Genetic manipulation of 11β-hydroxysteroid dehydrogenases in mice

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Paterson, Janice M., Jonathan R. Seckl, and John J. Mullins. Genetic manipulation of 11β-hydroxysteroid dehydrogenases in mice. Am J Physiol Regul Integr Comp Physiol 289: R642–R652, 2005; doi:10.1152/ajpregu.00017.2005.—11β-Hydroxysteroid dehydrogenases (HSDs) interconvert active 11-hydroxy glucocorticoids (cortisol, corticosterone) and their inert 11-keto derivatives (cortisone, 11-dehydrocorticosterone). 11β-HSD type 1 is a predominant reductase that generates active glucocorticoids in expressing cells, thus amplifying local glucocorticoid action, whereas 11β-HSD type 2 catalyzes rapid dehydrogenation, potently inactivating intracellular glucocorticoids. Both isozymes thus regulate receptor activation by substrate availability. Spatial and temporal regulation of expression are important determinants of the physiological roles of 11β-HSDs, with each isozyme exhibiting a distinct, tissue-restricted pattern together with dynamic regulation during development and in response to environmental challenges, including diet and stress. Transgenic approaches in the mouse have contributed significantly toward an understanding of the importance of these prereceptor regulatory mechanisms on corticosteroid receptor activity and have highlighted its potential relevance to human health and disease. Here we discuss current ideas of the physiological roles of 11β-HSDs, with emphasis on the key contributions made by studies of 11β-HSD gene manipulation in vivo.

MOLECULAR GENETICS AND BIOCHEMISTRY OF 11β-HYDROXYPEROID DEHYDROGENASES

TWO DISTINCT 11β-HYDROXYPEROID dehydrogenases (HSDs) identified thus far are products of distinct genes: HSD11B1, located on chromosome 1 in humans and in mice, and HSD11B2, located on chromosome 16 in humans and on chromosome 8 in the mouse genome. As the gene sequences suggest, both 11β-HSD types 1 and 2 are membrane-bound microsomal enzymes, each belonging to the large superfamily of short-chain alcohol dehydrogenase reductases (22) with conserved cosubstrate [NAD or NADP(H)] and steroid binding sites (36). Comparing the HSD11B1 and HSD11B2 genes to each other within a single species detects little significant sequence identity apart from a conserved domain present in all members of the short-chain alcohol dehydrogenase family (36) that encompasses the enzymatic active site (35 and 41% amino acid sequence identity over a 78- or 74-amino acid region comparing mouse or human 11β-HSD1 and 11β-HSD2, respectively). However, comparing HSD11B1 and HSD11B2 genes between human and mouse indicates a high level of conservation where the coding regions of each enzyme exhibit 81 and 83% identity, translated to 85 and 77% amino acid sequence identity, respectively. Relationships between 11β-HSDs and the conservation of polypeptide 11β-HSD sequences across species are illustrated in Fig. 1.

Originally 11β-HSD types 1 and 2, purified and cloned from liver (1, 38) and kidney (2, 4), respectively, are both reported to function as single-chain polypeptides localized to the endoplasmic reticulum (ER; see Refs. 57 and 62). However, dimerization of 11β-HSD1 has been shown to occur, consistent with its function as a reductase in vivo (49). Tagging of 11β-HSD1 and 11β-HSD2 polypeptides by fusion to a fluorescent protein or immunological epitope has revealed their intracellular localization within transfected cells and indicated that they exhibit opposing orientation when inserted in the ER membrane (57, 61, 62). The catalytic domain of 11β-HSD1 is exposed to the lumen of the ER, whereas the 11β-HSD2 active site and cofactor binding domain are exposed to the cytoplasm (49, 62). This may be relevant with respect to the cofactor dependence of 11β-HSDs, since intracellular concentrations of NADP(H) and NAD vary. NADP(H), the cofactor for 11β-HSD1, is elevated within the lumen of the ER relative to the cytoplasm where NAD, the cofactor for 11β-HSD2, is more abundant (49, 62). The potential importance of cofactor availability has been highlighted in a recent report of corticosteroid reductase deficiency in humans precipitated by compound heterozygous mutations in both the 11β-HSD1 gene and the hexose-6-phosphate dehydrogenase gene, encoding a major enzyme for synthesis of NADP(H) in the ER lumen (5, 18).

