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Intracellular Regeneration of Glucocorticoids by 11β-Hydroxysteroid Dehydrogenase (11β-HSD)-1 Plays a Key Role in Regulation of the Hypothalamic-Pituitary-Adrenal Axis: Analysis of 11β-HSD-1-Deficient Mice*

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ABSTRACT

11β-Hydroxysteroid dehydrogenases (11β-HSDs) catalyze interconversion of active corticosterone and inert 11-dehydrocorticosterone, thus regulating glucocorticoid access to intracellular receptors *in vivo*. 11β-HSD type 1 is a reductase, locally regenerating active glucocorticoids. To explore the role of this isozyme in the brain, we examined hypothalamic-pituitary-adrenal axis (HPA) regulation in mice homozygous for a targeted disruption of the 11β-HSD-1 gene. 11β-HSD-1-deficient mice showed elevated plasma corticosterone and ACTH levels at the diurnal nadir, with a prolonged corticosterone peak, suggesting abnormal HPA control and enhanced circadian HPA drive. Despite elevated corticosterone levels, several hippocampal and hypothalamic glucocorticoid-sensitive messenger RNAs were normally expressed in 11β-HSD-1-deficient mice, implying reduced effective glucocorticoid activity within neurons. 11β-HSD-1-deficient mice showed exaggerated ACTH and corticosterone responses to restraint stress, with a delayed fall after stress, suggesting diminished glucocorticoid feedback. Indeed, 11β-HSD-1-deficient mice were less sensitive to exogenous cortisol suppression of HPA activation. Thus 11β-HSD-1 amplifies glucocorticoid feedback on the HPA axis and is an important regulator of neuronal glucocorticoid exposure under both basal and stress conditions *in vivo*. (*Endocrinology* **142:** 114–120, 2001)

GLUCOCORTICOIDS REGULATE MYRIAD metabolic and homeostatic processes and mediate the response to stress (1). Glucocorticoid release is stimulated by the hypothalamic-pituitary-adrenal (HPA) axis, which is activated by diurnal cues and stress. These stimulate CRH and vasopressin release from the hypothalamic paraventricular nuclei (PVN), which induce ACTH release from the anterior pituitary, stimulating adrenal glucocorticoid secretion. However, both chronic glucocorticoid excess (Cushing's syndrome, pharmacotherapy) and deficiency (Addison's disease) produce deleterious effects on many tissues; and normally, glucocorticoid secretion is tightly regulated by the balance of HPA axis forward drive and glucocorticoid negative feedback. Glucocorticoid feedback control is mediated at the level of the PVN and anterior pituitary, as well as suprahypothalamic sites, notably the hippocampus (2). Glucocorticoids act via intracellular receptors of 2 types: glucocorticoid (GR) and mineralocorticoid (MR) receptors (3). Until recently, it was believed that glucocorticoid actions on target tissues, including the HPA axis, were determined solely by prevailing plasma steroid levels and the tissue-specific density of GR and MR. However, it has become apparent that prereceptor metabolism by 11β-hydroxysteroid dehydrogenases (11β-HSDs) potently regulates glucocorticoid access to receptors in some tissues, including perhaps the brain (4).

11β-HSD catalyzes interconversion of active glucocorticoids (cortisol, corticosterone) and inert 11-keto forms [cortisone, 11-dehydrocorticosterone (11-DHC)]. 11β-HSD type 2, a potent NAD-dependent 11β-dehydrogenase, rapidly inactivates glucocorticoids, thus allowing aldosterone-selective access to intrinsically nonselective MR in the distal nephron in the face of a large molar excess of circulating glucocorticoid (5, 6). The crucial physiological principle illuminated by the action of 11β-HSD-2 is that corticosteroid action on target cells is determined by enzyme action within the cells, rather than circulating steroid levels alone. Thus, patients or mice lacking 11β-HSD-2 show excess mineralocorticoid activity, despite having normal circulating levels of the glucocorticoids and very low levels of the mineralocorticoid aldosterone (7, 8). Hence, it is the intracellular concentration of the steroids that is important.

The 11β-HSD-2 isozyme is not expressed in the pituitary or most regions of the adult central nervous system (CNS), including the PVN and hippocampus (9, 10). In contrast, the brain and pituitary widely express 11β-HSD type 1 (11–14), a nicotinamide adenine dinucleotide phosphate [NADP(H)]-dependent isozyme, initially purified from liver. 11β-HSD-1 is bidirectional in tissue homogenates (15), but it acts as a predominant 11β-reductase in many intact cells in culture

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(16–19), including primary hippocampal neurons (20) and perhaps the brain (21). In principle, 11β-reductase is anticipated to amplify glucocorticoid action in target cells, exploiting circulating inert 11-keto steroids produced largely by renal 11β-HSD-2. 11β-HSD-1 is highly expressed in hippocampal (12, 14) and PVN neurons (13, 22). However, any role of 11β-HSD-1 in glucocorticoid feedback and HPA function is obscure, with studies hindered by the nonselectivity of available licorice-based inhibitors and their variable access to CNS subregions *in vivo* (23).

