# Enhanced 11β-hydroxysteroid dehydrogenase type 1 activity in stress adaptation in the guinea pig

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## Abstract

The 11β-hydroxysteroid dehydrogenases (11β-HSDs) convert cortisol to its inactive metabolite cortisone and vice versa. 11β-HSD type 1 (11β-HSD-1) functions as a reductase *in vivo*, regulating intracellular cortisol levels and its access to the glucocorticoid receptor. In contrast, 11β-HSD-2 only mediates oxidation of natural glucocorticoids, and protects the mineralocorticoid receptor from high cortisol concentrations. We investigated the *in vivo* and *in vitro* effects of ACTH on the recently characterized 11β-HSDs in guinea pig liver and kidney.

Tissue slices of untreated guinea pigs were incubated with $^{3}$H-labelled cortisol or cortisone and $ACTH_{1-24}$ ($10^{-10}$ and $10^{-9}$ mol/l). The 11β-HSD activities in liver and kidney slices were not influenced by *in vitro* incubation with $ACTH_{1-24}$.

In addition, guinea pigs were treated with $ACTH_{1-24}$ or saline injections s.c. for 3 days. Liver and kidney tissue slices of these animals were incubated with $^{3}$H-labelled cortisol or cortisone. *In vivo* ACTH treatment significantly increased reductase and decreased oxidase activity in liver and kidney. Furthermore, 11β-HSD-1 activity assessed by measurement of the urinary ratio of (tetrahydrocortisol (THF) + 5αTHF)/(tetrahydrocortisone) was significantly increased after ACTH treatment compared with the control group. Plasma levels of cortisol, cortisone, progesterone, 17-hydroxyprogesterone and androstenedione increased significantly following *in vivo* ACTH treatment.

The enhanced reductase activity of the hepatic and renal 11β-HSD-1 is apparently caused by cortisol or other ACTH-dependent steroids rather than by ACTH itself. This may be an important fine regulation of the glucocorticoid tonus for stress adaptation in every organ, e.g. enhanced gluconeogenesis in liver.

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## Introduction

The 11β-hydroxysteroid dehydrogenases (11β-HSDs) convert 11-hydroxysteroids like cortisol and corticosterone to their inactive metabolites cortisone and 11-dehydrocorticosterone and vice versa. Two different isoenzymes of 11β-HSD have been cloned in the human (Tannin *et al.* 1991, Albiston *et al.* 1994) and several other animal species. The $NAD^{+}$/(H)-dependent 11β-HSD type 2 (11β-HSD-2) enzyme is found in the placenta and in cells expressing the mineralocorticoid (MC) receptor (Albiston *et al.* 1994, Stewart 1996, Stewart & Krozowski 1999, Quinkler *et al.* 2001). With the endogenous glucocorticoids (GCs) cortisol and corticosterone as substrates, 11β-HSD-2 acts exclusively as a dehydrogenase, whereas with 9α-fluorinated steroids as substrates it is mainly a reductase (Oelkers *et al.* 1994, Diederich *et al.* 1997, Li *et al.* 1997). Further characteristics of 11β-HSD-2 are the strong end-product inhibition by 11-dehydro-GCs (Rusvai & Náray-Fejes-Tóth 1993) and the ability to oxidize dexamethasone (Best *et al.* 1997, Li *et al.* 1997). The main function of 11β-HSD-2 is the protection of the unselective MC receptor, which has similar affinity to cortisol and aldosterone (Arriza *et al.* 1987). This isoenzyme converts large amounts of cortisol to cortisone, thus allowing the lower concentrated aldosterone to bind to the MC receptor (Edwards *et al.* 1988, Funder *et al.* 1988, Stewart 1996, Stewart & Krozowski 1999, Quinkler *et al.* 2001).

The $NADP^{+}$/H-dependent 11β-HSD type 1 (11β-HSD-1) enzyme functions *in vitro* as a bidirectional oxidoreductase and is expressed ubiquitously. *In vivo*, the hepatic 11β-HSD-1 acts mainly as a reductase and activates inactive cortisone to cortisol (Jamieson *et al.* 1995, 2000, Voice *et al.* 1996). 11β-HSD-1 of the guinea pig has recently been cloned and was present in all tissues


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examined, with highest levels in liver, kidney and the adrenal gland (Pu & Yang 2000).

