

REVIEW

The Role of 11 β -Hydroxysteroid Dehydrogenases in the Cardiovascular System

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GLUCOCORTICOID action is mediated by nuclear steroid receptors, and by a prereceptor mechanism that interconverts cortisol and cortisone. The two enzymes that perform these reactions, 11 β -hydroxysteroid dehydrogenase (HSD)1 and 11 β HSD2, are pivotal in a wide variety of physiological processes including the regulation of blood pressure, obesity and memory [1].

The 11 β HSD1 enzyme functions mainly as a reductase converting cortisone to cortisol with the aid of the cofactor NADPH. It is a glycoprotein with a molecular size of 34 kilodaltons and is located in the membrane of the endoplasmic reticulum. 11 β HSD1 belongs to the short chain alcohol dehydrogenase superfamily of proteins, of which there are several thousand members. Other members of the superfamily include enzymes that metabolize sex steroids, sugars, polyols, antibiotics, prostaglandins and retinoids. Multiple alignment analysis of protein sequences within this family has identified up to six conserved domains, although 11 β HSD1 only shows four conserved regions [2]. The A domain contains the cofactor binding sequence and the D domain displays a highly conserved tyrosine and lysine containing sequence that make up the active site YXXXX motif. The other conserved domains are hydrophobic in nature and probably form part of the scaffolding required to maintain the correct three-dimensional structure of the enzyme. Mutation analysis of 11 β HSD1 has shown that glycosylation is important for enzymatic activity [3].

Although there are no known cases of deleterious mutations in the 11 β HSD1 gene in man which allow us to glean an insight into the importance of the enzyme in various physiological pathways, gene deletion studies in mice have shown a number of interesting phenotypes. 11 β HSD1 appears to play an important role in glucose homeostasis as the 11 β HSD1(–/–) mouse displays increased resistance to

hyperglycemia due to stress or obesity. Furthermore, there is an attenuated response in gluconeogenic enzymes in knockout mice, reflecting the relatively lower hepatic level of glucocorticoids [4], and an increase in hepatic insulin sensitivity and lipid catabolising enzymes resulting in lower plasma triglyceride levels [5]. 11 β HSD1 also plays an important role in the brain. Mineralocorticoid receptors (MR) are particularly abundant in the hippocampus where they set the sensitivity of the stress response system [6]. Intracerebroventricular administration of HSD inhibitors, at a dose which is less than required to produce an effect when administered peripherally, causes hypertension in rats, an effect that is abolished by MR inhibition [7]. That hippocampal glucocorticoids do not simply reflect circulating levels is demonstrated by the significantly lower levels in mice harboring the 11 β HSD1 deletion [8]. Furthermore, 11 β HSD1 knockout mice are less sensitive to cortisol suppression of the hypothalamic-pituitary-adrenal (HPA) axis [9]. Additional evidence that 11 β HSD1 increases brain glucocorticoid levels comes from studies where carbenoxolone attenuated neuronal damage in rats treated with kainic acid [10], and in water maze studies which showed ameliorated age-related learning impairments in knockout mice [11].

Glucocorticoids are known to be important modulators of obesity. The 11 β HSD1 enzyme is increased several orders of magnitude during differentiation of the preadipocyte and provides an additional source of glucocorticoid to the circulation. Over-expression of 11 β HSD1 in adipose tissue of transgenic mice promotes increased visceral adiposity and leads to insulin-resistant diabetes and dyslipidemia, characteristics also found in the metabolic syndrome [12]. This suggests that inhibition of 11 β HSD1 may be beneficial in diabetes. Indeed, when carbenoxolone was given to diabetic patients there was a decrease in plasma glu-

cose levels attributable to a reduction in glycogenolysis in the setting of hyperglucagonemia [13]. *In vitro* studies have shown inhibition of 11 β HSD1 gene expression and enzyme activity by the peroxisome proliferator-activated receptor (PPAR)-gamma agonist thiazolidinedione. Furthermore, *in vivo* studies with these compounds in diabetic mice show a decrease in adipose 11 β HSD1 together with a fall in plasma corticosterone levels. This suggests that some of the beneficial effects of the PPARgamma agonists in man may be mediated via adipose 11 β HSD1 [14]. Liver X receptor (LXR) agonists have also been shown to decrease 11 β HSD1 expression in brown adipose tissue *in vivo* and may also mediate beneficial effects in insulin resistant states [15]. In undifferentiated human omental adipose stromal cells 11 β HSD1 acts as a dehydrogenase to oppose the anti-proliferative effects of glucocorticoid and facilitate proliferation. A switch to oxoreductase activity occurs during early differentiation, generating cortisol and promoting adipogenesis [16]. However, *in vivo* studies in patients with central adiposity have shown an inverse relationship between obesity and total body 11 β HSD1 activity [17]. The most likely explanation would appear to be a reduction in hepatic 11 β HSD1 activity.

