasma ACE activity (pACE), blood was collected by orbital punction under light anesthesia just before start therapy (week 6), at the time of renal biopsy (week 9), and at the end of the study (week12).

The effect of dietary sodium on renal tissue ACE in untreated adriamycin nephrosis had been tested in a pilot study in a separate group of rats (n=12 for high and low sodium each). In these rats, sodium intake did not affect renal ACE and proteinuria, with median (interquartile range) values of 136 (92-203) and 159 (47-331) mg/day for proteinuria, and 152 (113-186) and 157 (88-180) nmol His-Leu/g/min for renal ACE in the low and high sodium animals, respectively.

Surgery procedure, tissue processing and (immunohistochemical) staining procedures

The renal biopsy procedure was performed via a dorsolateral incision under anaesthesia. Immediately after surgical removal of a small part of the renal lower pole, gelfoam was applied to reach haemostasis. The renal lower pole tissue was divided in 1) tissue for rACE activity measurement and immediately frozen in liquid nitrogen and 2) slices for histology which were fixed in 4% paraformaldehyde and processed for paraffin embedding. Paraffin sections (4μm) were stained with periodic acid-Schiff (PAS) to evaluate glomerular and interstitial damage. For staining procedures, paraffin sections were dewaxed and subjected to heat induced antigen retrieval by overnight incubation in 0.1 M Tris/HCl buffer on 80°C. Endogenous peroxidase was blocked with 0.075% H_2O_2 in phosphate-buffered saline (PBS) for 30 minutes. Alpha-smooth muscle actin (Alfa-SMA) and desmin (Desm) were detected using a murine monoclonal antibody (Alfa-SMA: clone 1A4, Sigma Chemical Co., St. Louis, MO, USA; Desm: Dakopatts, DAKO, Glostrup, Denmark). Binding for both antibodies was detected using sequential incubations with peroxidase labelled rabbit anti-mouse and peroxidase-

labeled goat anti-rabbit antibody (Dakopatts, DAKO, Glostrup, Denmark) for 30 min. Peroxidase activity was developed using 3,3'-diaminobenzidine tetrachloride (DAB) for 10 min.

Renal Morphology

Focal glomerulosclerosis (FGS), defined as glomerular areas with mesangial expansion and adhesion formation simultaneously present in one segment, was scored semi-quantitatively on a scale 0 to 4 (18;19). Alpha-smooth muscle actin (a-SMA) staining was measured using computer-assisted morphometry as a marker of pre-fibrotic damage. Twenty cortical interstitial images without vessels or glomeruli were selected and the total immunohistochemical-staining surface for a-SMA was measured and divided by the total surface of the image. To detect early glomerular damage, the outer cell layer of the glomerular tuft was evaluated separately as described by Joles et al. (20). Semiquantitative staining scores in these cases depend on the percentage of the glomerular edge showing positive staining 0 (0 to 5% stained); 1 (5 to 25%); 2 (25 to 50%); 3 (50 to 75% stained) and 4 (> 75%).

Urinary protein excretion and ACE activity

Proteinuria was determined in 24-hour urine of Adriamycin nephrotic rats by a biuret method (Bioquant, Merck, Darmstadt, Germany). ACE activity was determined as the cleavage of Hippuryl-His-Leu by renal cortical homogenates, and plasma, respectively, as described previously (21).

Statistical analysis

Data are presented as median (interquartile range). Mann-Whitney-U test (SPSS 10.0) was used for group comparison, Wilcoxon-signed-rank test (SPSS 10.0) for paired parameters.

Results
Healthy rats

The data in healthy rats are shown in Table I. As anticipated a considerable difference in sodium status was obtained by the different diets. In untreated rats, sodium intake did not affect blood pressure. Interestingly, in the untreated rats rACE was significantly higher in the high sodium group. Blood pressure, plasma ACE (pACE) and rACE were significantly lower in the lisinopril treated rats in both sodium groups. As anticipated, the reduction of SBP was significantly larger in the lisinopril group on low sodium diet. Residual plasma and renal ACE activity during ACE inhibition, on the other hand, were similar with low and high sodium.

Table I Healthy rats (median (interquartile range)) Urinary sodium excretion, blood pressure and ACE activity after three weeks of therapy.