Whereas the function of 11β-HSD-2 is widely accepted, the function of 11β-HSD-1 is not yet well understood. It is believed that 11β-HSD-1 modulates intracellular active GC concentration and occupancy of the GC receptor. In the fetal lung, the activation of cortisone to cortisol by 11β-HSD-1 is essential for the induction of surfactant synthesis (Hundertmark *et al.* 1995), in the ovary for modulation of follicular maturation during the follicular phase (Michael & Cooke 1994, Tetsuka *et al.* 1997), and in adipose tissue for differentiation of adipocytes (Tomlinson *et al.* 2001, Stewart & Tomlinson 2002). In the liver, 11β-HSD-1 plays a crucial role in GC-mediated effects such as gluconeogenesis, glycolysis and insulin sensitivity (Voice *et al.* 1996, Kotelevtsev *et al.* 1997), and it is probably involved in detoxification of nitrosamines (Maser *et al.* 1996).

Recently, we and others have suggested that the increased ratio of cortisol to cortisone in plasma and urine during adrenocorticotrophin (ACTH) infusion in man (Eisenschmid *et al.* 1987, Walker *et al.* 1992) is not caused by a direct inhibitory effect of ACTH (Diederich *et al.* 1996), but is due to an overload of 11β-HSD-2 substrates such as cortisol and corticosterone with subsequent 11β-HSD-2 inhibition (Ulick *et al.* 1992).

Stress activates the hypothalamo–pituitary–adrenal axis and the sympathetic nervous system. During stress, ACTH, corticosteroid and catecholamine concentrations are high, as are other stress hormones. The plasma corticosteroid levels are the most striking changes during stress, and are regarded as a sensitive index of stress. Corticosteroid levels are regulated by the 11β-HSD enzymes, but it is not known how the latter respond to stress situations. We have recently characterized the 11β-HSD isoenzymes in the guinea pig liver and kidney (Quinkler *et al.* 1997). In this paper we describe the effects of *in vivo* ACTH administration for 3 days on the 11β-HSDs and the *in vitro* effects of ACTH on these enzymes in guinea pig liver and kidney tissue slices.

## Materials and Methods

### *Chemicals and solutions*

Cortisol and cortisone were obtained from Sigma Chemical Co. (St Louis, MO, USA), [1,2,6,7-$^3$H(n)]-cortisol (specific activity: 70·0 Ci/mmol) from DuPont de Nemours GmbH (Bad Homburg, Germany), and [1,2(n)-$^3$H]-cortisone (specific activity: 41Ci/mmol) from Amersham International plc (Amersham, Bucks, UK). Both tritiated steroids were purified by high performance liquid chromatography (HPLC) prior to use. The steroids were dissolved in methanol and kept at −20 °C. Synacthen ($ACTH_{1–24}$) was purchased from Ciba-Geigy GmbH (Wehr, Germany), ketamine from Sanofi Ceva (Düsseldorf, Germany) and xylazine (Rompun) from Bayer AG (Leverkusen, Germany). Acetone, ethanol, n-hexane and 2-propanol (all LiChrosolv quality) were purchased from Merck GmbH (Darmstadt, Germany), methanol in LiChrosolv quality from J T Baker BV (Deventer, The Netherlands), Sep-Pak $C_{18}$ cartridges from Waters Millipore GmbH (Eschborn, Germany) and radioactivity scintillant 'Instant Scint Gel Plus' from Packard Instruments BV (Groningen, The Netherlands).