Glucocorticoids oppose the effects of proinflammatory signals and 11 β HSD1 has been shown to increase active glucocorticoid levels under these conditions. Using glomerular mesangial cells Escher *et al.* were able to show that proinflammatory cytokines, such as tumor necrosis factor and interleukin 1 β , stimulate production of phospholipase A2, while simultaneously inhibiting production of this enzyme via higher local levels of 11 β HSD1 and glucocorticoid [18]. The modulation of local glucocorticoid levels is also important in bone, where excess steroid can lead to osteoporosis. 11 β HSD1 has been demonstrated in bone and the effect of its inhibition on bone markers has been assessed in clinical studies. There was a significant decrease in urinary bone resorption indices without effects on bone formation markers, such as terminal peptides of type I collagen [19]. Osteoblast 11 β HSD1 reductase activity increases with age and is stimulated with exogenous steroid, suggesting that this enzyme may play a role in glucocorticoid induced osteoporosis [20]. 11 β HSD2 also plays a role in calcium homeostasis as evidenced by a retarded bone age in the syndrome of apparent mineralocorticoid excess (AME), and by an increase in urinary

calcium in studies where glycyrrhetic acid was administered. This suggests that calcium resorption in the distal tubule is compromised in the setting of glucocorticoid/mineralocorticoid excess [21].

The 11 β HSD2 enzyme is encoded by the HSD11B2 gene in man and is located on chromosome 16q22. It is five kilobases in length and is comprised of five exons. The enzyme displays high affinity for cortisol (K_m 50 nM) and is inhibited by glycyrrhetic acid [22]. 11 β HSD2 gene expression is highest in sodium transporting epithelia, such as distal tubule, colon and salivary gland, where inactivation of cortisol is a prerequisite for aldosterone binding to a non-selective mineralocorticoid receptor [23]. Mutations in the gene allow the high circulating levels of cortisol access to the receptor and result in AME, a homozygous recessive condition characterized by high blood pressure with low renin activity, hypokalemia, and high urinary cortisol to cortisone metabolite ratios [24, 25]. High 11 β HSD2 levels are also found in placental syncytiotrophoblasts and serve to protect the fetus from high circulating maternal glucocorticoids. AME patients are invariably low birthweight babies, as a result of the compromised placental 11 β HSD2 activity that allows the high levels of maternal glucocorticoids to impede intrauterine growth. Deletion of the 11 β HSD2 gene in mice recapitulates all of the characteristics of the syndrome of AME in man [26]. AME is one of a group of syndromes that are classified as monogenic causes of hypertension. Other syndromes include Gitelman's, Liddle's and the Bartter syndromes (types 1,2,3), and all have in common defects of ion transport in the distal tubule of the kidney [27].

The adrenal gland is an unexpected site of 11 β HSD2 expression. Studies in rat, sheep and man show that 11 β HSD2 is highly expressed in the zona reticularis/fasciculata, while 11 β HSD1 appears to be present in the glomerulosa and medulla [28, 29]. In man adrenal 11 β HSD2 is responsible for the production of cortisone and 11-dehydrocorticosterone. However, increasing steroid production with ACTH decreased production of the 11-keto metabolites, suggesting that corticosteroid intermediates, such as progesterone, may inhibit enzyme activity [30]. In a subsequent study from this group it was concluded that 11 β HSD1 is upregulated, and 11 β HSD2 down regulated by high intracellular concentrations of steroids and that these enzymes could contribute to the elevated hormonal secretion of cortisol producing tumors [31].

11 β HSD2 activity can be significantly inhibited, and blood pressure increased, by the ingestion of excessive amounts of licorice, which contains glycyrrhetic acid. Bile salts are also known to inhibit 11 β HSD2 *in vitro*, and increased plasma levels of bile acids during cholestasis have been shown to result in increased urinary cortisol metabolite ratios. This may explain the increased sodium retention and potassium loss over and above the changes expected due to modest increases in aldosterone levels in this condition. Resolution of the hepatic blockage results in normalization of plasma bile acid levels and urinary metabolite ratios [32].

The 11 β HSD1 and 11 β HSD2 enzymes are also thought to play a role in some forms of hypertension. Low birthweight has been associated with an increased risk of hypertension in the adult, and it has been suggested that decreased placental 11 β HSD2 activity may be the cause. Thus increased glucocorticoid exposure in utero may result in changes in fetal imprinting that result in altered patterns of response leading to hypertension in adult life [33]. There is evidence for both enzymes in the vasculature where glucocorticoids potentiate the effects of pressor agents. Studies on vascular 11 β HSDs have shown that the effects of these enzymes are mediated by endothelin-1 and nitric oxide [34]. This finding goes some way to explaining the immediate effect of glycyrrhetic acid on vascular reactivity when blood pressure rises are

only seen after chronic administration of the inhibitor. Clinical studies have proved less enlightening in terms of elucidating mechanisms by which 11 β HSDs may modulate blood pressure in essential hypertension. Studies in patients with essential hypertension have yielded divergent findings, a possible reflection of different subclasses of the disease. One study has shown an increase in cortisol half life in a sub-group of hypertensives without changes in metabolite ratios [35], while in another study it was found that there were increased cortisol to cortisone metabolite ratios together with other urinary steroid changes suggestive of altered 11 β HSD activities [36]. No evidence was provided for the mechanisms involved, but there is support for the presence of endogenous inhibitors of 11 β HSD2 that are modulated by sodium status [37]. These inhibitors are excreted in the urine but have proved refractory to isolation and identification.

In summary, the 11 β HSD1 and 11 β HSD2 enzymes are modulators of local glucocorticoid levels and play an important role in the physiology of many complex processes such as glucose homeostasis, memory, obesity, vascular reactivity, blood pressure and osteoporosis. There is currently considerable interest in developing specific inhibitors to 11 β HSD1 in an attempt to treat obesity. Other applications of this approach may deliver beneficial effects in the fields of diabetes, osteoporosis and memory loss.

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