Effects of growth hormone on steroid-induced increase in ability of urea synthesis and urea enzyme mRNA levels

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Gårdfte, Thorbjørn, Dorthe Svenstrup Jensen, Henning Grønbæk, Troels Wolthers, Søren Astrup Jensen, Niels Tystrup, and Hendrik Vilstrup. Effects of growth hormone on steroid-induced increase in ability of urea synthesis and urea enzyme mRNA levels. Am. J. Physiol. 275 (Endocrinol. Metab. 38): E79–E86, 1998.—Growth hormone (GH) reduces the catabolic side effects of steroid treatment due to its effects on tissue protein synthesis/degradation. Little attention is focused on hepatic amino acid degradation and urea synthesis. Five groups of rats were given 1) placebo, 2) prednisolone, 3) placebo, pair fed to the steroid group, 4) GH, and 5) prednisolone and GH. After 7 days, the in vivo capacity of urea synthesis (CUNS) was determined by saturating alanine infusion, in parallel with measurements of liver mRNA levels of urea cycle enzymes, N contents of organs, N balance, and hormones. Prednisolone increased CUNS (µmol·min⁻¹·100 g⁻¹, mean ± SE) from 9.1 ± 1.0 (pair-fed controls) to 13.2 ± 0.8 (P < 0.05), decreased basal blood α-amino N concentration from 4.2 ± 0.5 to 3.1 ± 0.3 mmol/l (P < 0.05), increased mRNA levels of the rate- and flux-limiting urea cycle enzymes by 20 and 65%, respectively (P < 0.05), and decreased muscle N contents and N balance. In contrast, GH decreased CUNS from 6.1 ± 0.9 (pair-fed controls) to 4.2 ± 0.5 (P < 0.05), decreased basal blood α-amino N concentration from 3.8 ± 0.3 to 3.2 ± 0.2, decreased mRNA levels of the rate- and flux-limiting urea cycle enzymes to 60 and 40%, respectively (P < 0.05), and increased organ N contents and N balance. Coadministration of GH abolished all steroid effects. We found that prednisolone increases the ability of amino N conversion into urea N and urea cycle gene expression. GH had the opposite effects and counteracted the N-wasting side effects of prednisolone.

HEPATIC UREA SYNTHESIS is the only “on demand” pathway for irreversible removal of excess N after catabolism of amino acids. Its rate in vivo is determined by the level of circulating substrate, i.e., the amino N of blood α-amino acids. Factors such as functional liver mass and hormones regulate the relation between process rate and substrate concentration (17, 18, 39). Once synthesized, urea accumulates in body water and is excreted into urine. Urea production reflects the net overall rate of total protein breakdown. If this rate exceeds the intake of dietary protein N, the subject is catabolic and wasting body proteins. Glucocorticoid treatment results in protein wasting, poor healing, and increased incidence of infection (6, 19, 27, 28). Intensive dietary regimens have been insufficient to counteract these side effects. With the key role of glucocorticoids as immunosuppressant in humans taken into consideration, the identification of therapeutic agents able to limit protein wasting during steroid treatment is a major therapeutic goal (12, 25, 26).

The steroid wasting is caused partly by an increase in tissue protein breakdown and net release of amino N and partly by accelerated hepatic conversion of amino N to urea N (6, 37, 40). In contrast, it has been shown that growth hormone (GH) is able to increase tissue net uptake of amino N and also to reduce hepatic conversion of amino N to urea N (15). It was recently shown that GH is able to prevent the catabolic side effects of steroids in humans (25).

We raise the hypothesis that this effect of GH in preventing steroid catabolism, besides a change in protein metabolism, also involves regulatory events in liver function as to urea synthesis, and that is related to regulation of gene functions.

In rats, it is possible to standardize urea synthesis in relation to substrate drive by measuring the capacity of urea N synthesis during saturating alanine loading (18), thereby allowing studies on specific hormonal effects on urea synthesis.

We examined the effect of 7 days of prednisolone and GH treatment singly and in combination on the capacity of urea N synthesis, the expression of mRNA levels of urea cycle enzymes in liver, tissue N contents in different tissues, and whole body N balance.

