PERGAMON

Journal of Steroid Biochemistry and Molecular Biology 69 (1999) 391–401

The Journal of Steroid Biochemistry & Molecular Biology

The type I and type II 11β-hydroxysteroid dehydrogenase enzymes ☆

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Abstract

Local tissue concentrations of glucocorticoids are modulated by the enzyme 11β-hydroxysteroid dehydrogenase which interconverts cortisol and the inactive glucocorticoid cortisone in man, and corticosterone and 11-dehydrocorticosterone in rodents. The type I isoform (11β-HSD1) is a bidirectional enzyme but acts predominantly as a oxidoreductase to form the active glucocorticoids cortisol or corticosterone, while the type II enzyme (11β-HSD2) acts unidirectionally producing inactive 11-keto metabolites. There are no known clinical conditions associated with 11β-HSD1 deficiency, but gene deletion experiments in the mouse indicate that this enzyme is important both for the maintenance of normal serum glucocorticoid levels, and in the activation of key hepatic gluconeogenic enzymes. Other important sites of action include omental fat, the ovary, brain and vasculature. Congenital defects in the 11β-HSD2 enzyme have been shown to account for the syndrome of apparent mineralocorticoid excess (AME), a low renin severe form of hypertension resulting from the overstimulation of the non-selective mineralocorticoid receptor by cortisol in the distal tubule of the kidney. Inactivation of the 11β-HSD2 gene in mice results in a phenotype with similar features to AME. In addition, these mice show high neonatal mortality associated with marked colonic distention, and remarkable hypertrophy and hyperplasia of the distal tubule epithelia. 11β-HSD2 also plays an important role in decreasing the exposure of the fetus to the high levels of maternal glucocorticoids. Recent work suggests a role for 11β-HSD2 in non-mineralocorticoid target tissues where it would modulate glucocorticoid access to the glucocorticoid receptor, in invasive breast cancer and as a mechanism providing ligand for the putative 11-dehydrocorticosterone receptor. While previous homologies between members of the SCAD superfamily have been of the order of 20–30% phylogenetic analysis of a new branch of retinol dehydrogenases indicates identities of >60% and overlapping substrate specificities. The availability of crystal structures of family members has allowed the mapping of conserved 11β-HSD domains A–D to a cleft in the protein structure (cofactor binding domain), two parallel β-sheets, and an α-helix (active site), respectively.

1. Introduction

The effects of glucocorticoids on target tissues are modulated by the enzyme 11β-hydroxysteroid dehydrogenase which interconverts cortisol in man, or corticosterone in rodents, and their inactive 11-keto metabolites cortisone and 11-dehydrocorticosterone, respectively. Two isoforms of the enzyme have been isolated. The type I enzyme (11β-HSD1) converts 11-keto metabolites into active glucocorticoids in the presence of NADPH [1], while the type II isoform (11β-HSD2) utilizes NAD [2] to metabolize glucocorticoids to 11-keto compounds with low affinity [3,4] for glucocorticoid and mineralocorticoid receptors (Fig. 1).

Recently, these enzymes have been the focus of

☆ Proceedings of the Xth International Congress on Hormonal Steroids, Quebec City, Quebec, Canada, 17–21 June 1998.

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0960-0760/99/$ - see front matter
PII: S0960-0760(99)00074-6

Fig. 1. Activities and major sites of action of the 11β-HSD enzymes. F, cortisol; E, cortisone.

intense investigation because localization studies suggest important roles in a variety of physiological processes including gluconeogenesis, vasoconstriction, sodium balance, development and reproduction. Murine gene deletion experiments and clinical studies have underlined the pivotal role played by both enzymes in some of these areas. In addition, an explosion in the identification and characterization of new members of the short chain alcohol dehydrogenase/reductase (SCAD or SDR) superfamily has provided new insights into the molecular structures and structure–function relationships of the 11β-HSDs. It is the purpose of this article to review these recent developments.

2. The 11β-HSD1 enzyme

The largest production of active glucocorticoids from inactive 11-keto metabolites by 11β-HSD1 occurs in the liver (Table 1). Cathaterization studies in patients clearly demonstrate high cortisol to cortisone ratios in the hepatic vein reflecting cortisol production [5]. The human liver has been shown to contain high amounts of the enzyme around the hepatic central vein while there are undetectable levels around the hepatic artery, hepatic portal vein and bile duct [6]. Significant amounts of 11β-HSD1 were also found in the human adrenal cortex. An understanding of the contribution that 11β-HSD1 makes to the production of active glucocorticoids in the adrenal is confounded by the presence of high amounts of the glucocorticoid inactivating 11β-HSD2 enzyme in sheep and rat adrenal cortex [7,8]. In contrast to the rat and sheep [9] where high amounts of 11β-HSD1 are present in the kidney, the human kidney has barely detectable levels [10].

