Introduction

Antihypertensive therapy has always been the cornerstone of renoprotective intervention. The last 20 years progress has been made in retarding the progression rate of renal function loss in both diabetic and non-diabetic renal diseases, since the successful introduction of agents that intervene in the renin-angiotensin-aldosterone system (RAAS). Several large clinical trials suggest that the renoprotective efficacy of RAAS blockade is not only due to the fact that RAAS-blockers are effective antihypertensives in the renal population, but also to specific renoprotective properties of RAAS-blockade, in particular their antiproteinuric properties. In non-diabetic patients, the AIPRI (1) and REIN-study (2) showed that angiotensin-converting enzyme (ACE) inhibitors attenuate renal function loss more effectively than a conventional antihypertensive regimen. These two studies included recommendations about sodium intake. In type 1 diabetes Lewis et al. (3) showed a renoprotective effect of ACE inhibition, whereas three studies in type 2 diabetic patients, RENAAL (4), IDNT (5) and IRMA-2 (6), demonstrated AII antagonists to be renoprotective.

However, other large studies could not confirm the renoprotective superiority of RAAS-blockade over other antihypertensives. Recently, the ALLHAT study (7), which is the largest trial ever comparing different classes of antihypertensive agents in more than 30,000 patients, failed to demonstrate additional benefit of ACE inhibitors over conventional antihypertensives in diabetic and in non-diabetic patients. However, the UKPDS study (8), a large, well-conducted trial in type 2 diabetic patients did not support better renoprotection by RAAS-blockade over beta-blockade either. Although the importance of blood pressure is reiterated by all of these trials, it is important to recognize that the ALLHAT showed a big difference in blood pressure between the comparator drugs in favor of the non-ACE inhibition therapies. In addition, comparison with beta-blockade may not be the best to answer the above question, since beta-blockade is known to also inhibit the RAAS. Thus, these trials do not necessarily negate the potential extra benefit of RAAS-blockade.

Sodium restriction has long since been shown to enhance the reduction of blood pressure by diuretics (9) and beta-blockers (10). This does not only hold true for older classes of antihypertensives that were associated with reactive sodium retention in themselves, but notable also for the newer classes of antihypertensives such as blockers of the RAAS (11). Unfortunately, in the recent large trials comparing the effects of various antihypertensive regimens on reduction of target organ damage, control of sodium status was not taken into account as a relevant modifier of therapeutic efficacy.

Despite the overall advances that have been made in renoprotective intervention by whatever regimen, chronic renal failure is still essentially a progressive condition, with - albeit retarded - still progressive renal function loss in many patients, leading to the need
for renal replacement therapy in many of them. The latter poses a tremendous burden on both the patient and society. Obviously, therefore, it would be crucial to improve the efficacy of our current renoprotective regimens. In this respect, different strategies stand out as important. In chapter 1 we already described several options. First, it would be important to fully exploit the potential of measures known to enhance therapy response, such as control of sodium status. Second, recent insights in the relevance of individual differences in genetic and environmental factors on therapeutic efficacy might allow new perspectives for improvement of renoprotective intervention.

In this final chapter, therefore, we will give an overview of the renoprotective effects of RAAS-blockade by ACE inhibition and AII antagonists from the perspective of optimizing renoprotective intervention. This will include a brief review of their efficacy in different populations, and of the determinants of differences in therapeutic efficacy, and summarize the new insights in the mechanisms of sodium-induced resistance to blockade of the RAAS by ACE inhibition that were obtained by the studies described in this thesis.

### Blood pressure and renal function loss

Blood pressure is an important risk factor for renal function loss, as discussed in chapter 1. Several studies pointed out that a more aggressive blood pressure control is beneficial on the course of renal function loss in renal patients.

Indeed, in patients with diabetic nephropathy the importance of aggressive blood pressure reduction for renal function preservation has long since been demonstrated (12;13). In non-diabetic patients several studies showed that blood pressure level was an important contributor to progression of chronic renal failure (14-16).

