REVIEW

5 α -Reduced glucocorticoids: a story of natural selection

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Abstract

5 α -Reduced glucocorticoids (GCs) are formed when one of the two isozymes of 5 α -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 5 α -dihydro steroids, e.g. 5 α -dihydrotestosterone, retain or have increased activity at their cognate receptors. However, recent findings suggest that 5 α -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. 5 α -Dihydro- and 5 α -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 5 α -reduced GCs to suppress inflammation have been demonstrated *in vitro* and *in vivo*. Thus, the balance of parent GC and its 5 α -reduced metabolite may critically affect the profile of GR signalling. 5 α -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, 5 α -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 5 α -reductase inhibitors, drugs that are commonly administered long term for the treatment of benign prostatic hyperplasia.

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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 $\sim 70\%$ 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.

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.* 1997b), 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- κ B and AP-1, thus preventing their actions activating transcription of pro-inflammatory cytokines such as TNF α 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 I κ B α 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 17 α -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 $\sim$ 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_d s of $\sim$ 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_d s similar to that of aldosterone, $\sim$ 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 $\sim$ 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.

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

Recently, the anti-inflammatory potential of some endogenous metabolites of corticosterone, namely 5 α -dihydro and 5 α -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, 5 α - and 5 β -tetrahydro steroids, following sequential reduction of the Δ^{4-5} double bond and then the 3-ketone group (Andrew & Walker 2005). 5 α -Reductases were initially

Figure 2 Metabolism of cortisol and corticosterone to generate 5 α -reduced glucocorticoids. Generic scheme of reaction catalysed by 5 α -reductases (5 α Rs) followed by 3 α -hydroxysteroid dehydrogenases (3 α HSDs). 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 5 α -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 5 α -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 5 α -reductases 1 and 2 (5 α R1 and 5 α R2) 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 5 α -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).

5 β -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, 5 α -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, 5 β -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 5 α -reduced GCs

5 α -Reduced GCs are formed by 5 α R1 and 5 α R2 (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. 5 α -Reduced metabolites of testosterone and androstenedione, 5 α -dihydrotestosterone or 5 α -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 5 α -reductase 2 (Imperato-McGinley *et al.* 1974, Walsh *et al.* 1974, Fisher *et al.* 1978). This condition, now termed 5 α -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 5 α R 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 5 α R1 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 5 α R 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).

5 α -Reductases are hydrophobic, membrane-bound enzymes with predicted molecular weights of ~28–29 kDa (Andersson & Russell 1990). Human 5 α R1 and 5 α R2 contain 259 (Andersson & Russell 1990) and 254 (Andersson *et al.* 1991) amino acids respectively. The two isozymes of 5 α -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 5 α -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 5 α Rs 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 5 α reductases in human, rat and mouse. In cases of differing evidence, those demonstrating isozyme-specific expression are shown

