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Phenotypic analysis of mice bearing targeted deletions of 11β-hydroxysteroid dehydrogenases 1 and 2 genes

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Abstract

The glucocorticoid metabolising enzymes, 11β-hydroxysteroid dehydrogenases (11β-HSD), play a critical role in determining the availability of glucocorticoids to activate their receptors and hence modulate target gene transcription. There are two isozymes, 11β-HSD-1 and -2, which act in opposing directions. 11β-HSD-2 acts as a dehydrogenase, converting active corticosterone (cortisol in humans) to its inactive 11-keto derivative (11-dehydrocorticosterone in rodents and cortisone in humans), whereas 11β-HSD-1 acts as a reductase, regenerating active glucocorticoids in a tissue-specific manner. Owing to the lack of specific inhibitors of these enzymes, it has been difficult to confirm the roles and determine the importance of these enzymes in vivo. Hence, to address this, we produced transgenic mice with null-mutations in the genes encoding the 11β-HSD-1 or 11β-HSD-2 enzymes. 11β-HSD-2 $-/-$ mice show signs of hypertension, hypotonic polyuria, hypokalemia and hypochloremia. These symptoms arise from illicit activation of mineralocorticoid receptors by glucocorticoids, in the absence of the protective action of 11β-HSD-2. The phenotype is directly comparable to the Syndrome of Apparent Mineralocorticoid Excess, seen in humans with mutations in the 11β-HSD-2 gene. Mice lacking 11β-HSD-1, however, show a more subtle phenotype with reduced activation of glucocorticoid-induced processes. They were unable to convert 11-dehydrocorticosterone to corticosterone in vivo, confirming 11β-HSD-1 as the sole 11-reductase in the mouse. They have elevated circulating levels of plasma corticosterone levels and adrenal hyperplasia, but they also have attenuated glucocorticoid-induced activation of gluconeogenic enzymes in response to fasting, and lower glucose levels in response to obesity or stress. Overall, these transgenic models have proved very useful for elucidating the roles of 11β-HSDs in vivo and will be a unique resource for investigating the importance of each enzyme in the diverse actions of glucocorticoids.

Keywords: Blood pressure; Corticosterone; Glucocorticoids; Gluconeogenesis; 11β-Hydroxysteroid dehydrogenase; Hypothalamo-pituitary adrenal axis; Mineralocorticoid; SAME; Transgenic

1. Glucocorticoids

Glucocorticoids exert multiple actions within the body to maintain homeostasis. They are released in response to 'stressors', both physical and psychological, by activation of the hypothalamo-pituitary adrenal (HPA) axis. Glucocorticoids alter their own release through a negative feedback action on the HPA axis and alter the transcription of many target genes. This stimulates adaptive processes such as gluconeogenesis, lipolysis and (immune response and sexual drive). One critical factor modulating the degree of glucocorticoid access to its receptors intracellularly is the presence of glucocorticoid metabolising enzymes within various tissues.

2. 11β-Hydroxysteroid dehydrogenases

11β-Hydroxysteroid dehydrogenases (11β-HSDs) are glucocorticoid metabolising enzymes that interconvert active glucocorticoids (cortisol in the human and corticosterone in the rodent) to the inactive 11-keto derivative (cortisone and 11-dehydrocorticosterone respectively). There are two isozymes of 11β-HSD, which apparently play different roles in the body as

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suggested by their function and distribution. 11β-HSD type 2 acts as a dehydrogenase, inactivating glucocorticoids, it uses NAD as a cofactor and has a K_m of 10 nM (Albiston et al., 1994; Brown et al., 1996a,b). 11β-HSD type 1, although being bidirectional in tissue homogenates, acts predominantly, if not exclusively, as a reductase in intact cells and tissues, regenerating active glucocorticoids from the circulating 11-ketosteroids. This enzyme uses NADP(H) as a cofactor and has a K_m of approximately 100 nM for ketosteroids (Agarwal et al., 1989; Lakshmi and Monder, 1988). The cofactor preference of these isozymes is consistent with the preferential directionality of the reaction they perform, compared with the 17-HSDs.

