SUMMARY

The studies described in this thesis focus on cortisol metabolism, and in particular on the activity of 11β-hydroxysteroid dehydrogenase (11BHSD), and their contribution to the pathogenesis of several clinical conditions, characterized by abnormalities in volume or glucose homeostasis.

Chapter 1 contains a general overview on cortisol metabolism and 11BHSD activity. The intracellular enzyme 11BHSD catalyses the interconversion between the hormonally active cortisol and inactive cortisone. In humans, two isozymes exist, designated as 11BHSD type 1 and 11BHSD type 2. In vivo, 11BHSD type 1 behaves as a NADPH-dependent reductase, thus activating cortisone towards cortisol. 11BHSD type 2 functions as a NAD+-dependent dehydrogenase, thereby inactivating cortisol into cortisone. In addition to differences in catalytic activity, these two isozymes also have a different tissue distribution pattern. 11BHSD type 1 is ubiquitously expressed, but is notably absent in human kidney. In contrast, expression of 11BHSD type 2 is more limited to the mineralocorticoid target organs (i.e. kidney, colon, salivary glands) and placental tissue. The 11BHSD isozymes can be regarded as molecular switches, regulating the bioavailability of cortisol for binding to its receptor. Besides activating the glucocorticoid receptor, cortisol also has the ability to activate the mineralocorticoid receptor, which binds cortisol with the same affinity as aldosterone in vitro. In vivo, stimulation of the mineralocorticoid receptor by cortisol is prevented by the cortisol inactivating effect of 11BHSD type 2. Thus, 11BHSD type 2 confers specificity to the mineralocorticoid receptor in the presence of glucocorticoids. The activity of 11BHSD in vivo is usually assessed by measurement of the ratios of plasma cortisol/cortisone and urinary (THF + allo-THF)/THE, reflecting the overall index of 11BHSD activity. In addition, the UFF/UFE ratio has been proposed to reflect more specifically the activity of 11BHSD type 2 in kidney. The impact of 11BHSD in several clinical conditions, such as AME syndrome, liquorice abuse, hypertension, diabetes mellitus and obesity, is discussed.

Chapter 2 describes the effect of acute administration of angiotensin on 11BHSD activity. We examined 21 male healthy subjects after 1 week of a low- and high-salt diet. Separate infusions of angiotensin-I and II were administered, both at two different doses. Glomerular filtration rate was accurately measured, in order to adjust for angiotensin-mediated changes in renal function.

Angiotensin-I and II infusion dose-dependently increased mean arterial blood pressure and plasma aldosterone, and decreased plasma renin activity and glomerular filtration rate at both diets. During angiotensin-I and II infusion, we observed a dose-dependent decrease in the excretion of UFF, UFE and of the UFF/UFE ratio
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(THF+allo-THF)/THE ratio. Salt restriction did not affect these 11BHSD parameters, but was accompanied by a decrease in UFF and UFE excretion.

These data suggest that acute angiotensin administration stimulates the activity of 11BHSD type 2 in human kidney. We therefore hypothesize that angiotensin exerts a dual effect on the mineralocorticoid receptor, i.e. an indirect agonistic effect by increasing aldosterone availability and a direct or indirect antagonistic effect by stimulation of renal 11BHSD type 2 activity resulting in a decreased cortisol-mediated receptor activation. The observed effect of dietary salt intake on UFF and UFE excretion is most likely due to sodium-induced alterations in cortisol elimination, as further described in chapter 4.

Chapter 3 addresses the question whether an altered setpoint of the 11BHSD mediated cortisol to cortisone interconversion towards cortisol is pathogenetically involved in the sodium retention of patients with the nephrotic syndrome. Therefore, we studied the serum cortisol/cortisone ratio and the ratios of urinary (THF+allo-THF)/THE and of UFF/UFE in 8 proteinuric patients and in 8 matched healthy subjects. In addition, the effect of angiotensin-II receptor blockade on 11BHSD activity was evaluated, by placebo-controlled administration of losartan 50 mg/day during 4 weeks.

