REVIEW

5α-Reduced glucocorticoids: a story of natural selection

Mark Nixon, Rita Upreti and **Ruth Andrew**

Endocrinology, Queen's Medical Research Institute, University/British Heart Foundation Centre for Cardiovascular Science, Edinburgh EH16 4TJ, UK
(Correspondence should be addressed to R Andrew; Email: ruth.andrew@ed.ac.uk)

Abstract

5α-Reduced glucocorticoids (GCs) are formed when one of the two isozymes of 5α-reductase reduces the Δ^{4-5} double bond in the A-ring of GCs. These steroids are largely viewed inert, despite the acceptance that other 5α-dihydro steroids, e.g. 5α-dihydrotestosterone, retain or have increased activity at their cognate receptors. However, recent findings suggest that 5α-reduced metabolites of corticosterone have dissociated actions on GC receptors (GRs) *in vivo* and *in vitro* and are thus potential candidates for safer anti-inflammatory steroids. 5α-Dihydro- and 5α-tetrahydro-corticosterone can bind with GRs, but interest in these compounds had been limited, since they only weakly activated metabolic gene transcription. However, a greater understanding of the signalling mechanisms has revealed that transactivation represents only one mode of signalling via the GR and recently the abilities of 5α-reduced GCs to suppress inflammation have been demonstrated *in vitro* and *in vivo*. Thus, the balance of parent GC and its 5α-reduced metabolite may critically affect the profile of GR signalling. 5α-Reduction of GCs is up-regulated in liver in metabolic disease and may represent a pathway that protects from both GC-induced fuel dyshomeostasis and concomitant inflammatory insult. Therefore, 5α-reduced steroids provide hope for drug development, but may also act as biomarkers of the inflammatory status of the liver in metabolic disease. With these proposals in mind, careful attention must be paid to the possible adverse metabolic effects of 5α-reductase inhibitors, drugs that are commonly administered long term for the treatment of benign prostatic hyperplasia.

Journal of Endocrinology (2012) **212,** 111–127

Introduction

Glucocorticoids (GCs) are steroid hormones synthesised in the adrenal cortex, which exert a plethora of effects in the body, ranging from modulation of metabolism and suppression of inflammation to regulation of stress responses and neuronal function. These hormones are vital for health and this is most vividly demonstrated in disorders characterised by GC deficiency or excess. Diminished GC production, as seen in Addison's disease, leads to critical illness and is life-threatening if inadequately treated. The converse is seen in Cushing's syndrome, where excess GC production manifests as insulin resistance, obesity, dyslipidaemia, altered immune responses (e.g. impaired wound healing) and disturbed central nervous system activity (e.g. depression).

While endogenous GCs play a key role in many aspects of health and disease, synthetic exogenous GCs have become widely used therapeutically, predominantly as anti-inflammatory agents. Many synthetic GC variants have been developed, aiming to maximise their clinical utility through optimisation of pharmacological properties. In the UK, it is estimated that ~40 million prescriptions for GCs are dispensed each year, with ~70% of these being either inhaled or topical preparations (http://www.isd.scot.nhs.uk/isd/1038.html, http://www.ic.nhs.uk/statistics-and-data-collections/primary-care/prescriptions). Despite their success in treating inflammatory conditions, synthetic GCs remain plagued by side effects wrought through widespread activation of the ubiquitous GC receptors (GRs). Receiving GCs therapeutically incurs a dose-dependent rise in the relative risk of cardiovascular morbidity (Souverein *et al.* 2004, Wei *et al.* 2004, Solomon *et al.* 2011), with an increase of up to threefold amongst current users of high-dose GCs. The relative risk for any fracture in those using GCs is 1·33, and this increase in risk is particularly marked for vertebral fractures with a relative risk of 2·60 (Canalis *et al.* 2007, van Staa *et al.* 2011). On this background, there is considerable interest in designing GR ligands that have the ability to suppress inflammation without modulating metabolism or adversely affecting bone remodelling. However, it has been difficult to achieve this goal.

DOI: 10.1530/JOE-11-0318
0022–0795/12/0212–111 *Printed in Great Britain*
Online version via http://www.endocrinology-journals.org

Therapeutic actions of GCs to relieve inflammation

GCs mediate many of their effects by binding with the GR (NR3C1; Hollenberg *et al.* 1985), a ligand-activated transcription factor belonging to the nuclear hormone receptor superfamily. The conventional model (Beato *et al.* 1995) of GR action is of GR homodimers modulating gene transcription through direct interactions with DNA. In the unbound state, monomeric GR is located in the cytosol in association with a complex of chaperone proteins. Ligand binding induces conformational changes resulting in dissociation of the chaperone complex, in turn enabling exposure of the DNA binding and dimerisation domains and permitting nuclear translocation. A GR homodimer interacts with GC response elements (GREs) within the promoter region of target genes in the nucleus, leading to up-regulation of gene transcription (transactivation). Multiple genes encoding critical proteins in metabolic pathways, such as tyrosine aminotransferase (TAT; Grange *et al.* 2001) or phosphoenolpyruvate carboxykinase (PEPCK; Petersen *et al.* 1988, Ruppert *et al.* 1990), are up-regulated in this manner. This mechanism of transactivation underpins many of the metabolic side effects of these drugs. In contrast, GR can also down-regulate gene transcription by binding with negative GRE sites. Examples of genes regulated in this way are POMC (Drouin *et al.* 1993) and osteocalcin (Meyer *et al.* 1997*b*), where GR multimers bind to negative GREs within promoter regions, repressing gene transcription and influencing negative feedback on the hypothalamic–pituitary–adrenal (HPA) axis and bone remodelling respectively.

However, not all responses to GCs are mediated in a manner solely dependent on GR dimers binding with DNA, and indeed, the ability of GCs to suppress inflammation involves many other modalities of receptor signalling. Those pro-inflammatory signalling pathways believed to be most important in GC action have been reviewed extensively (De Bosscher & Haegeman 2009) and are summarised in Fig. 1. GR monomers play a crucial role in the repression of pro-inflammatory genes. These actions are attributed to GR monomers tethering with DNA-bound pro-inflammatory transcription factors NF-κB and AP-1, thus preventing their actions activating transcription of pro-inflammatory cytokines such as TNFα and IL6 (Barnes 1998). This mechanism is distinct from those described above as it requires neither GR dimerisation nor direct DNA binding of the GR monomer. The relative importance of such dimerisation-independent mechanisms to suppress inflammation was elegantly demonstrated in $GR^{dim/dim}$ mice, which express a mutant GR incapable of dimerisation (Reichardt *et al.* 2001). These mice were still able to respond to dexamethasone, suppressing expression of pro-inflammatory cytokines.

Whilst the predominant anti-inflammatory actions of GCs are mediated through GR monomer-based repression of pro-inflammatory transcription factor activity, some of the actions of GR to reduce inflammation are mediated through

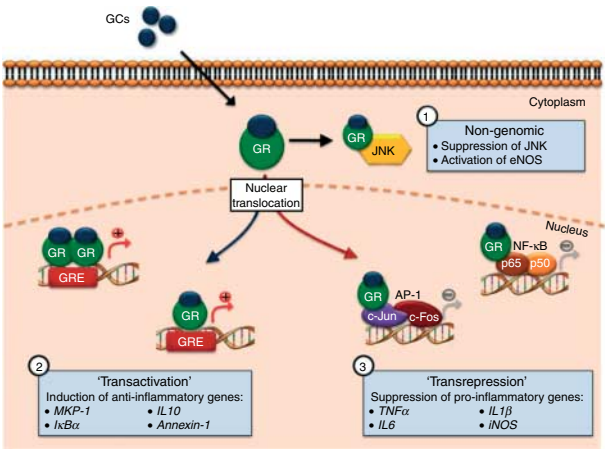

Figure 1 Glucocorticoid (GC) effects on inflammatory signalling. GCs act through several mechanisms to exert anti-inflammatory effects: 1) non-genomic pathways involve GC receptor (GR)-mediated direct interactions with second messenger proteins, including the MAPK protein JNK, inhibiting the activation of this signalling pathway. 2) GR-mediated transactivation of key anti-inflammatory genes involves direct DNA binding of both GR dimers and monomers/multimers to GC-response elements (GRE) in the promoter region of target gene. 3) Transrepression of pro-inflammatory genes does not require direct DNA binding of GR, but rather 'tethering' of GR monomers to DNA-bound pro-inflammatory transcription factors.

transactivation mechanisms. Here, GR DNA binding is essential, but dimerisation is not always required. For example, transcription of the anti-inflammatory protein IκBα results from GR dimers interacting with hormone response elements located within its promoter region (Heck *et al.* 1997, Deroo & Archer 2001). However, transactivation of other anti-inflammatory genes including activation of interleukin 10 (IL10) have been shown to occur independently of dimerisation (Unterberger *et al.* 2008). This has also been demonstrated for *MKP1* (also known as *DUSP1*) gene expression, which is induced by GC treatment even in the presence of the dimerisation-deficient GR mutant (Abraham *et al.* 2006). Since prevention of dimerisation does not abrogate all of the anti-inflammatory actions of GR, this gives hope that the independent mechanisms underlying these pathways may be tractable to pharmaceutical manipulation and aid rational design of new drugs. Simply dissociating transrepression from transactivation may not represent the best approach (Belvisi *et al.* 2001) and indeed to achieve tissue-specific selectivity, targeting specific interactions between GR and co-activator/co-repressor proteins may be more successful (Coghlan *et al.* 2011).

There have been numerous attempts to develop 'dissociated' steroids or 'selective GR modulators' with improved therapeutic indices (McMaster & Ray 2008). This strategy has been successful previously in the development of selective oestrogen receptor modulators, where careful manipulation of intracellular interactions of nuclear hormone receptors with co-regulators or co-repressors has led to selectivity of

actions (oestrogenic effects on reproductive tissues vs bone; Smith & O'Malley 2004). Endogenous steroids have been utilised as templates in the pharmaceutical development of synthetic GCs. In humans, the most abundant, naturally occurring GC is cortisol (Fig. 2), whereas in rodents (lacking adrenal 17α-hydroxylase), corticosterone is the active species formed (Fig. 2). Corticosterone is also produced in humans but at a rate approximately one-seventh of that of cortisol (Van der Straeten *et al.* 1963), achieving circulating levels ~10–30 times lower (Mattingly *et al.* 1989, Hariharan *et al.* 1992, Ghulam *et al.* 1999). These steroids are viewed as the only endogenous GCs, binding tightly to the GR with K_ds of ~3·5 and 10–30 nM respectively (Reul *et al.* 1990, Mulatero *et al.* 1997). However, they are not specific for these receptors and can also bind with other nuclear hormone receptors with high affinity, for example to MR (K_ds similar to that of aldosterone, ~1·3 nM (Arriza *et al.* 1987)). Corticosterone can interact with the pregnane X receptor (PXR) or rodent steroid X receptor (SXR) to induce transcription but activates this less potently than GR (Blumberg *et al.* 1998).

Synthetic GCs used clinically have structural modifications aimed at enhancing binding affinity with, and specificity for, GR. As with endogenous GCs, synthetic derivatives have varying degrees of action at other receptors, particularly MR. Dexamethasone has a K_d for GR of ~5 nM (Ray *et al.* 1999), but lacks significant agonist activity at MR and is devoid of adverse effects on electrolyte balance. While prednisolone strongly binds GR, it also has affinity for MR (Coghlan *et al.* 2011), thus retaining some mineralocorticoid activity. All synthetic GCs commonly used in clinical practice have potential metabolic and osteopenic side effects, since they were not designed to discriminate between the various facets of GR actions. A novel drug that successfully discriminates side effects from anti-inflammatory actions has not been established in clinical use. Indeed, although some earlier candidates did make it to the market, e.g. deflazacort, when dose equivalency was fully checked, they still possessed an adverse side effect profile (Markham & Bryson 1995). However, some agents have come to the fore in more recent pre-clinical studies, which validate the potential of the approach (De Bosscher & Haegeman 2009), e.g. compound A, a natural product derived from a Namibian desert plant (De Bosscher *et al.* 2011) and AL-438 (Coghlan *et al.* 2011) developed by Abbott. Although they bind selectively with GR, not all of these novel agents are steroidal in nature.

5α-Reduced GCs: the case for natural selection?

Recently, the anti-inflammatory potential of some endogenous metabolites of corticosterone, namely 5α-dihydro and 5α-tetrahydro-corticosterone (Fig. 2) have been investigated (McInnes *et al.* 2004, Yang *et al.* 2011). Endogenous GCs (cortisol and corticosterone) are principally inactivated in the liver, yielding polar metabolites that can be more rapidly cleared by the kidneys. The main routes of clearance are via A-ring reduction yielding two stereoisomeric metabolites, 5α- and 5β-tetrahydro steroids, following sequential reduction of the Δ^{4-5} double bond and then the 3-ketone group (Andrew & Walker 2005). 5α-Reductases were initially

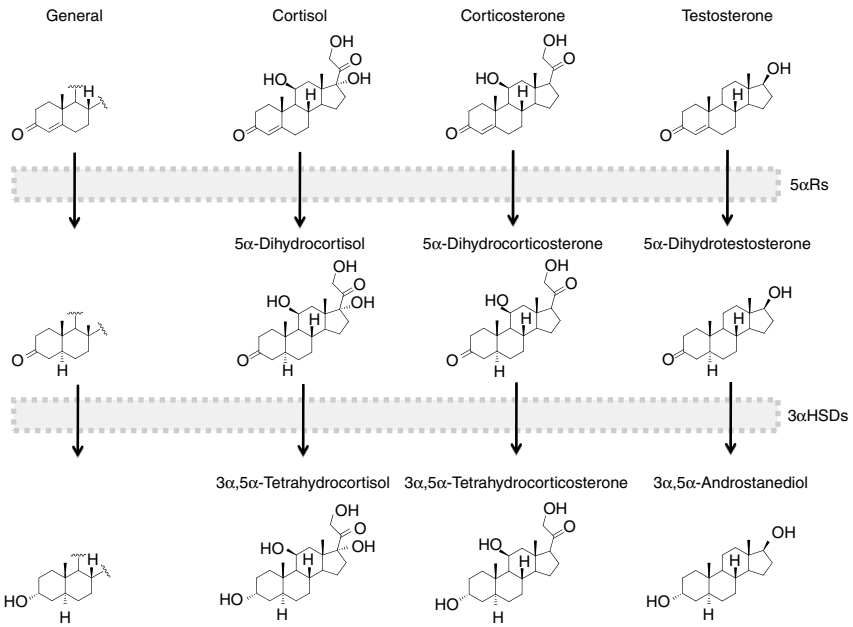

Figure 2 Metabolism of cortisol and corticosterone to generate 5α-reduced glucocorticoids. Generic scheme of reaction catalysed by 5α-reductases (5αRs) followed by 3α-hydroxysteroid dehydrogenases (3αHSDs). Specific reactions relating to cortisol, corticosterone and testosterone are shown.

described and characterised in the 1950s and 1960s stimulated by work in rodents revealing the presence of hepatic enzymes that catalysed 5α-reduction of steroids (Schneider & Horstmann 1951, Schneider 1952, Forchielli & Dorfman 1956). Although the presence of multiple enzymes was initially suggested (McGuire & Tomkins 1960, McGuire *et al.* 1960, Godoy *et al.* 2011), genes encoding two isozymes (*SRD5A1* and *SRD5A2)* were later cloned in the 1990s (Andersson & Russell 1990, Andersson *et al.* 1991).