We recently produced mice homozygous for a targeted disruption of the 11β-HSD-1 gene (24). These mice cannot regenerate active corticosterone from inert 11-dehydrocorticosterone *in vivo*, confirming that 11β-HSD-1 is the predominant or sole 11β-reductase in the body. Despite more than adequate levels of active corticosterone in the plasma, the 11β-HSD-1-deficient mice show reduced activation of glucocorticoid-sensitive hepatic gluconeogenic enzymes in response to stress or obesity, and consequently, attenuated plasma glucose elevations to these stimuli. These data clearly illustrate the principle that *intracellular* activation of corticosterone from circulating inert 11-DHC plays an important role in determining effective intracellular glucocorticoid action in the hepatocyte. Indeed, the 11β-HSD-1 null mice show elevated basal (morning) plasma levels of corticosterone, as well as adrenal hypertrophy. Though adrenal hypertrophy might be anticipated to reflect a requirement for increased steroid synthesis, in the light of a greater, net inactivation of corticosterone in the deficient mice (with no reactivation of 11-DHC by 11β-reductase), elevated basal levels of corticosterone would not occur if HPA axis regulation is functioning normally. In such a case, there would be elevated corticosterone production, over time, to maintain normal plasma corticosterone levels. We have now, therefore, investigated HPA activity and glucocorticoid feedback in 11β-HSD-1-deficient mice, to determine whether circulating levels of glucocorticoids are the only signal for central actions of glucocorticoids or whether the glucocorticoid signal is modified by 11β-HSD-1 in a tissue-specific manner.

Materials and Methods

Animals

Adult 11β-HSD-1 $^{-/-}$ male mice (MF1/129) were used for this study and were compared with age-matched wild-type controls (24). Animals were housed singly before experiments. The light-dark cycle was kept constant with lights on from 0700 h to 1900 h. Animals were given standard chow and water *ad libitum*, and all studies were carried out to the highest standards of humane animal care.

Materials

All chemicals were purchased from Sigma (Poole, UK), unless otherwise stated. Molecular biology reagents were obtained from Promega Corp. (Southampton, UK).

Circadian experiments

To investigate the diurnal rhythm of glucocorticoids, animals were killed by decapitation in stress-free conditions at 0800, 1200, 1600, 2000, and 2400 h. Trunk blood was collected in EDTA (0.1% final concentration), and plasma samples were stored at −80 C before analysis for corticosterone, 11-DHC, ACTH, and corticosteroid-binding globulin (CBG) levels. Brains, taken at 0800 h, were rapidly frozen on dry ice and stored at −80 C. Liver samples were removed into molybdate buffer for homogenization, and GR binding assays were performed on the extracts.

Restraint stress

For the acute restraint stress test, mice were placed in a restraint tube for 10 min and then decapitated immediately, 45 min, or 90 min after the start of restraint. Trunk blood was collected, as above. At the 90-min time point, brains were taken and stored, as above.

Glucocorticoid feedback inhibition experiments

To study HPA axis feedback, basal plasma samples were obtained by tail nick, and then mice received vehicle (corn oil) or cortisol (5 mg kg $^{-1}$; 100 μl ip, a dose chosen to cause near-complete HPA axis suppression in wild-type animals) and were left to recover for 2 h. Synthetic glucocorticoids, such as dexamethasone, were not used because these may poorly penetrate the CNS in rodents and are poor 11β-HSD-1 substrates. Animals were placed in a restraint tube for 10 min and then killed immediately or after 90 min, and trunk blood was collected and stored, as above.

Analysis of plasma hormones

Plasma corticosterone was measured by RIA, as described (25), modified for microtiter plate scintillation proximity assay. Cross-reactivity with cortisol was less than 8%. ACTH was quantified by RIA, as described (22), using commercially available rabbit anti-ACTH antisera (IgG Corp., Nashville, TN).