### *Animals*

Male Duncan–Hartley guinea pigs (weight 300 g) were provided by Moellegard Breeding Centre (Schönwalde, Germany), and kept under standard conditions and on a control diet. The study was approved by the local ethics committee and is in agreement with UK legal requirements. The animals were randomly divided into two groups. One group ($n=6$) was stimulated with 0·1 ml depot $ACTH_{1–24}$ (10 IU) by s.c. injection twice daily (at 0800 h and 1800 h) for the last 3 days before they were killed. The other group ($n=6$) received injections of 0·1 ml saline (0·9%) as a placebo at the same times. One hundred and fifty minutes after the last injection of $ACTH_{1–24}$ or saline in the morning, the animals were anaesthesized with ketamine combined with xylazine by intramuscular injection. Blood samples were obtained by heart puncture, and plasma was stored at −20 °C until analysis. The animals were killed, and the livers and kidneys quickly removed and kept in cold saline until the onset of incubation (maximum duration from death to onset of incubation was 70 min). Urine was collected over 24 h on the day before the injections started and before the animals were killed. The urine samples were stored at −20 °C until analysis. Plasma steroids were measured by HPLC or radioimmunoassay (RIA); tetrahydrocortisol (THF), 5αTHF and tetrahydrocortisone (THE) concentrations in 24-h urine samples were analyzed by RIA (Maser-Gluth *et al.* 2000). Guinea pig liver and kidney tissues were cut into 1 mm slices, and 70 mg wet tissue was weighed out for each incubation well. The incubation volume of 1 ml Krebs–Ringer–bicarbonate–glucose buffer contained 100 µl [$^3$H]-cortisol or [$^3$H]-cortisone (100 000 c.p.m. = $10^{-9}$ mol/l) and unlabelled cortisol or cortisone ($10^{-7}$ mol/l). The incubation time was 90 min, and four independent incubations for each tissue and reaction direction were performed. Incubations of tissue slices were carried out in a shaking preheated (37 °C) steel chamber with a 95% $O_2$ and 5% $CO_2$ gas supply as described previously (Oelkers *et al.* 1994, Diederich *et al.* 1996).

The possibility of a direct effect of $ACTH_{1–24}$ on renal or hepatic 11β-HSD of the guinea pig was tested by measuring the conversion of cortisol to cortisone and vice versa in the presence of two concentrations of $ACTH_{1–24}$ ($10^{-10}$ and $10^{-9}$ mol/l). The same amounts of tissue, radioactive tracer and unlabelled cortisol/cortisone were used as described above. For studies of enzyme kinetics we studied the renal and hepatic 11β-oxidation and

-reduction in guinea pig tissues by measuring the conversion of [$^3$H]-cortisol (100 000 c.p.m. = $10^{-9}$ mol/l) to [$^3$H]-cortisone and vice versa in the presence of increasing concentrations of unlabelled cortisol or cortisone ($10^{-9}$ to $10^{-5}$ mol/l). For each concentration, tissue and reaction direction, three independent incubations were performed (incubation time 90 min).

*Analytical procedure*

The incubation was stopped by transferring the incubation set on ice and by removing the supernatant fluid. Precipitation of protein and cleaning of samples with Sep-Pak $C_{18}$ cartridges and HPLC procedure with $^3$H measurement were performed as described previously (Diederich *et al.* 1996). In the *in vivo* experiment steroids were extracted as mentioned above, but were spotted on a thin-layer chromatography plate that was developed in dichloromethane/methanol (15:1, v/v). The bands containing cortisol and cortisone were identified by UV light of the unlabelled carriers, cut out, transferred into scintillation vials and analyzed in a beta-counter. The interconversion of 11-hydroxy- or 11-oxosteroids by tissue slices was expressed as percentage of the total steroid measured.

*Statistics*

Statistical calculations were done with an SPSS program from SPSS Inc. (Chicago, IL, USA). The Duncan multiple range and the Mann–Whitney rank sum tests as well as the independent *t*-test were used.

## Results

*Guinea pig* in vivo *experiment*

The mean weight of guinea pigs of the ACTH subgroup was 305·5 ± 11·3 g and that of the control group was 301·7 ± 15·4 g before the experiment. The animals of each group gained weight significantly during the experiment, resulting in slightly heavier ACTH-treated animals (377·5 ± 10·4 g) than control animals (370·5 ± 20·5 g). There was no significant difference in weight between the ACTH-treated animals and the control animals.

Serum cortisol concentrations rose significantly from 525 ± 194 nmol/l to 2679 ± 640 nmol/l following ACTH treatment for 3 days. Plasma cortisone concentrations rose likewise from 55 ± 18 nmol/l (control guinea pigs) to 336 ± 62 nmol/l in ACTH-treated animals (Table 1). The serum cortisol/cortisone ratio was not altered significantly in ACTH-treated or control animals. Other steroids measured (progesterone, 17α-hydroxyprogesterone and androstenedione) increased significantly following *in vivo* ACTH treatment (Table 1). Plasma dehydroepiandrosterone sulphate (DHEA-S) concentrations were below the assay detection limit in control animals and slightly above the limit in ACTH-treated animals.