Bakris et al performed a meta-analysis of long-term clinical trials of blood pressure lowering in both diabetic and non-diabetic patients (17). This analysis showed a linear relationship between the obtained blood pressure and the rate of decline of renal function across the different studies. Thus, the available evidence indicates that blood pressure reduction is prerequisite for renoprotection. Nevertheless, interesting differences in renoprotective potency between different regimens have also been observed, as outlined below.

### Renoprotective effect: does it depend on antiproteinuric potency of antihypertensive class?

Over the last decade, several studies found additional renoprotective benefits of ACE inhibition in comparison with conventional antihypertensive treatment. These observations were made both in diabetes (type 1 and type 2) as well as in a variety of non-diabetic renal
diseases. (3;18;19). In addition, three recent randomised double-blind placebo controlled studies investigated the renoprotective effect of RAAS intervention by AII antagonists (4) (5;6). Treatment with an additional AII antagonist was renoprotective and the changes or differences in blood pressure that were achieved could not explain these differences. Again proteinuria was reduced, and the amount of proteinuria reduction followed the dose-response in renoprotection.

In contrast, the UKPDS, in type 2 diabetic patients, demonstrated the long-term benefit of a lower than usual blood pressure goal (20), but an additional benefit of the ACE inhibitor captopril against conventional treatment with the beta-blocker atenolol could not confirmed in reaching the endpoint of renal failure (8). Also, a small randomized double blind parallel study comparing lisinopril and atenolol in hypertensive type 2 diabetic patients reported an identical blood pressure reduction and GFR decline after a follow-up of almost 3 years (21). In addition, the ABCD study did not show a larger benefit of ACE inhibition on renal function loss in either hypertensive and non-hypertensive type 2 diabetic patients (22;23). Likewise, in a relative small clinical study of Apperloo et al., patients were randomised to beta-blocker or ACE inhibitor therapy. This study could not demonstrate better renoprotection in patients treated with ACE inhibition. Of note, in this study, it was prospectively shown that the antiproteinuric effect during therapy independently predicted renal prognosis, independently of class of drug (24). The observation that proteinuria could be a key factor in the eventual renoprotection during RAAS blockade was subsequently confirmed by results from many studies in non-diabetic renal patients (1;2). A meta analysis of several trails in majority without a significant difference between different antihypertensive regimes indicate that the beneficial effect of ACE inhibitors is mediated by factors in addition to decreased blood pressure and urinary protein excretion and is greater in patients with proteinuria (25). Recently, the results from the COOPERATE trial, comparing the combination treatment with ACE inhibitor and AII antagonist versus both monotherapies were published (26). In this study blood pressure was kept almost equal in the three therapy arms during 3 year follow-up, and, moreover, the renoprotective benefit of dual RAAS blockade was accompanied with better reduction of proteinuria than with single treatment.

Thus, data available from the many trials strongly suggest that RAAS intervention has a renoprotective effect that goes beyond its antihypertensive effect in different renal diseases. However, in most studies, blood pressure was not similar in the tested arms, and notably lower in the RAAS intervention arm. Furthermore, as stated before, the ALLHAT trial did not demonstrate superiority of RAAS blockade in any particular subpopulation (7), with the remark that no equal blood pressure reduction was obtained in the different treatment arms, and the study not specifically was designed for renal patients. These studies re-emphasise not only once again the importance of blood pressure reduction as an important determinant
of renoprotection, but also the putative role of proteinuria in renoprotection. This was also confirmed by meta-analyses (25). To date, all studies with RAAS blocking therapy showed that intervention in the RAAS led to a reduction of mean urinary protein excretion with, however, large individual differences in antiproteinuric response between patients. This antiproteinuric effect was significantly higher than with all the other treatment strategies, and it was the single most important factor predicting subsequent renal prognosis.