3. Role of 11β-HSD-2

11β-HSD-2 is expressed highly in discrete areas, usually in mineralocorticoid target tissues such as the kidney, as well as the placenta (Brown et al., 1996a,b; Edwards et al., 1988; Funder et al., 1988). Both glucocorticoids and mineralocorticoids (aldosterone) bind to the mineralocorticoid receptor (MR) with equal high affinity [see de Kloet (1991)], yet only aldosterone is able to activate these receptors in vivo even though circulating corticosterone is in considerable excess (100-fold). The proposed mechanism for protection of MR from illicit activation by corticosterone is intracellular inactivation of corticosterone by 11β-HSD-2 (Edwards et al., 1988). In order to confirm this role for 11β-HSD-2 and to determine any other major functions of this enzyme, transgenic animals have been produced with a null mutation in the 11β-HSD-2 gene.

4. 11β-HSD-2 knockout mice

A null mutation of the 11β-HSD-2 locus was successfully generated by replacing the genomic fragment encompassing exons 2–5 with a neomycin resistance cassette through homologous recombination in mouse 129 ES cells (Kotelevtsev et al., 1999). Cells exhibiting homologous recombination were injected into blastocysts, which ultimately produced chimeras and 11β-HSD-2 ($-/-$) transgenic lines (intercrossed on an outbred MF1 background). When offspring of the heterozygote intercross ($+/-$) were monitored at weaning, a deviation from Mendelian distribution was observed, showing an unexpected low number of $-/-$ mice (16%). The ratios of the genotypes were as expected in the E18.5 foetuses, and hence the litters were studied more closely after birth. Indeed, approximately 50% of the 11β-HSD-null mice died within 48 h of birth. The death was preceded by motor weakness and reduced suckling. The reason underlying this dramatic phenotype in half the animals compared with the apparent normal development of the remaining offspring to adulthood is unknown, and necessitates more experimentation. However, suggestions of intestinal ileus and possible cardiac arrest resulting from the severe hypokalemia have been made.

The 11β-HSD-2 $+/-$ mice have reduced and the $-/-$ mice have no detectable 11β-HSD-2 mRNA in the kidney as determined by northern analysis (Kotelevtsev et al., 1999). 11β-HSD-2 activity was measured in placental extracts. As there is also 11β-HSD-1 in the placenta, enzyme assays were done in the presence of NAD and NADP cofactors to measure dehydrogenase and reductase activity respectively. 11β-HSD-1 activity was similar in wild-type ($+/+$), $+/-$ and $-/-$ mice; the 11β-HSD-2 activity was reduced in the $+/-$ and negligible in the $-/-$ mice (Fig. 1).

5. Syndrome of Apparent Mineralocorticoid Excess (SAME)

From the hypothesised function of 11β-HSD-2 to protect MR from illicit activation by glucocorticoids, the 11β-HSD-2 null mice would be expected to have abnormal mineralocorticoid activity and hence changes in electrolyte levels and blood pressure by over-activation of MR in the kidney. Indeed, humans homozygous for deleterious mutations of the 11β-HSD-2 gene, who display SAME (Mune et al., 1995; Stewart et al., 1988) exhibit hypertension, sodium retention and hypokalemia. We checked to see if there is a similar phenotype in the 11β-HSD-2 knockout mice. Firstly, the 11β-HSD-2 null mice showed severe hypertension in both males and females compared with wild-type con-