The consequences of 11β-HSD1 activity in the liver are perhaps best described by recent gene deletion studies in the mouse, although the degree to which metabolic pathways are perturbed during development in such mice is unknown [11]. Homozygous mutants fail to convert 11-dehydrocorticosterone, exhibit a weakened response to activation of key hepatic gluconeogenic enzymes, possibly due to intrahepatic glucocorticoid deficiency, and resist hyperglycemia normally provoked by stress or obesity [11]. Thus 11β-HSD1 activity promotes autocrine/paracrine effects of glucocorticoids in the liver. Further evidence for such intracrinology comes from studies on human adipose deposits where it has been proposed that the restricted distribution of 11β-HSD1 to omentum suggests central obesity results from relatively elevated levels of cortisol in this tissue [12].

The 11β-HSD1 enzyme is widely distributed in the brain with highest expression in hippocampus and cortex [13]. In vivo studies have shown that glucocorticoids regulate 11β-HSD1 [14], while inhibition of 11β-HSD activity has in turn been shown to alter glucose utilization in the brain [15]. Other tissues where the modulation of glucocorticoid levels by 11β-HSD1 has been shown to affect physiology are the ovary, where the presence of the enzyme is thought to mitigate against successful embryo transfer [16], and the vasculature where prostacyclin production may be affected in aortic endothelial cells [17].

3. The 11β-HSD2 enzyme

3.1. The kidney

11β-HSD2 plays a pivotal role in mineralocorticoid target tissues by allowing aldosterone access to a mineralocorticoid receptor which binds cortisol with equal affinity. Partial or complete abolition of 11β-HSD2 activity, as occurs in licorice abuse, (Table 2) as a side effect of the anti-ulcer drug Carbenoxolone (CBX), or results from mutations in the HSD11B2

Table 1
Proposed biological effects of 11β-HSD1 activity

Tissue	Biological effect	Reference
Liver	Major site of glucocorticoid production from 11-keto metabolites. Stimulates gluconeogenic enzymes.	[11]
Omental fat	Proposed to enhance accumulation of omental adipose tissue.	[12]
Brain	Inhibits glucose utilization.	[15]
Ovary	11β-HSD1 mitigates against successful embryo transfer.	[6,16]
Vasculature	Inhibits prostacyclin production in aortic endothelial cells. Potentiates effects of adrenalin.	[17,18]

Table 2
Proposed biological effects of 11β-HSD2

Tissue	Biological effect	Reference
Kidney	Prevents excessive retention of salt. Absence of activity associated with nephrocalcinosis.	[22]
Placenta	Substantially reduces amount of maternal cortisol crossing placenta. Decreased activity associated with low birthweight and development of hypertension later in life. Possible regulator of fetal ACTH and adrenal steroidogenesis.	[29,30]
Fetal brain	High expression during midgestation suggests neuroprotective effect.	[31]
Vasculature	Inhibition potentiates cutaneous blanching induced by cortisol.	[32]

gene leads to overstimulation of the mineralocorticoid receptor by cortisol, sodium retention and severe hypertension. The syndrome of apparent mineralocorticoid excess has been shown to be attributable to an autosomal recessive inheritance of faulty HSD11B2 alleles [19–21]. Characteristic features of this disease include a severe form of low-renin hypertension, excess sodium retention, hypokalemia and underdeveloped bone mass possibly due to the associated nephrocalcinosis [22]. An important diagnostic criterium is the elevated urinary tetrahydrocortisol to tetrahydrocortisone ratio which may exceed 50, compared to normal values of about 1.

Mutations in the HSD11B2 gene represented only the third monogenic cause of hypertension. Several other monogenic causes have since been identified and all affected genes are modulators of sodium transport [23]. Clinical studies have now examined the role of HSD11B2 in essential hypertension. An early study on black patients provided evidence of an association of the polymorphic marker D16S496 with hypertensive end-stage renal disease [24]. An Alu I polymorphism in exon 3 has also been reported to be associated with end stage renal disease in Caucasians (with an incidence in this cohort of 18%) but not with hypertension, diabetes mellitus or patients undergoing dialysis (all 4–5%) [25]. A more recent larger study on two independent French cohorts using both association with the Alu I site, and sibling pair linkage analysis of a microsatellite marker in the 3′ flanking region of the gene was also unable to implicate a role for 11β-HSD2 in essential hypertension [26].

The 11β-HSD2 enzyme has been studied in the kidneys of patients with hypertensive nephrosclerosis, or with hypertension secondary to renal disease. Collapsed tubules retained strong immunoreactivity but decreased staining was observed in dilated tubules. It was concluded that prominent changes in 11β-HSD2 expression are not a feature of these diseases [27]. Recently, it has been shown that inactivation of the 11β-HSD2 gene in mice results in a phenotype with similar features to AME in that -/- animals develop hypokalemia and hypertension. In addition, these mice show high neonatal mortality (40%) associated with marked colonic distention, and remarkable hypertrophy and hyperplasia of distal tubule epithelia [28]. This model should provide a valuable tool with which to study mineralocorticoid dependent hypertension.