11-DHC was measured by RIA. Plasma (20 μl) was incubated, in 50 μl, with rabbit anti-11-DHC antibody (1:10,000 dilution; a gift from Prof. Vecsei and Dr. Haack, Ruprecht-Karls-Universitat, Heidelberg) and [^{3}H]-11-DHC (10,000 cpm) in borate buffer, in a 96-well plate for 1 h at room temperature (RT). Antirabbit scintillation proximity assay (SPA) beads (Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, UK; 50 μl) were added, and the plate was sealed and incubated overnight at RT and counted in a Microbeta counter. The assay showed sensitivity to 11-DHC, down to 2.5 nM, and did not cross-react with corticosterone (in the range of 0.5–320 nM). To reduce the concentration of free corticosterone in samples and thus any low-level cross-reactivity, nondenatured samples were used because this preserves the specific binding of corticosterone to CBG, while allowing 11-DHC to remain unbound (26). [^{3}H]-11-DHC was generated from [1,2,6,7-^{3}H]-corticosterone (Amersham Pharmacia Biotech) as described (16). The efficiency of conversion of [1,2,6,7-^{3}H]-corticosterone to [^{3}H]-11-DHC was more than 98%.

Plasma corticosteroid binding capacity was assessed in plasma samples, as outlined (27). Briefly, plasma samples were stripped of endogenous steroids, using dextran-coated charcoal [DCC: methanol-washed charcoal (1 g) coated with dextran T-70 (0.1 g) in 100 ml molybdate buffer] and aliquots incubated with 1 pmol/100 μl [1,2,6,7-^{3}H]-corticosterone (Amersham Pharmacia Biotech), in the presence (nonspecific binding) and absence (total binding) of 2000-fold excess of unlabeled corticosterone, for 1 h at 22 C. Unbound corticosterone was removed using DCC (for 10 min, at 4 C), followed by centrifugation at 3000 × *g* for 15 min at 4 C. Bound [^{3}H]-corticosterone in the supernatant was counted. CBG in plasma was estimated from specifically-bound [^{3}H]-corticosterone. Results are presented as picomoles of corticosterone bound per milliliter of plasma.

Liver cytosol GR K_d and B_{max} measurements

Liver was homogenized in 3× vol ice-cold molybdate buffer (10 mM Tris-HCl, 2 mM dithiothreitol, 1.5 mM EDTA, 0.1 M sodium molybdate, 10% glycerol, pH 7.2) using 3 × 10-sec pulses of a Polytron homogenizer. The homogenate was centrifuged (20,000 × *g*, 20 min, 4 C), and the supernatant was further centrifuged (105,000 × *g*, 60 min, 4 C) to obtain samples for protein (Bradford assay; Bio-Rad Laboratories, Inc., Hemel Hempstead, UK) and receptor assays. For the receptor assays, samples (50 μl) were diluted to 4 mg/ml in molybdate buffer and incubated overnight with 25 μl [1,2,4,6,7-^{3}H]-dexamethasone (final concentration, 1.5 nM; 3000 cpm/assay tube; Amersham Pharmacia Biotech) and 25 μl nonradioactive dexamethasone or corticosterone (final concentration,

0.316 nM–100 μM) at 4 C in 96-well plates. Nonspecific binding was assessed in the presence of 200 μM cold dexamethasone. Free and bound steroids were separated, using 100 μl DCC, by centrifugation at 1,500 × *g* for 15 min at 4 C. Supernatants were removed to a 96-well plate and 2 vol scintillant (Optiphase-Supermix; Wallac, Finland) added. Plates were sealed, shaken, and counted. Liver cytosol GR K_d for dexamethasone (DEX; nM) and B_{max} (nmol DEX/g protein) were calculated using equilibrium binding analysis.

In situ hybridization for GR, MR, and CRH steady-state messenger RNA (mRNA) expression