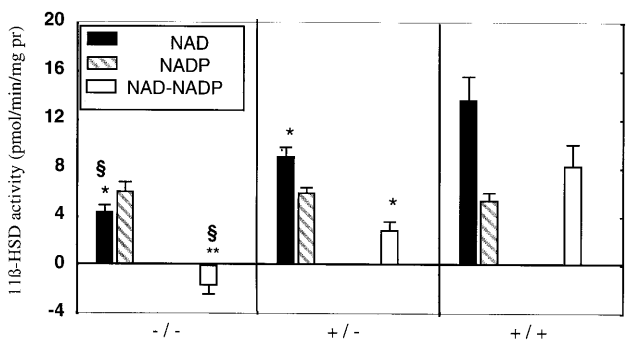

Fig. 1. 11β-HSD-1 and 11β-HSD-2 activities in placental extracts from 11β-HSD-2 mutant mice. Activities (mean ± SEM) are expressed as conversion of nmol [3H]-steroid substrate (either corticosterone for 11-dehydrogenase or 11-dehydrocorticosterone for 11β-reductase) to pmol product/mg protein/min. Black bars: NAD-dependent 11β-dehydrogenase activity; striped bars: NADP-dependent activity reflecting 11β-HSD-1 activity; open bars: NAD–NADP-dependent activity reflecting the residual activity due to 11β-HSD-2. $^{*}P < 0.05$ compared with 11β-HSD-2 $+/+$ and $^{\S}P < 0.05$ compared with 11β-HSD-2 $+/-$ mice.

Table 1
Blood pressure in 11β-HSD-2 transgenic mice[a]

	Pressure (mm Hg)		
	−/− $n=6$	+/− $n=5$	+/+ $n=4$
Systolic	162 ± 2.5*	127 ± 3.8	132 ± 2.4
Diastolic	138 ± 2.3*	107 ± 4.7	115 ± 2.6
Mean	145 ± 2.4*	110 ± 4.7	121 ± 2.6

[a] Blood pressures were measured in chronically cannulated, 3-month-old, conscious mice following microsurgical implantation of microrenathane catheter in the abdominal aorta. Measurements were made in 11β-HSD-2 +/+ wild type, 11β-HSD-2 +/− heterozygotes and 11β-HSD-2 −/− homozygotes. Values are mean ± SEM.
* $P<0.05$ compared with 11β-HSD-2 +/+ mice.

trols (Table 1), giving on average a 30 mmHg increase in mean blood pressure. Analysis of urine showed hypotonic polyuria, with reduced concentrations of sodium, potassium and creatinine (Fig. 2). This was coupled with marked hypokalemia and hypochloremia, but unchanged plasma levels of sodium and creatinine (Table 2). Consequently, the plasma aldosterone and renin activity were also significantly suppressed (Table 2). None of the above parameters showed any deviation from the wild type in the heterozygotes. The polyuria is likely caused by hypokalemia inducing nephrogenic diabetes insipidus and polydipsia, as is indeed seen in the humans with SAME (White et al., 1997).

The kidneys from the 11β-HSD-2 null mice were significantly enlarged compared with wild-type controls at 3 months of age (353 ± 13 mg, $n=6$ versus 277 ± 14 mg, $n=9$; $P=0.0003$). This increase in renal mass was associated with distal tubular enlargement which showed a two- to four-fold increase in diameter due to hyperplasia and hypertrophy of the epithelium (Kotelevtsev et al., 1999). The glomeruli, proximal tubules, medullary rays, medulla and collecting ducts were all normal under light and electron microsope analysis.

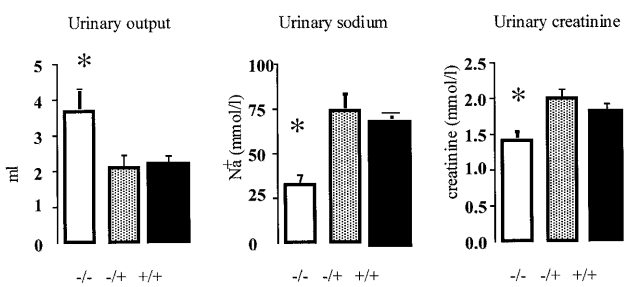

Fig. 2. Volume of urinary output and electrolyte concentrations in urine from 11β-HSD-2 mutant mice. 24 h urine samples were collected from mice in metabolic cages and the electrolyte concentrations measured. Samples were collected from 11β-HSD-2 −/− null mice (open columns), 11β-HSD-2 +/− heterozygous (stippled columns) and 11β-HSD-2 +/+ wild-type mice (black columns). Values are mean ± SEM; *$P<0.05$ compared with respective +/+ control.