TISSUE-SPECIFIC PROTECTION FROM CORTICOSTEROID RECEPTOR ACTIVATION: 11β-HSD2

Tissue distribution is an important factor in the physiological function of 11β-HSDs, summarized in Fig. 2. 11β-HSD2 exhibits a restricted pattern of expression where it is highly enriched in aldosterone (mineralocorticoid) target tissues. This distribution reflects the key role of this enzyme, which is to prevent glucocorticoid (GC) activation of mineralocorticoid receptors (MRs), which are otherwise nonselective between cortisol, corticosterone, and aldosterone. The function of pre-
receptor GC metabolism by 11β-HSD type 2, a high-affinity NAD-dependent dehydrogenase, was initially proposed as part of a mechanism conferring mineralocorticoid substrate specificity on otherwise nonselective high-affinity mineralocorticoid (type 1 corticosteroid) receptors (19, 21). This role was clearly demonstrated in the 11β-HSD2 knockout mouse, which serves as a model of the syndrome of apparent mineralocorticoid excess (AME) in humans (34). Major phenotypes of the 11β-HSD2 null mouse are summarized in Table 1. A physical association of 11β-HSD2 and the MR has recently been demonstrated through the use of confocal fluorescence microscopy with tagged proteins expressed in cell culture (61). This study indicated specificity in association of 11β-HSD2 with MR and not GC receptor (GR). However, 11β-HSD2 has also been suggested to protect GR against ligand activation, which may be particularly important during prenatal development. 11β-HSD2 is highly expressed in both placenta and the developing embryo where it is postulated to regulate activation of both MR and GR (9, 17, 83, 86, 87). 11β-HSD2 in the placenta may offer protection to GRs against the effects of abundant active GCs within the maternal circulation (76).

GC INACTIVATION BY 11β-HSD2 IN THE KIDNEY: AME IN 11β-HSD2 KNOCKOUT MICE

During postnatal development and in adults, 11β-HSD2 is pivotal in the regulation of electrolyte balance, which is conventionally controlled by mineralocorticoids acting predominantly in the kidney but also in the colon. “Escape” of MR or GR from control by 11β-HSD2 was proposed (19, 21) and subsequently determined as the underlying cause of the rare syndrome of AME in humans where mutations in the 11β-HSD2 gene were identified and found to compromise enzyme activity (16, 20, 32, 40, 41, 56, 60, 90, 91). AME is characterized by hypokalemia, hypernatremia, and hypertension, generally leading to death in infancy or childhood (58). In the few surviving adult AME patients, electrolyte imbalance and marked hypertension persist. The generation of 11β-HSD2 knockout mice, which closely models AME, allowed the molecular and cellular changes occurring in the kidney to be defined (34). Approximately one-half of affected homozygote 11β-HSD2 null mouse pups die within 48 h of birth; similar early perinatal deaths are observed in human AME (34). In 11β-HSD2 null mice surviving to adulthood, the phenotype is characterized by hypokalemia, hypocholesterolemia, hypotonic polyuria, and marked hypertension, primarily resulting from unregulated activation of MR by corticosterone but may also involve activation of GR (34). 11β-HSD2 null mice have also revealed striking renal histological changes, with marked hyperplasia and hypertrophy of the distal nephron consistent with persistent MR activation, leading to electrolyte imbalance and the AME disease phenotype (34). Subsequently, distal tubular hypertrophy and hyperplasia have been found to occur in AME patients, illustrating a rare example of predicting human pathology from an animal model. The potentially irreversible structural changes in 11β-HSD2 null mice may also explain why suppression of endogenous GC production does not always reverse the phenotype in human AME (8).