SRD5A1 and *SRD5A2* are members of a larger family of genes containing 5α-reductase domains, the other members being encoded by the genes *SRD5A3* and *GPSN2-SRD5A2L2*, whose roles remain less clear, but may involve reduction of non-steroidal substrates (Cantagrel *et al.* 2010). *SRD5A3* was identified through expression profiling of hormone refractory prostate cancer cells and its transcript is expressed in higher levels in malignancy in comparison with benign tissues (Uemura *et al.* 2008, Yamana *et al.* 2010, Godoy *et al.* 2011). Conservation in the C- and N-terminal regions between it and 5α-reductases 1 and 2 (5αR1 and 5αR2) supports a role for resultant SRD5A3 protein in the metabolism of steroids, a hypothesis was borne out using constructs expressing a protein capable of metabolising testosterone (Uemura *et al.* 2008) and androstenedione (Yamana *et al.* 2010) to their 5α-dihydro metabolites. To date, similar catalytic activities have not been demonstrated for GCs. However, a distinct role for this enzyme has been proposed to catalyse conversion of polyprenol to dolichol and mutations in *SRD5A3* give rise to a congenital disorder of glycosylation (Cantagrel *et al.* 2010, Morava *et al.* 2010, Kahrizi *et al.* 2011).

5β-Reductase, similarly cloned in the 1990s (Kondo *et al.* 1994), is a distinct enzyme and part of the aldo-keto reductase superfamily (AKR1D), being most highly expressed in liver (Kimura *et al.* 1998). It is best recognised for its vital role in bile acid synthesis (Kimura *et al.* 1998). Both configurations of A-ring-reduced metabolites of GCs were generally considered inert. However, recently, 5α-reduced metabolites of corticosterone have demonstrated attractive pharmaceutical properties *in vivo*, possessing anti-inflammatory properties, with an apparent paucity of adverse metabolic side effects (Yang *et al.* 2011). Although not the topic of this review, 5β-reduced GCs also possess some biological activities, having the ability to bind and activate the steroid (or pregnane) X receptor (Blumberg *et al.* 1998) and can lower intra-ocular pressure (Weinstein *et al.* 1983, Southren *et al.* 1985, 1986, 1987) through an uncharacterised mechanism.

Biosynthesis of 5α-reduced GCs

5α-Reduced GCs are formed by 5αR1 and 5αR2 (Andersson & Russell 1990, Andersson *et al.* 1991). Both isozymes have five exons and four introns and retain a high degree of homology, suggestive of a common origin (Russell & Wilson 1994). There is also an apparently non-functioning pseudogene mapped to the X chromosome (Jenkins *et al.* 1991). The *SRD5As* have received most attention for their critical role in the development of the male reproductive system. 5α-Reduced metabolites of testosterone and androstenedione, 5α-dihydrotestosterone or 5α-androstane-3-dione, respectively, possess higher affinity for the androgen receptor (AR) compared with the parent steroid (Wilson & French 1976, Chang *et al.* 1988, Lubahn *et al.* 1988). Identification and characterisation of these genes, therefore, led to an understanding of the aetiology of a condition termed pseudohermaphroditism, caused by lack of 5α-reductase 2 (Imperato-McGinley *et al.* 1974, Walsh *et al.* 1974, Fisher *et al.* 1978). This condition, now termed 5α-reductase 2 deficiency, occurs in individuals with 46 XY disorder of sexual development and is characterised by lack of, or reduction in, virilisation at birth, followed by the development of a male phenotype at puberty, though without associated prostatic growth. Polymorphisms in the type 2 gene may also influence the severity and predisposition to prostate cancer, although a recent meta-analysis argues against their importance in this disease (Li *et al.* 2011). Although the first cDNA clone of 5αR was the type 1 isozyme, a specific and unique role for this enzyme has not been recognised (Andersson & Russell 1990).

Disease states associated with inactivating mutations in 5αR1 have not been identified, although polymorphisms have been reported in polycystic ovarian syndrome (PCOS; Eminovic *et al.* 2005). One study implicates single nucleotide polymorphisms in both 5αR genes; a variant in *SRD5A2* (which encodes a protein with less activity) is protective against PCOS, and several different variants in *SRD5A1* are associated with the presence, and increased severity, of hirsutism (Goodarzi *et al.* 2006). However, others have failed to demonstrate relationships between genetic polymorphisms in *SRD5A1* and severity of either hyperandrogenic states in women (Eminovic *et al.* 2005) or androgenetic alopecia in men (Ha *et al.* 2003).

5α-Reductases are hydrophobic, membrane-bound enzymes with predicted molecular weights of ∼28–29 kDa (Andersson & Russell 1990). Human 5αR1 and 5αR2 contain 259 (Andersson & Russell 1990) and 254 (Andersson *et al.* 1991) amino acids respectively. The two isozymes of 5α-reductase have species-specific tissue distributions (summarised for human and rodents in Table 1), which in turn may have implications for formation and action of 5α-reduced GCs. For example, in the immune system, the type 1 isozyme is more widely expressed than type 2, although there are species differences (Milewich *et al.* 1983, Araneo *et al.* 1991, Normington & Russell 1992, Zhou *et al.* 1998, Borlak *et al.* 2004, Hammer *et al.* 2005, Samy *et al.* 2001); Yang *et al.* (2011) demonstrated an absence of transcripts of both isozymes in bone marrow-derived murine macrophages, compared with previous findings reporting the presence of enzyme in human and rat (Table 1). Activity and/or expression of 5αRs has also been demonstrated in the skin (Dube *et al.* 1975, Takayasu *et al.* 1980, Randall *et al.* 1982, Luu-The *et al.* 1994, Eicheler *et al.* 1995, 1998, Sawaya &

Table 1 Tissue distribution of 5α reductases in human, rat and mouse. In cases of differing evidence, those demonstrating isozyme-specific expression are shown

	5αR1			5αR2			Isozyme unconfirmed	
	mRNA	Protein/IC	Activity[a]	mRNA	Protein/IC	Activity[a]	Activity[b]	References
System								
Metabolic/cardiorespiratory								
Liver	H✓; R✓; M✓	H✓; R✓	R✓	H✓; R×; M✓	H✓; R×		H✓	22, 53, 65, 40, 32, 70
Adipose	H✓; R✓; M✓			H✓; M×			R✓	91, 13, 5, 83, 38, 33
Skeletal muscle	H×; R✓	H×	H×	H×; R×	H×	H×		78, 65
Heart/vessels	R×			R×			H✓; R✓	48, 53, 65, 7
Kidney	H×; R✓	H×	H✓	H×; R✓	H×	H✓	M±	51, 78, 65
Adrenal	H×; R✓; M±	H×; R✓	H✓	H×; R✓; M✓	H×	H×	R✓	10, 78, 65, 89, 37
Lung	H✓; R✓; M✓	H✓	H✓	H✓; R×	H×			53, 61, 28, 60
Immune								
Spleen	R✓			R×				53
Monocytes/ macrophages	H✓; M×			H±; M×			H✓; R✓; M✓	47, 35, 3, 20, 86
Lymphocytes	H✓		H✓	H±			M✓	90, 66
Thymus	R✓*			R✓*				8
Bone								
Cartilage	R✓		R:♂✓,♀×	R×				63
Bone	H✓		H✓	H✓		H±	R×	80, 23
Skin								
Genital skin	H✓	H×		H✓	H✓		H✓; R✓	14, 57, 65, 77
Non-genital skin	H✓	H✓		H±	H✓		H✓; R✓	14, 76, 62, 36, 65, 16
Hair follicle	H✓	H✓		H✓	H✓		H✓	16, 17, 68
Sweat gland	H✓							67
Gastrointestinal								
Stomach	R✓			R×				53
Intestine	R✓			R✓			H✓; R✓	52, 18, 53
Neurological								
Hypothalamus	H✓; R✓; M✓	H✓; R✓	R✓	H×; R×	H×		R✓	30, 78, 56, 65, 31, 27
Pituitary	H×; R✓	H×; R✓	R✓	H×; R×	H×		R✓	30, 65, 87, 54
Thalamus	M✓(G, g)	R✓; M✓ (G, g)					R✓	56, 31, 2
Hippocampus	H✓; M×(G); M✓(g)	R✓; M×(G); M✓(g)		H×			R✓	44, 56, 31, 74, 2
Cortex	H✓; R✓; M×(G); M✓(g)	H✓; R✓; M×(G); M✓(g)	H✓	H×; R✓	H×		R✓	44, 56, 31, 74, 73, 2, 79
Medulla oblongata	H✓	H✓		H✓	H✓		R✓	78, 31
Pons	H✓	H×		H×	H×		R✓	44, 78, 65, 31
Amygdala	M(g)✓	M(g)✓					R✓	31, 2
Corpus callosum	M×	M×					R✓	44, 2
Striatum	M(G)✓	M(G)✓						2
Cerebellum	H✓; M✓(G); M± (g)	H✓; M✓(G); M±(g)		H×	H×		R✓	44, 78, 65, 31, 2
Spinal cord	R✓	R✓		R✓	R✓		R✓; M✓	21, 39, 58, 55
PN/paraganglia		R✓					R✓	45, 88

(continued)

Table 1 *Continued*

	5αR1			5αR2			Isozyme unconfirmed	
	mRNA	Protein/IC	Activity[a]	mRNA	Protein/IC	Activity[a]	Activity[b]	References
Sensory								
Olfactory bulb	R✓; M±(G); M✓(g)	R✓; M±(G); M✓(g)					R✓	31, 29, 2
Eyes	H✓			H✓			H✓	84, 64
Reproductive								
Prostate	H✓; R✓	H×; R✓	H✓; R✓; M±	H✓; R✓	H✓; R✓	R✓; M✓	H✓	22, 53, 65, 41, 72
Testes	H×; R✓	H×	M✓	H✓; R✓	H✓	M×	M✓	71, 65, 42
Epididymis	H✓; R✓; M✓	H×; R✓	R✓; M✓	H✓; R✓; M✓	H✓; R✓	R✓; M✓	H✓	53, 78, 41, 11, 43, 81
Vas deferens	R✓			R✓			H✓; R✓; M✓	15, 69, 26, 65
Seminal vesicles	H×; R✓	H×; R✓	M×	H✓; R✓	H✓; R✓	M×		65, 41, 59
Ovary	H✓; R✓; M✓	H×	H✓	H✓; R×; M✓	H×	H✓		4, 53, 78, 65, 49, 25, 37
Vagina	H✓; M✓			H✓; M✓			R✓	19, 7, 6, 37
Uterus	H✓; M✓	H✓; M✓		H✓; M±	H✓		R✓	40, 7, 24, 37
Placenta	M✓	H✓; M✓	H✓		H✓	H✓	R✓	9, 46, 12, 40, 82
Breast	H✓	H✓		H✓	H✓		H✓; R✓; M✓	50, 34, 1, 85, 75

5αR1, 5α reductase type 1; 5αR2, 5α reductase type 2; H, human; R, rat; M, mouse; ✓, present; ×, absent; ±, very low levels; IC, either immunocytochemistry or immunohistochemistry; G, gamma aminobutyric acid; g, glutaminergic neurons; PN, peripheral nerves. Activity[a]=demonstration of substrate → product in conditions designed for optimal isozyme action. Activity[b]=*ex vivo* or *in vivo* demonstration of 5α reduction of substrate. *mRNA identified but isozyme not specified. [1]Abulhajj & Klang (1982); [2]Agis-Balboa *et al.* (2006); [3]Araneo *et al.* (1991); [4]Backstom *et al.* (1986); [5]Barat *et al.* (2007); [6]Berman *et al.* (2003); [7]Blom *et al.* (2001); [8]Borlak *et al.* (2004); [9]Chan & Leathem (1975); [10]Colby & Kitay (1972); [11]Delarminat *et al.* (2011); [12]Dombroski *et al.* (1997); [13]Drake *et al.* (2005); [14]Dube *et al.* (1975); [15]Dupuy *et al.* (1979); [16]Eicheler *et al.* (1995); [17]Eicheler *et al.* (1998); [18]Eiknes *et al.* (1983); [19]George (1993); [20]Hammer *et al.* (2005); [21]Hauser *et al.* (1987); [22]Houston *et al.* (1987); [23]Issa *et al.* (2002); [24]Ito *et al.* (2002); [25]Jakimiuk *et al.* (1999); [26]Jeanfaucher *et al.* (1986); [27]Karolczak *et al.* (1998); [28]Kimura *et al.* (2003); [29]Kiyokage *et al.* (2005); [30]Lephart (1993); [31]Li *et al.* (1997); [32]Livingstone *et al.* (2000); [33]Livingstone *et al.* (2009); [34]Lloyd (1979); [35]Lofthus *et al.* (1984); [36]Luu-The *et al.* (1994); [37]Luu-The *et al.* (2005); [38]MacKenzie *et al.* (2008); [39]Maclusky *et al.* (1987); [40]Mahendroo *et al.* (1997); [41]Mahendroo *et al.* (2001); [42]Mahendroo *et al.* (2004); [43]Mahoney *et al.* (2011); [44]Melcangi *et al.* (1988); [45]Melcangi *et al.* (1990); [46]Milewich *et al.* (1979); [47]Milewich *et al.* (1983); [48]Milewich *et al.* (1987); [49]Milewich *et al.* (1995); [50]Mori *et al.* (1978); [51]Mowszowi & Bardin (1974); [52]Nienstedt *et al.* (1980*a*,*b*); [53]Normington & Russell (1992); [54]Nowak (2002); [55]Patte-Mensah *et al.* (2004); [56]Pelletier *et al.* (1994); [57]Pinsky *et al.* (1978); [58]Pozzi *et al.* (2003); [59]Pratls *et al.* (2003); [60]Provost & Tremblay (2007); [61]Provost *et al.* (2002); [62]Randall *et al.* (1982); [63]Raz *et al.* (2005); [64]Rocha *et al.* (2000); [65]Russell & Wilson (1994); [66]Samy *et al.* (2001); [67]Sato *et al.* (1998); [68]Sawaya & Price (1997); [69]Seethalakshmi *et al.* (1982); [70]Seo *et al.* (2009); [71]Sheffield & O'Shaughnessy (1988); [72]Shirakawa *et al.* (2004); [73]Steckelbroeck *et al.* (2001); [74]Stoffel-Wagner *et al.* (2000); [75]Suzuki *et al.* (2001); [76]Takayasu *et al.* (1980); [77]Thiele *et al.* (2005); [78]Thigpen *et al.* (1993*b*); [79]Torres & Ortega (2006); [80]Turner *et al.* (1990); [81]Viger & Robaire (2011); [82]Vu *et al.* (2009); [83]Wake *et al.* (2007); [84]Weinstein *et al.* (1991); [85]Wiebe *et al.* (2000); [86]Yang *et al.* (2011); [87]Yokoi *et al.* (1996); [88]Yokoi *et al.* (1998*a*); [89]Yokoi *et al.* (1998*b*); [90]Zhou *et al.* (1998); [91]Zyirek *et al.* (1987)

Price 1997, Thiboutot *et al.* 2000, Thiele *et al.* 2005), lungs (Normington & Russell 1992, Provost *et al.* 2002, Kimura *et al.* 2003), and in the gastrointestinal tract (Nienstedt *et al.* 1980*a*,*b*, Normington & Russell 1992), all sites of importance in inflammatory diseases and GC therapies. In metabolic tissues, 5αR1 is highly expressed in the liver (Normington & Russell 1992, Livingstone *et al.* 2005, 2009) but is also present in lower abundance in adipose (Livingstone *et al.* 2005, 2009, Wake *et al.* 2007), but not in human skeletal muscle (Thigpen *et al.* 1993*b*). 5αR2 is more prevalent in reproductive tissues, although it is present in human liver (Thigpen *et al.* 1993*b*) and adipose (MacKenzie *et al.* 2008). Both isozymes have been reported in primary osteoblasts (Issa *et al.* 2002), though the significance of 5αRs in bone is not well understood at present. Within the brain, 5αR1 is more highly expressed than 5αR2 (Melcangi *et al.* 1993, 1998, Poletti *et al.* 1997*a*,*b*, Stoffel-Wagner *et al.* 1998, 2000, Steckelbroeck *et al.* 2001, Torres & Ortega 2006), particularly in the white matter, but notably also in the hypothalamus (Karolczak *et al.* 1998) and pituitary (Lephart 1993, Lephart & Husmann 1993, Lopes-Solache *et al.* 1996, Yokoi *et al.* 1996), key sites where GCs exert negative feedback on the HPA axis. It is conceptually possible that manipulation of, or abnormalities in, 5αRs may affect central GC feedback and regulation.