Table 2
Electrolyte and hormone levels in plasma from 11β-HSD-2 transgenic mice[a]

	−/−	+/−	+/+
Sodium (mM)	152.1 ± 1.35	149.5 ± 0.56	150.3 ± 0.84
Potassium (mM)	3.2 ± 0.31*	4.9 ± 0.27	4.74 ± 0.2
Chloride (mM)	109.3 ± 1.75*	117.2 ± 0.86	114.7 ± 1.67
Creatinine (μM)	19.1 ± 0.9	20.1 ± 0.74	19.8 ± 0.54
Sodium/potassium	50.2 ± 4.6*	31.1 ± 1.6	32.1 ± 1.5
Ang I (ng/mg pr/min)	<0.05*		0.75 ± 0.4
Aldosterone	232 ± 93*		3085 ± 397

[a] The concentration of electrolytes and hormones in 11β-HSD-2 −/− homozygous mutants, 11β-HSD-2 +/− heterozygous mutants and 11β-HSD-2 +/+ wild-type adult mice was measured in plasma separated from terminal cardiac blood samples of 3-month-old mice (mean ± SEM). $n=4$ for electrolyte measurements and $n=6$–8 for hormone measurements.
* $P<0.05$ compared with 11β-HSD-2+/+.

The kidney hypertrophy and hyperplasia is evident by 3 weeks of age but not at birth. Similar structural changes have been observed in patients with chronic furosemide administration (Kaissling et al., 1985), in which increased distal tubular sodium transport occurs as a consequence of increased sodium delivery to the distal tubule after inhibition of inward salt transport by the loop diuretic. However, in the 11β-HSD-2 null mice the pathology is likely due to increased mineralocorticoid activity within the distal nephron. Such changes have not yet been confirmed in patients with SAME.

Finally, to determine whether it was the endogenous glucocorticoids that were responsible for the symptoms of SAME, the effect of suppression of the hypothalamo-pituitary axis by dexamethasone, a synthetic glucocorticoid that is not a ligand for MR, was tested in these mice. Untreated 11β-HSD-2 −/− mice have low urinary Na/K ratio compared with +/+ controls. Upon administration of dexamethasone, the −/− mice Na/K ratios increased to the unchanged levels of the wild-type mice, and decreased again following exogenous administration of corticosterone (Fig. 3). The dexamethasone inhibits corticosterone release and, therefore, its illicit activation of MR. When corticosterone is returned, the electrolyte abnormality was recreated, whilst wild-type mice show no mineralocorticoid effects of any of the manipulations. Dexamethasone is used routinely to alleviate the symptoms of SAME, but the hypertension is not usually fully reversed. This suggests that the SAME patients may have structural differences in the kidney and heart that maintain the hypertension even though the mineralocorticoid activation is blocked. Indeed, chronic treatment of the 11β-HSD-2 null mice with the MR antagonist spironolactone failed to reverse the structural changes seen in the kidney.

The 11β-HSD-2 null mice have been confirmed as a good model for SAME and will allow investigation of the processes underlying the development of the phenotype.

6. Role of 11β-HSD-1

Now moving to 11β-HSD-1, which generally is thought to act as a reductase, regenerating active glucocorticoids from inactive 11 keto-derivatives. The phenotype from a null-knockout of this gene is less easy to predict, and as yet there are no human polymorphisms predicting decreased 11β-HSD activity to base the phenotype on.