GC INACTIVATION BY 11β-HSD2 IN THE VASCULATURE: ALTERED RESPONSES TO VASOACTIVE AGENTS IN 11β-HSD2 KNOCKOUT MICE

11β-HSD2 knockout mice show impaired endothelium-derived nitric oxide activity together with endothelium-dependent elevation of contractile responses of the aorta and also show altered endothelium-independent responses to vasodilatory stimuli (23). Such changes were not detected in vessels derived from a chemically induced model of apparent mineralocorticoid excess, suggesting that altered vascular function in 11β-HSD2 null mice is independent of altered circulating electrolyte balance resulting from loss of 11β-HSD2 function in the kidney in the knockout model (23). 11β-HSD2 expression within the mouse aorta is restricted to endothelium, whereas 11β-HSD1 has been detected within both endothelial and vascular smooth muscle layers (12). MR expression is enriched within the endothelial cell layer and may be protected by 11β-HSD2 in these cells against illicit activation by corticosterone. Meanwhile, GR is present at similar levels in both endothelium and vascular smooth muscle and may be regulated by 11β-HSD1 in both cell layers, as well as by 11β-HSD2 in endothelium (12). Testing the proposed hypothesis that lack of corticosteroid inactivation by 11β-HSD2 in the endothelium might underlie altered responses to vasoactive substances in 11β-HSD2 knockout mice, Christy et al. (12) found that these altered endothelial responses in aortic ring preparations in vivo could be reversed by incubation in medium containing L-arginine, suggesting that additional mechanisms must be responsible, perhaps related to changes in the kidney and the resultant hypertension or impaired nitric oxide production. Roles of 11β-HSDs within the vessel wall, therefore, remain to be fully elucidated. Involvement of GCs in the regulation of vascular development, structure, function, and inflammatory responses has been well documented (reviewed in Refs. 36, 76, 89, and 92) and may involve 11β-HSDs in vivo. Altered vascular structure may underlie sporadic sudden death in 11β-HSD2 null mice, particularly in females during pregnancy, which appears to be caused by rupture or dissection of the aorta, arising in the aortic arch region, possibly precipitated as a consequence of volume-expanded hypertension (unpublished observation, J. M. Paterson and S. Fleming, University of Dundee, UK; schematically represented in Fig. 3). The effects of 11β-HSD2 in the vasculature may contribute toward overall regulation of blood pressure. It will be interesting to determine whether potentiated vasoconstriction found in MF1 11β-HSD2 knockout mice also occurs on other mouse strain backgrounds or whether this is particular to MF1 11β-HSD2 null mutants and contributory toward the marked hypertension in this strain.

GC INACTIVATION BY 11β-HSD2 IN THE HEART: CARDIAC FIBROSIS AND HEART FAILURE IN MICE OVEREXpressing 11β-HSD2 SELECTIVELY IN CARDIOMYOCYTES

Although fibrosis within the heart is not a reported feature of AME, it is commonly associated with conditions of actual mineralocorticoid excess concomitant with elevated plasma sodium levels (75). MR antagonists are widely used in treatment (75). The role of MR per se in mediating cardiac fibrosis has been explored using transgenic approaches. Mice trans-
genetic for overexpression of human MR in kidney and heart exhibit renal abnormalities with mild dilated cardiomyopathy (39). Conditional knockdown of MR expression in mice bearing an inducible antisense MR transgene precipitates cardiac fibrosis and severe heart failure, reversible upon withdrawal of transgene induction (7). Findings from these studies suggest that increased or decreased MR abundance in cardiomyocytes can affect heart structure and function. Independent of adrenal hormone production, autocrine, paracrine, or intracrine regulation of receptor ligand may be important for MR action in the heart, as it increasingly appears to be for GR action in several tissues.

Although intracardiac synthesis of aldosterone remains highly controversial, a report describes increased activity of aldosterone synthase within the heart in human hypertrophic cardiomyopathy and in a transgenic mouse model of this disease (88). Elevated plasma aldosterone in a rodent model of cardiac fibrosis is associated with increased expression of both MR and 11β-HSD2 mRNA within the heart (33). GC occupancy of MR in the heart is normal under physiological conditions, with expression of 11β-HSD2 generally below the limits of detection (14, 42, 53). Overexpression of 11β-HSD2 in mice, selectively in cardiomyocytes under control of the mouse α-myosin heavy chain promoter (α-myosinHC-HSD2), precipitates marked cardiac hypertrophy and fibrosis, leading to early death from heart failure (69). Cardiac pathology in α-myosinHC-HSD2 transgenic mice is attenuated by MR antagonist treatment, whereas a GR antagonist does not rescue this phenotype, suggesting that the effects of inappropriate GC inactivation in this model are mediated through MRs (69). The findings from the α-myosinHC-HSD2 transgenic mouse model suggest that, in the absence of GCs, MR may then be occupied by aldosterone to deleterious effect. Although 11β-HSD2 expression in the heart is barely detectable, 11β-HSD1 is expressed and, if regenerating active GC, could potentially play a role in maintaining equilibrium between corticosterone (or

### Comparison of 11β-HSD1 and 11β-HSD2 polypeptides

**CLUSTAL W (1.82) multiple sequence alignment**

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Sequence identity: ✗
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#### Conservation of 11β-HSD1