With such a wide distribution of 5α-reductase expression, it follows that 5α-reduced steroids can be synthesised in many tissues, including sites that are pharmaceutical targets of GCs, as well as sites of their adverse effects. This implies that not only are these natural occurring GCs important in health and disease but 5α reduction may yield metabolites exerting effects in many organ systems.

Substrates

Both 5αRs irreversibly catalyse the reduction of the steroidal Δ^{4-5} double bond:

$$\text{3-oxo-}\Delta^{4}\text{-steroid} + \text{NADPH} + \text{H}^{+} \rightarrow \text{3-oxo-5}\alpha\text{-dihydrosteroid} + \text{NADP}^{+}$$

5αRs have an absolute requirement for NADPH and are unable to utilise NADH as an alternative (Frederiksen & Wilson 1971). Insights into the NADPH-binding domain and also interactions between substrates and the active site have been gained through site-directed mutagenesis and also investigation of the characteristics of proteins translated in patients with natural mutations in 5αR2 (Wigley *et al.* 1994, Wang *et al.* 1999). 5α-Reduced dihydrosteroids are rapidly reduced at the 3 keto position by 3α or 3β-hydroxysteroid dehydrogenase, enzymes of the aldo-keto reductase 1C family. The ratio of 3α vs 3β metabolites varies greatly in a tissue-dependent manner but, in the liver, 3α-reduced products are formed predominantly (Steckelbroeck *et al.* 2004). In human and rat tissues, the pH optima allows biochemical differentiation between these two isozymes *in vitro*, with 5αR1 having a pH optimum of 6–8·5 and 5αR2 having a pH optimum around 5 (Andersson *et al.* 1991). Half-lives of both enzymes are in the order of 20–30 h (Russell & Wilson 1994), but this may be altered in disease (Wigley *et al.* 1994).

Both 5α-R isozymes accept a range of 3-oxo-4-ene steroids as substrates (Andersson & Russell 1990, Thigpen *et al.* 1993*a*), including many hormonally active steroids (Russell & Wilson 1994), including GCs, androgens, progestogens (Purdy *et al.* 1990) and mineralocorticoids. They also catalyse formation of allo-bile acids (Tomkins 1956, Schefer *et al.* 1966), which are present in the foetus and, in the adult, more prevalent in liver disease (Mendoza *et al.* 2003). Using rat liver enzyme, McGuire *et al.* (1960) demonstrated chemical substituents that prevented catalysis including steroids unsaturated at C1-2, with a methyl substitution at C2 or C6 or halogenation at the 9α position. The last two modifications are typical of synthetic GCs (McGuire *et al.* 1960), suggesting that endogenous but not most pharmaceutical steroids are substrates for the enzymes.

Androgens are better substrates for 5αR2 than 5αR1, with low K_ms of $<0{\cdot}1$ μM (Normington & Russell 1992). Testosterone is also the most researched substrate of 5αR1, but progesterone is its preferred substrate, with a lower K_m (Andersson & Russell 1990, Normington & Russell 1992). GCs are poorer substrates for both human isozymes and thus unable to achieve high velocities of metabolism (Andersson & Russell 1990); in the case of human type 2, the apparent K_m for cortisol was measured as 3·6 μM in epididymal microsomes (Fisher *et al.* 1978). In the case of the rat, the K_m values for reduction of cortisol and corticosterone are 18 and 16 μM, respectively, for type 1 and 0·376 μM for type 2 (Normington & Russell 1992). The K_m for 5α-reduction of cortisol is slightly lower than that for 5β-reduction measured in human hepatic cytosol (27 μM; Iyer *et al.* 1990), but these routes appear to make balanced contributions to metabolic clearance of steroids by first pass metabolism. 3α,5α-Reduced cortisol metabolites represent just under half of urinary tetrahydro-metabolites of cortisol synthesised in the liver (Fukushima *et al.* 1960), with 3α,5β-reduced metabolites comprising the rest. Similarly, metabolites of corticosterone of both isomeric configurations are detected in rodent urine (Livingstone *et al.* 2000, Shackleton *et al.* 2007).

Cortisone can also be metabolised by 5αRs (McGuire & Tomkins 1960, Gold & Crigler 1972) and, while it is a better substrate than cortisol (McGuire & Tomkins 1960), its 5α-reduced metabolites are not readily found in urine (Bush & Mahesh 1959). 5α-Tetrahydrocortisone, if present, is rapidly converted into 5α-tetrahydrocortisol (Bush & Mahesh 1959), possibly by 11β-hydroxysteroid dehydrogenase 1. This contrasts with the more skewed 5β-tetrahydrocortisone, which is not reduced at the 11 position and is consequently abundant in urine. Precursors for the synthesis of corticosteroid, 11-deoxycortisol and 11-deoxycorticosterone are also metabolised (Forchielli *et al.* 1955, McGuire & Tomkins 1960, Frederiksen & Wilson 1971) by 5αRs, indeed more rapidly than cortisol; McGuire & Tomkins (1960) found

similar rates of metabolism of DOC as androstenedione using rat liver microsomes and this may help explain the suggestion that the activity of 5α-reductases in the adrenal gland plays part of a mechanism to regulate steroidogenesis (Carsia *et al*. 1984).

Actions of substrate vs product

Modification by 5α or 5β reduction results in distinct orientations of the A-ring, with the 5α confirmation adopting a planar 'trans' structure, whereas the 5β product assumes a skewed 'cis' orientation. The Δ^{4-5} double bond in the parent is more planar than the four to five single bonds in the 5α-reduced metabolite (Askew *et al*. 2007). These structural differences influence the ability of metabolites to interact with the cognate receptor (Askew *et al*. 2007) and metabolising enzymes (Bush & Mahesh 1959) and thereby critically influence their ability to trigger downstream signalling pathways. It has long been recognised that 5α-reduced androgens are more potent than the parent steroid, binding to the AR with greater affinity, and also dissociating from it three times more slowly (Wilson & French 1976, Chang *et al*. 1988, Lubahn *et al*. 1988, Askew *et al*. 2007). Likewise, 5α-dihydroprogesterone is a ligand for the progesterone receptor (Pasqualini & Nguyen 1980) and is the principle progestogen in some species (Meyer *et al*. 1997*a*). Its subsequent 3α-reduced metabolite, allopregnenolone (Uzunova *et al*. 2006), promotes inhibitory neuronal tone via an independent mechanism mediated by the GABA-A receptor (Puia *et al*. 1990). 5α-Reduced metabolites of aldosterone have weak mineralocorticoid activities (Sekihara *et al*. 1978, Kenyon *et al*. 1983, 1985, Gorsline *et al*. 1986, Morris 1986). In contrast to other steroids, metabolism of GCs by 5αR has generally been thought to inactivate these steroids prior to excretion and, throughout the years, there have only been sporadic reports of 5α-reduced GCs exerting effects through GR.

A few studies have suggested that the 5α-reduced metabolites of cortisol or corticosterone can bind GR, first demonstrated by Baxter & Tomkins (1971), who showed that 5α-dihydro-cortisol was able to displace tritiated dexamethasone from cytosolic GR, but more weakly than other endogenous steroids. In similar experiments, even weaker displacement of tritiated dexamethasone by 5α-dihydrocorticosterone (5αDHB) was demonstrated by Carlstedt-Duke *et al*. (1977). While McInnes *et al*. (2004) concurred that 5α-DHB was a weak GR ligand, they also demonstrated that 5α-tetrahydrocorticosterone (5αTHB) could displace dexamethasone with a similar K_d as corticosterone (Fig. 3A). Recently, 5α-DHB has also been suggested as a ligand for a putative, alternative DHB receptor (Sheppard *et al*. 1998).

The potency and efficacy of 5α-reduced corticosterone metabolites have been compared to the parent steroid *in vitro*, exploring functional endpoints that depend on different modalities of GR receptor signalling. Signalling by transactivation through conventional GR dimerisation can be modelled through activation of the MMTV promoter linked to a luciferase reporter (Grange *et al*. 2001). This assay is frequently used as a screening tool for metabolic side effects during development of dissociated GR ligands (Schake *et al*. 2004). 5αTHB is a weaker activator of this promoter than the parent steroid and stimulates transcription of the *TAT* gene in hepatocytes to a lesser extent than the parent steroid (McInnes *et al*. 2004; Fig. 3B). Similar early reports by Samuels & Tomkins (1970) classified 5α-dihydrocortisol as a 'sub-optimal inducer' (i.e. a partial agonist) of TAT activity in hepatoma cells. These findings were supported by limited responses of other hepatic genes transactivated by GC (Danesch *et al*. 1987, Ruppert *et al*. 1990); 5αTHB induces PEPCK weakly (McInnes *et al*. 2004), 5α-dihydrocortisol lacks efficacy to stimulate activity of tryptophan oxidase (Carlstedt-Duke *et al*. 1977) and achieved sub-optimal induction of hepatocyte adhesion (Ballard & Tomkins 1969). Overall, the weak effects of these metabolites caused interest in their potential biological role to wane.

These small inductions of gene transcription were corroborated by lack of metabolic side effects when 5αTHB was administered *in vivo* to mice, e.g. in TAT activity or expression unlike those observed when the endogenous active hormone, corticosterone was administered (Yang *et al*. 2011). The pathophysiological correlation of these changes is impaired insulin sensitivity, as demonstrated by increased plasma insulin levels during glucose tolerance testing, in the corticosterone-treated animals, which did not develop when 5αTHB was administered instead (Fig. 4A). This lack of metabolic disruption has also been demonstrated in rats, where administration of 5α-reduced dihydroglucocorticoids (5α-dihydrocortisol and 5α-DHB) for a week actually lowered plasma insulin and hepatic PEPCK activity (Golf *et al*. 1984). These latter effects may arise from suppression of synthesis of corticosterone, since, in both rats (McInnes *et al*. 2004) and mice (Yang *et al*. 2011), 5αTHB suppresses the HPA axis. *Ex vivo*, 5α-DHB impairs the development of long-term potential in neurones of the dentate gyrus (Dubrovsky *et al*. 1987). In contrast to their limited metabolic actions, a recent study has shown that 5α-reduced steroids possess anti-inflammatory abilities, *in vitro* and *in vivo*: 5αTHB suppressed release of pro-inflammatory cytokines in cultured bone marrow-derived macrophages, induced immune suppression when administered chronically, and also alleviated the induction of inflammation in response to challenge (Yang *et al*. 2011). This combination of activities suggests that 5α-reduced GCs have a different spectrum of biological actions from their parent steroids and poses the question of why changing the chemical nature in the steroidal A-ring might cause this change in profile.

Differential interactions between ligand and receptor can influence the spectrum of down-stream signalling and the ability of the occupied receptor to recruit co-activators and co-repressors. Dexamethasone (Bledsoe *et al*. 2002), but not corticosterone or its 5α-reduced metabolites, has been crystallised with human GR. Nonetheless, insights into

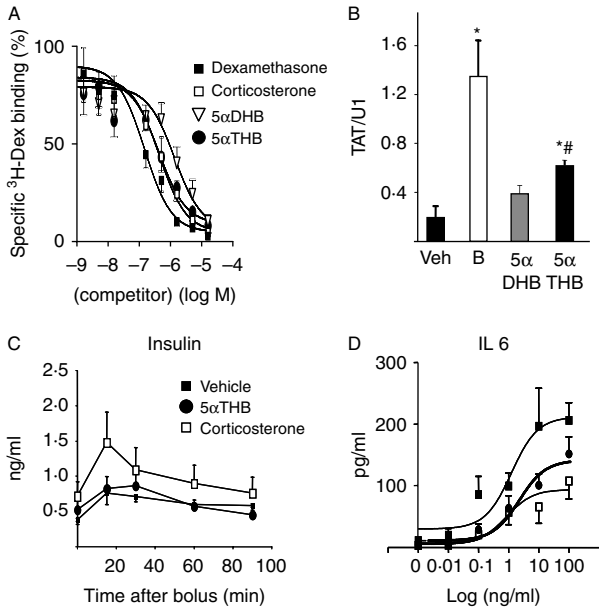

Figure 3 5α-Reduced glucocorticoid binds and selectively activates GR. (A) 5α-Tetrahydrocorticosterone (5αTHB) and 5α-dihydrocorticosterone (5αDHB) (overnight incubation with concentration range from 10^{-5} to 10^{-9} M) displaced tritiated dexamethasone (100 nM) from glucocorticoid receptors in rat hepatic cytosol. The K_ds for binding of 5αTHB (268 nM) and 5αDHB (336 nM) were similar to that of corticosterone (153 nM) and higher than dexamethasone (38 nM; $n=6$/treatment). (B) 5αTHB, but not 5αDHB, induced transcription of tyrosine aminotransferase (TAT) to a lesser extent than corticosterone (B) when incubated for 24 h in H4iiE cells (rat hepatoma cells). Abundance of transcript was quantified by northern blot and normalised for that of U1, $n=6$/treatment. $^*P<0{\cdot}05$ vs vehicle (veh), $^{\#}P<0{\cdot}05$ vs B. Following chronic infusion of steroids (50 μg/day, 2 weeks) to mice ($n=10$–12/group): (C) corticosterone, but not 5αTHB, impaired glucose tolerance, as demonstrated by increased insulin concentrations ($P<0{\cdot}001$ vs vehicle) after a glucose tolerance test (2 g/kg body weight i.p.) performed in mice following a 6 h fast. Insulin was quantified by immunoassay. (D) 5αTHB caused immune suppression to a similar extent to corticosterone, assessed by a significant reduction ($P<0{\cdot}01$) in the ability of lipopolysaccharide (LPS; 0·01–100 ng/ml)) to induce release of interleukin 6 (IL6) following incubation (24 h) from cells in whole blood harvested at cull. IL6 was quantified by immunoassay. Data are mean ± S.E.M. Data presented in A and B are adapted from those originally published in the McInnes KJ, Kenyon CJ, Chapman KE, Livingstone DEW, Macdonald LJ, Walker BR & Andrew R 2004 5α-Reduced glucocorticoid metabolites, novel endogenous activators of glucocorticoid receptors (GR). *Journal of Biological Chemistry* **279** 22908–22912 © the American Society for Biochemistry and Molecular Biology. Data presented in C and D were originally published in the Yang CA, Nixon M, Kenyon CJ, Livingstone DEW, Duffin R, Rossi AG, Walker BR & Andrew R 2011 5α-Reduced glucocorticoids exhibit dissociated anti-inflammatory and metabolic effects. *British Journal of Pharmacology*. (In press) (doi:10.1111/j.1476-5381.2011.01465.x).

potential, discriminant interactions of parent and metabolite can be inferred through parallels with 5α-reduced androgens, which have been studied bound with the recently crystalised AR (Askew *et al.* 2007). GR and AR belong to the same superfamily of nuclear receptors, sharing many structural and functional features (Bledsoe *et al.* 2002). The ligand-binding domain (LBD) contains a binding pocket specific for the cognate ligand, as well as the second activation function (AF2; Bledsoe *et al.* 2002, McMaster & Ray 2007). On ligand binding, interactions of AF2 with a broad range of motifs are permitted (Hur *et al.* 2004), including the p160 family of co-activators (Voegel *et al.* 1996, Heery *et al.* 1997, Ma *et al.* 1999, He *et al.* 2006), and differences have been proposed in the profile interactions with AF2 domain following binding of testosterone or 5αDHT with AR.