3.2. The placenta

The 11β-HSD2 enzyme is present in high concentrations in syncytiotrophoblast of the placenta where it is well situated to protect the fetus from high circulating levels of maternal glucocorticoids [33,34]. Most cortisol is inactivated on crossing the placenta and at birth about three quarters of the circulating glucocorticoid is derived from the fetal adrenal [35]. Abrogation of placental 11β-HSD2 activity has been reported to result in stillbirths in some families with mutations in the HSD11B2 gene [21]. Although 11β-HSD2 metabolizes dexamethasone it also converts 11-dehydrodexamethasone back to dexamethasone [36,37], enabling most of the synthetic glucocorticoid to cross the placenta intact [38]. In some animal models, notably rodents, placental 11β-HSD2 expression is high and correlates with fetal weight in midgestation, but drops dramatically toward term paralleling enzyme levels in fetal tissues [39,40].

3.3. The brain

There is widespread distribution of 11β-HSD2 in the fetal brain at midgestation, but levels fall drastically during the last trimester except in the thalamus and cerebellum. Glucocorticoid receptor is widely expressed throughout gestation and mineralocorticoid receptor is induced during the last few days before birth [31]. In situ studies show that 11β-HSD2 is present at high levels in the commissural portion of the nucleus tractus solitarius, subcommissural organ and ventrolateral ventromedial hypothalamus. Scattered cells containing 11β-HSD2 were also seen in the medial vestibular nucleus [41]. This pattern is distinct from that seen for the 11β-HSD1 enzyme which is highly expressed in the hippocampus [42]. Furthermore, the distribution of 11β-HSD2 bears little resemblance to that of the mineralocorticoid receptor, although, hypertensinogenic effects have been observed after intracerebroventricular injection of the 11β-HSD inhibitors

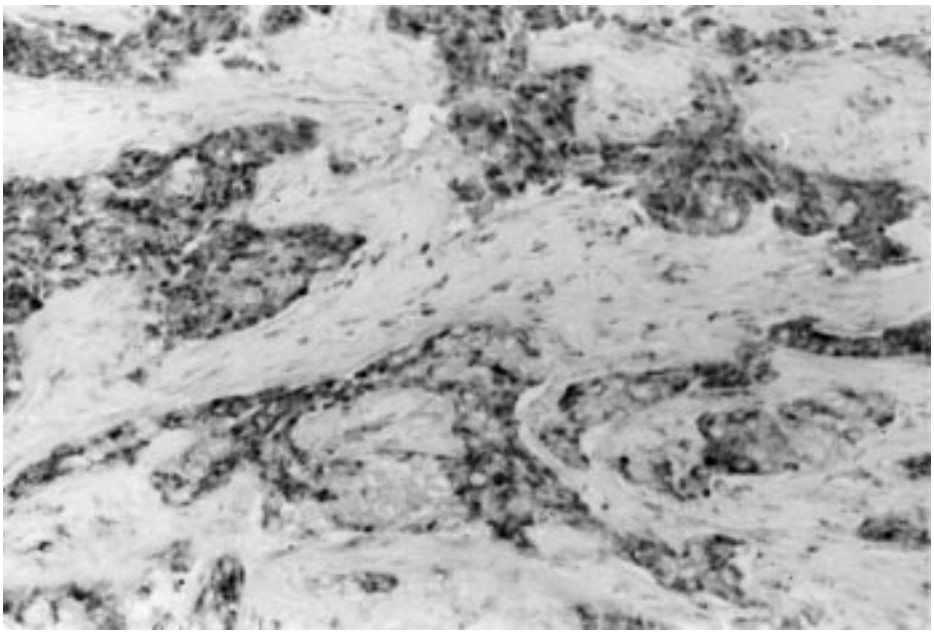

Fig. 2. Immunolocalization of 11β-HSD2 by the HUH23 antibody in invasive ductal carcinoma. Dark staining represents immunoreactivity in carcinoma cells ($\times$200).

glycyrrhizic acid and CBX [43]. This is consistent with high local concentrations of glucocorticoids leading to overstimulation of glucocorticoid/mineralocorticoid receptors. Some clinical and experimental forms of hypertension characterized by low circulating levels of mineralocorticoids respond to mineralocorticoid receptor antagonists, possibly reflecting glucocorticoid occupation and underlining the importance of this receptor in central mechanisms regulating blood pressure. Other ligands may also be involved as preliminary evidence suggests the de novo synthesis of mineralocorticoids in the brain [44]. Other studies indicate functional effects for both enzymes in the periventricular hypothalamus and limbic system [45].