MATERIALS AND METHODS

Animals

Female Wistar rats (body weight 200–210 g; Møllegaard Breeding Center, Eiby, Denmark) were housed at 22 ± 2°C, 55 ± 10% relative humidity, air change 8–10 times per hour, and on a 12:12-h light-dark cycle (0630–1830 light). The animals had free access to standard food (Altromin diet no. 1324; Chr. Petersen, Slagelse, Denmark) and tap water.

Each rat was weighed every day during treatment and before experimental procedures. Food and water intakes were recorded every day for every cage, each containing two rats. Two days before investigational procedures, all rats were housed singly in metabolic cages to determine N balance.

Protocols

There were five study groups: 1) control rats (n = 20), saline subcutaneously twice a day for 7 days; 2) prednisolone-treated rats (n = 20), 4 mg/kg of prednisolone (Delcortol; LEO) subcutaneously onethice each day for 7 days; 3) pair-fed control to prednisolone-treated rats (n = 20), saline subcuta-
necessarily twice a day for 7 days; 4) GH-treated rats (n = 20), 1 mg/kg of GH subcutaneously (Novo Nordisk) twice a day for 7 days; and 5) GH- and prednisolone-treated rats (n = 20), 4 mg/kg of prednisolone and 1 mg/kg of GH subcutaneously.

Pilot studies were performed to obtain prednisolone doses that would result in significant weight loss. The human dose equivalent would be the one used in, e.g., inflammatory bowel diseases. The GH doses used are equivalent to substitution doses in hypophysectomized rats. In eight animals of each study group, the basal blood α-amino N (AAN) concentration, the capacity of urea synthesis rate (CUNS), and whole body N balance were determined after an overnight fast. Basal insulin and glucose were determined to evaluate insulin sensitivity and total insulin-like growth factor I (IGF-I) for control of the treatment. N contents of liver, kidney, soleus, and extensor digitorum longus (EDL) were determined together with liver mRNA levels of the five urea cycle enzymes, carbamoyl phosphate synthetase (CPS), ornithine transcarbamoylase (OTC), argininosuccinate synthetase (ASS), argininosuccinate lyase (ASL), and arginase (ARG), in eight animals of each group. CUNS and mRNAs were measured in separate but otherwise identically treated animals, which ensures that the amino acid loading of the CUNS determinations did not influence gene expression.

Experimental Procedures

Immediately after decapitation, organs were isolated and weighed after blotting on filter paper, instantly frozen in toto in liquid N, and stored at −80°C until analysis. Muscular type I fibers are predominantly in soleus (84% type I, 16% type IIa, 0% type IIb), and type II fibers are mainly represented by EDL (3, 57, and 40% see Ref. 24); nonmuscular protein was examined by contents in liver and kidneys. N balance was assessed as follows. On the last 2 days of the investigation period, the animals were housed in metabolic cages, and the amount of food ingested by each animal was determined. Samples of quantitatively collected urine were analyzed for total N and urea contents, and samples of feces were analyzed for N contents. From these measurements, the N balance (mmol/24 h) was calculated as a mean of these 2 days, in order to minimize day-to-day variation.

CUNS. After anesthesia with a subcutaneous injection of 0.75 mg/kg Hypnorm (Janssen Pharmaceutica, Beerse, Belgium) and 4 mg/kg midazolam (Dormicum; La Roche, Basel, Switzerland), a catheter (Neoflon 0.6 mm; Viggo-Spectramed, Helsingborg, Sweden) was inserted into the femoral vein for continuous infusion. In all animals (n = 8 in all groups), retroperitoneal bilateral nephrectomy (to facilitate determination of urea synthesis, see below) was performed immediately before the investigation started (18). This procedure in itself does not acutely influence the CUNS (18), which remains unchanged until 12 h after hysterectomy and is at maximum after 24 h (21). Blood samples were taken from the retrobulbar venous plexus using heparinized micropipets (Vitrex; Horsens Laboratory Equipment).