There were no significant differences between proteinuric patients and healthy subjects in serum cortisol, serum cortisone, serum cortisol to cortisone ratio, or in the urinary excretions of THF, allo-THF, THE, sum of cortisol metabolites or (THF+allo-THF)/THE ratio. UFF excretion and the UFF/UFE ratio were lower in proteinuric patients than in healthy subjects. Losartan treatment resulted in significant reduction of mean arterial pressure and proteinuria, but did not affect cortisol metabolism.

In contrast to previous studies, our data suggest an increased renal inactivation of cortisol in proteinuric patients. Thus, this study does not support the contention that an altered 11BHSD activity contributes to sodium retention in patients with the nephrotic syndrome. Moreover, although a relatively low dose of losartan reduces mean arterial pressure and proteinuria, it does not exert a significant effect on the cortisol to cortisone interconversion.

Chapter 4 is directed at the question whether the sodium-dependency of blood pressure (i.e. salt sensitivity) is linked with changes in cortisol metabolism. As previous studies have suggested that salt loading can affect insulin sensitivity, we also examined whether changes in insulin sensitivity after salt loading are associated with alterations in cortisol metabolism. Therefore, we studied cortisol metabolism and insulin sensitivity in 28 healthy normotensive subjects after a low- and high-salt diet. Based on their blood pressure response to salt loading, 19 subjects were classified as salt-resistant and 9 subjects as salt-sensitive.
Interestingly, we found that the sum of urinary cortisol metabolite excretion after salt loading increased in salt-resistant and decreased in salt-sensitive subjects. This was accompanied by a decrease in morning plasma cortisol levels in salt-resistant subjects, which did not change significantly in salt-sensitive subjects. Moreover, changes in cortisol metabolite excretion after salt loading were inversely related to changes in plasma morning cortisol. The most likely explanation of these observations is that salt loading increases cortisol elimination rate in salt-resistant subjects, a response which is blunted in salt-sensitive individuals. We suggest that this variation in cortisol elimination is probably caused by sodium-induced alterations in liver hemodynamics, as has been demonstrated previously by others. Although liver hemodynamics were not studied, we did observe that a sodium-induced decrease in blood pressure was accompanied by an increase in renal vasodilatation. A similar hemodynamic response in the liver is expected to result in enhancement of cortisol elimination. Indeed, we did find that an increase in renal vasodilatation was accompanied by an increase in the sum of urinary cortisol metabolites. Although insulin sensitivity, as determined by homeostatic model assessment (HOMA), did not change significantly in either group, analysis of all subjects together revealed that a rise in insulin resistance was associated with an increase in plasma morning cortisol and a decrease in the sum of urinary cortisol metabolites excreted. Salt-resistant and salt-sensitive subjects demonstrated similar activities of 11βHSD, which was not affected by variation in dietary salt intake.

We conclude that salt loading increases cortisol elimination in salt-resistant normotensive subjects, a response which is blunted in salt-sensitive subjects. In addition, changes in circulating cortisol might explain individual variations in insulin sensitivity in response to alterations in dietary sodium intake.

Chapter 5 is aimed at evaluating cortisol production and the 11βHSD activity in type 1 diabetic patients. As several previous studies might have been confounded by gender distribution and differences in body mass index and dietary sodium intake, we studied cortisol metabolism during a low- and high-salt diet in 14 male, nonobese, normotensive type 1 diabetic patients without severe complications and a HbA₁c less than 8.5% and in 14 individually matched healthy subjects.

We found that the sum of urinary cortisol metabolite excretion during both diets was significantly lower in diabetic patients than in healthy subjects. In addition, the allo-THF excretion and allo-THF/THF ratio were lower in diabetic than in healthy males during both diets. The ratios of urinary (THF+alloTHF)/THF and UFF/UFE were similar in both groups and remained unchanged after salt loading.

Thus, the sum of urinary cortisol metabolite excretion as a measure of cortisol production is lower in nonobese, normotensive type 1 diabetic males with
adequate glycaemic control and without severe complications, irrespective of sodium intake. This might in part be explained by a diminished 5α reductase activity, resulting in a decreased cortisol metabolic clearance. Moreover, there is no difference in 11βHSD setpoint between type 1 diabetic and in healthy males, and this setpoint is not affected by physiological variations in sodium intake.