Testosterone is a less potent androgen than 5αDHT, with a tenfold higher concentration needed to achieve equipotent AR-mediated effects. Structural data suggest that the reduced number of protons present in testosterone compared with 5αDHT ensures a superior hydrophilic profile and greater H-bonding potential within the LBD of AR (Askew *et al.* 2007; Fig. 4). The altered conformation, with a more planar Δ^{4-5} double bond present in testosterone relative to 5αDHT, allows testosterone to accept a bridged H-bond with Met745, a residue in the AF2 domain of the AR that lies directly above the steroidal A-ring. In this setting, greater H-bonding is detrimental to agonist activity, with interactions weakened or absent in the presence of 5αDHT. The projection of this bridged H-bond is towards Leu712, a proximal residue in the AF2 domain crucial in mediating contacts with residues within accessory molecules (Ghali *et al.* 2003). 5αDHT has been suggested to impart greater structural integrity to Leu712, stabilising cofactor binding and thus enhancing AR activity (Askew *et al.* 2007).

Due to the homology between AR and GR, similar differential interactions may be proposed for 5α-reduced GCs and their parent steroid, potentially causing different subsets of co-activators and repressors to be recruited. Within the AF2 domain in GR, Arg611, Met605 and Gln711 are homologous to Arg752, Met745 and Gln711 in AR. However, in GR, a different hydrophobic amino acid, valine, is present in GR in place of Leu712 in AR, restricting flexibility of interactions. The abilities of ligands to induce different conformations in GR and recruit different proteins (e.g. TIF2 or NCoR) have been investigated for the agonist, dexamethasone and the antagonist, mifepristone (RU38486; Schoch *et al.* 2010). Taken together, these reports support the concept that the exact topography of the interactions of 5α-reduced steroids within GR-LBD may influence their profile of activity when bound with the receptor and may, in the future, provide an explanation for why 5α-reduced GCs retain some, but not all, actions of their parent GCs. Crystallisation of the metabolites with GR is required to add credence to this hypothesis and allow subsequent design of dissociated steroids using their structure and interactions as a template.

Roles in health and disease

While 5α-reduced GCs present a potential prototype for drug design, a question remains about their role *in vivo*. In the many

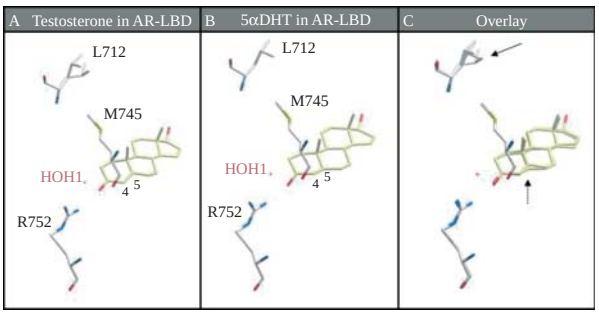

Figure 4 Binding of testosterone and 5α-dihydrotestosterone (5αDHT) in the androgen receptor ligand-binding domain (AR-LBD). When bound with AR, testosterone (A) and 5αDHT (B) are positioned near the side chain of Arg-752 (R752), a helix 5 residue required for ligand binding. The bond between the 3-keto-O on the steroid and R752 is influenced by the 4–5 double bond in testosterone, which imparts a more planar structure to testosterone than that seen in 5αDHT (C, dashed arrow), allowing more favourable H-bonding. The presence of structural water HOH1 permits a H-bond network between the steroid and key residues in the AF2 transactivation domain of the receptor. In particular, a bridged H-bond with Met-745 (M745), a residue that lies directly above the A-ring of the steroid, is formed. The structure of this bond is believed to be important as M745 projects towards Leu712 (L712), a proximal residue in the AF2 domain crucial in mediating contacts with residues within accessory molecules. Alterations in the structural integrity of L712 as a result of this projection (C, Block arrow) are believed to directly affect the binding of key cofactors essential for gene transcription. 5αDHT has been suggested to impart greater structural integrity to L712, stabilising cofactor binding and thus enhancing AR activity (Askew *et al.* 2007). Images were generated with ICM-Pro Software (MolSoft, San Diego, CA, USA) for testosterone with AR-LBD (Protein Data Bank Code 2Q7I) and 5αDHT with AR-LBD (Protein Data Bank Code 1T63).

tissues where 5α-reductases are expressed, this enzyme will regulate the proportions of the parent steroid and the metabolite, potentially modulating the balance of metabolic and anti-inflammatory actions. Given the high expression of the isozymes in liver and rapid inactivation of 5α-reduced GC by hepatic conjugation, circulating concentrations will be low (Furuta *et al.* 1998), predicting paracrine rather than endocrine signalling. Therefore, understanding the factors regulating the balance between parent and metabolite is crucial.

The ratio of urinary 5α- and 5β-reduced GC metabolites, primarily influenced by the liver, is altered in metabolic disease (Andrew *et al.* 1998, Fraser *et al.* 1999, Rask *et al.* 2001, 2002). Indeed, this was the fact that drew attention to the potential importance of these metabolic routes to regulate GC action. In obesity, the total daily production of GCs and the proportion of cortisol metabolised by 5α-reduction are increased (Andrew *et al.* 1998, Fraser *et al.* 1999, Rask *et al.* 2001, 2002); this can be corrected by weight loss (Johnstone *et al.* 2004, Tomlinson *et al.* 2008) and recapitulated in rats, where the liver can be identified as the major site of up-regulation (Livingstone *et al.* 2000). Again in rodents, insulin sensitisation ameliorates these changes (Livingstone *et al.* 2005). Interestingly, in humans with fatty liver, the proportion of cortisol metabolised by 5α-reduction is less (Westerbacka *et al.* 2003) and mice lacking 5α-reductase 1 develop fatty liver (Livingstone *et al.* 2008). These data suggest a pattern of hepatic metabolic dysfunction and susceptibility to inflammation under conditions of low activity of hepatic 5α-reduction of GCs.

Genetic variants in 5αRs have not been described in polygenic obesity or type 2 diabetes, and in particular, regulation of 5αR1 is poorly understood with little known about functional regulatory promoter elements. The most likely candidate for dysregulation in metabolic disease is insulin, although sex steroids and GCs also have powerful influences (Miller & Colas 1982, Horton *et al.* 1993, El-Awady *et al.* 2004). In hepatocytes, albeit under culture, insulin up-regulates the enzyme 5αR1, whereas 5αR2 is amplified primarily by feed-forward action of the metabolite (George *et al.* 1991).

Changes in GC clearance rates subsequent to up-regulation of hepatic metabolism cause adaptation in the set point of the HPA axis, which is fine-tuned to maintain circulating GC levels within a tight window. Increased GC production can be inferred from the adrenal hypertrophy evident in obese rodents, which is also ameliorated by insulin sensitisation. A similar association can be extrapolated in PCOS, where 5α-reduction of GCs is up-regulated (Stewart *et al.* 1990, Fassnacht *et al.* 2003, Tsilchorozidou *et al.* 2003) and insulin sensitisation normalises adrenal hyperactivity (Dunaif *et al.* 1996).

Conclusion

5α-Reduced GCs have received little attention in terms of their biological activity, despite other 5α-reduced steroids being well recognised as active hormones. They are formed in many tissues where GR is expressed and revisiting recent and previous literature allows one to propose a distinct profile of actions in comparison to the parent GC. They appear weak regulators of metabolism but retain other biological actions of pharmaceutical interest. To date, the spectrum of actions of 5α-reduced GC has not been investigated in bone.

A further avenue of interest in 5α-reduced GCs relates to the common pharmacological inhibition of 5αRs in clinical practice. 5α-Reductase inhibitors are used to treat benign prostate hyperplasia, a disease afflicting over half of elderly men. Although unlicensed, these drugs are also used to treat women with PCOS. It is possible that these patients will develop an imbalance in parent and 5α-reduced metabolites, leaving them susceptible to adverse metabolic effects and inflammatory changes, effects more marked with dutasteride, a new dual 5αR inhibitor. To date, there have not been any comprehensive metabolic studies in patients on these drugs (Amory *et al.* 2007).

Greater understanding of the role of 5α-reduced GCs in health, disease and therapeutics will provide important

insights into the effects in patients on 5α-reductase inhibitors, as well as providing an exciting focus of research in the quest for novel selective GR modulators.

Declaration of interest

R A is an inventor of a relevant patent held by the University of Edinburgh.

Funding

This work was supported by the Wellcome Trust (grant numbers 072217/Z/03/Z, GR060707FR), British Heart Foundation (grant number RG/05/008), Chief Scientist Office (grant number CZB/4/642), Diabetes UK (grant number RD 05/0003028), Society for Endocrinology and Medical Research Council for Funding. R U is a recipient of the Graham Aitken Nuffield Postgraduate Travelling Scholarship.

Author contribution statement

M N, R U and R A co-wrote the manuscript, and R A collated the material.

Acknowledgements

We are grateful to Diego Cobice and Gregorio Naredo for their help with chemical figures.

References

Abraham SM, Lawrence T, Kleiman A, Warden M, Medghalchi M, Tuckermann J, Sklatvala J & Clark AR 2006 Anti-inflammatory effects of dexamethasone are partly dependent on induction of dual specificity phosphatase 1. *Journal of Experimental Medicine* **203** 1883–1889. (doi:10.1084/jem.20060336)

Abulhajj YJ & Klang DT 1982 Metabolism of testosterone by GR mouse mammary tumours. *Cancer Research* **42** 3510–3513.

Agis-Balboa RC, Pinna G, Zhubi A, Maloku E, Veldic M, Costa E & Guidotti A 2006 Characterization of brain neurons that express enzymes mediating neurosteroid biosynthesis. *PNAS* **103** 14602–14607. (doi:10.1073/pnas.0606544103)

Agis-Balboa RC, Pinna G, Pibiri F, Kadriu B, Costa E & Guidotti A 2007 Down-regulation of neurosteroid biosynthesis in corticolimbic circuits mediates social isolation-induced behavior in mice. *PNAS* **104** 18736–18741. (doi:10.1073/pnas.0709419104)

Amory JK, Wang C, Swerdloff RS, Anawalt BD, Matsumoto AM, Bremner WJ, Walker SE, Haberer LJ & Clark RV 2007 The effect of 5alpha-reductase inhibition with dutasteride and finasteride on semen parameters and serum hormones in healthy men. *Journal of Clinical Endocrinology and Metabolism* **92** 1659–1665. (doi:10.1210/jc.2006-2203)

Andersson S & Russell DW 1990 Structural and biochemical properties of cloned and expressed human and rat steroid 5α-reductases. *PNAS* **87** 3640–3644. (doi:10.1073/pnas.87.10.3640)

Andersson S, Berman DM, Jenkins EP & Russell DW 1991 Deletion of steroid 5alpha-reductase 2 gene in male pseudohermaphroditism. *Nature* **354** 159–161. (doi:10.1038/354159a0)

Andrew R & Walker BR 2005 Glucocorticoid metabolism: regulation of tissue specific responses in obesity. In *Trends in Obesity Research*, pp 85–119. Eds Ling & Peter R. Hauppauge, NY: Nova Publisher.

Andrew R, Phillips DIW & Walker BR 1998 Obesity and gender influence cortisol secretion and metabolism in man. *Journal of Clinical Endocrinology and Metabolism* **83** 1806–1809. (doi:10.1210/jc.83.5.1806)

Araneo BA, Dowell T, Diegel M & Daynes RA 1991 Dihydrotestosterone exerts a depressive influence on the production of interleukin-4 (IL-4), IL-5 and gamma-interferon, but not IL-2 by activated murine T-cells. *Blood* **78** 688–699.

Arriza JL, Weinberger C & Cerelli G 1987 Cloning of human mineralocorticoid receptor complementary DNA; structural and functional kinship with the glucocorticoid receptor. *Science* **237** 268–275. (doi:10.1126/science.3037703)

Askew EB, Gampe RT Jr, Stanley TB, Faggart JL & Wilson EM 2007 Modulation of androgen receptor activation function 2 by testosterone and dihydrotestosterone. *Journal of Biological Chemistry* **282** 25801–25816. (doi:10.1074/jbc.M703268200)

Backstom T, Andersson A, Baird DT & Selstam G 1986 The human corpus luteum secretes 5 alpha-pregnane-3,20-dione. *Acta Endocrinologica* **111** 116–121.

Ballard PL & Tomkins GM 1969 Hormone induced modification of the cell surface. *Nature* **224** 344–345. (doi:10.1038/224344a0)

Barat P, Livingstone DE, Elferink CM, McDonnell CR, Walker BR & Andrew R 2007 Effects of gonadectomy on glucocorticoid metabolism in obese Zucker rats. *Endocrinology* **148** 4836–4843. (doi:10.1210/en.2007-0597)

Barnes PJ 1998 Anti-inflammatory actions of glucocorticoids: molecular mechanisms. *Clinical Science* **94** 557–572.

Baxter JD & Tomkins GM 1971 Specific cytoplasmic glucocorticoid hormone receptors in hepatoma tissue culture cells. *PNAS* **68** 932–937. (doi:10.1073/pnas.68.5.932)

Beato M, Herrlich P & Shutz G 1995 Steroid hormone receptors: many actors in search of a plot. *Cell* **83** 851–857. (doi:10.1016/0092-8674(95)90201-5)

Belvisi MG, Wicks SL, Battram CH, Bottoms SEW, Redford JE, Woodman P, Brown TJ, Webber SE & Foster ML 2001 Therapeutic benefit of a dissociated glucocorticoid and the relevance of *in vitro* separation of transrepression from transactivation activity. *Journal of Immunology* **166** 1975–1982.