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Sequence identity: ✗
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**Fig. 1. Amino acid sequence alignments using CLUSTAL W (1.82) software program comparing human 11β-hydroxysteroid dehydrogenase (HSD) 1 with human 11β-HSD2, 11β-HSD1 across species, and 11β-HSD2 across species. **Sequence identity. Conservative substitution.
Conservation of 11βHSD2

CLUSTAL W (L.42) multiple sequence alignment

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mouse_LbetaHSDD2
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cow_LbetaHSDD2
sheep_LbetaHSDD2
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Fig. 1. Continued.
cortisol) and aldosterone in this organ. However, this speculative hypothesis remains to be tested.

**GC INACTIVATION BY 11β-HSD2 IN BONE: ALTERED BONE FORMATION AND FUNCTION IN TRANSGENIC MICE OVEREXPRESSING 11β-HSD2 IN OSTEOBLASTS**

A role for intracellular GCs in differentiation from bone marrow stromal cells and the formation and/or maintenance of bone is supported by reports where levels of intraosteoblast GC have been reduced through introduction of transgene-derived 11β-HSD2 expressed within this cell type (59, 82). Recent studies of two transgenic mouse models selectively overexpressing 11β-HSD2 in osteoblasts under the control of either the rat Col1a1 promoter (Col2.3-HSD2 mice; see Ref. 82) or osteocalcin promoter (OG-2-HSD2 mice; see Ref. 59) have revealed the importance of sufficient GC signaling in the regulation of bone-forming cells in vivo. In Col2.3-HSD2 mice, bone volume and architecture are altered in transgenic females (82). In OG-2-HSD2 mice, although no differences in bone histomorphometry, density, or strength were identified under basal conditions, transgenic mice were protected against loss of bone strength and osteoblast apoptosis in response to high-dose GC challenge (59). These models demonstrate that high levels of 11β-HSD2 expression directly within bone-forming cells are detrimental and suggest that a threshold requirement for GCs may concur with optimal bone formation and/or maintenance. Recent studies of the 11β-HSD1 null mouse, discussed later in this review, suggest that local GC regeneration by 11β-HSD1 expressed within bone is important for normal cellular differentiation during bone development (30).

**TISSUE-SPECIFIC AMPLIFICATION OF CORTICOSTEROID RECEPTOR ACTIVATION: 11β-HSD1**

Although 11β-HSD2 acts exclusively as a dehydrogenase with high affinity for its substrate, the 11β-HSD1 enzyme, though found to act as a dehydrogenase in cell culture (10, 37), functions predominantly as a reductase in vivo (28). The role of 11β-HSD1 as a prereceptor device to regulate GR activation has been obscured by the fact that this enzyme has relatively...
low affinity for its substrate (49). However, recent biochemical analysis of $11\beta$-HSD1 dehydrogenase and reductase activities in vitro (49) has revealed that this enzyme exhibits Michaelis-Menton kinetics in its affinity for the dehydrogenase substrate (cortisol or corticosterone in humans or rodents, respectively) but cooperative kinetics in the binding of reductase substrate cortisone (in humans) or $11\beta$-dehydrocorticosterone (in rodents). Maser et al. (49) propose that these features are consistent with adaptation to low or high circulating levels of substrate, which vary across a broad range with stress or the circadian rhythm. These findings further support claims from earlier studies (28, 46) that the function of $11\beta$-HSD1 in vivo is primarily as a reductase. Unlike $11\beta$-HSD2, where a physical association of the enzyme with MR has been indicated (61), no such association of $11\beta$-HSD1 with GR has, thus far, been described.

In adults, $11\beta$-HSD1 mRNA expression is widespread but exhibits distinct tissue-specific abundance with highest transcript and enzyme activity levels observed in liver, adipose tissue, and brain (36, 78, 79). Genetic manipulations in vivo have greatly enhanced delineation of tissue-specific roles of $11\beta$-HSD1 and are summarized in Table 1.