**Table 1** Serum concentrations of different steroids in ACTH-treated and control guinea pigs. Values are means ± S.D. ($n$=6 in each group)

| | Control animals | ACTH-treated animals | Control vs ACTH (*t*-test) |
|---|---|---|---|
| **Serum concentration** (nmol/l) | | | |
| Cortisol | 525 ± 194 | 2679 ± 640 | $P$<0·001 |
| Cortisone | 55 ± 18 | 336 ± 62 | $P$<0·001 |
| Progesterone | 2·7 ± 0·3 | 10·9 ± 2·1 | $P$<0·001 |
| 17α-Hydroxyprogesterone | 0·35 ± 0·15 | 2·32 ± 0·6 | $P$<0·001 |
| Androstenedione | 2·3 ± 0·6 | 10·7 ± 4·7 | $P$<0·005 |
| DHEA-S | <0·135 | 0·189 ± 0·014 | $P$<0·001 |

The urinary excretion of cortisol, cortisone, THF, 5αTHF and THE increased significantly under ACTH treatment (Table 2). Surprisingly the urinary cortisol/cortisone ratio did not increase after ACTH treatment, whereas the urinary excretion of THF+5αTHF/THE showed a significant increase compared with the ratio before ACTH treatment and to the control group (Fig. 1). The urinary ratio 5αTHF/THF, representing the balance between 5α-reductases and 5β-reductase activities, remained unchanged in control and ACTH-treated animals before and after treatment (Table 2).

*Guinea pig* in vitro *experiments*

Incubations with [$^3$H]-cortisol to [$^3$H]-cortisone and vice versa in the presence of increasing concentrations of unlabelled cortisol or cortisone ($10^{-9}$ to $10^{-5}$ mol/l) in untreated guinea pig liver and kidney tissue slices showed typical enzyme kinetics with no substrate overload (data not shown).

Three days of *in vivo* ACTH treatment significantly increased the *in vitro* 11β-reductase activity in guinea pig liver (50·2% to 70·3%) and kidney (39·6% to 56·8%) slices, whereas the 11β-oxidative activity was significantly decreased in both liver (33·7% to 23·9%) and kidney (33·9% to 28·6%) (Fig. 2). Due to the high conversion of substrate, and consequent difficulties in accurately measuring absolute conversion rates, the results observed may underestimate the difference between control and treatment groups

*In vitro* incubations of liver and kidney slices with increasing concentrations of $ACTH_{1–24}$ had no significant effect on the interconversion of cortisol to cortisone (Table 3).

## Discussion

The pituitary–adrenal system of the guinea pig (*Cavia porcellus*) is different from other rodents in several respects (Keightley & Fuller 1996). The total plasma cortisol concentration in unstressed animals ranges between 260

**Table 2** Daily urinary excretion of free cortisol, cortisone, THF, 5αTHF and THE in ACTH-treated and control guinea pigs. Values are means ± S.D. ($n$=6 in each group). Paired and unpaired $t$-tests were used