In summary, there is increasing evidence that the antiproteinuric effect is the crucial factor in the renoprotective properties of RAAS blockade. In this respect, interesting inferences can be made from analysing the individual responses to RAAS blockade to those on comparator regimens. This shows that subjects with a poor antiproteinuric response – either on ACE inhibition or beta-blocker – have a more progressive renal function loss, whereas a good response leads to retarded renal function decline. These findings argue strongly in favor of proteinuria as an independent factor in renoprotection, that is, independent from class of drugs.

Proteinuria: independent risk factor for renal prognosis during therapy

Indeed, proteinuria is nowadays looked upon as an independent risk factor of progressive renal function loss, and not merely a consequence of renal disease, as pointed out first by Remuzzi et al. (27). In different renal conditions, both in man and experimental renal diseases, proteinuria consistently determines the rate of progression of renal function loss. Taken together with experimental data demonstrating a tubulotoxic effect of leaked proteins (28), these data support the pathogenic role of proteinuria in progressive renal damage.

The relation between proteinuria and renal function decline appears to be three-fold. First, baseline proteinuria is an important determinant of the renoprotective benefit after reduction of blood pressure. The additional benefit of a lower blood pressure goal was clearly more pronounced in patients with a higher baseline proteinuria (16). Second, also the extent to which proteinuria is lowered during antihypertensive treatment is of prognostic value, in diabetic as well as non-diabetic renal patients (29). Third, the residual proteinuria during treatment is correlated with the subsequent progression of renal function loss, no matter what drug was used, be it ACE inhibitor or beta-blocker (24). These statements have now been confirmed by the results from large randomised clinical trials with ACE inhibitors as well as AII antagonists. (26;30;31).

The observation that residual proteinuria is a main prognostic factor has considerable implications for therapy, as measuring residual proteinuria during therapy can serve as
short-term indicator of long-term renoprotective efficacy. This allows titration of therapy, especially using RAAS blockade, to obtain optimal renoprotection by reducing proteinuria as far as possible. Indeed, maximum reduction of proteinuria to the lowest possible levels as a treatment target for each individual renal patient has been recommended (32-34). It has to be noted, however, that prospective studies titrating for proteinuria have not yet been performed, and prospective evidence that titrating for proteinuria will improve renoprotection is still lacking. In this respect, it may be relevant that experimental data indicate that residual proteinuria may reflect a poor renal prognosis as such; however, considering the evidence for an independent nephrotoxic effect of leaked proteins into urine, the next step in optimising renoprotective strategies is obviously to titrate for proteinuria. Also, the COOPERATE trial indicates that the individual response to therapy should be considered, since this study demonstrated that, although much progress can be made by optimising RAAS blockade on group level, large differences between patients exist in their antiproteinuric response and that these differences contribute to the renal prognosis (26). Thus, optimization of renoprotection will require an individual approach, aimed at reduction of blood pressure and, in addition, proteinuria. This approach awaits confirmation in a prospective fashion, but some inference can be already be made from the results of small studies, as described below.

### Optimizing renoprotection by titrating to the antiproteinuric response

Based on the data from the MDRD and REIN study, it has been advocated that the treatment target for proteinuria should be below 1 g/day, and likely near zero, for each renal patient to ensure optimal renoprotection (16;33). Indeed, different strategies to optimize the antiproteinuric response during blockade of RAAS are available, as listed in Table 1.

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<tr>
<th>Table 1 Strategies for optimising the antiproteinuric response during RAAS blockade</th>
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<td>Correction of volume excess:</td>
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<td>- restriction of dietary sodium to 50 mEq/d (35)</td>
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<td>- combined therapy with diuretic (36)</td>
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<td>Dose-titration to reduce proteinuria further than needed for blood pressure control (37)</td>
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<tr>
<td>Combined treatment of both ACE inhibitor and AII antagonist (26)</td>
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<td>Combined treatment with statin (38;39)</td>
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<td>Intervention in prostaglandins:</td>
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<td>- combined treatment with NSAID (40)</td>
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First, it has long since been shown that the therapeutic effects of RAAS blockade depend on volume status (35;41). The antiproteinuric response of ACE inhibition, for example, was almost annihilated by an increase of sodium intake from 50mEq/d to 200mEq/d in patients with proteinuria in the nephrotic range (35), whereas the blood pressure response was blunted to a lesser extent. Notably, a small study from our group in non-diabetic proteinuric patients showed that this blunted antiproteinuric effect can be restored by the addition of diuretic therapy with hydrochlorothiazide (36), supporting the importance of control of volume status for an effective therapy response. Corresponding observations have been made for the combination of AII antagonists and sodium depletion (42;43).