Exposure of the fetus to excess glucocorticoids has been shown to affect fetal development and programming of adult physiology. Documented effects on the fetus include retarded fetal growth, elevated blood pressure and effects upon the hypothalamo-pituitary-adrenal (HPA) axis. Administration of modest doses of dexamethasone to pregnant rats results in elevated blood pressure in the offspring several months after cessation of treatment, underlining the critical role played by perinatal glucocorticoids in programming of the cardiovascular system [46]. These effects may be mediated in part by programming of the HPA axis via altered glucocorticoid receptor levels in the hippocampus, an important mediator of feedback control of glucocorticoid secretion [47].

3.4. The vasculature

Arterioles in human skin can be made to constrict on topical application of glucocorticoids [32]. This blanching phenomenon is potentiated by the addition of 11β-HSD inhibitors, consistent with the presence of an inactivating 11β-HSD in the skin [48]. Immunohistochemical studies have also provided evidence for 11β-HSD2 in cutaneous arterioles [49]. Thus 11β-HSD2 would appear to attenuate the response of resistance vessels to glucocorticoids, which in turn are known to amplify responses to vasoconstrictors. Indeed, hypertension appears to be reflected in increased skin blanching. The increased skin sensitivity to glucocorticoids in hypertension is thought to result from increased glucocorticoid receptor affinity coupled with decreased glucocorticoid inactivating capacity [50].

3.5. Cancer

There is now increasing evidence of 11β-HSD2 expression in cancer cells. Glucocorticoids have been shown to inhibit cell proliferation in some types of cancers, suggesting that inhibition of 11β-HSD2 activity may retard growth of the tumour. 11β-HSD2 plays an important role in the normal physiological function of the breast. Mineralocorticoid receptor and 11β-HSD have been demonstrated in pregnant and lac-

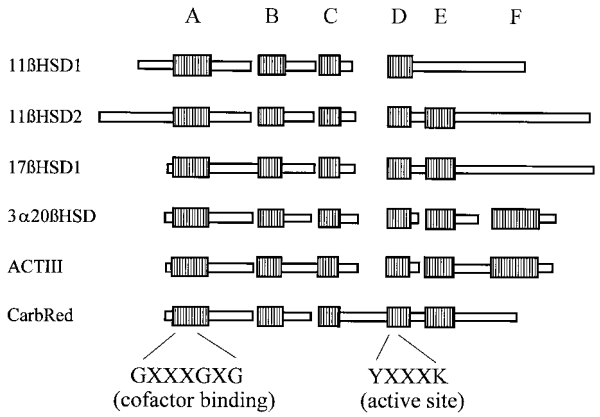

Fig. 3. Multiple alignment of SCAD family members with the 11β-HSD enzymes. Boxes A–F represent conserved domains. The ACTIII is involved in polyketide antibiotic synthesis in *Streptomyces coelicolor* and CarbRed is carbonyl reductase.

tating mammary gland where they are thought to modulate ion transport during milk production, and it has also been postulated that 11β-HSD2 activity could prevent premature milk production by decreasing local concentrations of corticosterone [51,52]. A study of normal and pathological human breast tissue shows colocalization of mineralocorticoid receptor and 11β-HSD2 in ductal epithelia with labeling indices significantly higher in invasive ductal carcinoma than invasive lobular carcinoma (Fig. 2), suggesting that the expression of these proteins may be related to ductal differentiation [53].

There is some evidence that targeting 11β-HSD2 activity may retard tumor growth. An in vitro study of MCF-7 and ZR-75-1 breast cancer cells showed that inhibition of activity enhanced the antiproliferative effects of glucocorticoids [54]. The prostatic cancer cell line LNCap has also been shown to possess high amounts of 11β-HSD2 together with a functional mineralocorticoid receptor [55]. High corticosterone levels have been shown to decrease prostatic weight, suggesting that 11β-HSD2 could play a protective role in this tissue [56]. Western blot analysis has shown evidence of a possible polymorphic 11β-HSD2 in the rat prostate [8].

4. Structural analysis of the SCAD superfamily

The three dimensional structure of 11β-HSD enzymes have thus far not been determined. However, five SCAD [57] proteins have been crystallised and had their structures elucidated. These are bacterial 3α,20β-HSD, human 17β-HSD1, rat liver dihydropteridine reductase, bacterial 7α-HSD and mouse lung carbonyl reductase. Duax and Ghosh [58] have analysed the conservation in sequence and structure in these enzymes and found that although only eleven residues are fully conserved the strucures are largely superimposable. Using the 17β-HSD1 numbering system the conserved residues are Thr8, Gly9, Gly15, Asp8, Ala91, Gly135, Tyr155, Lys159, Leu165, Ala170 and Thr190. The similarity of exon organization in ADP binding enzymes suggests that the nucleotide-binding domain evolved by gene duplication and was subsequently dispersed to different proteins through a process of intron-mediated recombination dividing the domain into characteristic β-sheets [59]. Thus most conservation is found at the core of the Rossmann fold in the cofactor binding domain; the lack of conservation in the C-terminal half is a reflection of the diversity of substrates metabolised. These include steroids, prostaglandins, antibiotics, chlorophylls/hemes, sugars, alcohols and polyols.