| | | **Control group (NaCl)** (μg/24 h) | **ACTH-treated group** (μg/24 h) | **Control vs ACTH** ($t$-test) |
|---|---|---|---|---|
| **Urinary steroid profiles** | | | | |
| Cortisol | Before injection | 44·5 ± 13·6 | 51·4 ± 17·9 | $P$=0·468 |
| | 3rd day of injection (ACTH or NaCl) | 30·1 ± 8·2 | 364·3 ± 88·5 | $P$<0·001 |
| | $t$-test (before vs after injections) | $P$=0·051 | $P$<0·001 | |
| Cortisone | Before injection | 1·7 ± 0·4 | 2·5 ± 0·8 | $P$=0·065 |
| | 3rd day of injection (ACTH or NaCl) | 1·2 ± 0·8 | 22·1 ± 10·7 | $P$<0·001 |
| | $t$-test (before vs after injections) | $P$=0·143 | $P$<0·001 | |
| Cortisol/cortisone | Before injection | 25·8 ± 7·1 | 20·7 ± 2·3 | $P$=0·125 |
| ratio | 3rd day of injection (ACTH or NaCl) | 35·9 ± 22·4 | 20·4 ± 10·2 | $P$=0·168 |
| | $t$-test (before vs after injections) | $P$=0·194 | $P$=0·943 | |
| THF | Before injection | 8·8 ± 2·7 | 8·9 ± 3·3 | $P$=0·940 |
| | 3rd day of injection (ACTH or NaCl) | 7·0 ± 1·7 | 41·2 ± 11·0 | $P$=0·001 |
| | $t$-test (before vs after injections) | $P$=0·272 | $P$=0·001 | |
| 5αTHF | Before injection | 4·6 ± 0·8 | 4·9 ± 0·8 | $P$=0·506 |
| | 3rd day of injection (ACTH or NaCl) | 3·6 ± 0·6 | 16·7 ± 3·2 | $P$<0·001 |
| | $t$-test (before vs after injections) | $P$=0·073 | $P$<0·001 | |
| THE | Before injection | 7·0 ± 1·5 | 8·2 ± 2·7 | $P$=0·385 |
| | 3rd day of injection (ACTH or NaCl) | 6·4 ± 2·1 | 24·8 ± 7·0 | $P$=0·001 |
| | $t$-test (before vs after injections) | $P$=0·605 | $P$<0·001 | |
| 5αTHF/THF | Before injection | 0·55 ± 0·13 | 0·65 ± 0·4 | $P$=0·572 |
| | 3rd day of injection (ACTH or NaCl) | 0·53 ± 0·11 | 0·42 ± 0·08 | $P$=0·058 |
| | $t$-test (before vs after injections) | $P$=0·812 | $P$=0·222 | |
| (THF+5αTHF)/ | Before injection | 1·90 ± 0·28 | 1·82 ± 0·54 | $P$=0·471 |
| THE | 3rd day of injection (ACTH or NaCl) | 1·72 ± 0·26 | 2·39 ± 0·33 | $P$=0·003 |
| | $t$-test (before vs after injections) | $P$=0·330 | $P$<0·045 | |

and 690 nmol/l (Fujieda *et al.* 1982, Fenske 1997), and free cortisol concentrations are at least three times higher than in humans (Fujieda *et al.* 1982). One reason for the high circulating cortisol concentration may be the low affinity of the guinea pig GC receptor to cortisol (Hodgson & Funder 1978, Kraft *et al.* 1979). In spite of high cortisol levels, the plasma ACTH concentration is comparable with that in other species (Martin *et al.* 1980), but the biological potency of guinea pig ACTH is increased (Keightley *et al.* 1991). The guinea pig MC receptor shows no differences from that of other species (Myles & Funder 1994). We found that the guinea pig possesses a very strong 11β-HSD-2 activity in the kidney (Quinkler *et al.* 1997), which could be the major protecting mechanism for the MC receptor.

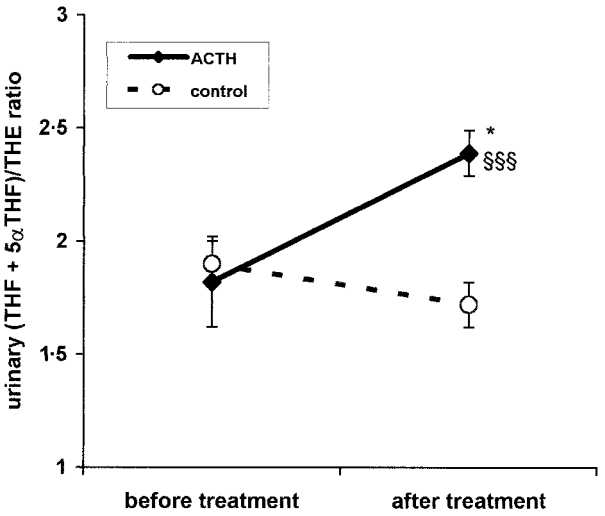


**Figure 1** Urinary (THF+5αTHF)/THE ratio of six ACTH- and NaCl-treated guinea pigs. Values are means ± S.E.M. Significant differences ($^{§§§}P$<0·005) of the ratios after treatment in ACTH-treated versus the control group. Significant increase ($^{*}P$<0·05) of the ratio in the ACTH-treated group before versus after the injections.

It is not known how 11β-HSD activity and its reaction directions are influenced in chronic stress situations. Until now, only a few studies have addressed this topic. Nwe *et al.* (2000) investigated the 11β-HSD-1 oxidative activity of testis and liver during stress in rats. They did not investigate reductase activity, which is the main function of 11β-HSD-1, and they used only homogenates. Tissue slices are preferrable to homogenates, because homogenization of tissue changes 11β-HSD-1 activity from reduction to oxidation (Oelkers *et al.* 1994, Bach *et al.* 1996). We therefore investigated the effect of 3 days of *in vivo* ACTH treatment on the interconversion of cortisol and cortisone in liver and kidney slices of the guinea pig.