Berman JR, Almeida FG, Jolin J, Paz S, Chaudhuri G & Gonzalez-Cadavid NF 2003 Correlation of androgen receptors, aromatase, and 5-alpha reductase in the human vagina with menopausal status. *Fertility and Sterility* **79** 925–931. (doi:10.1016/S0015-0282(02)04923-3)

Bledsoe RK, Montana VG, Stanley TB, Delves CJ, Apolito CJ, McKee DD, Consler TG, Parks DJ, Stewart EL, Willson TM *et al.* 2002 Crystal structure of the glucocorticoid receptor ligand binding domain reveals a novel mode of receptor dimerization and coactivator recognition. *Cell* **110** 93–105. (doi:10.1016/S0092-8674(02)00817-6)

Blom MJ, Wassink MG, Van Wijk F, Ederveen AGH, Kloosterboer HJ, Verhoeven CHJ, Lambert JGD & Goos HJT 2001 Metabolism of norethisterone and norethisterone derivatives in rat uterus, vagina, and aorta. *Drug Metabolism and Disposition* **29** 976–982.

Blumberg B, Sabbagh W, Juguilon H, Bolado J, van Meter CM, Ong ES & Evans R 1998 SXR, a novel steroid and xenobiotic sensing nuclear receptor. *Genes and Development* **12** 3195–3205. (doi:10.1101/gad.12.20.3195)

Borlak JI, Schulte I & Thum T 2004 Androgen metabolism in thymus of fetal and adult rats. *Drug Metabolism and Disposition* **32** 675–679. (doi:10.1124/dmd.32.6.675)

Bush IE & Mahesh VB 1959 Metabolism of 11-oxygenated steroids. Influence of the A/B ring junction on the reduction of 11-oxo steroids. *Biochemistry* **71** 705–717.

Canalis E, Mazziotti A, Giustina A & Bilezikian P 2007 Glucocorticoid-induced osteoporosis: pathophysiology and therapy. *Osteoporosis International* **18** 1319–1328. (doi:10.1007/s00198-007-0394-0)

Cantagrel V, Lefeber DJ, Ng BG, Guan Z, Silhavy JL, Bielas SL, Lehle L, Hombauer H, Adamowicz M, Swiezewska E *et al.* 2010 *SRD5A3* is required for the conversion of polyprenol to dolichol essential for N-linked protein glycosylation. *Cell* **142** 203–217. (doi:10.1016/j.cell.2010.06.001)

Carlstedt-Duke J, Gustafsson SA, Gustafsson J-A & Wrange O 1977 Interactions of corticosterone, 5α-dihydrocorticosterone and dexamethasone with proteins in rat-liver cytosol. *European Journal of Biochemistry* **73** 231–238. (doi:10.1111/j.1432-1033.1977.tb11311.x)

Carsia RV, Scanes CG & Malamed S 1984 Self-suppression of corticosteroidogenesis: evidence for a role of adrenal 5alpha-reductase. *Endocrinology* **115** 2464–2472. (doi:10.1210/endo-115-6-2464)

Chan SWC & Leathem JH 1975 Placental steroidogenesis in rat – progesterone production by tissue of basal zone. *Endocrinology* **96** 298–303. (doi:10.1210/endo-96-2-298)

Chang C, Kokontis K & Liao S 1988 Molecular cloning of human and rat complementary DNA encoding androgen receptors. *Science* **240** 324–326. (doi:10.1126/science.3353726)

Coghlan MJ, Jacobson PB, Lane B, Nakane M, Lin CW, Elmore SW, Kym PR, Luly JR, Carter GW, Turner R *et al.* 2011 A novel anti-inflammatory maintains glucocorticoid efficacy with reduced side effects. *Molecular Endocrinology* **17** 860–869. (doi:10.1210/me.2002-0355)

Colby HD & Kitay JI 1972 Effects of gonadal hormones on adrenocortical secretion of 5α-reduced metabolites of corticosterone in the rat. *Endocrinology* **91** 1523–1527. (doi:10.1210/endo-91-6-1523)

Danesch U, Gloss B, Schmid W, Schutz G, Schule R & Renkawitz R 1987 Glucocorticoid induction of the rat tryptophan oxygenase gene is mediated by two widely separated glucocorticoid-responsive elements. *EMBO Journal* **6** 625–630.

De Bosscher K & Haegeman G 2009 Latest perspectives on anti-inflammatory actions of glucocorticoids. *Molecular Endocrinology* **23** 281–291. (doi:10.1210/me.2008-0283)

De Bosscher K, Vanden Berghe W, Beck IM, Van Molle W, Hennuyer N, Hapgood J, Libert C, Staels B, Louw A & Haegeman G 2011 A fully dissociated compound of plant origin for inflammatory gene repression. *PNAS* **102** 15827–15832. (doi:10.1073/pnas.0505554102)

Delarminat MA, Hinrichsen MJ, Scorticati C, Ghirlanda JM, Blaquier JA & Calandra RS 2011 Uptake and metabolism of androgen by the human epididymis *in vitro*. *Journal of Reproduction and Fertility* **59** 397–402.

Deroo BJ & Archer TK 2001 Glucocorticoid receptor activation of the IκBα promoter within chromatin. *Molecular Biology of the Cell* **12** 3365–3374.

Dombroski RA, Casey ML & MacDonald PC 1997 5-Alpha-dihydroprogesterone formation in human placenta from 5alpha-pregnan-3beta/alpha-ol-20-ones and 5-pregnan-3beta-yl-20-one sulfate. *Journal of Steroid Biochemistry and Molecular Biology* **63** 155–163. (doi:10.1016/S0960-0760(97)00058-7)

Drake AJ, Livingstone DEW, Morton NM, Andrew R, Seckl JR & Walker BR 2005 Reduced adipose glucocorticoid reactivation and increased hepatic glucocorticoid clearance as an early adaptation to high fat feeding in rats. *Endocrinology* **146** 913–919. (doi:10.1210/en.2004-1063)

Drouin J, Sun YL, Chamberland M, Gauthier Y, DelLEan A, Nemer M & Schmidt TJ 1993 Novel glucocorticoid receptor complex with DNA element of the hormone-repressed POMC gene. *EMBO Journal* **12** 145–156.

Dube JY, Ngothi NH & Tremblay RR 1975 Testosterone 5alpha reductase in rat skin homogenates – measurement in skin at various anatomical sites in males and females. *Endocrinology* **96** 235–237. (doi:10.1210/endo-96-1-235)

Dubrovsky BO, Liquornik MS, Noble P & Gijsbers K 1987 Effects of 5α-dihydrocorticosterone on evoked responses and long-term potentiation. *Brain Research Bulletin* **19** 635–638. (doi:10.1016/0361-9230(87)90049-9)

Dunaif A, Scott D, Finegood D, Quintana B & Whitcomb R 1996 The insulin-sensitising agent troglitazone improves metabolic and reproductive abnormalities in the polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism* **81** 3299–3306. (doi:10.1210/jc.81.9.3299)

Dupuy GM, Boulanger P, Roberts KD, Bleau G & Chapdelaine A 1979 Metabolism of sex steroids in the human and canine vas-deferens. *Endocrinology* **104** 1553–1558. (doi:10.1210/endo-104-6-1553)

Eicheler W, Dreher M, Hoffman R, Happle R & Aumuller G 1995 Immunohistochemical evidence for differential distribution of 5alpha-reductase isozymes in human skin. *British Journal of Dermatology* **133** 371–376. (doi:10.1111/j.1365-2133.1995.tb02663.x)

Eicheler W, Happle R & Hoffman R 1998 5alpha-Reductase activity in the human hair follicle concentrates in the dermal papilla. *Archives of Dermatological Research* **290** 126–132. (doi:10.1007/s004030050277)

El-Awady MK, El-Garf W & El-Houssieny L 2004 Steroid 5α reductase mRNA type I is differentially regulated by androgens and glucocorticoids in the rat liver. *Endocrine Journal* **51** 37–46. (doi:10.1507/endocrj.51.37)

Eiknes KB, Stenstad P & Loftus R 1983 Metabolism in vitro of testosterone (T) to 17-beta-hydroxy-androstane-3-one (DHT) and 5-alpha-androstane-3-alpha, 17-beta-diol (3-alpha) by the 800-G supernatant fraction of ileum from rats. *Journal of Steroid Biochemistry and Molecular Biology* **19** 683–686.

Eminovic I, Komel R, Prezelj J, Karamehic K, Gavrankapetanovic F & Heljic B 2005 Gene analysis of steroid 5 alpha-reductase 1 in hyperandrogenic women. *Croatian Medical Journal* **46** 664–669.

Fassnacht M, Schlenz N, Schneider SB, Wudy SA, Allolio B & Arlt W 2003 Beyond adrenal and ovarian androgen generation: increased peripheral 5α-reductase activity in women with polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism* **88** 2760–2766. (doi:10.1210/jc.2002-021875)

Fisher LK, Kogut M, Moore R & Goebelsmann U 1978 Clinical, endocrinological, and enzymatic characterisation of two patients with 5α-reductase deficiency: evidence that a single enzyme is responsible for the 5α-reduction of cortisol and testosterone. *Journal of Clinical Endocrinology and Metabolism* **47** 653–664. (doi:10.1210/jcem-47-3-653)

Forchielli E & Dorfman RI 1956 Separation of $\Delta^{4-5}\alpha$- and $\Delta^{4-5}\beta$-hydrogenases from rat liver homogenates. *Journal of Biological Chemistry* **213** 443–447.

Forchielli E, Rosenkrantz H & Dorfman RI 1955 Metabolism of 11-deoxycortisol *in vitro*. *Journal of Biological Chemistry* **215** 715–722.

Fraser R, Ingram MC, Anderson NH, Morrison C, Davies E & Connell JMC 1999 Cortisol effects on body mass, blood pressure, and cholesterol in the general population. *Hypertension* **33** 1364–1368.

Frederiksen DW & Wilson JD 1971 Partial characterization of the nuclear reduced nicotinamide adenine dinucleotide phosphate: delta 4-3-keto-steroid 5 alpha-oxidoreductase of rat prostate. *Journal of Biological Chemistry* **246** 2584–2593.

Fukushima DK, Bradlow HL, Hellman L, Zumoff B & Gallacher TF 1960 Metabolic transformation of hydrocortisone-4-C-14 in normal men. *Journal of Biological Chemistry* **235** 2246–2252.

Furuta T, Namekawa T, Shibasaki H & Kasuya Y 1998 Simultaneous determination of tetrahydrocortisol and tetrahydrocortisone in human plasma and urine by stable isotope dilution mass spectrometry. *Journal of Chromatography. B* **706** 181–190. (doi:10.1016/S0378-4347(97)00562-8)

George FW 1993 Post-natal expression of high-rates of 5-alpha-reductase in the female rat urogenital tract. *Journal of Developmental Physiology* **19** 187–191.

George FW, Russell DW & Wilson JD 1991 Feed-forward control of prostate growth: dihydrotestosterone induces expression of its own biosynthetic enzyme, steroid 5alpha-reductase. *PNAS* **88** 8044–8047. (doi:10.1073/pnas.88.18.8044)

Ghali S, Gottlieb B, Lumbruso R, Beitel LK, Elhaji Y, Wu J, Pinsky L & Trifiro MA 2003 The use of androgen receptor amino/carboxyl-terminal interaction assays to investigate androgen receptor gene mutations in subjects with varying degrees of androgen insensitivity. *Journal of Clinical Endocrinology and Metabolism* **88** 2185–2193. (doi:10.1210/jc.2002-021324)

Ghulam A, Kouach M, Racadot A, Boersma A, Vantyghem MC & Briand G 1999 Quantitative analysis of human serum corticosterone by high-performance liquid chromatography coupled to electrospray ionization mass spectrometry. *Journal of Chromatography. B* **727** 227–233. (doi:10.1016/S0378-4347(99)00048-1)

Godoy A, Kawinski E, Li Y, Oka D, Alexiev B, Azzouni F, Titus MA & Mohler JL 2011 5α-Reductase type 3 expression in human benign and malignant tissues: a comparative analysis during prostate cancer progression. *Prostate* **71** 1033–1045. (doi:10.1002/pros.21318)

Gold NI & Crigler JF 1972 Suggested *in vivo* irreversible metabolism of labelled cortisol during distribution: effects on kinetics measurements and role of modes and routes of administration. *Journal of Clinical Endocrinology and Metabolism* **34** 1025–1038. (doi:10.1210/jcem-34-6-1025)

Golf SW, Bepperling F & Graef V 1984 Effect of 5α-dihydrocorticoids on enzymes of gluconeogenesis in rat liver. *Steroids* **43** 85–91. (doi:10.1016/0039-128X(84)90060-6)

Goodarzi MO, Shah NA, Antione HJ, Pall M, Guo X & Azziz R 2006 Variants in the 5α-reductase type 1 and type 2 genes are associated with polycystic ovary syndrome and the severity of hirsutism in affected women. *Journal of Clinical Endocrinology and Metabolism* **91** 4085–4091. (doi:10.1210/jc.2006-0227)

Gorsline J, Harnik M, Tresco PA & Morris DJ 1986 Hypertensinogenic activities of ring A-reduced metabolites of aldosterone. *Hypertension* **8** (Suppl 1) I-187–I-190.

Grange T, Cappabianca L, Flavin M, Sassi H & Thomassin H 2001 *In vivo* analysis of the model tyrosine aminotransferase gene reveals multiple sequential steps in glucocorticoid receptor action. *Oncogene* **20** 3028–3038. (doi:10.1038/sj.onc.1204327)

Ha SJ, Kim JS, Myung JW, Lee HJ & Kim JW 2003 Analysis of genetic polymorphisms of steroid 5alpha-reductase type 1 and 2 genes in Korean men with androgenetic alopecia. *Journal of Dermatological Science* **31** 135–141. (doi:10.1016/S0923-1811(02)00145-7)

Hammer F, Drescher DG, Schneider SB, Quinkler M, Stewart PM, Allolio B & Arlt W 2005 Sex steroid metabolism in human peripheral blood mononuclear cells changes with aging. *Journal of Clinical Endocrinology and Metabolism* **90** 6283–6289. (doi:10.1210/jc.2005-0915)

Hariharan M, Naga S, VanNoord T & Kindt EK 1992 Simultaneous assay of corticosterone and cortisol in plasma by reversed-phase liquid chromatography. *Clinical Chemistry* **38** 346–352.

