Published in final edited form as:

Mol Cell Endocrinol. 2007 February ; 265-266: 89–92.

HEXOSE 6-PHOSPHATE DEHYDROGENASE (H6PD) AND CORTICOSTEROID METABOLISM

Perrin C White¹, Daniela Rogoff¹, D Randy McMillan¹, and Gareth G Lavery^{1,2}

¹ Department of Pediatrics, University of Texas Southwestern Medical Center, Dallas, TX 75390

² Division of Medical Sciences, University of Birmingham, Edgbaston, Birmingham, B15 2TH, United Kingdom.

Abstract

Cortisone or (in rodents) 11-dehydrocorticosterone are reduced to cortisol or corticosterone, respectively, by the oxo-reductase activity of 11 β -hydroxysteroid dehydrogenase type 1 (11-HSD1). This requires NADPH, generated by hexose-6-phosphate dehydrogenase (H6PD), a component of the pentose phosphate pathway. H6PD is located along with 11-HSD1 in the lumen of the endoplasmic reticulum (ER). Increasing or decreasing expression levels of H6PD in cultured cells has corresponding effects on the reductase activity of 11-HSD1. Mice carrying a targeted mutation in H6PD have drastically decreased 11-HSD1 oxo-reductase activity, but their 11-dehydrogenase activity is increased. They have many phenotypic features in common with mice carrying a mutation of 11-HSD1 itself. Polymorphisms in both H6PD and 11-HSD1 were originally identified in patients with apparent cortisone reductase deficiency (who have signs of hyperandrogenism and decreased urinary excretion of cortisol versus cortisone metabolites). However, these polymorphisms do not have detectable biochemical or physiologic effects when prospectively ascertained.

Keywords

Endoplasmic reticulum; 11 β -hydroxysteroid dehydrogenase; pentose phosphate pathway; corticosterone; cortisol; polycystic ovarian syndrome

Biochemistry of 11HSD isozymes

Isozymes of 11 β -hydroxysteroid dehydrogenase (11-HSD) interconvert cortisol and cortisone (in rodents, corticosterone and 11-dehydrocorticosterone). Because cortisone and 11-dehydrocorticosterone are not active corticosteroids, 11-HSD activity is able to modulate access of active corticosteroids to their receptors in a tissue specific manner. The type 2 (kidney) isozyme (11-HSD2) is expressed mainly in mineralocorticoid target tissues such as the distal nephron. It has relatively high affinity ($K_m \sim 10$ nM) for corticosteroids, utilizes NAD⁺ as a cofactor, and functions almost exclusively as a dehydrogenase. Thus, it reduces concentrations of active glucocorticoids. Because the mineralocorticoid receptor has similar affinities for cortisol (or corticosterone) and aldosterone, 11-HSD2 activity is essential for maintaining the functional specificity of the mineralocorticoid receptor for aldosterone. This is demonstrated by the syndrome of apparent mineralocorticoid excess, an inherited form of

Correspondence to: Perrin C White, MD, UT Southwestern Medical Center, Dallas, TX 75390-9063, USA, Telephone 214-648-3501, Fax 214-648-9772, Perrin.white@utsouthwestern.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

hypertension caused by mutations in the HSD11B2 gene encoding 11-HSD2 (White et al., 1997). In this condition, cortisol acts as a mineralocorticoid; a similar phenotype is observed in mice carrying a mutation in this gene (Kotelevtsev et al., 1999).

In contrast, the type 1 (liver) isozyme (11-HSD1) is widely expressed, has a relatively low affinity ($K_m \sim 1 \mu\text{M}$) for corticosteroids, utilizes NADP⁺ or NADPH as cofactors, and catalyzes both dehydrogenation and the reverse oxoreduction reaction (cortisone and 11-dehydrocorticosterone to cortisol and corticosterone, respectively). The latter activity is more prominent *in vivo* but the enzyme behaves as a dehydrogenase in cellular homogenates or when purified. Oxo-reductase activity can be preserved under these conditions by generating NADPH using glucose-6-phosphate dehydrogenase, suggesting that co-factor availability dictates the direction of activity of 11-HSD1 (Agarwal et al., 1990).

Studies of mice with targeted inactivation of the corresponding HSD11B1 gene, as well as mice that overexpress this enzyme in liver or adipose tissue, suggest that this enzyme's main function is to increase glucocorticoid levels in glucocorticoid target tissues. Thus overexpression in adipose tissue leads to obesity (Masuzaki et al., 2001) whereas overexpression in the liver produces insulin resistance (Paterson et al., 2004); conversely, mice lacking 11-HSD1 are resistant to weight gain and have improved glucose tolerance on a high fat diet (Kotelevtsev et al., 1997). These studies have identified 11-HSD1 as a promising drug target for treating the metabolic syndrome (including type 2 diabetes) associated with obesity in humans (Tomlinson et al., 2004). The present paper reviews one mechanism regulating activity of 11-HSD1—provision of NADPH to 11-HSD1.