System	5 α R1			5 α R2			Isozyme unconfirmed		References
	mRNA	Protein/IC	Activity ^a	mRNA	Protein/IC	Activity ^a	Activity ^b		
Metabolic/cardiorespiratory									
Liver	H $\vee$; R $\vee$; M $\vee$	H $\vee$; R $\vee$	R $\vee$	H $\vee$; R $\times$; M $\vee$	H $\vee$; R $\times$		H $\vee$		22, 53, 65, 40, 32, 70
Adipose	H $\vee$; R $\vee$; M $\vee$			H $\vee$; M $\times$			R $\vee$		91, 13, 5, 83, 38, 33
Skeletal muscle	H $\times$; R $\vee$	H $\times$	H $\times$	H $\times$; R $\times$	H $\times$	H $\times$			78, 65
Heart/vessels	R $\times$			R $\times$			H $\vee$; R $\vee$		48, 53, 65, 7
Kidney	H $\times$; R $\vee$	H $\times$	H $\vee$	H $\times$; R $\vee$	H $\times$	H $\vee$	M $\pm$		51, 78, 65
Adrenal	H $\times$; R $\vee$; M $\pm$	H $\times$; R $\vee$	H $\vee$	H $\times$; R $\vee$; M $\vee$	H $\times$	H $\times$	R $\vee$		10, 78, 65, 89, 37
Lung	H $\vee$; R $\vee$; M $\vee$	H $\vee$	H $\vee$	H $\vee$; R $\times$	H $\times$				53, 61, 28, 60
Immune									
Spleen	R $\vee$			R $\times$					53
Monocytes/macrophages	H $\vee$; M $\times$			H $\pm$; M $\times$			H $\vee$; R $\vee$; M $\vee$		47, 35, 3, 20, 86
Lymphocytes									
Thymus	H $\vee$		H $\vee$	H $\pm$			M $\vee$		90, 66
	R $\vee$ *			R $\vee$ *					8
Bone									
Cartilage	R $\vee$		R: δ / α $\times$	R $\times$					63
Bone	H $\vee$		H $\vee$	H $\vee$		H $\pm$	R $\times$		80, 23
Skin									
Genital skin	H $\vee$								
Non-genital skin	H $\vee$	H $\times$		H $\vee$	H $\vee$		H $\vee$; R $\vee$		14, 57, 65, 77
Hair follicle	H $\vee$	H $\vee$		H $\pm$	H $\vee$		H $\vee$; R $\vee$		14, 76, 62, 36, 65, 16
Sweat gland	H $\vee$	H $\vee$		H $\vee$	H $\vee$		H $\vee$		16, 17, 68
Gastrointestinal									67
Stomach	R $\vee$			R $\times$					53
Intestine	R $\vee$			R $\vee$			H $\vee$; R $\vee$		52, 18, 53
Neurological									
Hypothalamus	H $\vee$; R $\vee$; M $\vee$	H $\vee$; R $\vee$	R $\vee$	H $\times$; R $\times$	H $\times$		R $\vee$		30, 78, 56, 65, 31, 27
Pituitary	H $\times$; R $\vee$	H $\times$; R $\vee$	R $\vee$	H $\times$; R $\times$	H $\times$		R $\vee$		30, 65, 87, 54
Thalamus	M $\vee$ (G, g)	R $\vee$; M $\vee$	(G, g)				R $\vee$		56, 31, 2
Hippocampus	H $\vee$; M $\times$ (G); M $\vee$ (g)	R $\vee$; M $\times$ (G); M $\vee$ (g)		H $\times$			R $\vee$		44, 56, 31, 74, 2
Cortex	H $\vee$; R $\vee$; M $\times$ (G); M $\vee$ (g)	H $\vee$; R $\vee$; M $\times$ (G); M $\vee$ (g)	H $\vee$	H $\times$; R $\vee$	H $\times$		R $\vee$		44, 56, 31, 74, 73, 2, 79
Medulla oblongata	H $\vee$	H $\vee$		H $\vee$	H $\vee$		R $\vee$		78, 31
Pons									
Amygdala	H $\vee$	H $\times$		H $\times$	H $\times$		R $\vee$		44, 78, 65, 31
Corpus callosum	M(g) $\vee$	M(g) $\vee$					R $\vee$		31, 2
	M $\times$	M $\times$					R $\vee$		44, 2
Striatum	M(G) $\vee$	M(G) $\vee$							2
Cerebellum	H $\vee$; M $\vee$ (G); M $\pm$ (g)	H $\vee$; M $\vee$ (G); M $\pm$ (g)		H $\times$	H $\times$		R $\vee$		44, 78, 65, 31, 2
Spinal cord	R $\vee$	R $\vee$		R $\vee$	R $\vee$		R $\vee$; M $\vee$		21, 39, 58, 55
PN/paraganglia							R $\vee$		45, 88

(continued)