Hauser KF, Maclusky NJ & Toranallerand CD 1987 Androgen action in fetal mouse spinal cord culture – metabolic and morphological aspects. *Brain Research* **406** 62–72. (doi:10.1016/0006-8993(87)90769-4)

He B, Gampe RTJ, Hnat AT, Faggart JL, Minges JT, French FS & Wilson EM 2006 Probing the functional link between androgen receptor coactivator and ligand-binding sites in prostate cancer and androgen insensitivity. *Journal of Biological Chemistry* **281** 6648–6663. (doi:10.1074/jbc.M511738200)

Heck S, Bender K, Kullmann M, Gottlicher M, Herrlich P & Cato ACB 1997 IκBα-independent downregulation of NF-kB activity by glucocorticoid receptor. *EMBO Journal* **16** 4698–4707. (doi:10.1093/emboj/16.15.4698)

Heery DM, Kalkhoven E, Hoare S & Parker MG 1997 A signature motif in transcriptional co-activators mediates binding to nuclear receptors. *Nature* **387** 733–736. (doi:10.1038/42750)

Hollenberg SM, Weinberger C, Ong ES, Cerilli G, Oro A, Lebo R, Thompson EB, Rosenfield MG & Evans RM 1985 Primary structure and expression of a functional human glucocorticoid receptor cDNA. *Nature* **318** 635–641. (doi:10.1038/318635a0)

Horton R, Pasupuletti V & Antonipillai I 1993 Androgen induction of steroid 5α-reductase may be mediated via insulin-like growth factor-1. *Endocrinology* **133** 447–451. (doi:10.1210/en.133.2.447)

Houston B, Chisholm GD & Habib FK 1987 A kinetic analysis of the 5-alpha reductases from human prostate and liver. *Steroids* **49** 355–370. (doi:10.1016/0039-128X(87)90010-9)

Hur E, Pfaff SJ, Payne S, Gron H, Buehrer BM & Fletterick RJ 2004 Recognition and accommodation at the androgen receptor coactivator binding interface. *PLoS Biology* **2** e274. (doi:10.1371/journal.pbio.0020274)

Imperato-McGinley J, Guerrero L, Gautier T & Peterson RE 1974 Steroid 5alpha-reductase deficiency in man: an inherited form of male pseudohermaphroditism. *Science* **186** 1213–1215. (doi:10.1126/science.186.4170.1213)

Issa S, Schnabel D, Feix M, Wolf L, Schaefer H-E, Russell DW & Schweikert H-U 2002 Human osteoblast-like cells express predominantly steroid 5 alpha-reductase type 1. *Journal of Clinical Endocrinology and Metabolism* **87** 5401–5407. (doi:10.1210/jc.2001-011902)

Ito S, Suzuki T, Akahira J, Moriya T, Kaneko C, Utsunomiya H, Yaegashi N, Okamura K & Sasano H 2002 Expression of androgen receptor and 5 alpha-reductases in the human normal endometrium and its disorders. *International Journal of Cancer* **99** 652–657. (doi:10.1002/ijc.10394)

Iyer RB, Binstock JM, Schwartz IS, Gordon GG, Weinstein BI & Southren AL 1990 Human hepatic cortisol reductase activities: enzymatic properties and substrate specificities of cytosolic cortisol Delta4-5beta-reductase and dihydrocortisol-3alpha-oxidoreductase. *Steroids* **55** 495–500. (doi:10.1016/0039-128X(90)90087-R)

Jakimiuk AJ, Weitsman SR & Magoffin DA 1999 5alpha-Reductase activity in women with polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism* **84** 2414–2418. (doi:10.1210/jc.84.7.2414)

Jeanfaucher C, Berger M, Gallon C, Deturckhelm M, Veyssiere G & Jean C 1986 Regional differences in the testosterone to dihydrotestosterone ratio in the epididymis and vas-deferens of adult mice. *Journal of Reproduction and Fertility* **76** 537–543. (doi:10.1530/jrf.0.0760537)

Jenkins EP, Hsieh C-L, Milatovich A, Normington K, Berman DM, Francke U & Russell DW 1991 Characterisation and chromosomal mapping of a human steroid 5α-reductase gene and pseudogene and mapping of the mouse homologue. *Genomics* **11** 1102–1112. (doi:10.1016/0888-7543(91)90038-G)

Johnstone AM, Faber P, Andrew R, Gibney ER, Elia M, Lobley G & Stubbs RJ 2004 Influence of short-term dietary weight loss on cortisol secretion and metabolism in obese men. *European Journal of Endocrinology* **150** 185–194. (doi:10.1530/eje.0.1500185)

Kahrizi K, Hu CH, Garhasbi M, Abedini SS, Ghadami S, Kariminejad R, Ullmann R, Chen W, Ropers H-H, Kuss AW *et al*. 2011 Next generation sequencing in a family with autosomal recessive Kahrizi syndrome (OMIM 612713) reveals homozygous frameshift mutation in *SRD5A3*. *European Journal of Human Genetics* **19** 115–117. (doi:10.1038/ejhg.2010.132)

Karolczak M, Küppers E & Beyer C 1998 Developmental expression and regulation of aromatase- and 5α-reductase type 1 mRNA in the male and female mouse hypothalamus. *Journal of Neuroendocrinology* **10** 267–274. (doi:10.1046/j.1365-2826.1998.00200.x)

Kenyon CJ, Brem AS, McDermott MJ, Deconti GA, Latif SA & Morris DJ 1983 Antinatriuretic and kaliuretic activities of the reduced derivatives of aldosterone. *Endocrinology* **112** 1852–1856. (doi:10.1210/endo-112-5-1852)

Kenyon CJ, Saccoccio NA, Harnik M & Morris DJ 1985 The effects of long-term infusions of the reduced derivatives of aldosterone on water and electrolyte metabolism. In *The Adrenal Gland and Hypertension*, 27th edn, pp 209–214. Eds F Mantero, EG Biglieri, JW Funder & BA Scoggins. New York: Raven Press.

Kimura A, Kondo K-H, Okuda K-I, Higashi S, Suzuki M, Kurosawa T, Tohma M, Inoue T, Nishiyori A, Yoshino M *et al*. 1998 Diagnosis of the first Japanese patient with 3-oxo-Δ4-steroid 5β-reductase deficiency by use of immunoblot analysis. *European Journal of Pediatrics* **157** 386–390. (doi:10.1007/s004310050835)

Kimura Y, Kaneko A, Darnel AD, Akahira J, Ebina M, Nukiwa T & Sasano H 2003 Expression of androgen receptor and 5 alpha-reductase types 1 and 2 in early gestation fetal lung: a possible correlation with branching morphogenesis. *Clinical Science* **105** 709–713. (doi:10.1042/CS20030236)

Kiyokage E, Toida K, Suzuki-Yamamoto T & Ishimura K 2005 Localization of 5alpha-reductase in the rat main olfactory bulb. *Journal of Comparative Neurology* **493** 381–395. (doi:10.1002/cne.20760)

Kondo K-H, Kai M-H, Setoguchi Y, Eggertsen G, Sjoblom P, Setoguchi T, Okuda K-I & Bjorkhem I 1994 Cloning and expression of cDNA of human Delta4-3-oxosteroid 5beta-reductase and substrate specificity of the expressed enzyme. *European Journal of Biochemistry* **219** 357–363. (doi:10.1111/j.1432-1033.1994.tb19947.x)

Lephart ED 1993 Pituitary and brain 5α-reductase messenger RNA levels in control, castrated and dihydrotestosterone treated rats. *Molecular and Cellular Neurosciences* **4** 526–531. (doi:10.1006/mcne.1993.1064)

Lephart ED & Husmann DA 1993 Altered brain and pituitary androgen metabolism by prenatal, perinatal or pre- and postnatal finasteride, flutamide or dihydrotestosterone treatment in juvenile male rats. *Progress in Neuro-Psychopharmacology & Biological Psychiatry* **17** 991–1003. (doi:10.1016/0278-5846(93)90026-O)

Li XD, Bertics PJ & Karavola HJ 1997 Regional distribution of cytosolic and particulate 5 alpha-dihydroprogesterone 3 alpha-hydroxysteroid oxido-reductases in female rat brain. *Journal of Steroid Biochemistry* **60** 311–318. (doi:10.1016/S0960-0760(96)00195-1)

Li J, Coates RJ, Gwinn M & Khoury MJ 2011 Steroid 5-α-reductase type 2 (SRD5a2) gene polymorphisms and risk of prostate cancer: a HuGE review. *American Journal of Epidemiology* **171** 1–13. (doi:10.1093/aje/kwp318)

Livingstone DEW, Jones GC, Smith K, Andrew R, Kenyon CJ & Walker BR 2000 Understanding the role of glucocorticoids in obesity: tissue-specific alterations of corticosterone metabolism in obese Zucker rats. *Endocrinology* **141** 560–563. (doi:10.1210/en.141.2.560)

Livingstone DEW, McInnes KJ, Walker BR & Andrew R 2005 Increased A-ring reduction of glucocorticoids in obese rats: attenuation by insulin sensitisation. *Obesity Research* **13** 1523–1526. (doi:10.1038/oby.2005.186)

Livingstone DEW, Walker BR & Andrew R 2008 Increased susceptibility to liver fat accumulation and insulin resistance, but not obesity induced by high fat diet, in 5alpha-reductase type 1 knock-out mice. *Proceedings of the Endocrine Society*, P2–P41.

Livingstone DEW, Grassick SL, Currie GL, Walker BR & Andrew R 2009 Dysregulation of glucocorticoid metabolism in murine obesity: comparable effects of leptin resistance and deficiency. *Journal of Endocrinology* **201** 211–218. (doi:10.1677/JOE-09-0003)

Lloyd RV 1979 Studies on the progesterone receptor content and steroid metabolism in normal and pathological human breast tissues. *Journal of Clinical Endocrinology and Metabolism* **48** 585–593. (doi:10.1210/jcem-48-4-585)

Lofthus R, Marthinsen ABL & Eiknes KB 1984 Metabolism of testosterone to 17-beta-hydroxy-5-alpha-androstane-3-one and 5-alpha-androstane-3-alpha, 17 beta-diol in alvealor macrophages from rat lung. *Journal of Steroid Biochemistry and Molecular Biology* **20** 1243–1248. (doi:10.1016/0022-4731(84)90152-3)

Lopes-Solache I, Luu-The V, Seralini G-E & Labrie F 1996 Heterogeneity of rat type 1 5 alpha-reductase cDNA: cloning, expression and regulation by pituitary implants and dihydrotestosterone. *Biochimica et Biophysica Acta* **1305** 139–144. (doi:10.1016/0167-4781(95)00198-0)

Lubahn DB, Joseph DR, Sullivan PM, Willard HF, French FS & Wilson EM 1988 Cloning of human androgen receptor complementary DNA and localisation to the X chromosome. *Science* **240** 327–330. (doi:10.1126/science.3353727)

Luu-The V, Sugimoto Y, Puy L, Labrie Y, Lopes-Solache I, Singh M & Labrie F 1994 Characterisation, expression and immunohistochemical localisation of 5-alpha-reductase in human skin. *Journal of Investigative Dermatology* **102** 221–226. (doi:10.1111/1523-1747.ep12371766)

Luu-The V, Pelletier G & Labrie F 2005 Quantitative appreciation of steroidogenic gene expression in mouse tissues: new roles for type 2 5α-reductase, 20α-hydroxy steroid dehydrogenase and estrogen sulfotransferase. *Journal of Steroid Biochemistry and Molecular Biology* **93** 269–276. (doi:10.1016/j.jsbmb.2005.01.003)

Ma H, Huang S-M, Irvine RA, Webb P, Kushner PJ, Coetzee GA & Stallcup MR 1999 Multiple signal input and output domains of the 160-kilodalton nuclear receptor coactivator proteins. *Molecular and Cellular Biology* **19** 6164–6173.

MacKenzie SM, Huda SS, Sattar N, Fraser R, Connell JMC & Davies E 2008 Depot-specific steroidogenic gene transcription in human adipose tissue. *Clinical Endocrinology* **69** 848–854. (doi:10.1111/j.1365-2265.2008.03262.x)

Maclusky NJ, Clark CR, Shanabrough M & Naftolin F 1987 Metabolism and binding of androgens in the spinal cord of the rat. *Brain Research* **422** 83–91. (doi:10.1016/0006-8993(87)90542-7)

Mahendroo MS, Cala KM, Landrum CP & Russell DW 1997 Fetal death in mice lacking 5alpha-reductase type 1 caused by estrogen excess. *Molecular Endocrinology* **11** 917–927. (doi:10.1210/me.11.7.917)

Mahendroo MS, Cala KM, Hess DL & Russell DW 2001 Unexpected virilization in male mice lacking steroid 5α-reductase enzymes. *Endocrinology* **142** 4652–4662. (doi:10.1210/en.142.11.4652)

Mahendroo MS, Wilson JD, Richardson JA & Auchus RJ 2004 Steroid 5 alpha-reductase 1 promotes 5 alpha-androstane-3 alpha,17 beta-diol synthesis in immature mouse testes by two pathways. *Molecular and Cellular Endocrinology* **222** 113–120. (doi:10.1016/j.mce.2004.04.009)

Mahoney MC, Swanlund DJ, Billeter M, Roberts KP & Pryor JL 2011 Regional distribution of 5 alpha-reductase type 1 and type 2 mRNA along the human epididymis. *Fertility and Sterility* **69** 1116–1121. (doi:10.1016/S0015-0282(98)00094-6)

Markham A & Bryson HM 1995 Deflazacort. A review of its pharmacological properties and therapeutic efficacy. *Drugs* **50** 317–333. (doi:10.2165/00003495-199550020-00008)

Mattingly D, Martin H & Tyler C 1989 Fluorimetric method for simultaneous estimation of cortisol, corticosterone and testosterone in plasma. *Journal of Clinical Pathology* **42** 661–666. (doi:10.1136/jcp.42.6.661)

McGuire JS & Tomkins GM 1960 The heterogeneity of Δ⋆-3-ketosteroid reductases (5α). *Journal of Biological Chemistry* **235** 1634–1638.

McGuire JS, Hollis VW & Tomkins GM 1960 Some characteristics of the microsomal steroid reductases (5α) of rat liver. *Journal of Biological Chemistry* **235** 3112–3117.

McInnes KJ, Kenyon CJ, Chapman KE, Livingstone DEW, Macdonald LJ, Walker BR & Andrew R 2004 5α-Reduced glucocorticoid metabolites, novel endogenous activators of glucocorticoid receptors (GR). *Journal of Biological Chemistry* **279** 22908–22912. (doi:10.1074/jbc.M402822200)

McMaster A & Ray DW 2007 Modelling the glucocorticoid receptor and producing therapeutic agents with anti-inflammatory effects but reduced side-effects. *Experimental Physiology* **92** 299–309. (doi:10.1113/expphysiol.2006.036194)

McMaster A & Ray DW 2008 Drug insight: selective agonists and antagonists of the glucocorticoid receptor. *Nature Clinical Practice. Endocrinology & Metabolism* **4** 91–101. (doi:10.1038/ncpendmet0745)

Melcangi RC, Celotti F, Ballabio M, Poletti A, Castano P & Martini L 1988 Testosterone 5-alpha reductase activity in the rat brain is highly concentrated in white matter structures and in purified myelin sheaths of axons. *Journal of Steroid Biochemistry and Molecular Biology* **31** 173–179. (doi:10.1016/0022-4731(88)90051-9)

Melcangi RC, Celotti F, Ballabio M, Poletti A & Martini L 1990 Testosterone metabolism in peripheral nerves – presence of the 5-alpha-reductase-3-alpha-hydroxysteroid-dehydrogenase enzymatic system in the sciatic nerve of adult and aged rats. *Journal of Steroid Biochemistry and Molecular Biology* **35** 145–148. (doi:10.1016/0022-4731(90)90159-P)

Melcangi RC, Celotti F, Castano P & Martini L 1993 Differential localization of the 5 alpha-reductase and the 3 alpha-hydroxysteroid dehydrogenase in neuronal and glial cultures. *Endocrinology* **132** 1252–1259. (doi:10.1210/en.132.3.1252)

Melcangi RC, Poletti A, Cavarretta I, Celotti F, Colciago A, Magnaghi B, Motta M, Negri-Cesi P & Martini L 1998 The 5alpha-reductase in the central nervous system: expression and modes of control. *Journal of Steroid Biochemistry and Molecular Biology* **65** 295–299. (doi:10.1016/S0960-0760(98)00030-2)

Mendoza M, Monte MJ, Serrano MA, Pastor-Anglada M, Steiger B, Meier PJ, Medarde M & Marin JJG 2003 Physiological characteristics of allo-cholic acid. *Journal of Lipid Research* **44** 84–92. (doi:10.1194/jlr.M200220-JLR200)

Meyer HH, Jewgenow K & Hdges JK 1997*a* Binding activity of 5alpha-reduced gestagens to the progestin receptor from African elephant (*Loxodonta africana*). *General and Comparative Endocrinology* **105** 164–167. (doi:10.1006/gcen.1996.6813)

Meyer T, Gustafsson J-A & Carlstedt-Duke J 1997*b* Glucocorticoid-dependent transcriptional repression of the osteocalcin gene by competitive binding at the TATA box. *DNA and Cell Biology* **16** 919–927. (doi:10.1089/dna.1997.16.919)

Milewich L, Gant NF, Schwarz BE, Chen GT & MacDonald PC 1979 5alpha-Reductase activity in human placenta. *American Journal of Obstetrics and Gynaecology* **133** 611–617.