Chapter 6 evaluates the relationship between insulin resistance and 11βHSD activity in type 2 diabetes mellitus. To this end, the urinary (THF+allo-THF)/THE and UFF/UFE ratios, as well as the plasma cortisol/cortisone ratio were measured in 8 male type 2 diabetic patients and 8 healthy male subjects without and after 24 h of insulin infused at a rate of 30 mU/kg/h to achieve moderate hyperinsulinaemia. Insulin was infused in the expectation that this would lower insulin sensitivity. Insulin sensitivity was assessed by measurement of whole body glucose uptake during a euglycaemic clamp (insulin infused at 30 mU/kg/h) directly after the 24 h insulin infusion and on a control day.

Baseline urinary (THF+allo-THF)/THE and plasma cortisol/cortisone at 0800h were similar in type 2 diabetic patients and healthy subjects, despite an impaired insulin sensitivity in the former group. Moreover, no correlation could be demonstrated between insulin sensitivity and baseline urinary (THF+allo-THF)/THE and plasma cortisol/cortisone. In type 2 diabetic patients, insulin sensitivity was further impaired by antecedent hyperinsulinaemia, but this impairment was not accompanied by changes in urinary (THF+allo-THF)/THE or in plasma cortisol/cortisone at 0800h. In healthy subjects, insulin sensitivity did not change significantly after 24 h insulin infusion, despite an increase in both urinary (THF+allo-THF)/THE and plasma cortisol/cortisone. The UFF/UFE ratio was not different between the two groups and was not affected by insulin administration.

In conclusion, this study does not support the hypothesis that insulin resistance in type 2 diabetes mellitus is associated with an overall change in the 11βHSD setpoint towards cortisol. The observed stimulation of 11βHSD reductase activity by insulin, as inferred from the increase in the urinary (THF+allo-THF)/THE and plasma cortisol/cortisone ratios, could further contribute to visceral obesity, in view of the stimulatory effects of insulin and cortisol on adipogenesis.

Chapter 7 focuses on recombinant human growth hormone (rhGH) replacement and its possible influence on the activity of 11βHSD and on the bioavailability of cortisone acetate tablets. A 12-month randomised, placebo-controlled rhGH replacement study was conducted. Twelve men and 12 women with GH deficiency of whom 17 received cortisone acetate participated.

No changes in urinary cortisol metabolites were observed after placebo. After 6 months rhGH the urinary (THF+allo-THF)/THE ratio was unaltered in cortisone acetate treated patients as well as in patients with intact adrenal function, whereas
after 12 months rhGH the (THF + allo-THF)/THE ratio decreased only in cortisone acetate treated patients. The sum of urinary cortisol metabolites did not change after rhGH in either group. The UFF/UFE ratio tended to decrease in cortisone acetate treated as well as in patients with intact adrenal function.

Thus, it is suggested that $\beta$HSD type 1 activity is decreased by rhGH replacement, which becomes manifest in patients receiving cortisone acetate substitution therapy. $\beta$HSD type 2 activity is unaltered or may even be increased. The bioavailability of conventional doses cortisone acetate is unlikely to be impaired by rhGH replacement in hypopituitary patients.

Chapter 8 describes a new assay, based on gas chromatography and mass spectrometry, to detect low concentrations of $\beta$-glycyrrhetinic acid ($\beta$GA) in urine, the hydrolytic product of glycyrrhizin. $\beta$GA inhibits the dehydrogenase activity of $\beta$HSD, and is therefore responsible for the mineralocorticoid side-effects of liquorice.

The detection limit for $\beta$GA of the gas chromatography assay was 10 µg/l, which was further lowered to 3 µg/l by subsequent mass spectrometry. The performance of this newly developed assay is demonstrated in a group of four volunteers consuming different amounts of liquorice. In addition, its clinical value is illustrated in two patients with hypokalaemic hypertension and suppressed plasma renin activity and aldosterone, in whom a suspicion of liquorice abuse could be confirmed by application of this new assay.