Biochemistry of hexose-6-phosphate dehydrogenase (H6PD)

The active site of 11-HSD1 is located within the lumen of the endoplasmic reticulum (Ozols, 1995). Because the reductase activity of 11-HSD1 depends on provision of NADPH and removal of NADP⁺ (Agarwal et al., 1990), it was hypothesized that the presence of hexose 6-phosphate dehydrogenase (H6PD) was required to maintain this activity. H6PD catalyzes the initial step (conversion of glucose-6-phosphate to 6-phosphogluconolactone) of the pentose phosphate pathway within the lumen of the endoplasmic reticulum (Clarke and Mason, 2003), thus generating NADPH from NADP⁺. This enzyme is distinguished biochemically from glucose-6-phosphate dehydrogenase (G6PD), which catalyzes the same reactions in the cytosol, by having much broader substrate specificity, including glucose-6-sulfate and galactose-6-phosphate (*in vitro*).

Co-transfection of mammalian cells with expression plasmids encoding H6PD and 11 β -HSD1 consistently increased oxo-reductase activity (Atanasov et al., 2004; Bujalska et al., 2005). Substituting an expression plasmid encoding the cytosolic enzyme G6PD failed to produce a similar increase in oxo-reductase activity, thus demonstrating that NADPH must be generated in the same cellular compartment as the active site of 11-HSD1; i.e., the lumen of the endoplasmic reticulum.

Conversely, decreasing H6PD expression with small interfering RNA (siRNA) decreased oxo-reductase activity in intact cells. Moreover, inter-individual variations in cortisone oxo-reductase activity in cultured primary human pre-adipocytes were positively correlated with H6PD mRNA expression, but not with HSD11B1 expression (Bujalska et al., 2005). In rat microsomes, inhibitors of glucose-6-phosphate transport into the lumen of the endoplasmic reticulum, or otherwise inhibiting the pentose phosphate pathway, reduced 11-HSD1 activity (Banhegyi et al., 2004; McCormick et al., 2006). Taken together, all of these functional studies supported the hypothesis that H6PD activity plays a critical role in regulating the oxo-reductase activity of 11-HSD1 by providing NADPH within the lumen of the endoplasmic reticulum.

Studies of mice lacking H6PD activity

To further elucidate the biological role(s) of H6PD, a null mutation was created in the murine *H6PD* gene via homologous recombination (Lavery et al., 2006). Gestation time and litter size were normal in heterozygote crosses. No gross morphological abnormalities were seen in mutant mice at birth.

No H6PD mRNA was detected in the liver of mutant mice, as assessed by RT-PCR. Heterozygote mice had reduced mRNA levels compared to wild type. No H6PD activity was measurable in liver microsomes from mutant mice.

11-HSD1 enzyme function was assessed by examining oxo-reductase and dehydrogenase activities in liver microsomes from wild type and mutant mice. In the presence of the H6PD substrate, glucose-6-phosphate (G6P, which can be transported across the membrane of the endoplasmic reticulum), intact wild type microsomes converted 32% of 11-dehydrocorticosterone to corticosterone, whereas mutant microsomes converted $\leq 5\%$ during the same period. Conversely, dehydrogenase activity in mutant microsomes was considerably higher than in wild type microsomes (29% vs. 10% conversion of corticosterone to 11-dehydrocorticosterone) indicating a switch from oxo-reductase to dehydrogenase activity in the absence of H6PD activity).

The oxo-reductase activity of 11-HSD1 was also affected in vivo. Wild type mice almost exclusively excreted steroids with an 11 β -hydroxyl group; only minor amounts of 11-oxo metabolites were detected (ratio of 0.06). In contrast, the 11-oxo/11 β -hydroxysteroid ratio in the H6PD mutant mice was 9.3 (i.e., >90% of total corticosterone metabolites were excreted as 11-oxo derivatives (Lavery et al., 2006).