Coronal cryostat sections (10 μm), at the level of the hippocampus and hypothalamic PVN, were mounted onto gelatin and poly-L-lysine-coated slides and stored at −80 C. *In situ* hybridization studies were performed according to Seckl *et al*. (28). Tissue sections were fixed in cold 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) containing 0.02% diethylpyrocarbonate for 10 min at RT, followed by 3 × 5 min in 2× SSC. For CRH mRNA, prehybridization buffer containing 50% formamide, 0.6 M NaCl, 0.01 M Tris HCl, 1× Denhardt's solution, 1 μM EDTA, 0.5 mg/ml denatured salmon sperm DNA, and 0.125 μg/ml yeast transfer RNA was applied to the sections; and the slides were incubated at 50 C for 2 h in sealed boxes. Plasmids containing fragments of complementary DNA (cDNA) for rat GR (673 bp *Pst*I-*Eco*RI fragment of rat cDNA), MR (513 bp *Eco*RI fragment of rat cDNA), and CRH (518 bp *Pvu*II-*Bam*HI fragment of rat cDNA) were used as templates to transcribe radiolabeled sense and antisense riboprobes using ^{35}S-uridine 5′-triphosphate (Amersham Pharmacia Biotech). cRNA probes were denatured, added to the hybridization mixture (10–15 × 10^6 cpm/ml; 50% formamide, 0.6 M NaCl, 0.01 M Tris HCl, 1× Denhardt's solution, 1 μM EDTA, 0.1 mg/ml denatured salmon sperm DNA, 0.125 μg/ml yeast transfer RNA, 10% dextran sulfate 10 min, at 75 C) and cooled to 55 C before addition of 10 mM dithiothreitol. Hybridization mixture (200 μl/slide) was then added to the sections and incubated overnight (16 h at 50 C) in sealed boxes. After hybridization, the slides were rinsed twice in 2× SSC for 30 min at RT before ribonuclease A digestion (0.09 μg/ml final concentration in 0.5 M NaCl, 0.01 M Tris HCl, 0.001 M EDTA for 1 h at 37 C). The slides were washed to a final stringency of 0.1× SSC at 60 C. After dehydration, sections were air-dried and exposed to autoradiographic film for 1 week at RT. Serial dilutions of ^{35}S-uridine 5′-triphosphate were made on filter paper and exposed, under identical conditions, to standardize signal strength. No signal was seen from ^{35}S-labeled sense RNA probes of similar specific activity hybridized under identical conditions to the antisense probes (not shown). Films were quantified by computer densitometry (Research Imaging, St. Catharines, Canada). For autoradiographs, specific optical density measurements were obtained after subtraction of background density (obtained over white matter). Five to 10 readings were taken from each region of each tissue section (3 sections per mouse). For GR, sections were dipped in NTB2 photographic emulsion (Eastman Kodak Co., Hemel Hempstead, UK) and stored at 4 C before development and counterstaining with pyronin (1%, wt/vol). Grain counting was performed using a microcomputer imaging device system (Research Imaging).

Statistics

For diurnal rhythms and stress experiments, groups were compared by genotype and time using 2-way ANOVA. Where significant differences and a genotype:time interaction were noted, one-way ANOVA with *post hoc* Scheffé F-tests was performed for within-group comparisons, and *t* tests were used to show significant differences between groups. For *in situ* hybridization histochemistry data, groups were compared by independent two-tailed Student's *t* tests. Some hormonal data were not normally distributed and were log-transformed before analysis, or, when this failed to normalize the distribution (*e.g.* basal ACTH), data were assessed by the nonparametric Mann-Witney test. Values are means ± SEM (n), and $P < 0.05$ was taken as significant.

Results

11-HSD-1-deficient mice have altered basal HPA parameters and diurnal rhythms

Wild-type mice showed a clear diurnal rhythm of plasma corticosterone, with a nadir of approximately 25 nM at 0800 h and a peak of approximately150 nM at 2000 h (Fig. 1A). Plasma corticosterone levels in 11β-HSD-1 $^{-/-}$ animals were elevated at 0800 h, compared with wild-type controls. The 11β-HSD-1 $^{-/-}$ mice also showed a diurnal rhythm of corticosterone, with a nadir at 0800 h, but had an earlier rise at 1200 h, resulting in a more extended corticosterone peak. The 11β-HSD-1-deficient mice showed significantly greater corticosterone secretion over the 24-h period (genotype:time interaction, $F(4,66) = 3.41$, $P = 0.0135$).

Plasma 11-DHC levels in wild-type mice also showed a significant diurnal variation, approximately in parallel with plasma corticosterone, with a nadir at 0800 h and a peak at 2000 h (Fig. 1B). 11-DHC levels in 11β-HSD-1-deficient mice