Second, different studies in both diabetic and non-diabetic proteinuric patients demonstrate that the dose-response curves of RAAS blocking agents for blood pressure and proteinuria may not always be similar, as apparent from the IRMA-2 study (6). In this trial, irbesartan 150 mg o.i.d. showed less protection against progression to overt diabetic nephropathy than irbesartan 300 mg, accompanied with a less effective proteinuria reduction, but equal efficacy on blood pressure reduction. However, different titration studies with AII antagonist, such as losartan and candesartan, show that the maximal recommended dose for blood pressure reduction also renders the maximal antiproteinuric response, and higher doses does not result in further proteinuria reduction (31;44;45). By contrast, increasing the ACE inhibitor dose seems not to show such flattening in the dose-response, indicating that higher doses of ACE inhibitor than needed for blood pressure control result in further proteinuria reduction (37).

Third, as mentioned earlier, the COOPERATE trial demonstrated better renoprotection of combined treatment with both an ACE inhibitor and an AII antagonist (26). Smaller clinical studies in diabetic as well as non-diabetic renal patients showed that dual RAS blockade led to further proteinuria reduction than with maximal recommended doses of monotherapy could be obtained (37;46;47).

Despite the demonstrated efficacy of these measures on group level, limited data are available on whether it is feasible to specifically pursue the lowest level of proteinuria by individual dose-titration with these measures. A small practice-based clinical study in non-diabetic patients with residual proteinuria during previous RAAS intervening therapy indicates that individual titration for maximal RAAS blockade has its limits. In this study, individual dose-titration of an ACE inhibitor on top of high dosed AII antagonist and diuretic therapy in order to lower proteinuria below 1 g/d induced as expected further reduction of the residual proteinuria on group level. However, in regard with the individual patient, the treatment target was reached in a low number of patients as compared with the increasing amount of patients that experienced adverse events for each titration step (Figure 1).
The increased occurrence of side effects during up-titration suggests that attempts to intensify the RAAS blockade based regimen by trial-and-error to overcome individual therapy resistance may not be a feasible solution for all patients. Again, despite increasing efficacy at group level, measures for optimization will hardly be of benefit to poor responders. This prompts for specific focus on the mechanisms of therapy resistance, in order to design specific measures that improve therapy response in high risk subjects.

Renal mechanisms underlying resistance to RAAS blockade

Experimental data could shed more light on several of the determinants of therapy resistance, i.e. the severity of pre-existing renal damage at onset of therapy, the role of the renal ACE activity, and the role of sodium status, particularly in relation to ACE activity. The role of pre-existent renal damage was prospectively studied in adriamycin-nephrotic rats. In this study, it was found that the efficacy of ACE inhibition in preventing development of focal glomerulosclerosis and macrophage influx was strongly set by prevalent damage before treatment, despite the fact that the extent of renal damage was still very mild and in fact did not exceed the stage of pre-fibrotic changes (48). A post hoc analysis of the REIN support these findings, as in this sub-study baseline GFR predicted renal outcome during ramipril...
treatment after 2.6 years follow-up (49). None of the patients within the highest tertile of baseline GFR reached the end-point of end-stage renal disease, whereas, importantly, in patients within the lowest tertile the risk of end-stage renal disease was lowered with 22% compared to those patients treated with conventional antihypertensives.