PERSPECTIVES

The central theme of the studies presented in this thesis is directed at the possible role of alterations in $\beta$HSD and cortisol metabolism in clinical conditions characterized by sodium and fluid retention or by changes in glucose homeostasis. In brief, our experiments do not support the contention that a diminished $\beta$HSD activity contributes to sodium retention in proteinuric patients or during acute administration of angiotensins. On the contrary, we found evidence for an enhanced activity of $\beta$HSD type 2 in these conditions, which is expected to diminish sodium retention. Although we were not able to demonstrate a direct relationship between alterations in $\beta$HSD activity and glucose homeostasis in either type 1 or type 2 diabetes mellitus, we did find that changes in cortisol metabolism might represent a link between sodium status and insulin sensitivity in healthy subjects. The next paragraphs will briefly discuss how future studies could deal with some questions raised by our studies, in particular with respect to insulin resistance, salt sensitivity and the renin-angiotensin-aldosterone system.
used only in the tissue-specific alterations in 11βHSD type 1 activity cannot be accurately determined by measurement of the commonly used urinary ratios of cortisol metabolites. Recent studies have provided additional evidence for the pathophysiological role of 11βHSD in the metabolic syndrome. Animal as well as human experiments have demonstrated that obesity is associated with tissue-specific alterations in 11βHSD type 1 activity. For example, 11βHSD type 1 activity in obese Zucker rats is reduced in liver but enhanced in adipose tissue. A similar distribution pattern of 11βHSD type 1 activity has been found in humans. The potential significance of 11βHSD in the pathophysiology of the insulin resistance syndrome has been substantiated further by the demonstration that the metabolic syndrome could be generated in transgenic mice selectively overexpressing 11βHSD type 1 in adipose tissue to an extent similar to that found in adipose tissue from obese humans. Thus it can be envisaged that a selectively enhanced 11βHSD type 1 activity will promote visceral obesity through the increased local production of glucocorticoids in visceral adipose tissue. This intriguing new pathophysiological mechanism of insulin resistance obviously provides an opportunity to develop specific therapies targeted at reduction of 11βHSD type 1 activity. Indeed, a recent experiment showed the efficacy of a selective inhibitor of mouse 11βHSD type 1, BVT 2733, in lowering blood glucose levels in spontaneously hyperglycaemic mice. Moreover, it has been demonstrated that fenofibrate, a PPARα agonist, has the ability to downregulate murine hepatic 11βHSD type 1 activity. Although hepatic 11βHSD type 1 activity seems to be decreased already in obese subjects, a further reduction of its activity could still improve glucose tolerance. Perhaps the previously observed improvement of insulin sensitivity during administration of fenofibrate is partly explained by its inhibition of hepatic 11βHSD type 1. Thiazolidinediones, which are PPARγ agonists, represent a new class of oral hypoglycaemic agents. Despite their proven benefit, the precise mode of action of thiazolidinediones is not completely understood. Interestingly, it has recently been demonstrated that rosiglitazone, a PPARγ agonist, can downregulate the activity of 11βHSD type 1 in adipose tissue. Therefore, the therapeutic efficacy of thiazolidinediones in the metabolic syndrome may at least in part be explained by inhibition of 11βHSD type 1 in adipose tissue.

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Chapter 8 describes a new assay, based on gas chromatography and mass spectrometry, to detect low concentrations of 18β-glycyrrhetinic acid (18βGA) in urine, the hydrolytic product of glycyrrhizin. 18βGA inhibits the dehydrogenase activity of 11βHSD, and is therefore responsible for the mineralocorticoid side-effects of liquorice.

The detection limit for 18βGA of the gas chromatography assay was 10 μg/l, which was further lowered to 3 μg/l by subsequent mass spectrometry. The performance of this newly developed assay is demonstrated in a group of four volunteers consuming different amounts of liquorice. In addition, its clinical value is illustrated in two patients with hypokalaemic hypertension and suppressed plasma renin activity and aldosterone, in whom a suspicion of liquorice abuse could be confirmed by application of this new assay.

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Future studies need to address the extent to which changes in 11βHSD activity contribute to the insulin resistance syndrome. In addition, it is to be expected...
that specific 11βHSD type 1 inhibitors will be developed, thus possibly providing a new class of drugs for treatment of the insulin resistance syndrome.