**GC REGENERATION IN METABOLIC AND CARDIOVASCULAR HOMEOSTASIS**

**Resistance to Metabolic Dysfunction in $11\beta$-HSD1 Knockout Mice**

Mice null for $11\beta$-HSD1 are viable and fertile (35, 55). Initial studies revealed that these mutants exhibit a number of physiological changes, including a failure to mount an increase in expression of mRNAs for key hepatic gluconeogenic enzymes phosphoenolpyruvate carboxykinase and glucose-6-phosphatase in response to novel environment stress or high-fat feeding (35). Subsequently, $11\beta$-HSD1 knockout animals have been found to exhibit increased hepatic and adipose insulin sensitivity, improved glucose tolerance, a favorable lipoprotein profile, and reduced hemostatic factor synthesis (54), all compatible with a “cardioprotective” metabolic state. $11\beta$-HSD1 null mice, when bred on an obesity-prone strain background (C57Bl/6J), show resistance to weight gain induced by high-fat feeding despite increased food intake (55). The differential in body weight is most likely attributable to lack of GC regeneration in adipocytes and a lack of adipocyte hypertrophy (55). However, reduced weight gain may be accounted for in part by the fact that the $11\beta$-HSD1 null mutants have a higher body temperature and therefore presumably increased energy expenditure (55). Findings from $11\beta$-HSD1 null mice to date suggest that lack of this enzyme confers protection against metabolic dysfunction.

**Full Metabolic Syndrome in Transgenic Mice Overexpressing $11\beta$-HSD1 Selectively in Adipose Tissue**

The generation of a mouse model overexpressing $11\beta$-HSD1 specifically in adipose tissue under control of the adipocyte P2 (aP2) gene promoter clearly demonstrated the importance of this enzyme in adipocyte function and the regulation of metabolism (aP2-HSD1 mice; see Ref. 50). A modest increase of GC regeneration specifically in adipocytes precipitated marked metabolic changes, including central obesity, glucose intolerance, and insulin resistant-diabetes (50) as well as hypertension (51). $11\beta$-HSD1 expression and activity are increased in adipose tissue of other rodent models of obesity (3, 44, 45), as well as obese humans (31, 43, 67, 70, 71). Therefore, $11\beta$-HSD1 activity in adipocytes may be a factor in the establishment and/or maintenance of the metabolically diseased state. The phenotypic features manifest in aP2-HSD1 transgenic mice resemble the grouping of disorders termed the insulin resistance syndrome, “Metabolic Syndrome,” or Syndrome X.
in humans, which commonly includes central (abdominal) obesity, insulin resistance/type 2 diabetes mellitus, dyslipidemia, and hypertension clustered in a single individual (25, 72). The circulating renin-angiotensin aldosterone system (RAAS) is chronically activated in aP2-HSD1 mice with increased expression of the renin substrate angiotensinogen in adipocytes, together with elevated plasma levels of renin and the potent vasoconstrictor peptide ANG II driving increased blood pressure in this model (51). It is important to note that circulating levels of corticosterone in aP2-HSD1 mice are normal, but, within portal blood, levels of both corticosterone and free fatty acids are elevated approximately threefold (50). This may have important consequences for hepatic function, with spillover of regenerated GC from adipocytes in the portal circulation contacting hepatocytes with the potential for hepatic GR activation. Thus the metabolic syndrome phenotype evident in aP2-HSD1 mice may be attributable, in part, to GC- or free fatty acid-induced changes in the liver. Intriguingly, obese humans and monogenic obesity models in rodents also show elevated 11β-HSD1 activity and mRNA levels in adipose tissue (31, 43, 67, 70, 71). Thus the aP2-HSD1 mouse mimics changes seen in obesity in a variety of settings and suggests that overexpression of 11β-HSD1 in adipose tissue contributes to pathogenesis of the metabolic syndrome in humans.

Metabolic Syndrome Without Obesity in Transgenic Mice Overexpressing 11β-HSD1 Selectively in the Liver

Elevated GC levels in portal blood in obesity, as occurs in aP2-HSD1 mice, may mediate changes in the liver that could contribute to the development of metabolic syndrome. To address directly the consequences of increased GC levels in the liver, a mouse model overexpressing 11β-HSD1 in hepatocytes has recently been generated (apoE-HSD1 mice; see Ref. 66). This transgenic model exhibits increased 11β-HSD1 activity in the liver but not in other tissues. ApoE-HSD1 mice do not exhibit obesity but display several subtle alterations of metabolism, including insulin resistance, fatty liver, dyslipidemia, and hypertension (66). The mechanism of their hypertension is likely to involve the RAAS (66). Although this axis is not chronically activated in apoE-HSD1 mice, they show increased mRNA expression for angiotensinogen within the liver, positively correlated with hepatic 11β-HSD1 activity and blood pressure in an apparently dose-dependent manner. Although morning plasma samples show only a trend for increased ANG II and aldosterone in apoE-HSD1 transgenic mice (66), hypertension in this model is most marked during the active period of the circadian rhythm, and diurnal and tissue level control of the RAAS awaits more detailed characterization. Hepatic 11β-HSD1 activity has been shown to vary widely in human populations and may be relevant to the risk of developing metabolic and/or cardiovascular disease. Increased levels of hepatic GC regeneration have been associated with insulin-resistant fatty liver syndromes, in some cases concurrent with hypertension (29, 47, 48). Decreased hepatic 11β-HSD1 expression, meanwhile (as well as increased expression of this enzyme in adipose tissue), has been correlated with obesity in humans (67, 70, 71, 84) and genetically obese rodents (44, 45).