Multiple alignment of members of the SCAD superfamly shows the existence of up to six conserved domains (designated A to F). The A domain contains the cofactor binding region with a characteristically

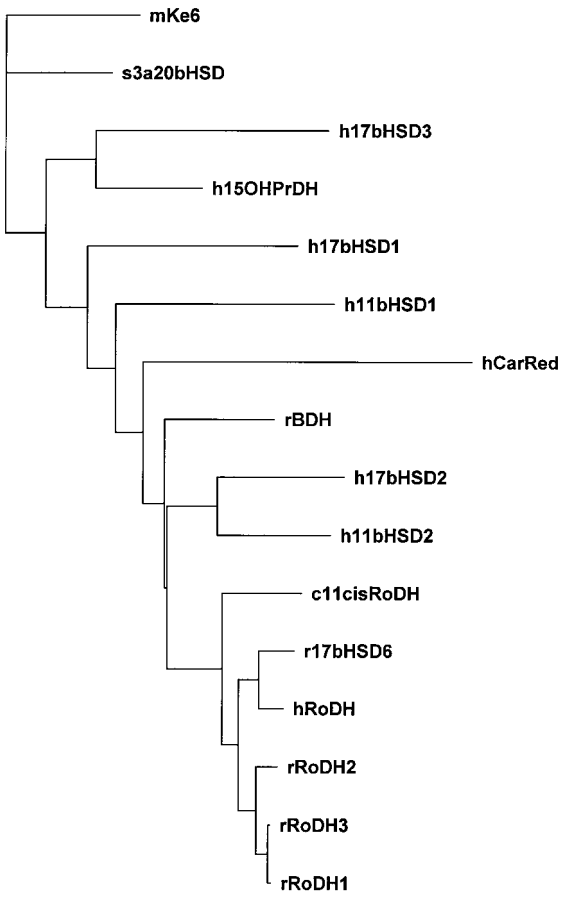

Fig. 4. Phylogenetic analysis of some SCAD family members. M, mouse; s, Streptomyces coelicolor; h, human, r, rat, c, cow. MKe6, a gene of unknown function; 15OHPrDH, 15-hydroxyprostaglandin dehydrogenase; rBDH, butyric acid dehydrogenase; RoDH, retinol dehydrogenase. Combined horizontal distances represent divergence.