For determination of CUNS, alanine was administered according to body weight as a bolus of 0.7–0.9 ml of a 1,120 mmol/l solution in sterile water followed by constant infusion of 2.8–3.5 ml/h of a 224 mmol/l solution for 70 min by means of an injectomat (Perfusor Secura; Braun, Melsungen, Germany). Steady-state blood AAN was defined as fluctuations below 10% during at least 50 min of the study, and the alanine infusion was aimed at obtaining a steady-state total blood AAN between 7.3 and 11.6 mmol/l; within this interval, urea synthesis is at maximum in rats, i.e., saturated, and thus independent of substrate concentration (18). Blood was sampled (100 μl) at 10-min intervals after an initial equilibration period of 20 min for determination of blood urea and total AAN. A total of 1 ml of blood was removed. This volume was compensated for by infusion of alanine.

Plasma IGF-I, glucose, and insulin were determined in all animals immediately after anesthesia and nephrectomy.

mRNA Determinations

About 200 mg of liver tissue from the left lobe of six separate but otherwise identically treated rats of each study group were immediately stored in liquid N. Total RNA was isolated with a Promega kit Z 5110 based on the triacylamid method according to the specification of the manufacturer. Specificity of all probes was ascertained by autoradiography of Northern blots, showing hybridization signals at the expected sites. Slot blots were used for quantification of mRNA levels using a Schleicher and Schuell Minifold. After blotting, the filters were ultraviolet cross-linked in a stratalinker (Stratagene). The standard error of loading, estimated by loading the same extract to 6–10 wells, was on average 7%. In recent measurements, this procedure has been changed so that we now use hybridization of ribosomal RNA as an index of mRNA signal. This change in procedure has not affected the results.

Hybridization. About 25 ng cDNA were labeled by random priming using the multiprime kit RPN 1601Z (Amersham) and eluted on NICK spin columns (Pharmacia). Prehybridization was performed at 42°C for 1 h in a solution of 50% formamide (Merck), 10× Denhart’s solution (Sigma), 0.05 M Tris, pH 7.4, 1 M NaCl, 1% SDS (wt/vol), 0.1% sodium pyrophosphate (wt/vol), and 0.25 mg/ml salmon sperm DNA (Sigma); sonicated and immersed in boiling water for 15 min; and then added to the solution. Hybridization was performed at 42°C for 16 h with the solution as above (except 0.25 mg/ml salmon sperm DNA) with the labeled probe added. Finally, filters were washed twice with 0.1× SSC and 0.5% SDS (wt/vol) at 65°C for 30 min.

Phosphoimaging was made on an Imaging Plate BASIII under leadshield, and the hybridization signal was analyzed in a Fujix Bioimaging Analyzer System BAS2000 (Fujifilm Co., Tokyo, Japan).

cDNA probes. cDNA probes were as follows: CPS I, pCP5SrPst 850 bp (8); OTC, pOTC1 Hind III 388 bp (32); ASS, pASr11 Pst I 1450 bp (38); ASL, AL-2 Eco R I 1000 bp (4); and ARG, 3B1 Pst I 650 bp (10).

DNA fragments were separated by agarose gel electrophoresis and eluted on Spinbind DNA Extraction Units (FMC).

Distribution Volume of Urea

The volume of distribution of urea (VD) was determined in four nephrectomized animals of each study group by an intravenous bolus injection (D) of ~1 mmol urea. The blood urea concentration was then followed for 60 min. After 20 min, the distribution of urea was completed. The volume of distribution was calculated as D = VD(Ct − ci), where D is the amount of urea injected, Ci is the mean blood urea concentration in the interval 20–60 min after injection of urea, and Ct is the blood urea concentration at time immediately before injection of urea. D in relation to body weight was 64 ± 2% in all control groups, 65 ± 4% in prednisolone-treated rats, 69 ± 3% in GH-treated animals, and 68 ± 2% in rats treated with both prednisolone and GH (mean ± SE). Neither were different from the 63% found earlier (2), which was used in all present calculations.
**RESULTS**

**Fodder Intake**

Prednisolone treatment for 7 days reduced fodder intake by 20% ($P < 0.01$), whereas treatment with GH increased intake by 15% compared with free-fed controls ($P < 0.01$). The combination of prednisolone and GH initially decreased food intake by 20% during the first 3 days of treatment, but it quickly returned toward normal and exceeded that during the last 2 days of treatment. Pair feeding was done only to treatment groups with a reduced overall food intake.