Also, the extent of inhibition of ACE activity specifically in renal tissue has been proposed to be an important determinant for the eventual renoprotection, as also suggested by the earlier mentioned observation that the dose-response for proteinuria differs between ACE inhibitors and AII antagonists (37). In this respect, a recent study in adriamycin-induced proteinuria reported that both proteinuria and focal glomerulosclerosis strongly correlate with the amount of intra-renal ACE activity, in the untreated condition, as well as during renoprotective treatment with ACE inhibition or AII antagonist. However, plasma ACE activity was negatively associated with renal damage in this study. These findings indicate, first, the importance of intra-renal ACE as opposed to plasma ACE for the development of renal damage, second, that it is unlikely that the higher renal ACE in the animals with more damage during ACE inhibition is due to less effective dosing, as this would presumably have been reflected in plasma ACE as well. The latter assumption is supported by the findings in the animals treated with AII antagonist therapy. Together, these data suggest that intra-renal ACE activity may be a permissive or promoting factor in the processes by which proteinuria eventually leads to renal structural damage.

These data point towards the relevance of the regulation of renal ACE as a factor involved in renal damage. In this respect it is important to note that, apparently, intra-renal ACE activity during ACE inhibition (at least within the context of these well-established high doses) is not primarily governed by the pharmacologic properties of the drug. Individual differences in regulation of renal ACE due to genetic factors may be relevant in this respect (50;51). Moreover, renal ACE may be modified by environmental factors. Considering the consistent role of sodium as a cause of therapy resistance, it would be important to investigate the role of sodium status in the regulation of renal ACE as well, especially in the setting of renoprotective treatment.

This issue is explored in chapter 3 (High residual ACE activity). First, in normal Wistar rats we found that a high sodium diet was associated with a higher renal ACE activity than a low sodium diet, whereas no difference in plasma ACE activity was observed (Figure 2) which re-emphasizes the importance of studying renal tissue ACE - rather than just plasma ACE. When these healthy animals were treated with an ACE inhibitor, renal ACE was reduced to a similar low level during high and low sodium diet.

Subsequently, we studied the effects of changes in sodium intake on renal ACE activity...
(measured in renal cortical homogenate obtained by renal biopsy) in relation to therapy response during ACE inhibition in rats with adriamycin-induced proteinuria. In these proteinuric rats, the shift from low to high sodium intake during maintenance treatment ACE inhibitor led to a pronounced rise in residual renal ACE activity, paralleled by the anticipated rise in proteinuria. When shifting sodium-intake the other way round, a pronounced fall in proteinuria occurred, with a less pronounced fall in renal ACE activity (Figure 3).

These data support the assumption that sodium-induced effects on renal ACE activity are linked to therapy response. As proteinuria as such, however, could also lead to elevation of renal ACE (62, 64), it cannot be derived from these data whether the changes in renal ACE activity are primarily mediated by the change in sodium status, by the change in proteinuria, or both. This issue clearly requires further study. Nevertheless, these data support the assumption that a rise in sodium intake can elicit a vicious circle of ongoing renal damage, with a rise in proteinuria that is associated with an increase in renal ACE activity, which in turn can aggravate renal damage. As organ-specific ACE activity and its functional consequences are difficult to measure in man, further studies in experimental models are warranted.