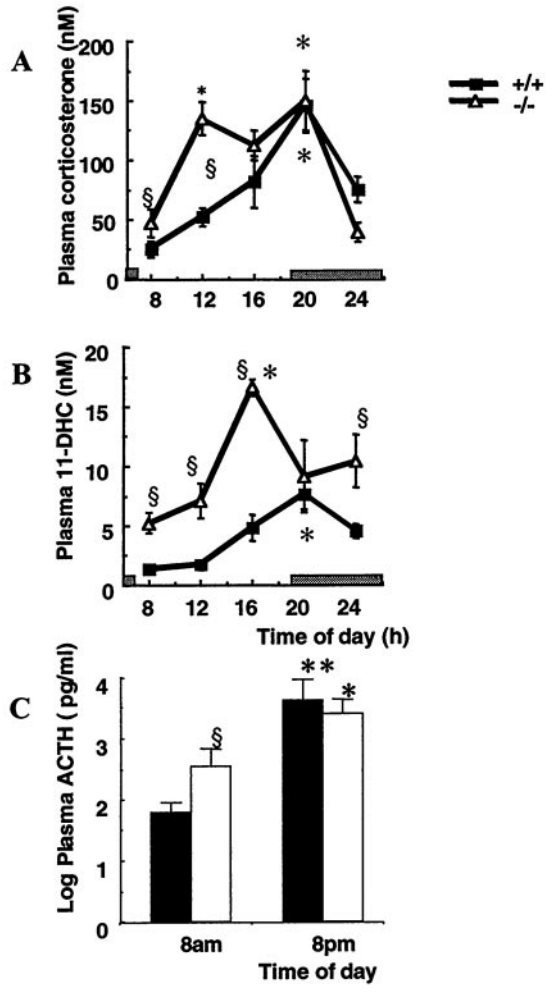

FIG. 1. Plasma levels of corticosterone (nM) (A), 11-DHC (nM) (B), and Log plasma levels of ACTH (pg/ml) (C) from wild-type (+/+, *filled squares*) and 11β-HSD-1 null mice (−/−, *open triangles*) secreted throughout the day (lights on, 0700 h to 1900 h). Values are mean ± SEM, n = 6–9 mice for each time point. *, $P < 0.05$, **, $P < 0.01$, compared with 0800 h time point; §, $P < 0.05$, compared with wild-type at that time point.

were significantly elevated, compared with wild-type animals, over the 24-h period (genotype:time interaction, $F(4,76) = 4.678$ $P = 0.002$). 11β-HSD-1$^{-/-}$ animals maintained the diurnal rhythm of 11-DHC, with levels significantly elevated above morning basal levels by 1200 h and an earlier peak than wild-type animals.

11β-HSD-1-deficient mice showed a striking reduction in the amplitude of the diurnal ACTH rhythm, compared with the wild-type mice. Although peak levels were similar, nadir plasma ACTH levels were higher in 11β-HSD-1$^{-/-}$ mice ($P = 0.02$, Mann-Witney test; Fig. 1C).

Plasma CBG levels (Table 1) and hepatic GR binding characteristics (Table 1) were similar in wild-type and 11β-HSD-1$^{-/-}$ animals, demonstrating that alterations in these parameters cannot underlie the changes in basal HPA activity in the 11β-HSD-1$^{-/-}$ mice. GR and MR mRNA levels in the hippocampus were also identical between the genotypes (Table 2). Whereas CRH mRNA expression in the hypothalamic PVN was similar in wild-type and 11β-HSD1$^{-/-}$ mice, GR mRNA levels in the PVN were significantly reduced in 11β-HSD-1$^{-/-}$ mice ($P < 0.01$; Table 2).

11-HSD-1-deficient mice show elevated responses to acute restraint stress

After 10 min of restraint stress, both wild-type and 11β-HSD-1$^{-/-}$ mice showed significant elevation of plasma corticosterone, but the peak corticosterone response of the 11β-HSD-1$^{-/-}$ mice was significantly greater than in wild-type (Fig. 2A); and overall, the 11β-HSD-1$^{-/-}$ mice had a greater corticosterone response to stress, compared with wild-type (genotype:time interaction, $F(3,54) = 5.48$, $P = 0.0023$). Though the initial fall in plasma corticosterone levels was rapid in both genotypes, 90 min after stress, plasma corticosterone levels were significantly higher in 11β-HSD-1$^{-/-}$ mice than in wild-type ($P < 0.05$).

In wild-type mice, restraint stress produced a gradual increase in plasma 11-DHC, over the sampling period, the increase only reaching significance at 90 min (Fig. 2B). In marked contrast, 11-DHC levels in 11β-HSD-1-deficient mice increased dramatically, peaking 10 min after restraint stress and falling thereafter. The peak levels achieved were significantly increased in the mutant mice, above those seen in wild-type mice (genotype:time interaction, $F(3,59) = 4.467$, $P = 0.0068$).

Both 11β-HSD-1-deficient and wild-type mice showed an immediate ACTH response to restraint stress. However, the overall ACTH response to stress was significantly greater in the 11β-HSD-1$^{-/-}$ mice (genotype:time interaction, $F(3,29) = 6.059$, $P = 0025$). Moreover, ACTH levels remained elevated above basal values for a longer period (90 min) in the 11β-HSD-1-deficient animals (Fig. 2C).