Three days of ACTH treatment increased adrenal steroidogenesis. Besides increased cortisol and cortisone

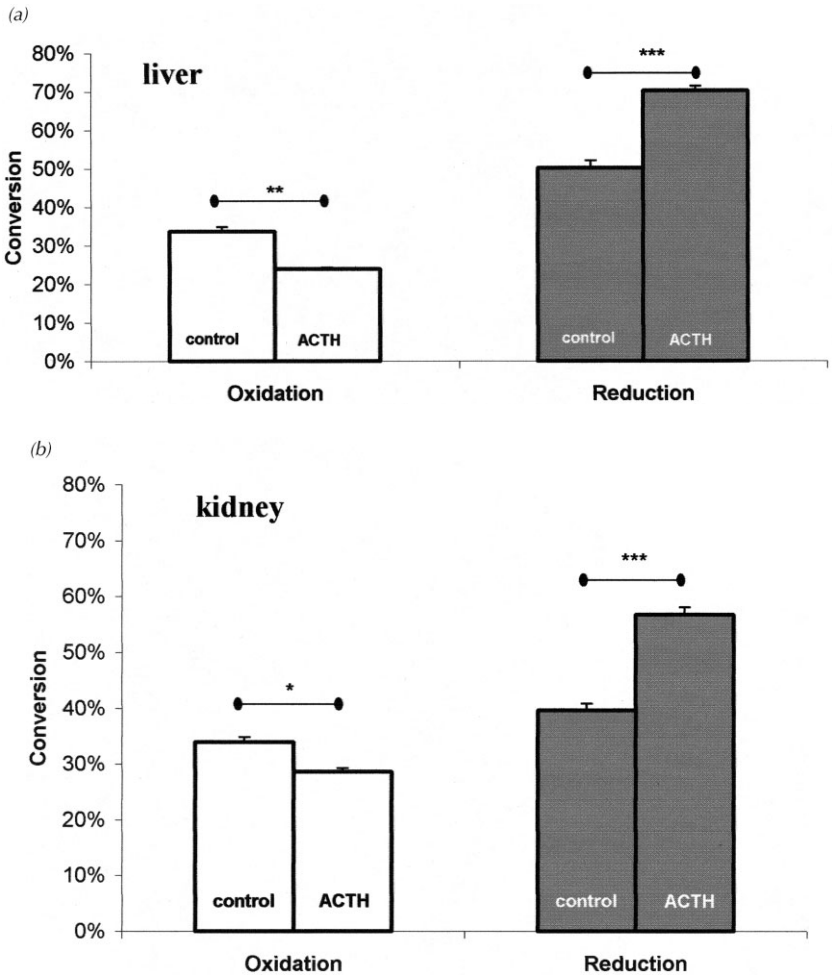


**Figure 2** Conversion (%) of [$^3$H]-cortisol and [$^3$H]-cortisone in guinea pig (*a*) liver tissue slices ($n=6$) and (*b*) kidney tissue slices ($n=6$) of sham- and ACTH-treated animals; 100 000 c.p.m. [$^3$H]-cortisol or [$^3$H]-cortisone and $10^{-7}$ mol/l unlabelled cortisol or cortisone were used. The incubation time was 90 min. Values are means ± S.E.M. (a) $^{**}P<0{\cdot}005$, $^{***}P<0{\cdot}001$; (b) $^{*}P<0{\cdot}05$, $^{***}P<0{\cdot}001$.

plasma concentrations (Table 1), we observed an increased production of progesterone, 17α-hydroxyprogesterone and androstenedione suggesting an activated 17α-hydroxylase and 17,20-lyase activity of P450c17 enzyme by ACTH treatment (Provencher *et al.* 1992). The DHEA-S plasma concentration was undetectable in untreated animals and was just measureable in ACTH-treated animals. The P450c17 enzyme of the guinea pig seems to prefer the progesterone–17α-hydroxyprogesterone–androstenedione pathway similar to rats (Shinzawa *et al.* 1985). This is in contrast to the human P450c17, which favours the pregnenolone–17α-hydroxypregnenolone–DHEA pathway.