7. 11β-HSD-1 knockout mice

The 11β-HSD-1 null mice were prepared following replacement of exons 3 and 4 with a neomycin resistance cassette (Kotelevtsev et al., 1997). The resulting null mice were fertile, had unaltered birth weight and litter size, and appeared generally normal. There was no detectable 11β-HSD-1 mRNA or activity in the liver of the 11β-HSD-1 $-/-$ mice (Kotelevtsev et al., 1997). We then checked to see if these animals were able to convert 11-dehydrocorticosterone to corticosterone. Both $+/+$ and $-/-$ 11β-HSD-1 mice were adrenalectomised and small pellets of 15 mg 11-DHC or cholesterol (control) were placed subcutaneously. After 3 days the circulating plasma corticosterone levels were measured and the thymuses were weighed as an assessment of active glucocorticoids present. The wild-type mice had no detectable plasma corticosterone in the animals with the cholesterol pellets, but the mice with the 11-DHC pellets had high levels of corticosterone, suggesting that 11-DHC is easily converted to active glucocorticoids; this is confirmed by an atrophied thymus being observed in these mice (Fig. 4). On the contrary, the 11β-HSD-1 $-/-$ mice were unable to perform this conversion, suggesting that 11β-HSD-1 is the sole 11β-reductase in the mouse.

11β-HSD-2 activity in the kidney of the $-/-$ was similar to that of the $+/+$ mice, plus there were no changes in blood pressure or urinary electrolytes. These results suggest there are no compensatory changes in 11β-HSD-2 expression or activity and there is no apparent mineralocorticoid excess.

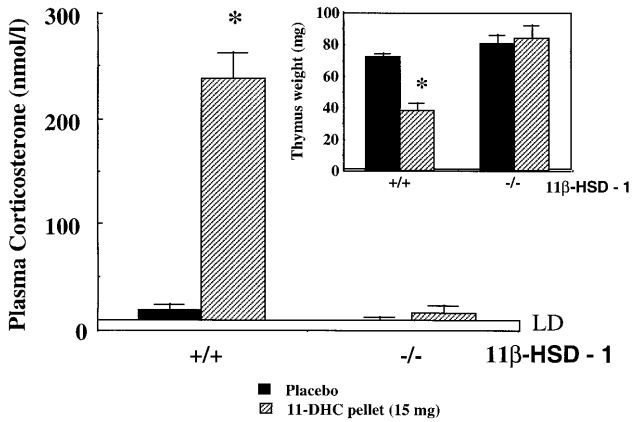

Fig. 4. Analysis of 11β-reductase activity in 11β-HSD-1 transgenic mice. The effect of dehydrocorticosterone (11-DHC) or cholesterol pellets in vivo (3 days) on plasma corticosterone and thymus weight (inset) in adrenalectomised 11β-HSD-1 $+/+$ and $-/-$ mice. Values are from mice with vehicle (black columns) and 11-DHC (striped columns); mean ± SEM; $n = 5–6$. LD, limit of detection of assay. $^*P < 0.05$ compared with vehicle control.

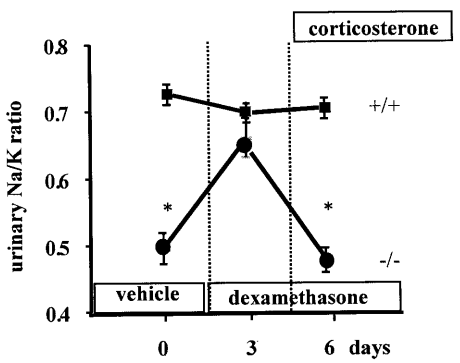

Fig. 3. Determination of the mineralocorticoid effects of corticosterone in 11β-HSD-2 null mice. 11β-HSD-2 $+/+$ wild type (squares) and 11β-HSD-2 $-/-$ null mice (circles) were injected with vehicle (3 days), followed by dexamethasone (100 μg/kg; 0–3 days) and finally dexamethasone was administered with replacement corticosterone (10 mg/kg; 3–6 days). Urine was collected for the last 24 h of each treatment and electrolytes measured by automated analyser. Values are mean ratio of sodium:potassium levels ± SEM; $n = 4$. $^*P < 0.001$ compared with corresponding wild-type value.