TABLE 1. Plasma CBG levels and liver GR K_d for dexamethasone (DEX, nM) and B_{max} (nmol DEX/g protein) from wild-type (+/+) and 11β-HSD-1 null mice (−/−). Values are mean ± SEM, n = 9

	+/+	−/−
Plasma CBG (pmol corticosterone/ml)	217 ± 23	174 ± 15
Liver GR binding		
kDa (nM)	1.9 ± 0.6	2.0 ± 0.5
B_{max} (nmol/g protein)	0.14 ± 0.02	0.17 ± 0.03

Reduced effect of exogenous glucocorticoids on HPA axis activity in 11-HSD-1-deficient mice

Wild-type mice were pretreated, 2 h before stress, with vehicle or cortisol in a dose chosen (on the basis of pilot studies) to cause near-full inhibition of the subsequent corticosterone response to restraint stress (cortisol-treated corticosterone peak,66 ± 17 nM; n = 5). In age- and weight-matched 11β-HSD-1$^{-/-}$ mice, this dose of cortisol was significantly less effective in suppressing the corticosterone rise, 10 min post stress (cortisol, 114 ± 8 nM; n = 4). Thus, at 10 min, immediately on cessation of stress, wild-type animals pretreated with cortisol had significantly lower corticosterone levels, compared with 11β-HSD-1$^{-/-}$ mice pretreated with cortisol (Fig. 3).

Discussion

Here, we show that deficiency of 11β-HSD-1 alters HPA function, both basally and in response to stress. 11β-HSD-1 has been shown to be the only enzyme to convert inactive 11-deoxycorticosterone (11-DHC) to active corticosterone in the mouse (24). Therefore, although normal conversion of corticosterone to 11-DHC occurs in the 11β-HSD-1$^{-/-}$ mice, no regeneration of corticosterone within tissues occurs, because of the lack of 11-reductase activity. This may be predicted to result in lower glucocorticoid levels; and hence, a compensatory increase in basal corticosterone secretion would ensue to maintain the normal corticosterone levels. The maintenance of basal corticosterone levels seems to be critical for normal functioning of the HPA axis and its response to various stressors (29). Indeed, 11β-HSD-1-deficient mice show adrenocortical hyperplasia and increased adrenal sensitivity to ACTH *in vitro* (24). However, basal plasma corticosterone levels should be unaltered, providing HPA sensitivity to glucocorticoids is unchanged. Instead, 11β-HSD-1-deficient mice have clear basal glucocorticoid hypersecretion alongside increased basal ACTH levels. These data imply increased basal HPA activity, which might be attributable to either increased drive and/or attenuated feedback control.

Several lines of evidence demonstrate that 11β-HSD-1-deficient mice have reduced sensitivity to glucocorticoid negative feedback upon the HPA axis. First, despite elevated circulating glucocorticoid levels, there is no down-regulation of several key HPA-associated glucocorticoid target genes and their products, including GR and MR mRNAs in the hippocampus, CRH mRNA in the PVN, and plasma ACTH levels. The latter is particularly unexpected, given the increased adrenal sensitivity to ACTH in these mice (24). Second, after stress, the rate at which plasma corticosterone levels return to basal (post peak) is dependent on the ability of glucocorticoids to terminate the central activation of HPA activity at various sites (pituitary, hypothalamus, and higher centers of the brain) by negative feedback. Despite elevated peak glucocorticoid levels in response to stress and the more efficient clearance of corticosterone, in the absence of 11β-reductase regeneration from 11-DHC, postpeak (60–90 min)

TABLE 2. Basal (nadir) steady-state mRNA levels of GR, MR and CRH in wild-type (+/+) and 11β-HSD-1 null mice (−/−) in the hypothalamic PVN, and dentate gyrus (DG), CA1, and CA3 regions of the hippocampus. *In situ* hybridization studies assessed by grain counting over individual neurons (GR) or optical density measurements over autoradiographically identified areas (MR, CRH). Data are means ± SEM (n); n.d., Not determined

	GR (grains/cell)		MR (OD; as % +/+ DG)		CRH (OD; as % +/+)	
	+/+	−/−	+/+	−/−	+/+	−/−
PVN	46 ± 6 (7)	25 ± 3 (8)[a]	n.d.	n.d.	100 ± 6 (10)	89 ± 5 (12)
DG	37 ± 6 (8)	31 ± 4 (8)	100 ± 11 (9)	83 ± 16 (9)	n.d.	n.d.
CA1	38 ± 9 (8)	32 ± 4 (8)	96 ± 10 (10)	107 ± 19 (9)	n.d.	n.d.
CA3	11 ± 3 (8)	13 ± 2 (8)	180 ± 23 (10)	196 ± 43 (10)	n.d.	n.d.