In liver and kidney tissue slices, 11β-HSD dehydrogenase activity decreased after *in vivo* ACTH treatment, whereas the 11β-HSD reductase activity increased considerably compared with the control group. The equilibrium of the reaction was shifted towards the active 11-hydroxy side (Fig. 2). In guinea pig liver, 11β-HSD1 mRNA expression and enzyme activity is predominant (Quinkler *et al.* 1997, Pu & Yang 2000). Therefore, the increase of the reduction of cortisone is most likely due to an increase in 11β-HSD-1 activity conferred by GCs (Jamieson *et al.* 1995, Liu *et al.* 1996). The preference for reduction could be caused by an increase of reduced cosubstrate (NADPH) and a lower intracellular pH, due to anaerobic glycolysis in stress situations. At lower pH (6·0) the reductase reaction is favoured over the dehydrogenase reaction (pH 8·0) (Brown *et al.* 1993). A direct transcriptional regulation could also be a possibility, but would not explain the reduced oxidation and increased reductive activity of one enzyme. Therefore, this change in enzyme

**Table 3** Percentage conversion of cortisol (F) to cortisone (E) and vice versa in kidney and liver tissue slices with increasing concentrations of $ACTH_{1-24}$ *in vitro*. Values are means ± S.E.M. ($n=3$). Percentage conversion of controls was set at 100%

| | | $ACTH_{1-24}$ | |
|---|---|---|---|
| | **Control** (%) | $10^{-10}$ mol/l | $10^{-9}$ mol/l |
| **Kidney** | | | |
| Oxidation (F to E) | 100 ± 7·8 | 96·9 ± 8·8 | 87·1 ± 3·6 |
| Reduction (E to F) | 100 ± 13·9 | 83·6 ± 9·6 | 91·7 ± 1·7 |
| **Liver** | | | |
| Oxidation (F to E) | 100 ± 3·7 | 112 ± 13·5 | 104·6 ± 6·6 |
| Reduction (E to F) | 100 ± 11·1 | 117 ± 6·9 | 106·4 ± 10·4 |

activity seems to correlate better with a shift of the equilibrium to the reductive side than with direct transcriptional changes.

We also observed marked oxidative activity in liver tissue slices. This can be explained by the activity of a third isoenzyme in guinea pig liver acting as a dehydrogenase (Quinkler *et al.* 1997). Since the oxidative activity was significantly decreased after *in vivo* ACTH treatment, an inhibition of this third 11β-HSD isoenzyme by ACTH treatment seems possible. This 11β-HSD isoenzyme could constitute a compensatory metabolic pathway for the poor cortisol metabolism by other enzymatic systems (e.g. ring A reduction) in the guinea pig liver (Abel *et al.* 1993).

Besides 11β-HSD-2, the guinea pig kidney expresses 11β-HSD-1 similar to rat and mouse kidney (Quinkler *et al.* 1997, Pu & Yang 2000). *In vivo* ACTH treatment increases renal reductive activity, possibly due to an activated 11β-HSD-1 activity. Although it is known that in the rat kidney 11β-HSD-2 is induced by GCs (Li *et al.* 1996), it seems as if the activation of type 1 isoenzyme would predominate in the guinea pig. It is also possible that the type 2 isoenzyme is inhibited by ACTH-induced steroids, e.g. progesterone and its metabolites, or by corticosterone (Diederich *et al.* 1996, Quinkler *et al.* 1999). Plasma cortisone concentration rises to over 300 nmol/l after ACTH treatment (Table 1) and cortisone has a lower corticosteroid-binding globulin affinity than cortisol. Therefore, end-product inhibition of 11β-HSD-2 by cortisone, which was described previously (Rusvai & Náray-Fejes-Tóth 1993, Albiston *et al.* 1994, Stewart *et al.* 1995), may also be an important reason for the decrease of renal 11β-HSD-2 activity *in vivo*. The physiological role of a decreased 11β-HSD-2 activity in response to stress is not clear. On the one hand, the body would need higher levels of oxidase activity in cells expressing the MC receptor to cope with increased cortisol levels and protect the MC receptor. But, on the other hand, even these cells may need a higher intracellular level of active GCs to respond adequately to stress. In addition, fluid retention is critical in stress, as we see it clinically in shock situations like septic or haemorrhagic shock, and traumata. Therefore an increased activation of the MC receptor would make sense.