	Control		ACE i	
	LS (n=10)	HS (n=10)	LS (n=9)	HS (n=10)
UNaV (mg/day)	0.56 (0.48-0.80)	3.68# (2.69-3.92)	0.41 (0.37-0.78)	4.78*# (3.46-2.69)
SBP (mmHg)	152 (146-154)	140 (132-148)	100* (98-109)	124*# (117-138)
pACE (nmol His-Leu/ml/min)	71 (59-81)	74 (58-91)	15* (13-33)	30* (24-33)
rACE (nmol His-Leu/g/min)	73 (62-88)	105# (90-129)	18* (9-30)	16* (9-25)

*$p<0.01$ for ACEi vs Control, # $p<0.01$ for HS vs LS pACE: plasma ACE activity, rACE: renal ACE activity

Table II Adriamycin rats (median (interquartile reange)) Baseline and during treatment (ACEi) on high (HS) and low (LS) dietary sodium intake

	Group I (n=14);			Group II (n=12)		
	Week 6 Baseline	Week 9 LS ACEi	Week 12 HS ACEi	Week 6 Baseline	Week 9 HS ACEi	Week 12 LS ACEi
UnaV (mg/day)	0.69(0.44-0.83)	0.41(0.32-0.50)	3.27#(2.01-4.20)	0.69(0.63-0.89)	3.99*(2.66-5.53)	0.51#(0.45-0.62)
SBP (mmHg)	153(141-163)	105(92-118)	105(102-111)	153(135-162)	111(101-130)	84#(74-91)
UprotV (mg/day)	266(73-422)	42(35-61)	132#(47-271)	270(137-475)	332*(122-585)	22#(11-40)
pACE	60(55-68)	25(22-37)	35(31-43)	61(53-65)	40*(28-46)	31(17-45)
rACE		19(17-32)	36#(28-46)		32*(25-44)	31(22-34)
FGS (score 0-400)		2(0-5)	3(0-12)		3(1-14)	8(1-14)
a-SMA (%)		1.35(0.71-1.98)	0.88(0.38-2.54)		0.39*(0.23-0.77)	0.71(0.38-1.52)
Desmin (score 0-4)		2.51(2.23-2.75)	3.26#(2.82-3.36)		2.47(2.16-2.61)	2.45(2.04-2.72)

*$p<0.05$ for Group I vs Group II (Week 9); # $p<0.01$ for HS vs LS (Week 9 vs Week 12) pACE: plasma ACE, rACE: renal ACE, FGS: focal glomerular sclerosis, a-SMA: alpha smooth muscle actin score
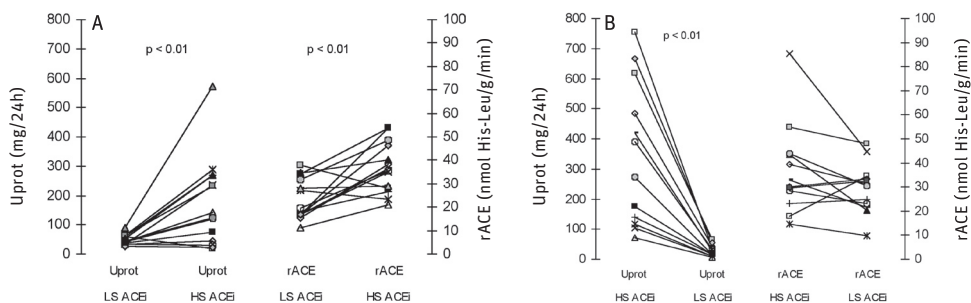
Proteinuric rats

Data on the proteinuric rats are given in Table II. It shows first, that both groups had similar blood pressure and proteinuria before start of lisinopril (Week 6, baseline). With lisinopril blood pressure fell in both sodium groups similarly. After three weeks of therapy on high sodium (week 9), proteinuria and residual renal ACE in group II was significantly higher than in group I, on low sodium diet (p<0.01 and p=0.03 respectively).

In the rats starting on high sodium, group II, a further fall in blood pressure occurred after the switch to low sodium, whereas in the rats starting on low sodium, group I, blood pressure did not increase after increasing dietary sodium. In group I lisinopril induced a pronounced reduction of proteinuria that was followed by a significant rise - albeit not to pretreatment values - after the shift to high sodium. This rise in proteinuria was paralleled by a significant increase in renal ACE activity. In group II, the rats starting on high sodium, lisinopril did not affect proteinuria during the first three treatment weeks, but resulted in a pronounced fall in proteinuria only after the shift to low sodium. The shift from high to low sodium intake did lower the residual renal ACE activity in this group but this did not quite reach statistical significance (p=0.07). The residual plasma ACE activity (pACE) remained similar in both groups after the switch in sodium intake. Individual data on proteinuria and renal ACE activity are given in Figure 3.