8. 11β-HSD-1 and regulation of the HPA axis

The 11β-HSD-1 mice, lacking 11-reductase activity would be unable to provide the tissue component of active glucocorticoids, above that of the circulating corticosterone levels. First, we investigated whether the circulating levels of corticosterone are markedly altered in the 11β-HSD-1 $-/-$ mice. The plasma corticosterone levels in the $-/-$ mice were nearly double the normal ($+/+$) basal levels at 8 a.m., the diurnal nadir (Fig. 5), whereas the values at the diurnal peak seemed similar. Indeed, the whole profile of the circadian rhythmicity of corticosterone appeared different in the $-/-$ compared with the $+/+$ mice (unpublished observations). In general, there is a hypersecretion of corticosterone over the 24 h period and this is accompanied by a hypertrophied adrenal (Fig. 5) in the $-/-$ compared with the $+/+$ mice. It is likely that the hypertrophy and hyperplasia is in the adrenal cortex, as

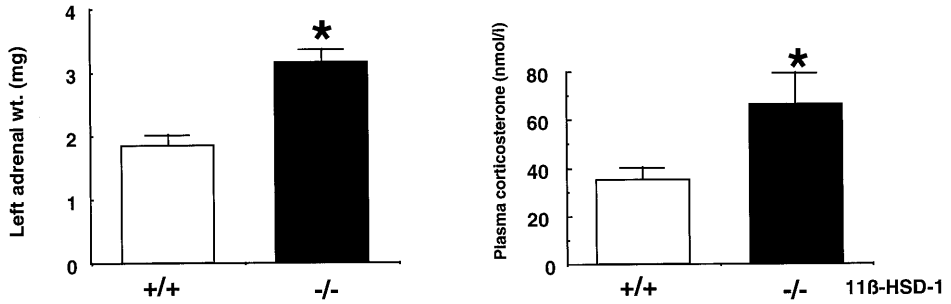

Fig. 5. Adrenal size and plasma corticosterone levels in 11β-HSD-1 transgenic mice. Left adrenals were removed and weighed from 11β-HSD-1 $+/+$ (open columns) and $-/-$ mice (black columns), and trunk blood (basal, unstressed samples) collected for plasma corticosterone analysis. Values represent mean ± SEM; $n = 8$–10. *$P < 0.05$ compared with $+/+$ control.

stimulation of the adrenals incubated in vitro with different doses of ACTH stimulated more corticosterone for each concentration of ACTH in the adrenals from the $-/-$ animals compared with the $+/+$. Hence the lack of 11β-HSD-1 activity has resulted in a compensatory increase in corticosterone secretion and altered regulation of the HPA axis. In the absence of the regenerated glucocorticoid signal, it would be expected that the refined compensatory ability of the HPA axis would be induced to secrete more corticosterone to reset the plasma corticosterone levels back to normal. However, the surprising finding is that the axis is overcompensating, producing elevated circulating plasma corticosterone levels. This could be explained by an absence of 11β-HSD-1 activity in CNS sites of normal negative feedback sites of glucocorticoids, which normally tightly regulate their own secretion. Results from stress experiments and feedback experiments support this hypothesis, showing attenuated negative feedback of glucocorticoids in the $-/-$ mice compared with the $+/+$ mice. Hence, these mice are unable to fully compensate for loss of 11β-HSD-1 activity in the regulation of the HPA axis.

9. 11β-HSD-1 and gluconeogenesis

Glucocorticoids play a critical role in induction of gluconeogenic enzymes during fasting. Measurements of fasting glucose levels in the 11β-HSD-1 knockout mice were similar to those observed in the wild type. The activity of two gluconeogenic enzymes, phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) were measured in liver microsomal fractions of ad lib fed and 72 h starved mice. These two enzymes catalyse the rate-limiting steps of gluconeogenesis, are induced upon fasting and transcription of the genes encoding both enzymes is regulated by classical glucocorticoid inducible promoters (Pilkis and Granner, 1992). In the wild-type mice there is a substantial induction of both G6Pase (152%) and PEPCK (296%) activity in the livers upon starvation, but in the 11β-HSD-1-deficient mice there is no induction of G6Pase and a reduced induction of PEPCK levels following starvation (Table 3). The attenuated response of the gluconeogenic enzymes may reflect an attenuated glucocorticoid-induced transcription due to a deficiency in glucocorticoid regeneration in the absence of 11β-HSD-1 activity in the liver, even in the face of elevated circulating glucocorticoid levels. The PEPCK gene appears to be less influenced by altered 11β-HSD-1 activity, possibly due to a complex regulation by both glucocorticoids and cAMP response elements (Imai et al., 1990) also noted in glucocorticoid receptor knockout mice (Cole et al., 1995).