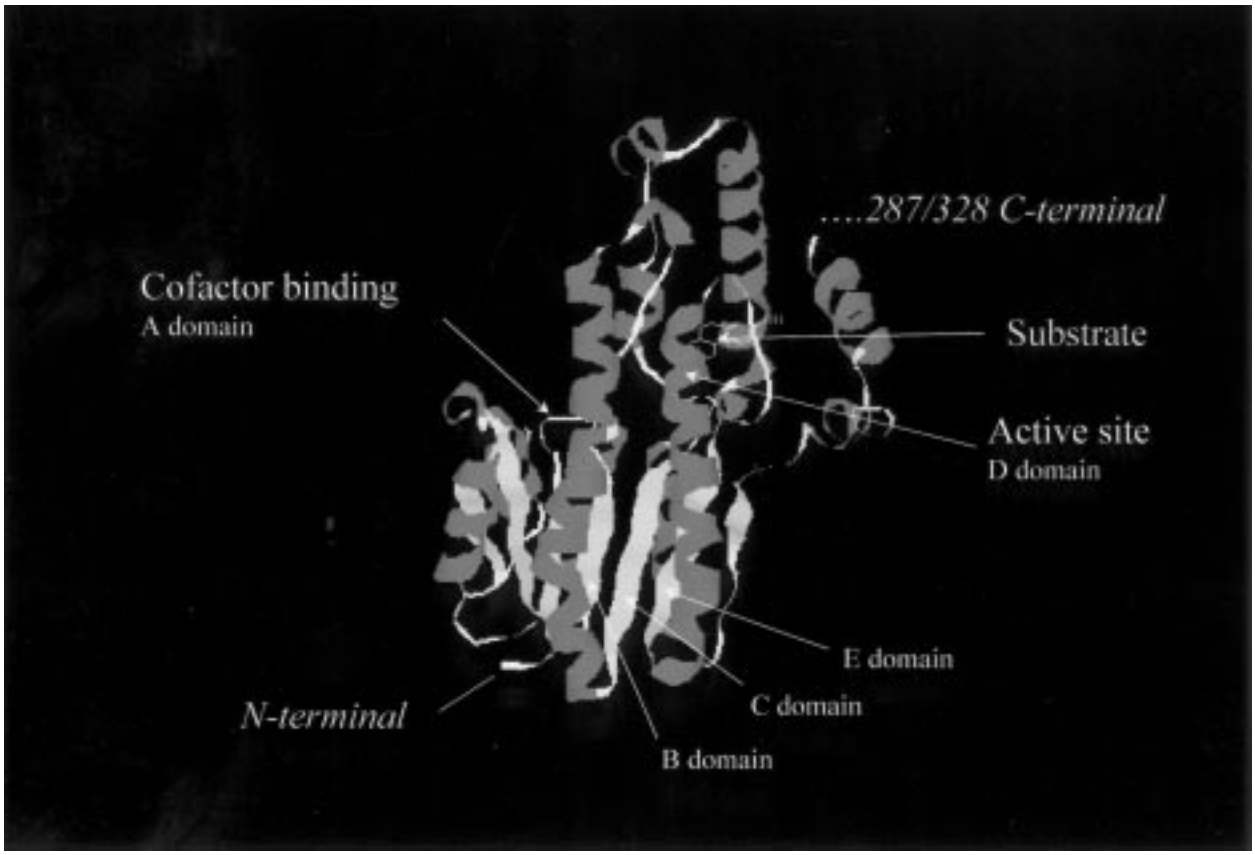

Fig. 5. Ribbon representation of crystal structure of 17β-HSD1 showing the positions of conserved domains identified by multiple alignment in Fig. 3. The three dimensional structure was determined by Azzi et al. [63].

conserved GXXXGXG motif, while the active site containing the absolutely conserved YXXXK motif resides in the D domain (Fig. 3). Within this domain the Y + 1 and especially the Y + 3 positions often contain a Serine or Threonine residue [60]. As in the aldo-keto reductase (AKR) superfamily the Tyrosine residue is thought to act as a general acid, with its pK_a being lowered by the Lysine. The distance between domains is also conserved, with the notable exception of carbonyl reductase which has an extra 41 residues inserted between the C and D domains. A large F domain can be identified in the C-teriminal region of a number of enzymes. In 7α-HSD this domain stretches from Pro212 to Gly247; F domains start with a conserved proline, contain highly conserved acidic and ThrGly motifs and end with the GlyGly dipeptide. Many of the enzymes containing this motif are present in procaryotes, suggesting that this is an ancient domain which evolved into a nonconserved region with high selectivity for substrates. Unlike the AKR family whose members in general have over 60% sequence identity, the SCAD family members are usually 20–30% conserved, although notable exceptions are 11β-HSD2 and 17β-HSD2 (45% identity), and an new emerging arm of the phylogenetic tree containing closely related retinol dehydrogenases (Fig. 4). Interestingly this new branch also contains 17β-HSD6 which is 65% identical with retinol dehydrogenase type I; the latter enzyme also converts 3α-diol to testosterone [61].

Inspection of the 17β-HSD1 crystal structure shows that domains B, C and E form β-sheets at the core of the protein, with the active site contained on an α-helix looping out between domains C and E (Fig. 5). Given the apparent similarity in structure between crystallised family members one can predict that the 11β-HSD's will have superimposable structures. Furthermore, given the dimeric form of 11β-HSD2 one can also expect that the structure of this enzyme will be similar to that determined for the dimeric 7α-HSD (Fig. 6). In the latter enzyme the protein chains appear to interact via anti-parallel α-helices. The two polypeptide chains comprising the dimer thus have opposite orientations. Mutations which disrupt dimer formation may also impact enzymatic activity and there is preliminary evidence that some heterozygotes with mutations in 11β-HSD2 may have compromised activity [62].