The intracellular level of GC regulated by 11β-HSD1 may be intrinsic to the molecular mechanisms governing several aspects of metabolic and cardiovascular function. The transgenic tissue-selective 11β-HSD1-overexpressing models, aP2-HSD1 and apoE-HSD1 mice, both present a phenotype of hypertension coupled with a distinct profile of metabolic disease. The findings are consistent with the differential 11β-HSD1 activity in specific tissues may underlie a spectrum of metabolic and cardiovascular disease, the severity of which may be related to the level and location of enzyme activity. These findings, taken together with the findings from the 11β-HSD1 null mutant, confer strong support for the importance of 11β-HSD1 in homeostatic control of metabolism in mice and are likely to have relevance for human metabolic and cardiovascular disorders.

GC REGENERATION AND COGNITION: AMELIORATION OF AGE-RELATED COGNITIVE IMPAIRMENT IN 11β-HSD1 KNOCKOUT MICE

Elevated circulating levels of GCs have been demonstrated to exert deleterious effects on brain neuronal biochemistry and function, particularly affecting the hippocampus, a region important for cognition (77, 80). 11β-HSD1 is widely expressed in brain, with high levels present within the hippocampus (93). In contrast, 11β-HSD2 expression in the brain is low, confined to a few nuclei thought to be involved in the central control of blood pressure and salt appetite (80). Addressing brain function in 11β-HSD1 null mice has revealed significantly decreased hippocampal tissue corticosterone content together with maintained ability with aging in tests of GC-associated cognitive function, which decline with normal senescence in mice (93). Therefore, despite maintained or even mildly elevated circulating corticosterone levels, arising from a dysregulated hypothalamic-pituitary-adrenal (HPA) axis (24, 35), lack of local GC regeneration within the hippocampus of 11β-HSD1 knockout mice exerts a protective effect on cognitive function with aging (93) and implies that intracellular GC content is more important than circulating corticosterone in mediating control of this function.

GC REGENERATION AND REGULATION OF THE HPA AXIS: IMPAIRED GC FEEDBACK IN 11β-HSD1 KNOCKOUT MICE

Along with their improved metabolic profile, 11β-HSD1 knockout mice exhibit an altered rhythm of corticosterone secretion and hyperplasia of the adrenal gland (24, 35), indicating dysregulation of the HPA axis. Impairment of GC feedback is suggested with increased basal plasma corticosterone and an exaggerated release of corticosterone in response to restraint stress. Although stress-induced levels of corticosterone are rapidly reduced to prestress levels in 11β-HSD1 knockout mice, plasma ACTH levels remain high poststress (24), again suggesting that GC negative feedback control is impaired. Altered GC responses and feedback may be attributable to increased clearance of corticosterone from the circulation and/or may indicate that a central nervous system defect exists in 11β-HSD1 knockout mice, leading to increased HPA axis activation and perhaps contributing to adrenal hyperplasia in this mutant. Dysregulation of the HPA axis in 11β-HSD1 null mice may be directly attributable to loss of enzyme activity. GC feedback control might be expected to involve expression of enzyme in the brain, but peripheral sites of expression may also be important. In the periphery, the lack of
GC regeneration may drive compensatory activation of the HPA axis. Within the brain in 11β-HSD1 null mice, expression of GR is significantly reduced within the paraventricular nucleus of the hypothalamus, a region intrinsic to regulation of the HPA axis (24). This is consistent with a reduced sensitivity to GCs in this mutant, possibly contributing to reduced negative feedback and/or sustained activity of the HPA axis (24).