**Effects of sodium intake on the vascular effects of ACE inhibition**

The above study demonstrated the importance of studying ACE at tissue level in order to
understand its role in sodium-induced therapy resistance. The vascular bed is another main target tissue relevant to the therapeutic effects of ACE inhibition. Interestingly, well-established in vitro vascular pharmacological methods are available to assess the functional local effects of changes in ACE activity in vascular tissue - by comparing the vasoconstrictor effects of angiotensin II and angiotensin I in the isolated blood vessel. In this set-up the structural integrity of the target tissue is preserved - which contrasts with the measurements tissue ACE as the cleavage of an artificial substrate (Hip-His-Leu) by renal cortical homogenates as used in our studies on renal ACE. Therefore, to investigate the functional effects of differences in sodium intake on tissue ACE activity during ACE inhibition, we studied the effects of ACE inhibitor treatment during different sodium intakes in isolated aortic rings of normal rats, as described in chapter 4 (High dietary sodium blunts effects of ACE inhibition on vascular angiotensin-I to angiotensin-II conversion in rats). In this study we investigated the functional conversion of angiotensin I-to-II in rings of the rat aorta. As reduction of angiotensin-II formation is usually considered to be the main mechanism of action of ACE inhibition, the effect of ACE inhibition on angiotensin-I induced responses at the site of action could be considered as an index of the ultimate effects of ACE activity. By this method we found a response to angiotensin I that was blunted relative to angiotensin II (i.e: functional inhibition of local ACE activity) in isolated aortic rings of rats that had been treated with ACE inhibition during low dietary sodium, but not in rats treated with ACE inhibition during a high sodium intake. Based on these data we conclude that high dietary sodium blunts the effect of ACE inhibition on the functional vascular angiotensin I to angiotensin II conversion in rats. Thus, insufficient suppression of local angiotensin II formation in the vascular bed might play a role in the reduced therapy response during high dietary sodium.
In this study, the increase in functional angiotensin II formation in the rat aorta of high sodium treated with ACE inhibition was not accompanied with an increase in cleavage of an artificial substrate (Hip-His-Leu) by vascular tissue homogenate. This might reflect the difference in methodological approach but might also indicate that factors other than local presence of ACE are involved in the sodium-induced differences in vascular responses to angiotensin I. First, non-ACE mediated angiotensin I-converting pathways might be present. Second, non-RAAS vasoactive pathways might also be involved in the interaction between sodium status, ACE inhibition and the vascular bed. In our studies we did not address non-ACE pathways, as these are assumed not to be relevant in rat vasculature. However, with respect to the second possibility, many studies showed improved vessel wall structure and improved endothelial dependent vasodilation in cardiovascular disease after chronic ACE inhibition therapy, supporting the importance of effects on endothelial function in the therapeutic efficacy of ACE inhibition. Therefore, in Chapter 5 (Low sodium modifies the vascular effects of ACE inhibitor therapy in healthy rats) we tested the effects of dietary sodium, ACE inhibition and the combination on endothelial function in renal and mesenteric arteries. In accord with our hypothesis, we found that dietary sodium restriction modifies the vascular effects of maintenance treatment with ACE inhibitors. This was either due to a direct effect or due to the potentiated blood pressure reduction. First, sodium and ACE inhibition changed basal vessel characteristics differently in small renal and mesenteric arteries. Whereas ACE inhibition alone increased the lumen diameter and the decreased adrenergic contractility in the interlobar renal arteries, ACE inhibition alone had no effect on mesenteric arteries. Additional dietary sodium restriction had no effect on the renal arteries. Mesenteric arteries however, were more prone to constrict after the combination of ACE inhibition and low dietary sodium. This may illustrate the different function of both vessels. Whereas mesenteric arteries are considered resistance vessels regulating blood pressure and become constricted during reduced blood pressure, the renal vessels ensure renal blood flow and remain dilated due to the autoregulatory properties of the renal vascular bed. Therefore, the effect of additional sodium restriction reveals the heterogeneity of vascular function rather than explaining enhanced therapy response. The effect of adding sodium restriction to ACE inhibition on blood pressure and proteinuria can be classified as favorable. However, whether the vascular effects observed in this study can be interpreted as favorable from the perspective of organ protection is questionable. In fact, in these healthy animals the endothelial effects might reflect an appropriate compensatory reaction of the vascular bed to the considerable reduction in blood pressure. On the other hand, they might also be considered as an unwanted side effect that limits the therapeutic benefit of the potentiated fall in blood pressure. However, the clinical conditions that respond to ACE inhibition (i.e. cardiovascular and renal conditions) are invariably characterized by endothelial dysfunction. This is likely to be of relevance to the endothelial effects of low sodium superimposed to ACE inhibition. Whether and how the effects of sodium status on endothelial function
will eventually modulate the effect on target organ protection should be subject of further study in disease models. For the moment, our results in this study should definitely not be taken to discard low sodium as an adjunct to ACE inhibition. They do provide a rationale for the further exploration of the mechanism of action of the vascular effects of ACE inhibition combined with low sodium therapy.