**Body Weight**

All groups had identical initial body weight (Fig. 1). Free-fed controls increased their body weight by 8 g during the study. Prednisolone-treated rats lost 14 g, primarily during the first 3 days, whereas pair-fed controls to steroid-treated rats maintained their weight ($P < 0.01$). GH-treated rats increased their body weight by 28 g ($P < 0.01$). Combined treatment with steroids and GH resulted in a diphasic pattern, with weight loss of 7 g during the first 3 days followed by accelerated growth; thus the rats regained their initial weight ($P < 0.01$; Fig. 2).

**Organ N Contents (mg N)**

Changes in organ weight and organ N contents were parallel, and only the latter is given. Prednisolone decreased N contents of heart and skeletal muscles, whereas liver and kidney N contents remained the same. GH treatment increased organ N contents of all measured organs. Combined treatment increased N contents of the liver and kidney and normalized heart and EDL N contents (Table 1).

**N Loss and N Balance**

In control rats, 50% of N was excreted as urea, 10% as nonurea N in the urine, and 40% as fecal N. In prednisolone-treated rats, total N excretion doubled due to a doubling in urinary urea N excretion ($P < 0.01$).

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**Gut Urea Hydrolysis**

Newly synthesized urea is lost in the gut by bacterial hydrolysis, and the resulting ammonia is recycled in the liver to reproduce urea. To determine this fraction (L), four rats from each group were nephrectomized and injected intravenously with 2 µCi [14C]urea. After 20 min, the distribution of [14C]urea was completed. Blood was then sampled every 10 min for 70 min to determine the blood activity of [14C]urea. Radioactivity in the blood samples was measured by counting in hisafe 2 (Wallac) using a liquid scintillation counter (Wallac 1409–2). The slope of decay of blood radioactivity reflects microbiotic urease-dependent intestinal loss of urea (L). L was $0.14 \pm 0.08$ in controls, $0.13 \pm 0.09$ in prednisolone-treated rats, $0.12 \pm 0.1$ in GH-treated animals, and $0.12 \pm 0.09$ in rats treated with both prednisolone and GH (mean ± SE). The control value was used in all present calculations.

**Analyses**

Blood urea concentration was measured by the urease-Berthelot method (11) and total blood AAN by the dinitrofluorobenzene method (13). Serum IGF-I was measured after acid-ethanol extraction by radioimmunoassay (RIA) using a polyclonal rabbit antibody (Nichols Institute Diagnostics, San Capistiano, CA) and recombinant human IGF-I as standard (Amersham International, Amersham, Bucks, UK). Serum glucose was measured in duplicate by the glucose oxidase method (Beckman oxidative glucose analyzer 700). Serum insulin was measured by RIA using recombinant human 125I-insulin as tracer, purified rat insulin as standard, and polyclonal guinea pig antibody (all reagents from Novo Nordisk, Bagsværd, Denmark). All samples were analyzed in triplicates in one assay. Organ N contents were determined by the micro-Kjeldahl technique as previously described (3).

**Calculations**

N balance was calculated as

\[
\text{N balance} = \text{food N} - (\text{urine total N} + \text{faeces total N})
\]

The alanine-stimulated CUNS ($\mu$mol·min$^{-1}$·kg body wt$^{-1}$) was calculated as the body accumulation of urea corrected for intestinal hydrolysis (18)

\[
\text{CUNS} = \frac{\text{dcu/d}t}{\text{tr}} \times 0.63BW \times 1/(1 - 0.14)
\]

where $\text{dcu/d}t$ is the slope of the linear regression analysis of blood urea concentration on time during steady state, 0.63BW is the distribution volume of urea (2), and $1/(1 - 0.14)$ is the correction factor for intestinal hydrolysis and recycling of N from newly synthesized urea.

**Statistical Methods**

Results are given as means ± SE. Statistics were performed using the Solo Statistical Software packet (BMDP Statistical Software, Los Angeles, CA). Data were analyzed using one-way analysis of variance followed by Student-Newman-Keuls method for multiple comparisons when appropriate. A two-tailed P value below 0.05 was considered significant.
Fecal N and urinary nonurea excretion were the same. GH almost cut total N excretion in half, due to a decrease in urinary urea N excretion \((P < 0.01)\). In the combined treatment group, total N excretion was similar to control and had the same contribution of N sources (Fig. 2).