[a] $P < 0.01$, compared with wild-type value.

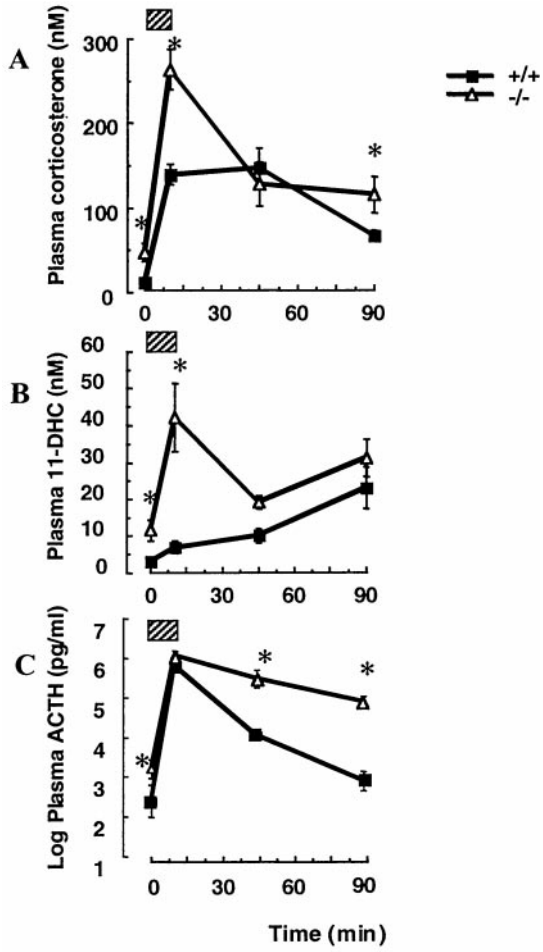

FIG. 2. Plasma levels of corticosterone (nM) (A), 11-DHC (nM) (B), and Log plasma levels of ACTH (pg/ml) (C) secreted over 90 min from wild-type (+/+, *filled squares*) and 11β-HSD-1 null mice (−/−, *open triangles*) in response to 10 min of restraint stress (*hatched bar*) given at 0900 h. Values are mean ± SEM, n = 7–10 mice for each time point. *, $P < 0.05$, compared with wild-type at that time point.

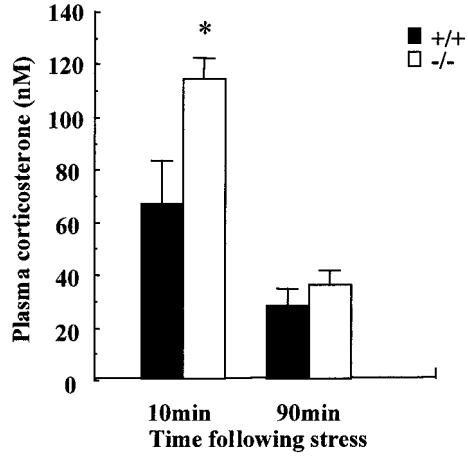

FIG. 3. Plasma levels of corticosterone (nM) secreted, over 90 min, from wild-type (+/+, *filled column*) and 11β-HSD-1 null mice (−/−, *open column*) in response to 10 min of restraint stress given at 1100 h, 2 h after pretreatment with cortisol (5 mg/kg ip). Values are mean ± SEM, (n). *, $P < 0.05$, compared with wild-type.

plasma ACTH and corticosterone levels are significantly higher in 11β-HSD-1-deficient mice than in wild-type controls. Hence, elevated postpeak plasma corticosterone and ACTH implies insensitivity to feedback control. Third, directly to address this, mice were given cortisol in a dose which greatly attenuated the HPA response to a subsequent stress in wild-type mice. 11β-HSD-1-deficient mice similarly pretreated with cortisol had a significantly greater corticosterone response to stress, despite increased metabolic clearance of cortisol (no regeneration by hepatic 11β-HSD-1 after metabolism to cortisone by renal 11β-HSD-2, which is unaltered in the 11β-HSD-1-deficient mice (24)), confirming a lack of sensitivity to glucocorticoid feedback in these animals.

