Altered corticosteroid metabolism differentially affects pituitary corticotropin response

JUNKO HANAFUSA,1 TOMOATSU MUNE,1 TETSUYA TANAHASHI,1 YUKINORI ISOMURA,1 TETSUYA SUWA,1 MAKO ISAI,1 HISASHI DAIJO,1 HIROYUKI MORITA,1 MASANORI MURAYAMA,2 AND KEIGO YASUDA1

1Third Department of Internal Medicine, Gifu University School of Medicine, Gifu 500-8705; and 2Gifu Prefectural Hospital, Gifu 500-8717, Japan

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IT IS WELL KNOWN that endogenous corticosteroid levels affects pituitary corticotropin response. Am J Physiol Endocrinol Metab 282: E466–E473, 2002. First published October 10, 2001; 10.1152/ajpendo.00065.2001.—To evaluate the effects of altered corticosteroid metabolism on the hypothalamic-pituitary-adrenal axis, we examined rats treated with glycyrhizic acid (G rats) or rifampicin (R rats) for 7 days. The half-life of exogenously administered hydrocortisone as a substitute for corticosterone was longer in G rats and shorter in R rats, with no differences in basal plasma levels of ACTH or corticosterone. The ACTH responses to human corticotropin-releasing factor (CRF) or insulin-induced hypoglycemia were greater in G rats and tended to be smaller in R rats compared with those in the control rats, whereas the corticosterone response was similar. No difference was observed in the content and mRNA level of hypothalamic CRF among the groups. The number and mRNA level of CRF receptor and type 1 11β-hydroxysteroid dehydrogenase (11-HSD1) mRNA level in the pituitary were increased in G rats but not changed in R rats, suggesting that chronically increased intrapituitary corticosterone upregulates pituitary CRF receptor expression. In contrast, CRF mRNA levels in the pituitary were increased in R rats. Our data indicate novel mechanisms of corticosteroid metabolic modulation and the involvement of pituitary 11-HSD1 and CRF in glucocorticoid feedback physiology.

corticosterone-releasing factor; corticotropin-releasing factor receptor; type 1 11β-hydroxysteroid dehydrogenase; glucocorticoid feedback

Reagents and animals. Hydrocortisone sodium succinate, glycyrhizic acid, hCRF, 125I-labeled-Tyr-CRF, and [1,2,6,7-3H]corticosterone (sp act 74 Ci/mmol) were purchased from...
Quantitation of mRNA levels of CRF, CRF receptor, and type 1 11-HSD in pituitary and hypothalamus. Because only a small amount of RNA could be obtained from an anterior lobe of the pituitary or a piece of the hypothalamus, we employed the quantitative competitive RT-PCR method to measure mRNA levels of CRF, CRF receptor, and 11-HSD1. Furthermore, the mRNA values were normalized for glycer-aldehyde-3-phosphate dehydrogenase (GAPDH), a housekeeping gene, to minimize the variations in DNA digestion and reverse transcription between samples. Total RNA was prepared from frozen tissues by use of Isogen (Nippon Gene, Toyama, Japan), and 1.8 μg of total RNA was treated with DNase I (GIBCO-BRL) and reverse transcribed with 160 units of Superscript II reverse transcriptase (GIBCO-BRL) in a 20-μl reaction volume containing 2.5 mM random 9 mers, 1 mM each dNTP, 8 units of placentar RNase inhibitor, and the manufacturer’s buffer. Each reaction was allowed to proceed at room temperature for 10-15 min followed by incubation at 42°C for 1.5 h. Competitors for each cDNA were prepared following the PCR MIMIC KIT protocol (Clontech, Palo Alto, CA). Specific primers for each cDNA were as follows: CRF (24), 5'-CCG CCT GGG GAA CCT CAA C-3' (sense, nuclear transcript (nt) 1393–1411) and 5'-CCC TGA CTT CCA TTT ACA C-3' (antisense, nt 1682–1700); CRF receptor (17), 5'-CTC CTT GCG GCC TTT GTC CTC-3' (sense, nt 478–498) and 5'-GGG GCC CTT GTC GTA GAT GTA GTC-3' (antisense, nt 883–903); 11-HSD1 (1) 5'-GAC ATG ATC ATT CTT AA-3' (sense, nt 415–431) and 5'-GGT GTT TCT GTG TCT ATG A-3' (antisense, nt 725–743); GAPDH (26), 5'-GGC AAG GTC ATC CAT GAC AAC-3' (sense, nt 482–497) and 5'-AGT GGC CAG CAG GGC CTT-3' (antisense, nt 812–832). Quantitative competitive PCR was performed by addition of 2.5 pmol of a sense and an antisense primer to 0.5 μl of reverse-transcribed samples with 0.5 μl of various ranges of 2.5× serially diluted competitor in 5 μl of 67 mM Tris-HCl (pH 8.5), 16 mMol/l (NH4)2SO4, 2 mMol/l MgCl2, 17 μg/ml BSA, 5% glycerol, and 0.25 units of Ex Taq DNA polymerase (TaKaRa, Osaka, Japan) for CRH, 11-HSD1, or GAPDH. For CRF receptor, MasterAmp Taq DNA polymerase (Epicentre Technologies, Madison, WI) was used with premixed buffer E supplied by the manufacturer. Samples were subjected to initial denaturation at 96°C for 2.5 min, followed by 36–52 cycles of 96°C denaturation for 20 s, 62°C annealing for 20 s, and 72°C extension for 30 s. PCR products were subjected to electrophoresis in 2.5% agarose gels, stained with ethidium bromide, scanned with a GT-9000 image scanner (EPSON, Nagano, Japan), and analyzed using NIH Image 1.60 (W. Rasband, NIH). All of the mRNA levels of CRF, CRF receptor, and 11-HSD1 are calculated by normalizing each quantitated mRNA values for those of GAPDH to control for inefficiency in the reverse transcription steps. 