**Individual patient factors underlying resistance to RAAS blockade**

As noted earlier, the individual differences in responsiveness to RAAS-blockade are large. In a prior study of our group it was demonstrated in diabetic and non-diabetic proteinuric patients (55) that increasing the dose of the ACE inhibitor or AII antagonist did not make the patients with a relatively poor response catch up with the good-responders - and neither did a shift from ACE inhibition to AII antagonist therapy or vice versa (Figure 4).

Obviously, it would be of interest whether combined blockade would specifically be of benefit in these non-responders. Data from the COOPERATE trial (26), however, suggest that this would not be the case, as this study showed that a poor therapy response during prior treatment with ACE inhibitors was independently predictive for the eventual renal outcome. However, to specifically answer this question, the appropriate design would be to stratify subjects as being poor versus good responders to ACE inhibitors and subsequently test the effects of dual blockade. No human studies have been performed according to this design, but experimental data have been obtained in adriamycin-nephrotic rats, i.e. a model of proteinuria-related renal damage. In this study addition of AII antagonist therapy to the ACE inhibitor, or doubling the dose of the ACE inhibitor to a supramaximal dose could not overcome therapy resistance to monotherapy ACE inhibition therapy (56). Thus, individual factors appear to be highly important in the responsiveness to therapy.

Several lines of evidence suggest that individual factors may also be relevant to the modifying effects of sodium intake on therapy response. First, it is well-established that the homeostatic responses to altered sodium intake differ widely between individuals. The differences in the response of blood pressure to altered sodium intake in man have given rise to the concept of sodium sensitivity of blood pressure. Whereas genetic factors appear to be involved in sodium sensitivity (57), superimposed renal factors, such as renal function impairment can also modify sodium sensitivity of blood pressure (58). Second, studies analyzing the interaction between sodium status and the response to RAAS-blockade have shown that the impact of altered sodium status on the responses of blood pressure and proteinuria to RAAS-blockade can also vary widely between different individuals (figure 5) (36;59).
Whereas a large body of data is available on the individual differences in the response of blood pressure to altered sodium status, the renal response to altered sodium status, however, has attracted much less interest, and has only been documented in subjects with essential hypertension. (60;61). These studies revealed distinct differences in the renal hemodynamic responses to altered sodium intake, that are assumed, however, to be specific for hypertension. Therefore, in chapter 6 (High sodium intake can induce an unfavorable renal hemodynamic response in healthy, normotensive man) we investigated the renal hemodynamic responses to a change in sodium intake in normotensive volunteers, with specific emphasis on the individual differences in response. In this study in health young adults we found the expected rise in ERPF and GFR at group level, with however a wide interindividual range that amounted to a drop in ERPF during high sodium in approximately one third of these subjects. In these subjects, moreover, GFR did not increase during high sodium, which suggests that high sodium induces an unfavorable intrarenal pressure profile in these subjects. Studies that are currently ongoing will elucidate whether these individual differences are also relevant to the modifying effect of sodium on the response to ACE inhibitor therapy.

Figure 4 Antiproteinuric effect of the ACE inhibitor enalapril 10mg o.i.d. and the AII antagonist losartan 100mg o.i.d. administered to the same patients. Close correlation is observed between the effect of both interventions in the same patient, be it in a non-diabetic (NDRD, triangles) or in diabetic (IDDM) proteinuric patient (Adapted from Bos H, Andersen S, Rossing P et al. The role of patient factors in therapy resistance to antiproteinuric intervention in non-diabetic and diabetic nephropathy. Kidney Int Suppl 2000;75:32-37).
Effects of sodium status and ACE inhibition on angiotensin (1-7)