The H6PD mutant mice have metabolic changes similar to those seen in HSD11B1 mutant mice (unpublished observations). They have increased serum corticosterone and ACTH levels at diurnal peak and nadir, but normal corticosterone levels upon ACTH stimulation. This suggests relative insensitivity of the HPA axis to feedback inhibition, possibly owing to reduced hypothalamic 11-HSD1 activity. The mice have fasting hypoglycemia and gain weight slowly. Hepatic glucocorticoid-sensitive enzymes (phosphoenolpyruvate carboxykinase, glucokinase, tyrosine aminotransferase) do not increase on fasting and have a blunted response to corticosterone in cultured mutant primary hepatocytes, consistent with decreased intrahepatic corticosterone levels. As measured by homeostatic model assessment (HOMA) values, mutant mice are more sensitive to insulin; similarly, both basal and insulin-stimulated glucose uptake are increased in normally insulin-sensitive extensor digitorum longus muscle. The mechanisms underlying the changes in insulin sensitivity cannot be assumed to be due solely to impaired glucocorticoid action, and require further study (unpublished observations).

Genetics of ACRD

Studies of humans who apparently lack 11-HSD1 activity have yielded confusing results. Apparent cortisone reductase deficiency (ACRD) is a rarely ascertained condition characterized by signs of androgen excess in women or children and decreased urinary excretion of cortisol metabolites compared with cortisone metabolites (Jamieson et al., 1999; Phillipov et al., 1996). It was originally assumed that ACRD was caused by mutations in HSD11B1. Lack of 11-oxoreductase activity would lead to decreased cortisol half-life due to the unopposed action of 11-HSD2. In turn, the adrenal cortex would secrete more cortisol to compensate for the decreased half-life, leading to increased secretion of adrenal androgens as well. In affected women, this would cause signs of androgen excess similar to polycystic ovarian syndrome (PCOS) but without the obesity or signs of insulin resistance that usually accompany PCOS. It is worthy of note that androgen excess cannot be a feature of HSD11B1

mutations in mice, which lack 17-hydroxylase/17,20-lyase (CYP17) activity in the adrenal cortex and consequently do not synthesize androgens in their adrenal glands.

However, no mutations in coding sequences or intron-exon junctions of HSD11B1 have been detected in any ACRD patient (Nikkila et al., 1993; Jamieson et al., 1999). Instead, two polymorphisms in intron 3 of HSD11B1 were found in complete linkage disequilibrium: 83,557insA (actually 1931 bp downstream of the initial A in the coding sequence) and 83,597T>G (1971 bp from the initial A; this polymorphism is hereafter referred to “intron 3 G”). Although these polymorphisms were associated with decreased HSD11B1 expression *in vivo* and after transfection of minigene constructs in cultured cells, the polymorphic haplotype had an allele frequency of 0.14 in normal individuals (thus being homozygous in 2% of the population), whereas ACRD is ascertained very rarely. Moreover, there was no association of homozygosity for these polymorphisms with ACRD (Draper et al., 2003).

Mutations were then sought in H6PD (this study predated, and inspired, most of the biochemical studies discussed above). In fact, two patients with ACRD were homozygous for a missense mutation in H6PD, R453Q, whereas one patient was heterozygous for a 29 bp insertion/frameshift mutation. When mutant enzymes were expressed in cultured cells, the R453Q mutation decreased enzymatic activity to <50% of normal, whereas the 29 bp insertion abolished it. The allele frequency of R453Q was 0.20, suggesting that approximately 4% of the population was homozygous. Thus this polymorphism could not by itself explain ACRD either. However, the patients who were homozygous for R453Q in H6PD were also heterozygous for the intron 3 polymorphisms in HSD11B1, and the patient carrying the 29 bp insertion in H6PD was homozygous for the HSD11B1 intron 3 polymorphism. It was therefore concluded that ACRD resulted from simultaneous allelic variation in the HSD11B1 and H6PD loci, requiring at least 3 out of a possible 4 affected alleles (also referred to as ‘minor’ alleles) at the two loci. The combined genotype of H6PD R453Q homozygosity and HSD11B1 intron 3 G heterozygosity was predicted to occur in approximately 0.8% of the population, which did not seem inconsistent with the apparent rarity of ACRD if the disease was poorly ascertained (Draper et al., 2003).

Because of the *post hoc* nature of the results and the small number of ascertained ACRD patients, we attempted to confirm these results by ascertaining additional ACRD cases through genetic screening of a large population-based sample, the Dallas Heart Study; it is a probability-based sample of Dallas County residents aged 18 to 65 years.

We genotyped 3551 individuals (White, 2005). The minor allele at each locus (HSD11B1 intron 3 G and H6PD R453Q) occurred more frequently than had been previously observed (Draper et al., 2003). In particular, blacks had a much higher frequency of H6PD R453Q ($p < 0.0001$) compared with other ethnic groups.