**GC REGENERATION IN DEVELOPMENT**

*Impaired Lung Maturation in 11β-HSD1 Knockout Mice*

11β-HSD1 mRNA expression has been detected within the developing mouse embryo in the heart at midgestation (17, 83, 86, 87), followed shortly thereafter, from middle through late gestation, by strong expression within the liver and lung together with lower-level expression within the kidney, adrenal gland, gastrointestinal tract, and thymus (83, 86). GCs are known to induce surfactant synthesis in fetal lung during late gestation and improve biophysical properties of the lungs (52). GCs stimulate expression of surfactant proteins SP-A, SP-B, and SP-C and increase lamellar body density in type II cells (27). Indeed, the importance of GCs for lung function has been starkly illustrated by knockout of GR in mice, which precipitates respiratory failure within a few hours of birth (13). 11β-HSD1 expression has been detected in mouse fetal lungs during late gestation (83, 86). Inhibition of pulmonary 11β-HSD1 in rats by glycyrrhetic acid treatment during gestation causes modest impairment of lung maturation (26), suggesting that 11β-HSD1 contributes to local GC activation in the lungs before parturition. Although 11β-HSD1 knockout mice showed no deviation from controls with respect to survival, fetal or neonatal body weight, or evidence of overt respiratory distress, they express reduced amounts of SP-A mRNA and protein and exhibit impaired lung morphology (27), consistent with the effects of enzyme inhibition in rats, thus supporting a role for 11β-HSD1 in amplifying GC action in normal prenatal lung development.

*Altered Bone Marrow Differentiation in 11β-HSD1 Knockout Mice*

GCs have an essential role in skeletal development and maintenance (15, 73). Escape from balanced levels, particularly where exposure to active GCs is increased, is associated with bone loss and fragility, characteristic of osteoporosis (11, 74). GCs active in the development and maintenance of bone in vivo may be recruited from the circulation or supplied locally through regeneration by 11β-HSD1. The case for involvement of 11β-HSD1 in these processes is supported by the fact that this enzyme is expressed in osteoblasts of both mice and humans.

Although 11β-HSD1 knockout mice do not exhibit significant changes in bone mass, adipocytes are entirely absent from 11β-HSD1 null bone marrow (30). A requirement for local GC regeneration in the differentiation of the adipocyte lineage from bone marrow stem cells is suggested (30). Studies in cultured bone marrow stromal cells (6, 68) indicate that GC-mediated promotion of adipocyte differentiation may competitively oppose osteoblast differentiation from these precursor cells and so lead to loss of bone mass and/or the capacity for bone regeneration in response to stress or injury.

Although adipocyte differentiation from bone marrow stem cells is clearly altered in 11β-HSD1 knockout mice, potentially affecting bone function, hematopoietic and immune cell lineages remain to be fully investigated in this model.

**LACK OF PRERECEPTOR CORTICOSTEROID REGULATION: 11β-HSD1/11β-HSD2 DOUBLE KNOCKOUT MICE AND, POTENTIALLY, 11β-HSD2 NULL MICE**

Compound null 11β-HSD1/11β-HSD2 [11β-HSD double knockout (DKO)] mice are viable and can survive to a minimum of one year of age, indicating that these enzymes are not an obligate requirement for life in mice (Y. Kotelevtsev and J. M. Paterson, unpublished observations). Survival of 11β-HSD DKO mice indicates that GC interconversion may either 1) not occur, 2) be mediated by one or more alternative steroid metabolizing enzymes, or 3) be mediated by an as yet undefined third member of the 11β-HSD family. Steroid binding activity, detected in the rodent intestine, described as distinct from corticosteroid receptors or 11β-HSDs but similar to that characteristic of 11β-HSD2, has been reported (81). Studies of 11β-HSD1 knockout mice suggest that residual 11β-HSD activity, distinct from 11β-HSD2, exists in the lung of these mutants (R. Brown and S. Morley, unpublished observations). Although there is no evidence for a novel 11β-HSD at present, 11β-HSD DKO mice provide the ideal resource from which to isolate one or more, should they exist. 11β-HSD DKO mutants have recently been generated by intercross of 11β-HSD1 and 11β-HSD2 null mice on mouse strain C57Bl/6J genetic background and appear outwardly normal. Kidneys of adult 11β-HSD DKO mice show histopathology of the distal convoluted tubule similar to that observed in 11β-HSD2 null mice. Similarly, from husbandry observations, polyuria, characteristic of the 11β-HSD2 knockout, is also noted in 11β-HSD DKO mutants, and detailed physiological analyses of these mice continue.