CRF receptor binding assay in the pituitary. The CRF receptor assay was performed with a modification of the method previously described by Wynn et al. (29). After rats were decapitated, pituitary glands were immediately removed and separated into the neural- intermediate and anterior lobes. The latter were placed in ice-cold 50 mM Tris·HCl (pH 7.4) containing 5 mM MgCl2, 2 mM EGTA, and 1 mM dithiothreitol (buffer A). For the assay of CRF receptors, 10 anterior lobes per experiment were mechanically homogenized in buffer A with a glass homogenizer. Homogenates were centrifuged at 800 g for 10 min, and the supernatant was centrifuged further at 30,000 g for 30 min. The pellet (crude membrane fraction) was resuspended in buffer A to give a protein concentration of 100–300 μg/100 μl. For the binding assay, 100-μl aliquots of the membrane suspension were incubated with 70,000 cpm [3H]Tyr-CRF (0.14 nM) and

Steroid Metabolism and ACTH

Male Wistar rats weighing 200–250 g were housed individually in a controlled temperature (22°C) and light environment (12:12-h light-dark cycle; lights on from 0630 to 1830) and allowed to consume standard chow ad libitum. The animals were divided into three groups. The first group received 0.5 ml of distilled water twice per day by gavage and distilled water ad libitum for 7 days (control group). The second group received 30 mg/kg glycyrrhizic acid in 0.5 ml distilled water twice per day by gavage and 600 mg/l glycyrrhizic acid in distilled water ad libitum for 7 days (G group). The third group received 50 mg/kg rifampicin in 1.0 ml of distilled water daily by gavage and distilled water ad libitum for 7 days (control group). The animals were allowed to move freely. Each cannula was connected to a 30-cm length of PE-50 tubing 1 h before the acute experiments.
various amounts of unlabeled CRF (0 to 10⁻⁶ M) in a total 300-μl volume of 50 mM Tris-HCl buffer (pH 7.4) containing 5 mM MgCl₂, 2 mM EDTA, 0.15% BSA, 150 KIU/ml aprotinin, and 1 mM dithiothreitol. Tubes were covered with 1.0 ml of oil made of dibutylphthalate and dinonyloleate (ratio, 2:3). After incubation for 60 min at 22°C, the tubes were centrifuged at 10,000 g for 3 min, and the supernatants were removed. After separation of the membrane pellet, residual-bound radioactivities were counted. The protein concentration was measured by using a BCA protein assay kit (Pierce Chemical). Under these conditions, the total bound radioligand was 8.4–24.4%, and nonspecific binding, determined in the presence of 10⁻⁶ M unlabeled CRF, was 52.1 ± 2.1% of total radioactivity. These results are consistent with a previous report by Wynn et al. (29).

Calculation of the peak and basal values. The peak value was slower than that for ACTH (data not shown). There were no significant differences in the basal concentration of plasma ACTH and corticosterone among the three groups (Table 2). The plasma ACTH response to hCRF in the G group was significantly higher than that in the control group (P < 0.05) or the R group (P < 0.01). The ACTH response in the R group tended to be lower, but with no significance, compared with that in the control group (Fig. 1A). The peak incremental response (Δpeak) and net AUC of plasma ACTH were increased in the G group. In the R group, the net AUC of plasma ACTH was smaller than in the control group (Table 2). There were no significant differences in peak, Δpeak, or net AUC of the plasma corticosterone response to hCRF between the control group and the G or R groups (Table 2).