Salt sensitivity and cortisol metabolism
The inverse relationship between changes in cortisol metabolite excretion and blood pressure after salt loading in normotensive subjects, as described in chapter 4, has not been reported before. Our hypothesis that these changes depend on sodium-induced alterations in cortisol elimination needs to be confirmed by additional studies. For instance, the cortisol elimination rate could be assessed after a low- and high-salt diet by determination of the plasma half-life of labelled cortisol. If our hypothesis is correct, then salt-resistant subjects would demonstrate a shortened half-life of plasma cortisol after salt loading, as a result of an increased metabolic clearance. Alternatively, our hypothesis could be tested by continuous cortisol administration to adrenalectomized animals (e.g., Dahl salt-sensitive rats) at different salt intakes. In this model, cortisol elimination rate can be assessed indirectly as the amount of infused cortisol needed to stabilize plasma cortisol. Clearly, such a model has the advantage that compensatory activity of the HPA-axis is circumvented. As pointed out in chapter 4, alterations in circulating cortisol as well as in 11βHSD setpoint are unlikely to represent a major determinant of salt sensitivity. These changes in circulating cortisol might, however, be directly involved in the sodium-induced alterations of insulin sensitivity. It should be noted that this relationship was observed in a group of healthy, normotensive individuals. It would be of interest to know whether this relationship can also be demonstrated in hypertensive subjects, in whom salt sensitivity as well as insulin resistance are more prevalent than in normotensive subjects. Obviously, the demonstration of a relationship between sodium-induced changes in circulating cortisol levels and insulin sensitivity does not mean that these two phenomena are also causally related. For proof of concept, it would be necessary to evaluate whether insulin sensitivity also changes after salt loading if sodium-induced alterations in plasma cortisol are prevented. This could, for instance, be achieved in adrenalectomized animals, with immediate adjustment of the sodium-induced plasma cortisol alterations through a continuous infusion of cortisol.

Thus, future studies should be able to elucidate whether or not variation in circulating cortisol levels represent a causal link between salt sensitivity and insulin resistance.

11βHSD and the renin-angiotensin-aldosterone system
It is suggested in chapter 2 that renal 11βHSD type 2 can be acutely stimulated by angiotensin-I and II, as inferred from the observed decrease in UFF/UFE ratios during their respective administration. Additional studies are needed to confirm this hypothesis. In view of their widespread use, it would be interesting to know whether angiotensin-converting enzyme inhibitors and angiotensin-II receptor

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possibly providing some benefit in terms of enhancing renal sodium excretion and potentially increasing plasma cortisol. Indeed, the methodology described in chapter 3 has been confirmed by several additional studies. It should be assessed whether the short-half-life of labelled 11β-HSD could be prolonged by concomitant administration of amlodipine (a Ca2+-sensitive agent) which can be assessed by the increase in plasma cortisol. The activity of the HPA-axis stimulating cortisol release and its role as a determinant of the individual variation in phenotype can also be related to parameters well as insulin sensitivity. Obviously, the understanding of the role of circulating cortisol and the in vivo phenomena related to this axis will be achieved if the effect of different angiotensin II receptor blockers can be assessed similarly to the study described in chapter 3. We did not observe any effect of losartan on parameters of 11β-HSD activity, this might have been due to the relatively low dose of this angiotensin-II receptor blocker that was administered. Therefore, additional dose-effect studies should provide the answer whether these drugs inhibit renal 11β-HSD type 2 or not. As yet, no other agents than angiotensin have been identified to stimulate renal 11β-HSD type 2. Future studies may characterize other substances with a similar stimulating effect on renal 11β-HSD type 2, thus potentially creating a new class of natriuretic agents.

The physiological importance of 11β-HSD in regulating the cortisol to cortisone interconversion has been clearly demonstrated. There is growing evidence establishing the role of 11β-HSD in a number of clinical conditions. Thus, an altered 11β-HSD activity has been implicated in neoplasia, osteoporosis, low birth weight, glaucoma and cognitive dysfunction (13). Individual susceptibility to the consequences of an altered 11β-HSD activity might be in part genetically determined (14). Data on the genomics of 11β-HSD are still limited, but could augment our insight into the effects of 11β-HSD at the molecular level in the near future. In addition, it might generate useful information for designing drugs that will be able to modulate selectively the activity of 11β-HSD type 1 and type 2.

In conclusion, these many aspects of 11β-HSD will continue to offer a relevant field of research. Further studies will undoubtedly provide additional information with important consequences for daily clinical practice.

REFERENCES