In both groups, the extent of renal structural damage was mild after three weeks of treatment as well as at the end of the study, as apparent from the virtual absence of FGS on routine morphology either group. However, early markers of renal damage in this model, i.e. alpha-smooth muscle actin and desmin expression, were increased in both groups to a modest extent.

Discussion

In this study on the role of renal ACE activity in sodium-induced therapy resistance to ACE inhibition we identified several associations between sodium intake and renal - but not plasma ACE activity. First, in healthy rats we found that high sodium intake increased renal ACE activity. This effect is likely to be due to the feedback effect of lower angiotensin II levels during high sodium on ACE expression (22). At variance with our findings, a study in Wistar-Kyoto rats found no significant effect of dietary sodium (23) on renal ACE activity. Differences between rats strains and disease model may be involved in this discrepancy, as both factors can affect ACE activity (24;25). It has not previously been assessed whether a sodium-induced rise in renal ACE activity might hamper the pharmacological efficacy of ACE inhibition. In the normal rats we observed the anticipated blunting of the blood pressure

Figure 3 Individual cross-over data on the modulating effect of dietary sodium shift on proteinuria (Uprot) and renal ACE (rACE) during ACE inhibition (ACEi) in two parallel treated groups of adriamycin rats. 3A: the shift from low sodium (LS; 0.05%NaCl) to high sodium diet (HS; 2.0%NaCl) resulted in an increase of both Uprot and rACE; 3B: the switch from HS to LS resulted in a reduction of Uprot and rACE activity - with a less pronounced or in some rats no reduction of rACE. The change of rACE was of borderline significance.

response to ACE inhibition by high sodium without, however, blunting of the effect on plasma or renal ACE activity. Apparently, sodium-induced effects on plasma or renal ACE do not mediate the effect of high sodium on the blood pressure response to ACE inhibition in normal rats. We recently showed a blunted inhibition of vascular functional angiotensin I to angiotensin II conversion during high sodium in normal rats (12). Taken together with the present data, this suggest that it may be the effects of high sodium on vascular tissue ACE function, rather than effects on plasma ACE, that could be involved in the blunting of the blood pressure response during high sodium.

Second, in proteinuric rats the antiproteinuric response to ACE inhibition was blunted during high sodium, coinciding with a high renal ACE activity under ACE inhibition compared to low sodium (Group I vs. II at week 9). In these nephrotic ACE inhibition treated rats the effect of sodium intake on proteinuria was impressive. The effect on blood pressure, on the other hand, was less pronounced. In this respect it should be noted that also in human proteinuria we have observed that the effects of altered sodium intake during ACE inhibition were more pronounced for proteinuria than for blood pressure (7).

It is known from prior studies from our lab as well as others (26;27) that proteinuria-associated renal damage itself can modify renal ACE activity in proportion to the severity of renal damage. Moreover, individual differences in proteinuria and renal ACE activity can be large. Therefore, for this part of the study we used a crossover design, to eliminate the effects of between-individual differences in proteinuria and renal damage as much as possible.

The shift from low to high sodium during ACE inhibition led to the expected significant rise

in proteinuria, paralleled by a rise in residual renal ACE activity, supporting the assumption that sodium induced effects on renal ACE activity are relevant to therapy response (figure 3A). The pronounced fall in proteinuria after the shift from high to low sodium was accompanied by a less pronounced and borderline significant fall in renal ACE activity. However, caution is warranted in interpreting the data on renal ACE activity. ACE activity was assessed as the cleavage of the substrate Hip-His-Leu in renal cortical homogenates - that is, after distortion of the normal renal architecture. The major part of ACE activity in renal homogenates is presumably derived from tubular cells - and subtle differences at other - possibly pathophysiologically relevant sites such as the vessel wall - may go unnoticed in this set-up. It would be relevant to know whether the rise in renal ACE activity observed here has pathophysiological consequences, but this cannot be derived from our data. It has been suggested that, a proteinuria-associated increase in renal tubular ACE activity is involved in the tubular toxicity of leaked proteins (26). Thus, a proteinuria-induced rise in renal ACE activity might be involved in the vicious circle of progressive proteinuria-induced renal damage and therefore exert a deleterious effect. Further prospective studies with long term follow-up of renal damage would be needed to test this assumption for our experimental set-up.

We conclude that a high dietary sodium intake is associated with a higher renal ACE activity under several experimental conditions. Notably, during antiproteinuric treatment a higher renal ACE activity during high sodium is associated with a poor therapy response to ACE inhibition. The pathophysiological consequences of the sodium-associated increase in renal ACE activity should be subject of further study.

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