The N balances were positive in free-fed rats and in controls pair fed to steroid rats, negative in steroid rats, positive and increased by 33% above free-fed controls in GH-treated rats \((P < 0.01)\), and positive and increased by 20% compared with pair-fed controls in the steroid plus GH group \((P < 0.01)\).

CUNS

Prednisolone treatment doubled CUNS compared with free-fed controls and increased CUNS by 35% in relation to pair-fed controls \((P < 0.01)\). GH decreased CUNS by 35% compared with free-fed control rats \((P < 0.01)\). Prednisolone combined with GH normalized CUNS (Fig. 3).

Basal Blood AAN

Prednisolone decreased basal blood AAN by 15 and 33% compared with free- and pair-fed controls, respectively \((P < 0.05)\). Pair feeding to prednisolone-treated rats increased basal blood AAN by 20%. GH decreased basal blood AAN by 15% \((P < 0.05)\), and the combined treatment normalized it (Fig. 3).

mRNA Levels

In the steroid-treated rats, mRNA abundance for the CPS enzyme rose by 20%, that for ASS rose by 68%, that for ASL was cut in half, and that for ARG rose by 80% compared with pair-fed controls \((P < 0.05)\). In the GH-treated rats, the mRNAs for all except ARG fell markedly compared with free-fed controls \((P < 0.05)\). In the group treated with both hormones, mRNAs for ASL fell to 80% and ARG rose by 30% compared with pair-fed controls. GH thus counteracted most of the steroid-induced increases in mRNAs.

Table 1. Organ N contents

<table>
<thead>
<tr>
<th></th>
<th>Liver</th>
<th>Kidney</th>
<th>Heart</th>
<th>Soleus Muscle</th>
<th>EDL Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>235 ± 10</td>
<td>20.4 ± 0.8</td>
<td>16.5 ± 0.9</td>
<td>3.33 ± 0.2</td>
<td>3.66 ± 0.2</td>
</tr>
<tr>
<td>Steroid group</td>
<td>229 ± 13</td>
<td>21.0 ± 0.8</td>
<td>13.1 ± 0.7*</td>
<td>2.76 ± 0.2*</td>
<td>2.33 ± 0.1*</td>
</tr>
<tr>
<td>Pair fed to steroid group</td>
<td>217 ± 9</td>
<td>21 ± 0.9</td>
<td>15.9 ± 0.8</td>
<td>3.14 ± 0.2</td>
<td>3.43 ± 0.2</td>
</tr>
<tr>
<td>GH group</td>
<td>282 ± 11†</td>
<td>24.8 ± 1.0†</td>
<td>18.4 ± 1.0†</td>
<td>4.41 ± 0.3†</td>
<td>4.27 ± 0.3†</td>
</tr>
<tr>
<td>Combined GH and steroid group</td>
<td>264 ± 12†</td>
<td>23.2 ± 1.0†</td>
<td>16.7 ± 0.9†</td>
<td>2.92 ± 0.07</td>
<td>3.40 ± 0.2†</td>
</tr>
</tbody>
</table>

Values are means ± SE; \(n = 8\) rats in all groups. Units are mg N. EDL, extensor digitorum longus; GH, growth hormone. * \(P < 0.05\), treatment groups vs. matched controls. † \(P < 0.05\), treatment groups vs. steroid group.
Plasma Glucose, Serum Insulin, and Serum IGF-I

Serum insulin increased fivefold and glucose threefold upon steroid treatment (P < 0.01). GH alone did not change serum insulin or glucose levels. Combined treatment with steroid and GH increased insulin levels nearly threefold (P < 0.01), whereas glucose levels were similar to controls. The insulin-to-glucose ratio doubled after steroid treatment, indicative of decreased insulin sensitivity, and was not restored by concomitant GH treatment (Fig. 4 and Table 2).

Serum IGF-I (µg/l) fell by 20% in the steroid-treated group compared with pair-fed control rats (P < 0.05) and increased by 66% after GH treatment compared with free-fed controls (P < 0.01). IGF-I levels rose by 30% after combined treatment (P < 0.01).