In the previous paragraphs we focused on tissue and end organs as target for the effects of ACE inhibition on angiotensin I-to-II conversion and endothelial effects but this is hard to address in man. In man we therefore focused on possible relevant changes in circulating components of the RAAS during ACE inhibition and low sodium as described in chapter 7. In human volunteers we tested our concept of an increased angiotensin (1-7) concentration during a combined ACE inhibition and low dietary sodium (Sodium status and ACE inhibition: effects on plasma Angiotensin-(1-7) in healthy man). Angiotensin-(1-7) has vasodilator and antiproliferative properties, and may therefore be a physiological antagonist to angiotensin II (62). In this line of reasoning, an altered balance between the vasodilator angiotensin-(1-7) and the vasoconstrictor angiotensin II has been proposed to be involved in the mechanism of action of ACE inhibition (63). We found the expected rise in plasma angiotensin I concentration after ACE inhibition and a further increase during low sodium. The angiotensin II concentration was significantly decreased after 1 week of ACE inhibition without a significant difference between a high and low dietary sodium diets. In accordance with our hypothesis, the concentrations of angiotensin (1-7) followed those of Angiotensin-I and, consequently were significantly increased during ACE inhibition. Additional dietary sodium restriction further increased the angiotensin I and angiotensin (1-7) concentration furthering a parallel fashion. Thus the beneficial effect of additional dietary sodium restriction could be partly explained by higher Angiotensin (1-7) levels. In this respect it may be of interest that a homologue of ACE, ACE-2, was recently discovered (71). ACE 2 appears to counteract effects of ACE by cleaving angiotensin I into vaso-inactive angiotensin(1-9) and cleaving angiotensin II into the vasodilative angiotensin (1-7). Moreover, in the kidney it co-localises with ACE, a finding consistent with an alleged counteracting function (65). Future functional studies will have to elucidate the role of ACE-2 in the development and prevention of progressive renal damage.

Conclusive remarks and look towards the future

Large advances in renoprotection have been afforded by the availability of effective classes of drugs interfering with RAAS activity. Nevertheless, chronic renal disease is still essentially a progressive condition, which prompts for further improvement of renoprotective efficacy. When pursuing optimization of the response to RAAS-blockade, it is important to consider that between-patients differences in therapy response by far exceed the differences between different RAAS-blockers, and moreover, that within-patient changes in therapy response induced by shifts in sodium intake are large as well. Considering the quantitative impact of these effects, specifically targeting the mechanisms underlying these forms of therapy resistance might afford considerable improvement in renoprotective efficacy. The
explore-ration of the mechanisms underlying the effects of sodium-status on the response to ACE inhibition pursued here identified two relevant candidate mechanisms. First, effects of sodium status on tissue ACE in the vessel wall and the kidney, and second, effects of sodium status on the vasodilator angiotensin angiotensin (1-7). These data prompt further explore-ration of the regulation of tissue ACE, in relation to sodium status and ACE inhibition as a possible modulator of therapy response. As ACE gene polymorphism is an important determinant of the regulation of plasma and tissue ACE level in man, it would be important to explore the interaction between sodium intake, ACE gene polymorphisms and therapy response to ACE inhibition. Considering the possible role of ACE, it would be of interest, furthermore, to investigate whether add-on therapy with AT1 receptor blockade might restore the therapy response to ACE inhibition during high sodium.

Further study of the role of smaller angiotensins in the response to ACE inhibition may also allow possibilities to improve therapy response, by devising strategies to stimulate the vasodilator angiotensins as endogenous vasodilator and antiproliferative substances. Their regulation is still incompletely understood, and involves effects of enzymes other than ACE, such as NEP, and notably, the recently discovered ACE-2. Further exploration of these newer components of the RAAS may allow making better use of their protective properties. It is remarkable indeed that - more than 20 years after the first introduction of ACE inhibitors - the window of opportunity for the therapeutic potential of these drugs is still increasing, allowed by a better insight in the physiology of the RAAS.

Finally, whereas these possibilities may open new avenues towards improvement of reno-protection, obviously, in clinical practice for the moment proper consideration for correction of sodium excess is warranted!
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