Both type 1 and type 2 11β-HSD null mutants display distinct early life growth patterns compared with C57Bl/6J controls (J. M. Paterson and M. C. Holmes, unpublished observations). 11β-HSD2 knockout mice show lower body weight consistently throughout life. Although developmental and nutritional factors may be important, equally relevant to consider is that, with loss of 11β-HSD2 in mice, inactivation of corticosterone does not occur, and, consequently, the production of inactive 11-keto metabolites, i.e., substrate for 11β-HSD1, is lost. Without substrate generation, the ability of 11β-HSD1 to regenerate active GC in vivo in 11β-HSD2 null mice will be denied. Although steroid metabolism in 11β-HSD knockout mice remains to be characterized in detail, some features of the 11β-HSD2 null mouse suggest that, as in 11β-HSD DKO mice, the shuttle mechanism of corticosteroid metabolism may be entirely absent in this model also. In AME, metabolism of administered cortisone to cortisol confirms competency of the reductase activity of 11β-HSD1 (85). However, urinary free cortisone has been reported to be almost absent in AME (63–65), suggesting that substrate availability for 11β-HSD1 may be limited in humans with compromised 11β-HSD2 activity as is proposed for 11β-HSD2 null mice. The AME phenotype, therefore, may be attributable to the compound loss of 11β-HSD1 and 11β-HSD2. Administration of substrate for 11β-HSD1 to 11β-HSD2 null mice will permit...
pharmacological dissection of the impact of 11β-HSD1 loss in the AME phenotype of this mutant in vivo and may allow further insight to the mechanism of AME in humans.

CONCLUDING REMARKS AND FUTURE DIRECTIONS

Genetic manipulation of 11β-HSDs in the mouse has confirmed several hypotheses on the function of these enzymes and elucidated previously unknown roles, revealing the sensitivity of many intracellular mechanisms to local GC concentration, modulated through their intracrine action. In the 11β-HSD2 knockout mouse model, a role has been defined for the enzyme in regulating ligand access to the MR. The syndrome of AME, precipitated by a lack of 11β-HSD2 activity in both humans and 11β-HSD2 null mice, reflects the pivotal position of this enzyme in the control of electrolyte balance in the kidney, and hence blood pressure. Loss of 11β-HSD1 by gene targeting in mice has revealed involvement of this enzyme in a number of physiological processes. Cognition with aging is improved in 11β-HSD1 null mice, most likely attributable to loss of enzyme function in the brain. Regulation of the HPA axis is disrupted and may be attributable to altered brain and peripheral functions in 11β-HSD1 null mice. Lack of 11β-HSD1 activity affects many aspects of metabolism, notably improving glucose tolerance and conferring resistance to obesity. Tissue-restricted overexpression of 11β-HSD1 in the mouse has revealed the importance of amplified GC signaling in adipose tissue and liver, with the enzyme performing distinct functions in these tissues to regulate adiposity, glucose, and lipid homeostasis, as well as blood pressure through activation of the RAAS, suggesting that 11β-HSD1 may be intrinsic to the regulation of local renin-angiotensin systems in these and other cell types. In parallel with observations in humans, excess 11β-HSD1 activity in specific tissues in mice precipitates distinct but related profiles of metabolic disease copresenting with cardiovascular dysfunction. The dynamic regulation of 11β-HSD1 in response to environmental, particularly dietary, challenge is likely to be an important factor in maintaining metabolic and cardiovascular homeostasis.

Genetic manipulation of 11β-HSDs in mice has provided much insight into the physiological roles of these enzymes and highlighted their importance as regulators of corticosteroid hormone receptor action. Currently developed models can continue to delineate the mechanisms important for the regulation and function of 11β-HSDs in vivo through pharmacological, gene-expression profiling and genetic mapping approaches. It would certainly be advantageous to study models where the function of 11β-HSD genes might be controlled spatially and/or temporally to differentiate relative contributions of 11β-HSD activity in particular tissues to physiological processes, as well as to explore, for example, the multitude of roles these enzymes are likely to perform during early development, both within the developing fetus and the placenta where complex and dynamic regulation of GR, MR, and 11β-HSD expression has been demonstrated. With the benefit of many recent advances in genomic information and technology, it seems likely that genetic manipulations of 11β-HSDs will continue.

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