DISCUSSION

The aim of this study was to evaluate the acute influence of prednisolone and GH singly and in combination on the CUNS, gene expression of urea cycle enzymes, N balance, and organ N contents. The main findings were that glucocorticoid exposure markedly increased and GH exposure markedly decreased the CUNS and that the combined use of both agents counteracted the effects of one another. These in vivo results were paralleled by changes in hepatic gene expression of urea cycle enzymes in the three treatment regimens. Correspondingly, body weight, N balances, and organ N contents fell after glucocorticoid treatment, rose after GH treatment, and remained the same after combined treatment. These results suggest the presence of a new hepatic mechanism whereby GH limits protein loss during glucocorticoid catabolism.

The changes in CUNS measured in the present study reflect substrate-independent hepatic changes in urea production due to altered urea cycle enzyme activity (18, 39). The CUNS method has been validated in terms of correlation with established liver function tests (17, 39) and has been applied in studies of experimental disease states (2, 7) as well as investigations of the effects of several hormones on urea synthesis (15, 16, 36, 37). The increase in CUNS and the decrease in N balance and organ N contents show that the catabolic effects of glucocorticoid treatment involve regulation of liver function as to conversion of amino acid N into urea N. The decrease in basal AAN shows that the accelerated removal of AAN by liver exceeded the release of AAN into the bloodstream. This difference was even larger when the steroid-treated animals were compared with their pair-fed controls. GH had the opposite effects; it decreased CUNS and increased N balance and organ N contents. Because basal AAN did not change in this situation, the decrease in liver removal of AAN and decrease in tissue release of AAN were in balance.

Table 2. Glucose and hormones

<table>
<thead>
<tr>
<th></th>
<th>Serum Insulin, ng/dl</th>
<th>Plasma Glucose, mmol/l</th>
<th>Blood Urea, mmol/l</th>
<th>Serum IGF-I, ng/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>54 ± 8</td>
<td>8.8 ± 0.3</td>
<td>4.2 ± 0.3</td>
<td>630 ± 42</td>
</tr>
<tr>
<td>Steroid group</td>
<td>204 ± 43*</td>
<td>14.5 ± 1.1*</td>
<td>6.2 ± 0.4*</td>
<td>454 ± 68†</td>
</tr>
<tr>
<td>Pair fed to steroid</td>
<td>37 ± 6</td>
<td>4.4 ± 1.0</td>
<td>4.0 ± 0.3</td>
<td>568 ± 34</td>
</tr>
<tr>
<td>GH group</td>
<td>65 ± 11†</td>
<td>9.2 ± 1.1†</td>
<td>3.8 ± 0.4</td>
<td>990 ± 64†</td>
</tr>
<tr>
<td>Combined GH and steroid group</td>
<td>142 ± 44†</td>
<td>8.3 ± 1.0†</td>
<td>4.6 ± 0.5†</td>
<td>723 ± 30†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 rats in all groups. IGF-I, insulin-like growth factor I. *P < 0.05 compared with pair-fed controls. †P < 0.05 compared with steroid group.

Serum IGF-I (µg/l) fell by 20% in the steroid-treated group compared with pair-fed control rats (P < 0.05) and increased by 66% after GH treatment compared with free-fed controls (P < 0.01). IGF-I levels rose by 30% after combined treatment (P < 0.01).
We did not measure amino acid profiles in this study. Horber and Haymond (25) found a decrease in nonessential amino acids and an increase in essential amino acids after prednisolone treatment and normalization after combination with GH. GH alone did not change levels of essential and nonessential amino acids (9, 5). Welbourne et al. (41) found that GH treatment to hypophysectomized rats had no effect on alanine and glutamine levels, whereas a significant increase in glutamate levels was recorded. This was accompanied by a significant reduction in urea synthesis rate (41). Taken together, it is not probable that changes in blood amino acid composition by GH are marked or contribute toward changes in urea synthesis.

Measurement of urea synthesis rate by accumulation of urea is subject to the assumption that body water is the same in all experimental conditions. This was confirmed by our study. Also, gut hydrolysis is presumed to be a constant in the calculations of urea synthesis in all groups. Our study confirmed that this is the case. There are no previous data on the effect of prednisolone and GH treatment on gut urease activity.