Table 1. Serum potassium level, adrenal weight, and half-life of exogenous hydrocortisone

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<tr>
<td>Potassium, meq/l</td>
<td>3.74 ± 0.16</td>
<td>3.66 ± 0.14</td>
<td>4.09 ± 0.15</td>
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<tr>
<td>Adrenal wt, mg</td>
<td>49.0 ± 3.8</td>
<td>47.7 ± 1.9</td>
<td>49.5 ± 3.1</td>
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<tr>
<td>Half-life, min</td>
<td>119.6 ± 5.5</td>
<td>158.3 ± 5.8*</td>
<td>92.1 ± 7.5†</td>
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Values are means ± SE. Each group was comprised of 12 rats for potassium and adrenal weight and 9 rats for half-life. C, control; G, glycyrhrizic acid treated; R, rifampicin treated. †P < 0.01 and *P < 0.05, significant difference from C.
the G group was significantly higher than that in the control group (P < 0.01) or the R group (P < 0.01). The ACTH response in the R group tended to be lower, but with no significance, compared with that in the control group (Fig. 1B). The net AUC of plasma ACTH in the G group was greater than that in the control group, and the peak and Δpeak tended to be high compared with those in control group. Peak, Δpeak, and net AUC of plasma ACTH in the R group tended to be low but not significantly different from those in the control group. There were no significant differences in peak, Δpeak, or net AUC of plasma corticosterone among the three groups (Table 3).

Changes in mRNA levels of CRF, CRF receptor, and type 1 11-HSD in pituitary and hypothalamus. As shown in Fig. 2, left, the hypothalamic mRNA levels of CRF receptor (top), which was about 1/10 of that in the pituitary, CRF (middle), and 11-HSD1 (bottom), did not differ among the three groups. However, in the pituitary (Fig. 2, right), the mRNA level of CRF receptor (top) was increased in the G group but not changed in the R group (control, 0.41 ± 0.03; G group, 1.18 ± 0.19; R group, 0.69 ± 0.11 × 10⁻³ CRF receptor/GAPDH, n = 4). The mRNA level of CRF in the pituitary (Fig. 2, right), which was about 1/12,000 of that in the hypothalamus, was increased fourfold in the R group compared with the control group (control, 2.6 ± 0.5; G group, 3.4 ± 0.8; R group, 10.2 ± 0.9 × 10⁻⁸ CRF/GAPDH, n = 4). Finally, the mRNA level of 11-HSD1 in the pituitary was increased in the G group (control, 4.9 ± 0.6; G group, 14.5 ± 1.0; R group, 6.8 ± 0.5 × 10⁻⁸ of 11-HSD1/GAPDH, n = 4).

Hypothalamic CRF content and pituitary CRF receptor binding assay. CRF content in the hypothalamus did not differ among the three groups (control, 2.50 ± 0.19; G group, 2.17 ± 0.18; R group, 2.21 ± 0.17 pg/mg wt, n = 12).

Pituitary CRF receptor concentration in the G group was statistically greater (P < 0.01) than that in the control or R groups (control, 57.4 ± 1.3; G group, 83.7 ± 4.7; R group, 51.6 ± 5.1 fmol/mg protein, mean ± SE of 5 experiments; Fig. 3, top). Although there were no significant differences in the dissociation constant of binding (Kₐ) between the control group and the G or R groups, Kₐ in the G group was higher (P < 0.05) than Kₐ in the R group (control, 0.88 ± 0.09, G group, 1.16 ± 0.11; R group, 0.75 ± 0.07 nM, mean ± SE of 5 experiments; Fig. 3, bottom).

Type 1 11-HSD activity in intact pituitary. In the intact pituitary halves, 11-reduction clearly predomi-