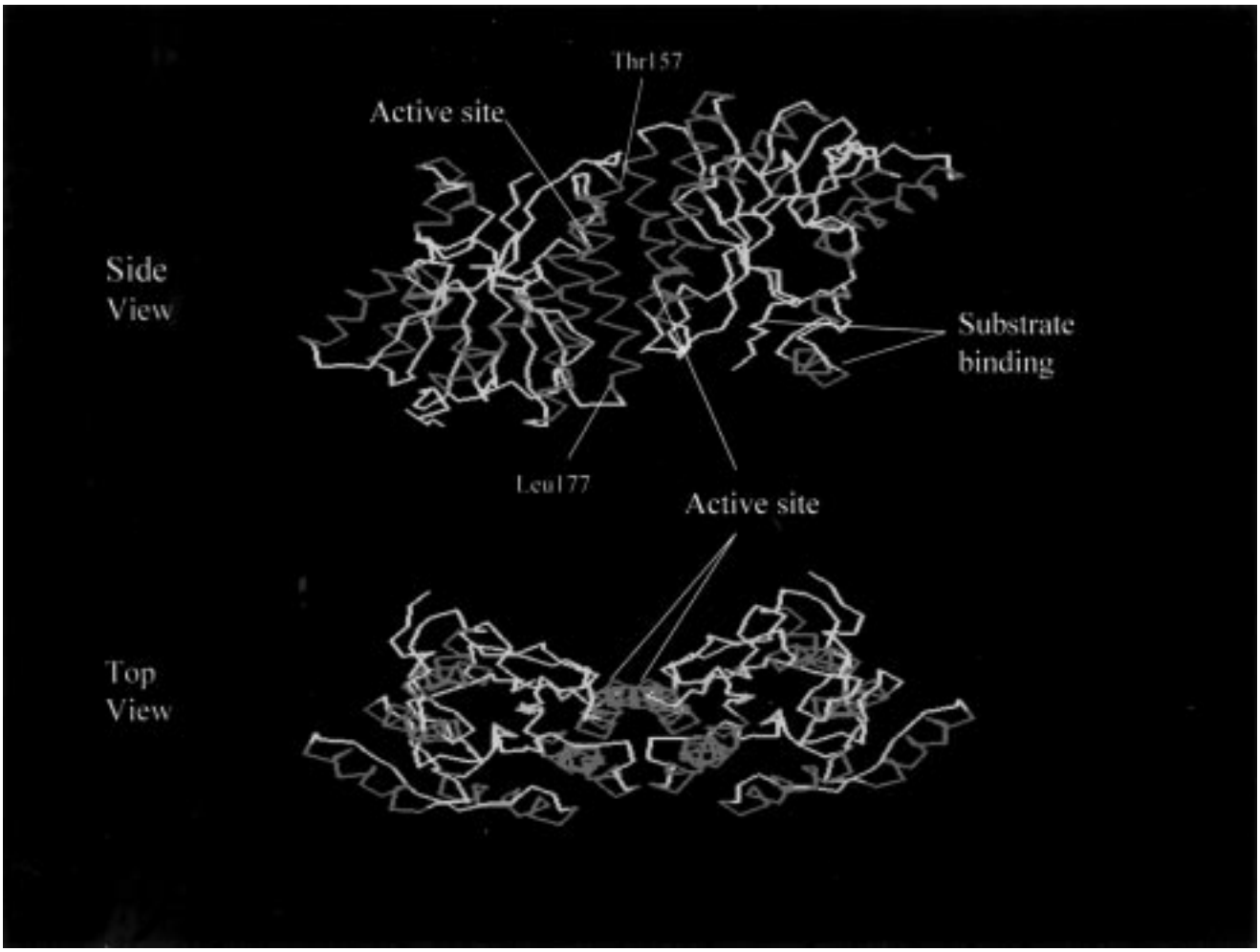

Fig. 6. Two views of the 7α-HSD dimer showing interacting helices. The original structure was determined by Tanaka et al. [64].

5. New members of the SCAD superfamily

There is accumulating evidence of further isoforms of 11β-HSD. The sheep kidney has been shown to contain an NADP dependent enzyme with affinity more than two orders of magnitude higher than 11β-HSD1 [65]. There is also evidence that Leydig cells possess an enzyme with similar characteristics [66], while in the ovary two distinct 11β-HSD activities have been found with K_m values of 490 nM and 2.6 μM [67]. Thus, there may be at least two additional enzymes which metabolize glucocorticoids at the 11-hydroxyl position. Recently, studies in toad bladder cells suggest that 6β-hydroxylation may also protect mineralocorticoid receptors from glucocorticoid occupation [68].

We have recently isolated a new member of the SCAD superfamily from a human lung cDNA library. The Pan1b enzyme possesses conserved domains A to D, is 300 residues in length and is highly expressed in adrenal carcinoma, liver, lung and small intestine. Expression in CHOP cells showed that it did not metabolize glucocorticoids despite interesting sequence similarities to 11β-HSD1 outside the classical conserved domains. Phylogenetic analysis revealed that Pan1b was most closely related to 11β-HSD1 and 17β-HSD3. A screen of potential radiolabeled substrates suggests that Pan1b may metabolize 17β-hydroxysteroids.

6. The putative dehydrocorticosterone receptor

The presence of the glucocorticoid receptor in cells containing a protected mineralocorticoid receptor is paradoxical given the inactivation of glucocorticoids by 11β-HSD2. A potential explanation is a novel putative steroid receptor recently identified in colonic crypt cells which binds 11-dehydrocorticosterone (11-DHB), the 11-keto metabolite of corticosterone [69]. The affinity of this putative DHB receptor for DHB is <10 nM and it has negligible affinity for aldosterone, dexamethasone, estradiol, RU38486, 5α-dihydrotestosterone, classical ligands for the family of steroid receptors. Evidence that binding is not to the