Because of the higher allele frequencies observed in the larger study, the frequencies of genotypes postulated to cause ACRD were higher than previously predicted. The frequency of genotypes carrying any 3 of the possible 4 minor alleles at the HSD11B1 and H6PD loci was 6.4% (4.3% whites+hispanics), almost ten times the genotype frequency previously predicted. Additionally, 0.6% of subjects (0.5% of whites+hispanics) carried four affected alleles. Therefore, 7.0% (4.8% whites+hispanics) of subjects had “ACRD genotypes” (i.e., at least 3 of 4 possible minor alleles at the two loci), which is not consistent with the apparent rarity of ACRD, even postulating a very low level of ascertainment. Moreover, subjects with ACRD genotypes had no abnormalities in urinary corticosteroid excretion (ratio of urinary free cortisol to cortisone, and ratio of the cortisol metabolites, tetrahydrocortisol and allo-tetrahydrocortisol to tetrahydrocortisone ((THF+aTHF)/THE)). There was no increased risk of PCOS (diagnosed on a population basis by MRI) with the ACRD genotypes, or with either of the loci alone. There

was no genotype effect on waist:hip ratio, BMI, blood pressure, visceral adiposity, glucose or insulin after ethnicity was taken into account. There were also no genotype effects on any relevant biochemical parameters in females such as testosterone, FSH, LH, or sex hormone binding globulin.

A similar lack of genotype effect on 11-HSD1 activity was also reported in a small sample of PCOS patients (Millan et al., 2005), and the absence of risk for PCOS has recently been confirmed in a larger study (Draper et al., 2006). Therefore, the initial results must be due to ascertainment bias. These results do not rule out the existence of other mutations in H6PD or HSD11B1 contributing to the ACRD phenotype.

In conclusion, studies utilizing cell culture and mutant mice have unequivocally demonstrated the importance of NADPH, generated by H6PD, in controlling the direction of the reactions catalyzed by 11-HSD1, reductase versus dehydrogenase. Genetic polymorphisms, or non-genetic interindividual variations in H6PD activity, have not yet been demonstrated to be associated with human disease. Whereas 11-HSD1 inhibitors are being studied as potential agents for treating metabolic syndrome, H6PD is likely to have additional functions within the endoplasmic reticulum. Efforts to develop H6PD inhibitors as drugs should therefore be approached with caution.

Acknowledgements

This work was supported by grant R01 DK068101 to PCW from the National Institutes of Health.

Reference List

- Agarwal AK, Tusie-Luna MT, Monder C, White PC. Expression of 11 beta-hydroxysteroid dehydrogenase using recombinant vaccinia virus. *Mol Endocrinol* 1990;4:1827–1832. [PubMed: 2082184]
- Atanasov AG, Nashev LG, Schweizer RA, Frick C, Odermatt A. Hexose-6-phosphate dehydrogenase determines the reaction direction of 11beta-hydroxysteroid dehydrogenase type 1 as an oxoreductase. *FEBS Lett* 2004;571:129–133. [PubMed: 15280030]
- Banhegyi G, Benedetti A, Fulceri R, Senesi S. Cooperativity between 11beta-hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase in the lumen of the endoplasmic reticulum. *J Biol Chem* 2004;279:27017–27021. [PubMed: 15090536]
- Bujalska IJ, Draper N, Michailidou Z, Tomlinson JW, White PC, Chapman KE, Walker EA, Stewart PM. Hexose-6-phosphate dehydrogenase confers oxo-reductase activity upon 11 beta-hydroxysteroid dehydrogenase type 1. *J Mol Endocrinol* 2005;34:675–684. [PubMed: 15956339]
- Clarke JL, Mason PJ. Murine hexose-6-phosphate dehydrogenase: a bifunctional enzyme with broad substrate specificity and 6-phosphogluconolactonase activity. *Arch Biochem Biophys* 2003;415:229–234. [PubMed: 12831846]
- Draper N, Powell BL, Franks S, Conway GS, Stewart PM, McCarthy MI. Variants implicated in cortisone reductase deficiency do not contribute to susceptibility to common forms of polycystic ovary syndrome. *Clin Endocrinol (Oxf)* 2006;65:64–70. [PubMed: 16817821]
- Draper N, Walker EA, Bujalska IJ, Tomlinson JW, Chalder SM, Arlt W, Lavery GG, Bedendo O, Ray DW, Laing I, Malunowicz E, White PC, Hewison M, Mason PJ, Connell JM, Shackleton CH, Stewart PM. Mutations in the genes encoding 11beta-hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase interact to cause cortisone reductase deficiency. *Nat Genet* 2003;34:434–439. [PubMed: 12858176]
- Jamieson A, Wallace AM, Andrew R, Nunez BS, Walker BR, Fraser R, White PC, Connell JM. Apparent cortisone reductase deficiency: a functional defect in 11beta-hydroxysteroid dehydrogenase type 1. *J Clin Endocrinol Metab* 1999;84:3570–3574. [PubMed: 10522997]
- Kotelevtsev Y, Brown RW, Fleming S, Kenyon C, Edwards CR, Seckl JR, Mullins JJ. Hypertension in mice lacking 11beta-hydroxysteroid dehydrogenase type 2. *J Clin Invest* 1999;103:683–689. [PubMed: 10074485]