Table 2. Responses of plasma ACTH and corticosterone to CRF

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<tr>
<td>Basal, pmol/l</td>
<td>29.9 ± 1.7</td>
<td>32.2 ± 3.2</td>
<td>32.2 ± 1.7</td>
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<tr>
<td>Peak, pmol/l</td>
<td>116.0 ± 5.9</td>
<td>193.6 ± 24.7†</td>
<td>102.2 ± 6.1</td>
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<tr>
<td>ΔPeak, pmol/l</td>
<td>86.1 ± 4.7</td>
<td>160.4 ± 24.1†</td>
<td>70.3 ± 6.5</td>
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<tr>
<td>Net AUC, nmol·min⁻¹·l⁻¹</td>
<td>3.8 ± 0.2</td>
<td>6.5 ± 1.2†</td>
<td>2.6 ± 0.4‡</td>
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Values are means ± SE. Each group was comprised of 6 rats. ΔPeak, incremental value between peak and basal value; AUC, area under the curve. P < 0.01, significant difference from C (†) and significant difference from R (‡). P < 0.05, significant difference from C (§) and significant difference from R (§).
nated 11β-dehydrogenation. 11β-Dehydrogenase activities in the G group (0.051 ± 0.004% conversion·mg⁻¹·h⁻¹) were lower (P < 0.01) than those in the control group (0.138 ± 0.003), whereas 11-oxo-reductase activities in the G group (0.498 ± 0.026) were higher (P < 0.01) than those in the control group (0.316 ± 0.033).

DISCUSSION

We demonstrated that the half-life of exogenous cortisol (which is presumably correlated with that of corticosterone) was longer in glycyrrhizic acid-treated rats (G group) and shorter in rifampicin-treated rats (R group) than in control rats, whereas basal concentra-

Fig. 2. Expression of corticotropin-releasing factor (CRF), CRF receptor, and 11β-hydroxysteroid dehydrogenase (11-HSD1) mRNA in hypothalamus and pituitary in control (C), glycyrrhizic acid-treated (G), and rifampicin-treated (R) groups. Bars are mean and SE values obtained in 4 rats. Each mRNA level is expressed as the ratio to the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA level in the same sample. *P < 0.05.
tions of plasma corticosterone were similar among the three groups. Using these models of decreased and increased metabolism of corticosteroids, we performed two provocative tests (insulin tolerance and CRF tests) to assess the action of altered corticosterone metabolism on the hypothalamic-pituitary-adrenal axis.

We found that plasma ACTH responses to CRF and insulin-induced hypoglycemia were greater in the G group than in the control group, with no differences in plasma corticosterone responses. When the hypothalamic-pituitary-adrenal axis is driven in Addison’s disease or metyrapone treatment, the ACTH response to CRF is enhanced (25). Hence, the increased ACTH responses to CRF in delayed corticosterone metabolism in our glycyrrhizic acid-treated rats seems to be paradoxical. However, plasma cortisol levels in Addison's disease or during metyrapone treatment are presumably low, unlike in our study.

CRF synthesis in hypothalamic paraventricular nuclei and its release into hypophysial portal blood undergo negative feedback regulation by glucocorticoids and are activated under various stresses (15). CRF secretion from the hypothalamus is suspected to be modulated by altered peripheral corticosterone metabolism and/or direct effects of drugs on the hypothalamus. Seckl et al. (22) reported that acute administration of 25 mg/kg glycyrrhetinic acid, the active component of glycyrrhizic acid, decreased CRF release into hypophysial portal blood without changing circulating glucocorticoid levels, which is probably the result of negative feedback of corticosterone that accumulates as a consequence of decreased conversion to its inactive 11-dehydro product at hypothalamic paraventricular nuclei. In our chronic study, however, neither hypothalamic content and mRNA expression of CRF nor mRNA expression of 11-HSD1 differed among the groups with increased or decreased metabolism of corticosteroids.

Chronic treatment with glycyrrhizic acid in our study revealed upregulation of pituitary CRF receptor by both binding assays and mRNA levels, consistent with the increased ACTH response to CRF and insulin-induced hypoglycemia. The CRF receptor in the anterior pituitary is known to be downregulated acutely by CRF and glucocorticoids (5, 18, 29), but recent studies demonstrated that acute stress causes biphasic changes in pituitary CRF receptor mRNA expression with an early decrease followed by an increase and that chronic stress causes a sustained increase in pituitary CRF receptor mRNA with a permissive role of glucocorticoids (14, 19). Interestingly, 11-HSD1 mRNA levels were increased by three times in the pituitary glands of our glycyrrhizic acid-treated rats. A previous report (28) described that 75 mg/kg of glycyrrhizic acid administration for 5 days inhibited 11β-dehydrogenase activity in kidney and liver and reduced 11-HSD1 mRNA levels in kidney, liver, and pituitary. Our glycyrrhizic acid-treated rats did show the decrease in 11β-dehydrogenase activity but an increase in oxoreductase activity in the intact pituitary. The mRNA expression of 11-HSD1 in the kidney of our glycyrrhizic acid-treated rats was also increased (T. Mune, unpublished observation). Because the dose or term of glycyrrhizic acid treatment seems to be similar, the discrepancy about changes in pituitary or kidney 11-HSD1 expression is not fully explained. There might be differences in rat strain or in peripheral corticosterone concentrations.