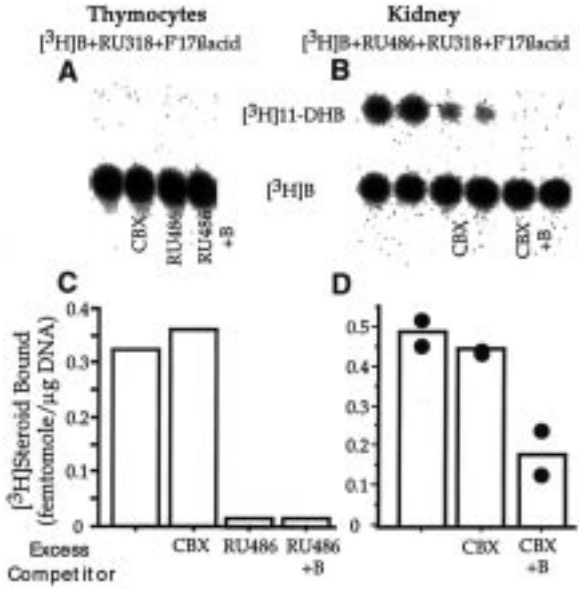

Fig. 7. Binding of 11-DHB to the putative DHB receptor. Rat kidney papilla slices and thymocytes were incubated (90 min, 22°C) with [^{3}H]B (30 nM) and a 200-fold excess of the indicated steroids of CBX. At the end of the incubation period media was extracted with ethyl acetate, steroids separated by TLC, and [^{3}H]-labelled bands quantified with a Fujix bio-Imaging Analyzer (A,B). Nuclei were separated from cytoplasm as previously described [69], and data expressed as fmol bound per μg DNA in the nuclear pellet (C,D). $n=2$ for kidney papilla slices and $n=1$ for thymocytes.

11βHSD2 enzyme is that CBX, progesterone, and deoxycorticosterone, all competitive inhibitors of 11βHSD2, do not displace DHB binding. Furthermore, 11β-HSD2 transfected cells express nuclear 11β-HSD2 but not a nuclear DHB receptor [70]. To determine if the putative DHB receptor colocalizes with 11βHSD activity, we measured [^{3}H]B binding and 11β-HSD activity in slices of rat kidney and thymocytes. Thymocytes showed no 11β-HSD activity (Fig. 7A), whereas kidney papilla slices converted 34% of [^{3}H]B to [^{3}H]11-DHB, a process which could be inhibited by CBX (Fig. 7B). When binding to mineralocorticoid receptor and corticosteroid binding globulin (CBG) is blocked specific nuclear binding of [^{3}H]B in thymocytes is totally displaced by RU38486, indicating the presence of a glucocorticoid receptor but not a DHB receptor (Fig. 7C). In contrast, in kidney papilla when mineralocorticoid receptor, glucocorticoid receptor and CBG are blocked, CBX does not compete for binding whereas B does, indicating that a DHB receptor is present (Fig. 7D). In addition, we have found evidence for the DHB receptor in rat lung which also exhibits 11β-HSD activity, but not in jejunum epithelial cells which have negligible enzyme activity (K.E. Sheppard, unpublished data). These data suggest that the DHB receptor is not ubiquitously expressed and may colocalize with 11β-HSD2.

The physiological function of a DHB receptor is yet to be defined but, given its apparent colocalization with 11β-HSD activity, it may mediate glucocorticoid effects in cells expressing high levels of this enzyme since 11βHSD2 compromises binding of endogenous glucocorticoids to both mineralocorticoid and glucocorticoid receptors [71]. In addition, since cellular levels of 11-DHB will reflect circulating corticosterone the receptor would respond to both stress and diurnal variations in corticosteroids. Thus, a DHB receptor may allow mineralocorticoid target tissues to respond to circulating glucocorticoids without compromising the aldosterone selectivity of the mineralocorticoid receptor.

7. Conclusion

The 11β-HSD enzymes provide mechanisms for the activation and inactivation of glucocorticoids. In the liver the large reservoir of 11β-HSD1 serves to produce active glucocorticoid which contributes to both endocrine and paracrine processes; in other tissues, such as the ovary, the effects appear to be primarily paracrine. 11β-HSD2 on the other hand unidirectionally inactivates glucocorticoids. The high levels of 11β-HSD2 expression in the distal tubule, placenta and fetus during midgestation underline the importance of glucocorticoid inactivation for normal physiological processes at these sites. In addition, 11β-HSD2 also plays a role in programming with fetal and placental 11β-HSD2 insufficiency suggested as a cause of hypertension in adulthood. The absence of mineralocorticoid receptor in a number of tissues expressing significant amounts of 11β-HSD2 suggests modulation of glucocorticoid access to the glucocorticoid receptor. 11β-HSD2 containing cancer cells that are growth-inhibited by glucocorticoids may thus be responsive to enzyme inhibitors. In some cells 11β-HSD2 activity may provide ligand for the putative 11-DHB receptor. The availability of a number of three dimensional structures of family members underlines the feasibility of such studies on the crystal structure of the 11β-HSD enzymes, studies which should facilitate the design of specific 11β-HSD inhibitors.

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