There was a diphasic response to the combined treatment of prednisolone and GH on body weight changes. Initially, body weight decreased, but after 3 days this was reversed. This may be due to either the normal delay of 2–3 days in the GH-mediated rise in IGF-I, the peptide that mediates some of the growth-promoting effects of GH, and/or the fact that the effect of prednisolone seems to be most pronounced and overrules the effects of GH the first 3 days of treatment. With declining steroid effects, the growth-promoting effects of GH become more marked. These mechanisms are not yet, however, elucidated.

The relation between regulation of urea synthesis and tissue N homeostasis has been demonstrated earlier in experimentally hyperglucagonemic rats (23): the CUNS rises, and carcass muscle protein contents fall. Correspondingly, in fasting humans, the efficacy of urea synthesis rises, and positive forearm amino acid balance is reversed (42). In the present context, the same and the opposite relations were observed during prednisolone and GH treatment, respectively; the increase in CUNS by prednisolone was accompanied by organ protein breakdown, and the reduction in urea synthesis by GH was followed by protein buildup. The combined treatment more or less neutralized these effects.

We suggest that the regulation of urea synthesis is one of the mechanisms of the actions of glucocorticoid and GH on body composition.

Studies by Horber and Haymond show that coadministration of GH during glucocorticoid treatment counteracts the protein catabolic effects as judged by measurements of N balance and isotope dilution techniques (25). The underlying mechanism could not be established, although the data suggested that GH increased whole body protein synthesis and decreased whole body protein oxidation (20). Our data of this study and of another recently published study (14) indicate that the mechanism involves regulation of the ability of urea synthesis. Data concerning hepatic amino acid metabolism during GH and glucocorticoid treatment are scarce. Receptors for both GH (30) and glucocorticoids (34) have been localized in hepatocytes. Regarding urea synthesis, it has been reported that GH administration and adrenalectomy decrease the activity of urea cycle enzymes (33), whereas glucocorticoid treatment increases the CUNS and the induction of urea cycle enzymes (31, 37).

The present study makes it possible to compare in vivo metabolic events with determinations of expression of the genes for the urea cycle enzymes. Prednisolone increased gene expression of both the flux-controlling feeder enzyme CPS and of the rate-limiting one, the ASS. Conversely, GH treatment decreased gene expression of both CPS and of ASS. The combined treatment resulted in near-normal expression of all the genes for the enzymes, including the CPS and ASS. The effects of prednisolone and GH and their combination on gene level thus corresponded qualitatively, and for the rate-limiting enzyme thus quantitatively, to the effects in vivo on the CUNS.

This may suggest that the urea synthesis capacity was determined by expression of the genes and, if so, that major posttranslational changes did not happen. This indicates that both prednisolone and GH exerted an effect on regulation of urea synthesis on the gene level, although an influence on other steps from transcription of the gene to the physiological effect of the enzyme can not be excluded.

Not all of the effects reported on regulation of urea synthesis may depend solely on prednisolone and GH but may also be related to secondary changes in other hormones. The most powerful hormonal upregulator of ureagenesis is glucagon, which was not measured, but steroids are known to stimulate glucagon secretion from the isolated pancreas (5), whereas GH does not influence glucagon levels in rats (9, 29). Thus glucagon may be responsible for some of the reported steroid effects. Furthermore, the CUNS is decreased by IGF-I, glucose, and possibly insulin (1, 15, 22). In this study, prednisolone increased both insulin and glucose concentrations and the insulin-to-glucose ratio markededly and decreased IGF-I levels. These changes indicate insulin resistance, probably also at the hepatic level, and may contribute toward an increased rate of urea synthesis and whole body catabolism (43). The combined treatment decreased both insulin and glucose levels, probably due to the rise in IGF-I, but did not restore the insulin-to-glucose ratio to control values.

In conclusion, we found that prednisolone increased the ability of urea synthesis and decreased AAN, N balance, and organ N contents. GH had the opposite effects. The N-wasting effects of prednisolone were neutralized by concomitant administration of GH. These effects were paralleled by regulation of urea cycle enzyme gene expression. This may be a new hepatic mechanism of the prevention of steroid catabolism by GH.
REFERENCES