Insensitivity to negative feedback regulation by glucocorticoid hormones could be obtained in several ways: by decreased free levels of circulating glucocorticoids by elevation of plasma CBG levels, by decreased glucocorticoid levels in relevant tissues caused by altered 11β-reductase activity, or by changes in tissue GR affinity or numbers at sites of negative feedback regulation (particularly the hippocampus and hypothalamus). However, no changes were observed in CBG levels in 11β-HSD-1-deficient mice. Indeed, CBG is a hepatic glucocorticoid-inhibited transcript (30). The maintenance of CBG levels in 11β-HSD-1-deficient mice further supports the notion of reduced effective glucocorticoid action in cells despite plasma glucocorticoid excess. The insensitivity to feedback cannot readily be ascribed to alterations in GR affinity for glucocorticoids, which was unaltered (at least in liver) or in GR or MR mRNA levels in the hippocampus. Hence, the implication is that lack of 11β-reductase in feedback sites (such as hippocampus, pituitary, and perhaps PVN) leads to attenuated glucocorticoid regeneration within these cells and therefore a reduced effective intracellular glucocorticoid signal *in vivo*.

However, there is a tissue-specific heterogeneity of glu-

cocorticoid effects in the 11β-HSD-1-deficient mice. Notably, the animals show reduced GR mRNA in the PVN. This contrasts with maintained GR in the hippocampus and indeed maintained CRH mRNA in the same region of the PVN. These findings suggest that there is a differential sensitivity to corticosterone in different brain areas. Activity of 11β-HSD-1 is lower in the hypothalamus than the hippocampus and pituitary (12, 14), which is in accord with these findings and supports the notion that the enzyme provides tissue-specific glucocorticoid modulation. Lower GR in PVN might attenuate feedback sensitivity too and may underpin the maintenance of CRH transcripts in the face of hypercorticosteronemia. It is likely that a combination of two processes, reduced GR expression in PVN and loss of 11β-reductase with consequent decrease in ligand availability to GR, underlies the negative feedback insensitivity in 11β-HSD-1-deficient mice. The importance of GR in negative feedback sensitivity of the HPA axis has been demonstrated in mice that lack GR throughout the CNS (31). These animals show elevated CRH and corticosterone, but reduced ACTH levels, suggesting the expected dominant role for the receptor, but illustrating the analogous (though less potent) effect of 11β-HSD-1. However, reduction of GR by antisense expression (32) has less effect on basal corticosterone and ACTH (33) than loss of 11β-HSD-1, suggesting that absence of enzyme activity in 11β-HSD-1-deficient mice is the more critical factor contributing to the HPA abnormalities than the modest reduction in GR seen in the PVN in the 11β-HSD-1-deficient mice.

Interestingly, circulating levels of 11-DHC (which are little bound to plasma proteins) in mice are 2–7 nM and thus similar to, if not greater than, basal free corticosterone levels (~1 nM), assuming approximately 95% of circulating corticosterone is usually bound to CBG. Cortisone levels in humans are even higher, at around 50 nM (34). Moreover, approximately 50% of 11-DHC is converted to active corticosterone on a single pass through the intact rat liver (35). It is therefore feasible that 11β-reductase activation of 11-DHC contributes an appreciable proportion of intracellular active glucocorticoid. The progressive accumulation of 11-DHC after stress in wild-type mice is presumably a consequence of increased corticosterone substrate for renal 11β-HSD-2 conversion to 11-DHC, which is then back-converted to corticosterone by 11β-HSD-1 in liver and elsewhere, fueling further renal conversion to 11-DHC. The rising 11-DHC levels presumably also represent an increasing substrate for reduction in feedback sites, amplifying the glucocorticoid effect.

What of forward HPA drive? The 11β-HSD-1-deficient mice show maintained circadian rhythmicity and good HPA responses to stress, suggesting that stimulatory pathways are intact. The exaggerated early stress responses of corticosterone are consistent with increased adrenal sensitivity to ACTH with paradoxically maintained ACTH release, presumably caused by the attenuated central sensitivity to the elevated basal glucocorticoid levels. Similar central glucocorticoid insensitivity (but not adrenal hypersensitivity) is seen in transgenic mice with antisense reduced GR expression (33, 36). However, there is a diurnal early peak of HPA activation in the 11β-HSD-1-deficient mice, suggesting more fundamental alterations in rhythm generation, perhaps at the suprachiasmatic nucleus, which expresses some 11β-HSD-1 (12) and is sensitive to glucocorticoid modulation. Future studies will address the nature of this finding and the possible relevance of 11β-HSD-1 in the human CNS and its disorders. Overall, the data suggest a novel and important role for 11β-reductase in regulation of the HPA axis. The importance of this enzyme in other CNS systems remains to be determined. This is of particular interest in the hippocampus and cerebellum, where 11β-HSD-1 expression is highest and where glucocorticoids have important effects upon electrophysiological parameters, behavior, and cognition, as well as neuronal development, structure, and survival (3, 37, 38).

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