A study in 11β-HSD-1 knock-out mice showed the importance of the 11β-HSD-1 isoenzyme for GC action: a lack of this enzyme leads to a relative intracellular GC deficiency (Kotelevtsev *et al.* 1997). Since ACTH had no effect on the bidirectional enzyme activities when added *in vitro* to liver or kidney slices (Table 3), the activation of the 11β-HSD-1 after *in vivo* ACTH application is most likely mediated by cortisol itself or by other ACTH-induced steroids (Hammami & Siiteri 1991, Walker *et al.* 1994, Jamieson *et al.* 1995, Voice *et al.* 1996). This is in accordance with the finding that 11β-HSD-1 activity is increased by dexamethasone in a rat hepatoma cell line (2S FAZA) (Voice *et al.* 1996), in primary culture of rat hepatocytes (Liu *et al.* 1996) and in rat liver *in vivo* (Jamieson *et al.* 1999). It was recently demonstrated that GC treatment caused a time- and dose-dependent increase in 11β-HSD-1 mRNA and activity in primary cultures of human osteoblasts (Cooper *et al.* 2002).

Nevertheless, this activation of 11β-HSD-1 in stress situations could be an important regulatory principle in many organ systems. This enhanced 'GC tonus' is essential for stress adaptation, e.g. GC-induced increase of gluconeogenesis in the liver. Due to the increased conversion of inactive cortisone to active cortisol, the liver seems to be an important cortisol production site in stress situations, besides the adrenals.

This observation was underlined by the significantly elevated urinary THF+5αTHF/THE ratio in the ACTH-treated group of guinea pig as a marker of 11β-HSD-1 activity (Fig. 2a). It has recently been proposed that the urinary ratio of THF+5αTHF/THE may also be an accurate marker for renal 11β-HSD-2 in humans (Ferrari *et al.* 2001). Due to different isoenzyme expression in the guinea pig kidney (11β-HSD-1 in addition to 11β-HSD-2) (Quinkler *et al.*1997, Pu & Yang 2000), this cannot be easily transferred to the guinea pig. We rather interpret this ratio as a marker for hepatic 11β-HSD-1 activity (Palermo *et al.* 1996, Quinkler *et al.* 2000). The urinary 5αTHF/THF ratio, representing the balance between 5α-reductases and 5β-reductase activities, did not change under ACTH treatment (Table 2). This implies that the increased THF+5αTHF/THE ratio was not influenced by 5α-reductases or 5β-reductase activities but represents an increased 11β-HSD-1 reductase activity. Surprisingly, the total amount of urinary tetrahydrometabolites was rather small compared with the human in relation to the cortisol concentration. This can be explained by the relatively poor cortisol metabolism to ring A-reduced metabolites in the guinea pig liver (Abel *et al.* 1993). Therefore these ratios need further assessment in this species.

The urinary cortisol/cortisone ratio in the guinea pig is high (approximately 20), whereas in humans it is low (approximately 0·5). The urinary ratio cortisol/cortisone is an excellent marker for 11β-HSD-2 in humans, because the human kidney expresses only 11β-HSD-2. But the ratio is a poor index for 11β-HSD-2 activity in the guinea pig, because both 11β-HSD enzymes are expressed in the kidney and contribute to the urinary cortisol/cortisone ratio. Therefore, it is difficult to compare human and guinea pig urinary cortisol/cortisone ratios. In situations of unchanged urinary cortisol/cortisone ratio in ACTH-treated animals, the increase in THF+5αTHF/THE ratio reflects an increase in 11β-HSD-1 activity in the liver.

In summary, we have shown that *in vivo* ACTH treatment for 3 days increases the hepatic and renal 11β-reductase activity in the guinea pig. This activation is probably due to increased concentrations of cortisol or of other ACTH-dependent GCs rather than to ACTH itself. The stress-induced activation of the hormonally inactive cortisone to active cortisol in liver, kidney and other 11β-HSD-1-expressing organs seems to be an important local tissue regulatory mechanism besides the adrenal cortisol *de novo* synthesis. Up to now, this mechanism, which could also be present in other species such as the human, has not been noted to be involved in the elevation of cortisol by ACTH in humans.

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