Although there was no significant alteration in 'unstressed' fasting blood glucose levels, if the animals are stressed the glucose response is substantially greater in the 11β-HSD-1 $+/+$ mice compared with the $-/-$ mice (Fig. 6). Lower glucose levels were also observed in the $-/-$ mice that had been fed a high-fat diet for 8 weeks even though the $-/-$ mice had gained a similar amount of body weight to the $+/+$ controls

Table 3
Gluconeogenic enzyme activity in livers from 11β-HSD-1 transgenic mice[a]

	Enzyme activity			
	$-/-$		$+/+$	
	Fed	Starved	Fed	Starved
G6Pase (μmol/min mg)	0.42 ± 0.04	0.42 ± 0.05*	0.43 ± 0.07	0.63 ± 0.04*
PEPCK (nmol/min mg)	3.5 ± 0.5	11.3 ± 1.4*,#	5.3 ± 0.5	15.7 ± 1.7*

[a] Enzyme activity in livers from fed or starved (72 h) 11β-HSD-1 $-/-$ homozygous mutant and 11β-HSD-1 $+/+$ wild-type mice. Values are means ± SEM, $n = 8$.

* $P < 0.05$ compared with corresponding fed animals.

# $P < 0.05$ compared with corresponding 11β-HSD-2 $+/+$ value.

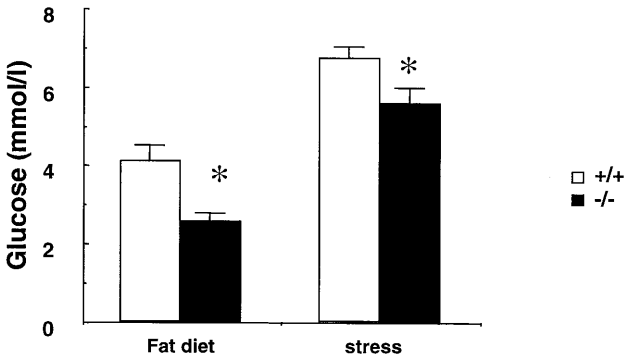

Fig. 6. Plasma glucose levels in 11β-HSD-1 mutant mice following a fat diet or stress. Glucose levels in blood from mice on a high-fat diet (8 weeks) or following a novel environment stress were measured in 11β-HSD-1 +/+ wild-type (open columns) and −/− transgenic mice (black columns). Values are mean ± SEM; $n = 8–10$. $P < 0.05$ compared with corresponding +/+ control.

(Fig. 6). The 11β-HSD-1 null mice, therefore, appear more resistant to hyperglycaemia upon stress or obesity, which is consistent with the hypothesis that these mice have lower intracellular glucocorticoid levels in the absence of the glucocorticoid regenerating activity.

10. The relevance of transgenics in assessing the role of steroid metabolising enzymes in vivo

We have successfully produced transgenic mice with null mutations in genes for two metabolising enzymes, namely 11β-HSD-1 and -2. These mice have enabled us to confirm the predicted role of these enzymes in vivo. The mice lacking 11β-HSD-2, glucocorticoid inactivating enzyme, showed a phenotype very similar to that observed in humans exhibiting SAME due to mutations in the 11β-HSD-2 gene. The phenotype for the 11β-HSD-1 null mouse was less easy to predict, but it is consistent with this enzyme acting as a reductase and regenerating active glucocorticoids from their inactive 11-keto derivatives. Now that the initial phenotype has been described for each transgenic mouse line, we have the opportunity to investigate the development of the phenotype and the importance of each enzyme in regulating/modifying the multiple and diverse actions of glucocorticoids at a cell-specific level.

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