Milewich L, Kaimla V & Toews GB 1983 Androstenedione metabolism in human alveolar macrophages. *Journal of Clinical Endocrinology and Metabolism* **56** 920–924. (doi:10.1210/jcem-56-5-920)

Milewich L, Kaimai V & Johnson AR 1987 Steroid 5-alpha-reductase in endothelial cells from human umbilical cord vessels. *Journal of Steroid Biochemistry and Molecular Biology* **26** 561–567. (doi:10.1016/0022-4731(87)90008-2)

Milewich L, Mendonca BB, Arnhold IP, Wallace AM, Donaldson MD, Wilson JD & Russel DW 1995 Women with steroid 5 alpha-reductase 2 deficiency have normal concentrations of plasma 5 alpha-dihydroprogesterone during the luteal phase. *Journal of Clinical Endocrinology and Metabolism* **80** 3136–3139. (doi:10.1210/jc.80.11.3136)

Miller MA & Colas AE 1982 Multihormonal control of microsomal 5α-reductase activity in cultured adult female rat hepatocytes. *Endocrinology* **111** 136–143. (doi:10.1210/endo-111-1-136)

Morava E, Wevers RA, Cantagrel V, Hoefsloot LH, Al-Gazali L, Schoots J, van Rooij A, Huijben K, van Ravenswaaij-Arts CMA, Jongmans MCJ *et al.* 2010 A novel cerebello-ocular syndrome with abnormal glycolsylation due to abnormalities in dolichol metabolism. *Brain* **133** 3210–3220. (doi:10.1093/brain/awq261)

Mori MT, Tominaga T & Tamaoki BI 1978 Steroid metabolism in normal mammary gland and in dimethylbenzthracene-induced mammary tumor of rats. *Endocrinology* **102** 1387–1397. (doi:10.1210/endo-102-5-1387)

Morris DJ 1986 Further studies on aldosterone metabolism. *Annals of Clinical and Laboratory Science* **16** 94–102.

Mowszowi I & Bardin CW 1974 *In vitro* androgen metabolism in mouse kidney – high 3-keto-reductase (3alpha-hydroxysteroid dehydrogenase activity) relative to 5alpha-reductase. *Steroids* **23** 793–807. (doi:10.1016/0039-128X(74)90167-6)

Mulatero P, Panarelli M, Schiavone D, Rossi A, Mengozzi G, Kenyon CJ, Chiandussi L & Veglio F 1997 Impaired cortisol binding to glucocorticoid receptors in hypertensive patients. *Hypertension* **30** 1274–1278. (doi:10.1161/01.HYP.30.5.1274)

Nienstedt WA, Ahotupa M & Hietanen E 1980*a* Metabolism of testosterone and androstenedione by human adult and fetal gastrointestinal tissues *in vitro*. *Journal of Steroid Biochemistry* **13** 1421–1425. (doi:10.1016/0022-4731(80)90054-0)

Nienstedt WA, Ojanotko A & Toivonen H 1980*b* Metabolism of progesterone, 17-hydroxyprogesterone and deoxycorticosterone by human small intestine *in vitro*. *Journal of Steroid Biochemistry* **13** 1417–1420. (doi:10.1016/0022-4731(80)90053-9)

Normington K & Russell DW 1992 Tissue distribution and kinetic characteristics of rat steroid 5α-reductase isozymes. Evidence for distinct physiological functions. *Journal of Biological Chemistry* **267** 19548–19554. (doi:10.1172/JCI116665)

Nowak FV 2002 Distribution and metabolism of 20 alpha-hydroxylated progestins in the female rat. *Journal of Steroid Biochemistry and Molecular Biology* **80** 469–479. (doi:10.1016/S0960-0760(02)00039-0)

Pasqualini JR & Nguyen BL 1980 Progesterone receptors in the fetal uterus and ovary of the guinea pig: evolution during fetal development and induction and stimulation in estradiol-primed animals. *Endocrinology* **106** 1160–1165. (doi:10.1210/endo-106-4-1160)

Patte-Mensah C, Penning TM & Mensah-Nyagan AG 2004 Anatomical and cellular localization of neuroactive 5 alpha/3 alpha-reduced steroid-synthesizing enzymes in the spinal cord. *Journal of Comparative Neurology* **477** 286–299. (doi:10.1002/cne.20251)

Pelletier G, Luu-The V & Labrie F 1994 Immunocytochemical localization of 5α-reductase in rat brain. *Molecular and Cellular Neurosciences* **5** 394–399. (doi:10.1006/mcne.1994.1049)

Petersen DD, Magnuson MA & Granner DK 1988 Location and characterisation of two widely separated glucocorticoid response elements in the phosphoenolpyruvate carboxykinase gene. *Molecular and Cellular Biology* **8** 96–104.

Pinsky L, Kaufman M, Straisfeld C, Zilahi B & Hall CSG 1978 5Alpha-Reductase activity of genital and non-genital skin fibroblasts from patients with 5alpha-reductase deficiency, androgen insensitivity, or unknown forms of male pseudohermaphroditism. *American Journal of Medical Genetics* **1** 407–416. (doi:10.1002/ajmg.1320010404)

Poletti A, Celotti F, Rumio C, Rabuffetti M & Martini L 1997*a* Identification of type 1 5alpha-reductase in myelin membranes of male and female rat brain. *Molecular and Cellular Endocrinology* **129** 181–190. (doi:10.1016/S0303-7207(97)04056-2)

Poletti A, Negri-Cesi P, Melcangi RC, Colciago A, Martini L & Celotti F 1997*b* Expression of androgen-activating enzymes in cultured cells of developing rat brain. *Journal of Neurochemistry* **68** 1298–1303. (doi:10.1046/j.1471-4159.1997.68031298.x)

Pozzi P, Bendotti C, Simeoni S, Piccioni F, Guerini V, Marron TU, Martini L & Poletti A 2003 Androgen 5-alpha-reductase type 2 is highly expressed and active in rat spinal cord motor neurones. *Journal of Neuroendocrinology* **15** 882–887. (doi:10.1046/j.1365-2826.2003.01074.x)

Pratis K, O'Donnell L, Stanton PG & McLachlan RI 2003 Differential regulation of rat testicular 5α-reductase type 1 and 2 isoforms by testosterone and FSH. *Journal of Endocrinology* **176** 393–403. (doi:10.1677/joe.0.1760393)

Provost PR & Tremblay Y 2007 Mouse 3 alpha-hydroxy steroid dehydrogenase mRNA: a marker of lung maturity. *Journal of Steroid Biochemistry and Molecular Biology* **103** 61–64. (doi:10.1016/j.jsbmb.2006.07.002)

Provost PR, Blomquist CH, Drolet R, Flamand N & Tremblay Y 2002 Androgen inactivation in human lung fibroblasts: variations in levels of 17 beta-hydroxysteroid dehydrogenase type 2 and 5 alpha-reductase activity compatible with androgen inactivation. *Journal of Clinical Endocrinology and Metabolism* **87** 3883–3892. (doi:10.1210/jc.87.8.3883)

Puia G, Santi MR, Vicini S, Pritchett DB, Purdy RH, Paul SM, Seeburg PH & Costa E 1990 Neurosteroids act on recombinant human GABAA receptors. *Neuron* **4** 759–765. (doi:10.1016/0896-6273(90)90202-Q)

Purdy RH, Morrow AL, Blinn JR & Paul SM 1990 Synthesis, metabolism, and pharmacological activity of 3 alpha-hydroxy steroids which potentiate GABA-receptor-mediated chloride ion uptake in rat cerebral cortical synaptoneurosomes. *Journal of Medicinal Chemistry* **33** 1572–1581. (doi:10.1021/jm00168a008)

Randall VE, Ebling FJ & Hargreaves G 1982 *In vivo* uptake and metabolism of testosterone by the skin and other tissues of the rat. *Journal of Endocrinology* **93** 253–266. (doi:10.1677/joe.0.0930253)

Rask E, Olsson T, Söderberg S, Andrew R, Livingstone DEW, Johnson O & Walker BR 2001 Tissue-specific dysregulation of cortisol metabolism in human obesity. *Journal of Clinical Endocrinology and Metabolism* **86** 1418–1421. (doi:10.1210/jc.86.3.1418)

Rask E, Walker BR, Söderberg S, Livingstone DEW, Eliasson M, Johnson O, Andrew R & Olsson T 2002 Tissue-specific changes in peripheral cortisol metabolism in obese women: increased adipose 11β-hydroxysteroid dehydrogenase type 1 activity. *Journal of Clinical Endocrinology and Metabolism* **87** 3330–3336. (doi:10.1210/jc.87.7.3330)

Ray DW, Suen C-S, Brass A, Soden J & White A 1999 Structure/function of the human glucocorticoid receptor: tyrosine 735 is important for transactivation. *Molecular Endocrinology* **13** 1855–1863. (doi:10.1210/me.13.11.1855)

Raz P, Nasatzky E, Boyan BD, Ornoy A & Schwartz Z 2005 Sexual dimorphism of growth plate prehypertrophic and hypertrophic chondrocytes in response to testosterone requires metabolism to dihydrotestosterone (DHT) by steroid 5-alpha reductase type 1. *Journal of Cellular Biochemistry* **95** 108–119. (doi:10.1002/jcb.20298)

Reichardt HM, Tuckermann JP, Gottlicher M, Vujic M, Weih F, Angel P, Herrlich P & Schutz G 2001 Repression of inflammatory responses in the absence of DNA binding by the glucocorticoid receptor. *EMBO Journal* **20** 7168–7173. (doi:10.1093/emboj/20.24.7168)

Reul JMHM, de Kloet ER, van Sluijs FJ, Rijnberk A & Rothuizen J 1990 Bonding characteristics of mineralocorticoid and glucocorticoid receptors in dog brain and pituitary. *Endocrinology* **127** 907–915. (doi:10.1210/endo-127-2-907)

Rocha EM, Wickham LA, da Silveira LA, Krenzer KL, Yu FS, Toda I & Sullivan DA 2000 Identification of androgen receptor protein and 5 alpha-reductase mRNA in human ocular tissues. *British Journal of Ophthalmology* **84** 76–84. (doi:10.1136/bjo.84.1.76)

Ruppert S, Boshart M, Bosch FX, Schmid W, Fournier RE & Schutz G 1990 Two genetically defined trans-acting loci coordinately regulate overlapping sets of liver specific genes. *Cell* **61** 895–904. (doi:10.1016/0092-8674(90)90200-X)

Russell DW & Wilson JD 1994 Steroid 5α-reductase: two genes/two enzymes. *Annual Review of Biochemistry* **63** 25–61. (doi:10.1146/annurev.bi.63.070194.000325)

Samuels HH & Tomkins GM 1970 Relation of steroid structure to enzyme induction in Hepatoma tissue culture cells. *Journal of Molecular Biology* **52** 57–74. (doi:10.1016/0022-2836(70)90177-4)

Samy TSA, Knoferl MW, Zheng R, Schwacha MG, Bland KI & Chaudry IH 2001 Divergent immune responses in male and female mice after

trauma-haemorrhage: dimorphic alterations in T lymphocyte steroidogenic enzyme activities. *Endocrinology* **142** 3519–3529. (doi:10.1210/en.142.8.3519)

Sato T, Sonoda T, Itami S & Takayasu S 1998 Predominance of type I 5 alpha-reductase in apocrine sweat glands of patients with excessive or abnormal odour derived from apocrine sweat (osmidrosis). *British Journal of Dermatology* **139** 806–810. (doi:10.1046/j.1365-2133.1998.02504.x)

Sawaya ME & Price VH 1997 Different levels of 5 alpha-reductase type I and II, aromatase, and androgen receptor in hair follicles of women and men with androgenetic alopecia. *Journal of Investigative Dermatology* **109** 295–300. (doi:10.1111/1523-1747.ep12335779)

Schake H, Schottelius A, Docke W-D, Strehike P, Jaroch S, Schmees N, Rehwinkel H, Hennekes H & Asadullah K 2004 Dissociation of transactivation from transrepression by a selective glucocorticoid receptor agonist leads to separation of therapeutic effects from side effects. *PNAS* **101** 227–232. (doi:10.1073/pnas.0300372101)

Schefer S, Hauser S & Mosbach EF 1966 Studies on the biosynthesis of 5α-cholestan-3β-ol I. Cholestenone 5α-reductase of rat liver. *Journal of Biological Chemistry* **241** 946–952.

Schneider JJ 1952 Conversion of desoxycorticosterone to four allopregnane metabolites by rat liver *in vitro*. *Journal of Biological Chemistry* **199** 235–244.

Schneider JJ & Horstmann PM 1951 Effects of incubating desoxycorticosterone with various rat tissues. *Journal of Biological Chemistry* **191** 327–338.

Schoch GA, D'Arcy B, Stihle M, Burger D, Bar D, Thoma R & Ruf A 2010 Molecular switch in the glucocorticoid receptor: active and passive antagonist conformations. *Journal of Molecular Biology* **395** 568–577. (doi:10.1016/j.jmb.2009.11.011)

Seethalakshmi L, Rodriguezrigau LJ & Steinberger A 1982 Testosterone metabolism by proximal and distal regions of the vas deferens from immature and mature rats. *Biology of Reproduction* **26** 584–590. (doi:10.1095/biolreprod26.4.584)

Sekihara H, Island DP & Liddle GW 1978 New mineralocorticoids:5α-dihydroaldosterone and 5α-dihydro-11-deoxycorticosterone. *Endocrinology* **103** 1450–1452. (doi:10.1210/endo-103-4-1450)

Seo YK, Zhu B, Jeon TI & Osborne TF 2009 Regulation of steroid 5-alpha reductase type 2 (Srd5a2) by sterol regulatory element binding proteins and statin. *Experimental Cell Research* **315** 3133–3139. (doi:10.1016/j.yexcr.2009.05.025)

Shackleton CHL, Hughes BA, Lavery GG, Walker EA & Stewart PM 2007 The corticosteroid metabolic profile of the mouse. *Steroids* **282** 27036.