- Kotelevtsev Y, Holmes MC, Burchell A, Houston PM, Schmoll D, Jamieson P, Best R, Brown R, Edwards CR, Seckl JR, Mullins JJ. 11 β -hydroxysteroid dehydrogenase type 1 knockout mice show attenuated glucocorticoid-inducible responses and resist hyperglycemia on obesity or stress. *Proc Natl Acad Sci U S A* 1997;94:14924–14929. [PubMed: 9405715]
- Lavery GG, Walker EA, Draper N, Jeyasuria P, Marcos J, Shackleton CH, Parker KL, White PC, Stewart PM. Hexose-6-phosphate dehydrogenase knock-out mice lack 11 β -hydroxysteroid dehydrogenase type 1-mediated glucocorticoid generation. *J Biol Chem* 2006;281:6546–6551. [PubMed: 16356929]
- Masuzaki H, Paterson J, Shinyama H, Morton NM, Mullins JJ, Seckl JR, Flier JS. A transgenic model of visceral obesity and the metabolic syndrome. *Science* 2001;294:2166–2170. [PubMed: 11739957]
- McCormick KL, Wang X, Mick GJ. Evidence that the 11 β -hydroxysteroid dehydrogenase (11 β -HSD1) is regulated by pentose pathway flux. Studies in rat adipocytes and microsomes. *J Biol Chem* 2006;281:341–347. [PubMed: 16234247]
- Millan JL, Alvarez-Blasco JJ, Luque-Ramirez F, Sancho M, Moghetti J, HF P. A study of the Hexose-6-Phosphate Dehydrogenase Gene R453Q and 11{ β }-Hydroxysteroid Dehydrogenase Type 1 Gene 83557insA Polymorphisms in the Polycystic Ovary Syndrome. *J Clin Endocrinol Metab* In press. 2005
- Nikkila H, Tannin GM, New MI, Taylor NF, Kalaitzoglou G, Monder C, White PC. Defects in the HSD11 gene encoding 11 β -hydroxysteroid dehydrogenase are not found in patients with apparent mineralocorticoid excess or 11-oxoreductase deficiency. *J Clin Endocrinol Metab* 1993;77:687–691. [PubMed: 8370690]
- Ozols J. Lumenal orientation and post-translational modifications of the liver microsomal 11 β -hydroxysteroid dehydrogenase. *J Biol Chem* 1995;270:2305–2312. [PubMed: 7836463]
- Paterson JM, Morton NM, Fievet C, Kenyon CJ, Holmes MC, Staels B, Seckl JR, Mullins JJ. Metabolic syndrome without obesity: Hepatic overexpression of 11 β -hydroxysteroid dehydrogenase type 1 in transgenic mice. *Proc Natl Acad Sci U S A* 2004;101:7088–7093. [PubMed: 15118095]
- Phillipov G, Palermo M, Shackleton CH. Apparent cortisone reductase deficiency: a unique form of hypercortisolism. *J Clin Endocrinol Metab* 1996;81:3855–3860. [PubMed: 8923828]
- Tomlinson JW, Walker EA, Bujalska IJ, Draper N, Lavery GG, Cooper MS, Hewison M, Stewart PM. 11 β -hydroxysteroid dehydrogenase type 1: a tissue-specific regulator of glucocorticoid response. *Endocr Rev* 2004;25:831–866. [PubMed: 15466942]
- White PC. Genotypes at 11 β -hydroxysteroid dehydrogenase type 11B1 and hexose-6-phosphate dehydrogenase loci are not risk factors for apparent cortisone reductase deficiency in a large population-based sample. *J Clin Endocrinol Metab* 2005;90:5880–5883. [PubMed: 16091483]
- White PC, Mune T, Agarwal AK. 11 β -hydroxysteroid dehydrogenase and the syndrome of apparent mineralocorticoid excess. *Endocr Rev* 1997;18:135–156. [PubMed: 9034789]