Type 1 11-HSD has bidirectional (dehydrogenase and oxoreductase) activities in vitro and mainly dehydrogenase activity in tissue homogenates (10), but this isozyme mainly has oxoreductase activity in many tissues in vivo (6, 20). Indeed, our conversion assays showed an oxoreductase predominance in the intact pituitary. These observations indicate the role of 11-HSD1 as a potential enhancer of glucocorticoid action, whereas the role of 11-HSD2 is a protective mechanism for the mineralocorticoid receptor, because 11-HSD2 has only dehydrogenase activity and is expressed in mineralocorticoid target tissues. Recently described 11-HSD1 knockout mice have elevated basal levels and...
greater stress responses of plasma ACTH and corticosterone, together with diminished glucocorticoid feedback (4, 7). CRF mRNA expression in the hypothalamic paraventricular nuclei is similar in wild-type and 11-HSD1-deficient mice, but glucocorticoid receptor mRNA expression is reduced in 11-HSD1-deficient mice (4). Although glucocorticoid receptor expression was not examined in the present study, no changes in hypothalamic CRF, CRF receptor, and 11-HSD1 suggested that the pituitary gland was the primary lesion in our glycyrrhizic acid-treated rats, whereas 11-HSD1 knockout mice possibly have abnormalities also in higher central organs such as hypothalamus and hippocampus. In any case, the increased oxoreductase activity resulting from increased 11-HSD1 mRNA expression in the pituitary of our glycyrrhizic acid-treated rats should lead to intracellular accumulation of corticosterone within the cells, even under unchanged circulating corticosterone levels. Considering the sustained increase in pituitary CRF receptor mRNA under chronic stress (14, 19), it is plausible that chronically increased intrapituitary corticosterone with no detectable changes in hypothalamic CRF and 11-HSD1 upregulates pituitary CRF receptor expression.

The lack of differences in corticosterone responses compared with the differences in ACTH responses may indicate a kind of adrenal insensitivity, especially in glycyrrhizic acid-treated rats. This is in contrast to the increased adrenal sensitivity to ACTH reported in 11-HSD1 knockout mice (4) that have elevated plasma levels of ACTH and corticosterone as well as adrenal hypertrophy (7), none of which was seen in our glycyrrhizic acid-treated rats. These discrepancies might be because of the difference in affected sites of the central nervous system or the difference between supraphysiological changes, as in 11-HSD1 knockout mice, and physiological changes in our study. In our preliminary examination, the adrenal mRNA levels of 11-HSD1 and 11-HSD2 in our glycyrrhizic acid-treated rats tended to be increased and decreased, respectively. Further elucidation, including the changes in ACTH receptor or steroidogenic enzymes, will be necessary to clarify the role of adrenal 11-HSD isozymes in rats treated with glycyrrhizic acid.

Finally, our rifampicin-treated rats showed diminished plasma ACTH responses to CRF assessed by net AUC. Other parameters of ACTH responses showed similar tendencies but with no significance. Considering the milder decreases in cortisol half-life (compared with the greater increases by glycyrrhizic acid), the doses of rifampicin we used might not be enough to induce significant changes. Under these conditions, however, CRF expression in the pituitary was increased fourfold compared with the control group, although its level was only 1/12,000 that in the hypothalamus. Giraldi and Cavagnini (2) recently reported the production of CRF in rat corticotropes as in our study and suggested that intrapituitary CRH acts to maintain basal ACTH secretion. Increased CRF expression in our rifampicin-treated rats should cause a relative insensitivity to exogenous CRF. Because rifampicin is a prokaryotic RNA polymerase inhibitor and was recently shown to induce CYP3A4 via the orphan pregnane X receptor (3), one can speculate that rifampicin might directly affect CRF transcription, but CRF transcription was not examined in the present study.

In conclusion, our results suggest that chronic treatment with glycyrrhizic acid or the consequent decrease in metabolism of corticosterone upregulates CRF receptors in the pituitary concomitantly with induction of 11-HSD1 expression, resulting in an increased plasma ACTH response to stimuli. In contrast, rifampicin or the resulting increased metabolism of corticosterone might upregulate pituitary CRF expression. Our study suggests a possible novel mechanism of corticosteroid metabolic modulation and the involvement of pituitary 11-HSD1 and CRF in glucocorticoid feedback physiology.

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