Sheffield JW & O'Shaughnessy PJ 1988 Testicular steroid metabolism during development in the normal and hypogonadal mouse. *Journal of Endocrinology* **119** 257–264. (doi:10.1677/joe.0.1190257)

Sheppard KE, Khoo K, Krozowski ZS & Li KZ 1998 Steroid specificity of the putative DHB receptor: evidence that the receptor is not 11betaHSD. *American Journal of Physiology. Endocrinology and Metabolism* **275** E124–E131.

Shirakawa T, Okada H, Acharya B, Zhang ZJ, Hinata N, Wada Y, Uji T, Kamidono S & Gotoh A 2004 Messenger RNA levels and enzyme activities of 5 alpha-reductase types 1 and 2 in human benign prostatic hyperplasia (BPH) tissue. *Prostate* **58** 33–40. (doi:10.1002/pros.10313)

Smith CL & O'Malley BW 2004 Coregulator function: a key to understanding tissue specificity of selective receptor modulators. *Endocrine Reviews* **25** 45–71. (doi:10.1210/er.2003-0023)

Solomon DH, Goodson NJ, Katz JN, Weinblatt ME, Avorn J, Setoguchi S, Canning C & Schnessweiss S 2011 Patterns of cardiovascular risk in rheumatoid arthritis. *Annals of Rheumatic Disease* **65** 1608–1612. (doi:10.1136/ard.2005.050377)

Southren AL, Gordon GG, l'Hommedieu D, Ravikumar S, Dunn MW & Weinstein BI 1985 5β-Dihydrocortisol: possible mediator of the ocular hypertension in glaucoma. *Investigative Ophthalmology and Visual Science* **26** 393–395.

Southren AL, Hernandez MR, l'Hommedieu D, Gordon GG & Weinstein BI 1986 Potentiation of collagen synthesis in explants of the rabbit eye by 5β-dihydrocortisol. *Investigative Ophthalmology & Visual Science* **27** 1757–1760.

Southren AL, l'Hommedieu D, Gordon GG & Weinstein BI 1987 Intraocular hypotensive effect of a topically applied cortisol metabolite: 3α,5β-tetrahydrocortisol. *Investigative Ophthalmology & Visual Science* **28** 901–903.

Souverein PC, Berard A, van Staa TP, Cooper C, Leufkens HGM & Walker BR 2004 Use of oral glucocorticoids and risk of cardiovascular and cerebrovascular disease in a population-based case–control study. *Heart* **90** 859–865. (doi:10.1136/hrt.2003.020180)

van Staa TP, Leufkens HGM & Cooper C 2011 The epidemiology of corticosteroid-induced osteoporosis: a metaanalysis. *Osteoporosis International* **13** 777–787. (doi:10.1007/s001980200108)

Steckelbroeck S, Watzka M, Reichelt R, Hans VHJ, Stoffel-Wagner B, Heidrich DD, Schramm J, Bidlingmaier F & Klingmuller D 2001 Characterization of the 5 alpha-reductase-3 alpha-hydroxysteroid dehydrogenase complex in the human brain. *Journal of Clinical Endocrinology and Metabolism* **86** 1324–1331. (doi:10.1210/jc.86.3.1324)

Steckelbroeck S, Jin Y, Gopishetty S, Ovesanmi B & Penning TM 2004 Human cytosolic 3 alpha-hydroxysteroid dehydrogenases of the aldo-keto reductase superfamily display significant 3 beta-hydroxysteroid dehydrogenase activity – implications for steroid hormone metabolism and action. *Journal of Biological Chemistry* **279** 10784–10795. (doi:10.1074/jbc.M313308200)

Stewart PM, Shackleton CHL, Beastall GH & Edwards CRW 1990 5alpha-Reductase activity in polycystic ovarian syndrome. *Lancet* **335** 431–433. (doi:10.1016/0140-6736(90)90664-Q)

Stoffel-Wagner B, Watzka M, Steckelbroeck S, Wickert L, Schramm J, Romalo G, Klingmuller D & Schweikert H-U 1998 Expression of 5alpha-reductase in the human temporal lobe of children and adults. *Journal of Clinical Endocrinology and Metabolism* **83** 3636–3642. (doi:10.1210/jc.83.10.3636)

Stoffel-Wagner B, Beyenburg S, Watzka M, BluEmcke I, Bauer J, Schramm J, Bidlingmaier F & Elger CE 2000 Expression of 5 alpha-reductase and 3 alpha-hydroxysteroid oxidoreductase in the hippocampus of patients with chronic temporal lobe epilepsy. *Epilepsia* **41** 140–147. (doi:10.1111/j.1528-1157.2000.tb00133.x)

Suzuki T, Darnel AD, Akahira J, Ariga N, Ogawa S, Kaneko C, Takeyama J, Morley T & Sasano H 2001 5alpha-Reductases in human breast carcinoma: possible modulator of *in situ* androgenic actions. *Journal of Clinical Endocrinology and Metabolism* **86** 2250–2257. (doi:10.1210/jc.86.5.2250)

Takayasu S, Wakimoto H, Itami S & Sano S 1980 Activity of testosterone 5alpha-reductase in various tissues of human skin. *Journal of Investigative Dermatology* **74** 187–191. (doi:10.1111/1523-1747.ep12541698)

Thiboutot D, Bayne E, Thorne J, Gilliland K, Flanagan J, SHao Q, Light J & Helm K 2000 Immunolocalization of 5alpha-reductase isozymes in acne lesions and normal skin. *Archives of Dermatology* **136** 1125–1129. (doi:10.1001/archderm.136.9.1125)

Thiele S, Hoppe U, Holterhus PM & Hiort O 2005 Isoenzyme type 1 of 5alpha-reductase is abundantly transcribed in normal human genital skin fibroblasts and may play an important role in masculinization of 5alpha-reductase type 2 deficient males. *European Journal of Endocrinology* **152** 875–880. (doi:10.1530/eje.1.01927)

Thigpen AE, Cala KM & Russell DW 1993*a* Characterisation of Chinese Hamster Ovary cell lines expressing human steroid 5α-reductase isozymes. *Journal of Biological Chemistry* **268** 17404–17412.

Thigpen AE, Silver RI, Guileyardo JM, Casey ML, McConnell JD & Russell DW 1993*b* Tissue distribution and ontogeny of steroid 5α-reductase isozyme expression. *Journal of Clinical Investigation* **92** 903–910. (doi:10.1172/JCI116665)

Tomkins GM 1956 The enzymatic reduction of Δ4-3-ketosteroids. *Journal of Biological Chemistry* **225** 13–24.

Tomlinson JW, Finney J, Hughes BA, Hughes S & Stewart PM 2008 Reduced glucocorticoid production rate, decreased 5α-reductase activity and adipose tissue insulin sensitization following weight loss. *Diabetes* **57** 1536–1543. (doi:10.2337/db08-0094)

Torres JM & Ortega E 2006 Steroid 5 alpha-reductase isozymes in the adult female rat brain: central role of dihydrotestosterone. *Journal of Molecular Endocrinology* **36** 239–245. (doi:10.1677/jme.1.01907)

Tsilchorozidou T, Honour JW & Conway GS 2003 Altered cortisol metabolism in polycystic ovary syndrome: insulin enhances 5α-reduction but not the elevated adrenal steroid production rates. *Journal of Clinical Endocrinology and Metabolism* **88** 5907–5913. (doi:10.1210/jc.2003-030240)

Turner RT, Bleiberg B, Colvard DS, Keeting PE, Evans G & Spelsberg TC 1990 Failure of isolated rat tibial periosteal cells to 5alpha reduced testosterone to 5 alpha-dihydrotestosterone. *Journal of Bone and Mineral Research* **5** 775–779. (doi:10.1002/jbmr.5650050715)

Uemura M, Tamura K, Chung S, Honma S, Okuyama A, Nakamura Y & Nakagawa H 2008 Novel 5α-steroid reductase (SRD5A3, type 3) is overexpressed in hormone refractory prostate cancer. *Cancer Science* **99** 81–86. (doi:10.1111/j.1349-7006.2007.00656.x)

Unterberger C, Staples KJ, Smallie T, Williams L, Foxwell B, Schaefer A, Kempke B, Hofer TPJ, Koeppel M, Lohrum M *et al*. 2008 Role of STAT3 in glucocorticoid-induced expression of the human IL-10 gene. *Molecular Immunology* **45** 3230–3237. (doi:10.1016/j.molimm.2008.02.020)

Uzunova V, Sampson L & Uzunov DP 2006 Relevance of endogenous 3alpha-reduced neurosteroids to depression and antidepressant action. *Psychopharmacology* **186** 351–361. (doi:10.1007/s00213-005-0201-6)

Van der Straeten M, Vermeulen A & Orie N 1963 The determination of cortisol and corticosterone production rates by an isotope dilution method. *Acta Endocrinologica* **43** 430–438. (doi:10.1530/acta.0.0430430)

Viger RS & Robaire B 2011 Immunocytochemical localization of 4-ene steroid 5-alpha reductase type 1 along the rat epididymis during postnatal development. *Endocrinology* **134** 2298–2306. (doi:10.1210/en.134.5.2298)

Voegel JJ, Heine MJS, Zechel C, Chambon P & Gronemeyer H 1996 TIF2, a 160 kDa transcriptional mediator for the ligand-dependent activation function AF-2 of nuclear receptors. *EMBO Journal* **15** 3667–3675.

Vu TT, Hirst JJ, Stark M, Wright IMR, Palliser HK, Hody N & Clifton VL 2009 Changes in human placental 5 alpha-reductase isoenzyme expression with advancing gestation: effects of fetal sex and glucocorticoid exposure. *Reproduction, Fertility, and Development* **21** 599–607. (doi:10.1071/RD08224)

Wake DJ, Strand M, Rask E, Westerbacka J, Livingstone DEW, Soderberg S, Andrew R, Olsson T, Yki-Jarvinen H & Walker BR 2007 The influence of intra-adipose enzymes generating estrogens and androgens on body fat distribution in idiopathic human obesity. *Clinical Endocrinology* **66** 440–446. (doi:10.1111/j.1365-2265.2007.02755.x)

Walsh PC, Madden JD, Harrod MJ, Goldstein JL, MacDonald PC & Wilson JD 1974 Familial incomplete male pseudohermaphroditism, type 2. Decreased dihydrotestosterone formation in pseudovaginal perineoscrotal hypospadias. *New England Journal of Medicine* **291** 944–949. (doi:10.1056/NEJM197410312911806)

Wang M, Bhattacharyya AK, Taylor MF, Tai HH & Collins DC 1999 Site-directed mutagenesis studies of the NADPH-binding domain of rat steroid 5α-reductase (isozyme-1) I: analysis of aromatic and hydroxylated amino acid residues. *Steroids* **64** 356–362. (doi:10.1016/S0039-128X(99)00010-0)

Wei L, MacDonald TM & Walker BR 2004 Taking glucocorticoids by prescription is associated with subsequent cardiovascular disease. *Annals of Internal Medicine* **141** 764–770.

Weinstein BI, Gordon GG & Southren AL 1983 Potentiation of glucocorticoid activity by 5β-dihydrocortisol: its role in glaucoma. *Science* **222** 172–173. (doi:10.1126/science.6623065)

Weinstein BI, Kandalaft N, Ritch R, Camras CB, Morris DJ, Latif SA, Vecsei P, Vittek J, Gordon GG & Southren AL 1991 5alpha-Dihydrocortisol in human aqueous humor and metabolism of cortisol by human lenses *in vitro*. *Investigative Ophthalmology & Visual Science* **32** 2130–2135.

Westerbacka J, Yki-Jarvinen H, Vehkavaara S, Häkkinen A-M, Andrew R, Wake DJ, Seckl JR & Walker BR 2003 Body fat distribution and cortisol metabolism in healthy men: enhanced 5β-reductase and lower cortisol/-cortisone metabolite ratios in men with fatty liver. *Journal of Clinical Endocrinology and Metabolism* **88** 4924–4931. (doi:10.1210/jc.2003-030596)

Wiebe JP, Muzia D, Hu JC, Szwajcer D, Hill SA & Seachrist JL 2000 The 4-pregnene and 5 alpha-pregnane progesterone metabolites formed in nontumorous and tumorous breast tissue have opposite effects on breast cell proliferation and adhesion. *Cancer Research* **60** 936–943.

Wigley WC, Prihoda JS, Mowszowicz I, Mendonca BB, New MI, Wilson JD & Ruseell DW 1994 Natural mutagenesis study of the human steroid 5α-reductase 2 isozyme. *Biochemistry* **33** 1265–1270. (doi:10.1021/bi00171a029)

Wilson EM & French FS 1976 Binding properties of androgen receptors. *Journal of Biological Chemistry* **251** 5620–5629.

Yamana K, Labrie F & Luu-The V 2010 Human type 3 5α-reductase is expressed in peripheral tissues at higher levels than types 1 and 2 and its activity is potently inhibited by finasteride and dutasteride. *Hormone Molecular Biology and Clinical Investigation* **2** 293–299. (doi:10.1515/HMBCI.2010.035)

Yang CA, Nixon M, Kenyon CJ, Livingstone DEW, Duffin R, Rossi AG, Walker BR & Andrew R 2011 5α-Reduced glucocorticoids exhibit dissociated anti-inflammatory and metabolic effects. *British Journal of Pharmacology* (In press). (doi:10.1111/j.1476-5381.2011.01465.x)

Yokoi H, Tsuruo Y, Miyamoto T, Kitagawa K, Futaki S & Ishimura K 1996 Steroid 5α-reductase type 1 immunolocalized in the anterior pituitary of intact and castrated male rats. *Histochemistry and Cell Biology* **106** 359–366. (doi:10.1007/BF02473294)

Yokoi H, Tsuruo Y & Ishimura K 1998*a* Steroid 5alpha-reductase type 1 immunolocalized in the rat peripheral nervous system and paraganglia. *Histochemical Journal* **30** 731–739. (doi:10.1023/A:1003482512567)

Yokoi H, Tsuruo Y, Miyamoto T & Ishimura K 1998*b* Steroid 5alpha-reductase type 1 immunolocalized in the adrenal gland of normal, gonadectomized, and sex hormone-supplemented rats. *Histochemistry* **109** 127–134. (doi:10.1007/s004180050210)

Zhou ZF, Shackleton CH, Pahwa S, White PC & Speiser PW 1998 Prominent sex steroid metabolism in human lymphocytes. *Molecular and Cellular Endocrinology* **138** 61–69. (doi:10.1016/S0303-7207(98)00052-5)

Zyirek M, Flood C & Longcope C 1987 5alpha-Reductase activity in rat adipose tissue. *Proceedings of the Society for Experimental Biology and Medicine* **186** 134–138. (doi:10.3181/00379727-186-42592)

Received in final form 11 August 2011
Accepted 6 September 2011
Made available online as an Accepted Preprint
8 September 2011