Table 1 Continued

	5αR1		5αR2		Isozyme unconfirmed		References
	mRNA	Protein/IC	Activity ^a	mRNA	Protein/IC	Activity ^b	
Sensory							
Olfactory bulb	R✓; M±(G); M✓(g)	R✓; M±(G); M✓(g)					
Eyes	H✓			H✓		R✓ H✓	31, 29, 2 84, 64
Reproductive							
Prostate	H✓; R✓	HX; R✓	H✓; R✓; M±	H✓; R✓	H✓; R✓	H✓	22, 53, 65, 41, 72
Testes	HX; R✓	HX	M✓	H✓; R✓	H✓	M✓	71, 65, 42
Epididymis	H✓; R✓; M✓	HX; R✓	R✓; M✓	H✓; R✓; M✓	H✓; R✓	H✓	53, 78, 41, 11, 43, 81
Vas deferens	R✓			R✓		H✓; R✓; M✓	15, 69, 26, 65
Seminal vesicles	HX; R✓	HX; R✓	MX	H✓; R✓	H✓; R✓		65, 41, 59
Ovary	H✓; R✓; M✓	HX	H✓	H✓; R✓; M✓	HX		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✓	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; X, 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. ¹Abulhajj & Klang (1982); ²Agis-Balboa et al. (2006); ³Araneo et al. (1991); ⁴Backstrom et al. (1986); ⁵Barat et al. (2007); ⁶Berman et al. (2003); ⁷Bloom et al. (2001); ⁸Borlak et al. (2004); ⁹Chan & Leatham (1975); ¹⁰Colby & Kitay (1972); ¹¹Delarminat et al. (2011); ¹²Dombroski et al. (1997); ¹³Drake et al. (2005); ¹⁴Dube et al. (1975); ¹⁵Dupuy et al. (1979); ¹⁶Eicheler et al. (1998); ¹⁷Eicheler et al. (1995); ¹⁸Eiknes et al. (1983); ¹⁹George (1993); ²⁰Hammer et al. (2005); ²¹Hauser et al. (1987); ²²Houston et al. (1987); ²³Issa et al. (2002); ²⁴Ito et al. (2002); ²⁵Jakimiuk et al. (1999); ²⁶Jeanfaucher et al. (1986); ²⁷Karolczak et al. (1998); ²⁸Kimura et al. (2003); ²⁹Kiyokage et al. (2005); ³⁰Leiphart (1993); ³¹Li et al. (1997); ³²Livingstone et al. (2000); ³³Livingstone et al. (2009); ³⁴Lloyd (1979); ³⁵Lofthus et al. (1984); ³⁶Luu-The et al. (1994); ³⁷Luu-The et al. (2005); ³⁸Mackenzie et al. (2008); ³⁹MacLusky et al. (1987); ⁴⁰Mahendroo et al. (1997); ⁴¹Mahendroo et al. (2001); ⁴²Mahendroo et al. (2004); ⁴³Mahoney et al. (2011); ⁴⁴Melcangi et al. (1988); ⁴⁵Melcangi et al. (1990); ⁴⁶Milewich et al. (1979); ⁴⁷Milewich et al. (1983); ⁴⁸Milewich et al. (1987); ⁴⁹Milewich et al. (1995); ⁵⁰Mori et al. (1978); ⁵¹Mowszowi & Bardin (1974); ⁵²Nienstedt et al. (1980a,b); ⁵³Normington & Russell (1992); ⁵⁴Nowak (2002); ⁵⁵Patte-Mensah et al. (2004); ⁵⁶Pelletier et al. (1994); ⁵⁷Pinsky et al. (1978); ⁵⁸Pozzi et al. (2003); ⁵⁹Pralls et al. (2003); ⁶⁰Provost & Tremblay (2007); ⁶¹Provost et al. (1982); ⁶²Randall et al. (1982); ⁶³Raz et al. (2005); ⁶⁴Rocha et al. (2000); ⁶⁵Russell & Wilson (1994); ⁶⁶Samy et al. (2001); ⁶⁷Sato et al. (1998); ⁶⁸Sawaya & Price (1997); ⁶⁹Seethalakshmi et al. (1982); ⁷⁰Seo et al. (2009); ⁷¹Sheffield & O'Shaughnessy (1988); ⁷²Shirakawa et al. (2004); ⁷³Steckelbroeck et al. (2001); ⁷⁴Stoffel-Wagner et al. (2000); ⁷⁵Suzuki et al. (2001); ⁷⁶Takayasu et al. (2001); ⁷⁷Thiele et al. (1993b); ⁷⁸Torres & Ortega (2006); ⁷⁹Turner et al. (1990); ⁸⁰Viger & Robaire (2011); ⁸¹Vu et al. (2009); ⁸²Wake et al. (2007); ⁸³Weinstein et al. (1991); ⁸⁴Wiebe et al. (1991); ⁸⁵Yokoi et al. (2011); ⁸⁶Yang et al. (1998a); ⁸⁷Yokoi et al. (1998b); ⁸⁸Zhou et al. (1998); ⁸⁹Zyrek 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.* 1980a,b, Normington & Russell 1992), all sites of importance in inflammatory diseases and GC therapies. In metabolic tissues, 5 α R1 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.* 1993b). 5 α R2 is more prevalent in reproductive tissues, although it is present in human liver (Thigpen *et al.* 1993b) and adipose (MacKenzie *et al.* 2008). Both isozymes have been reported in primary osteoblasts (Issa *et al.* 2002), though the significance of 5 α R in bone is not well understood at present. Within the brain, 5 α R1 is more highly expressed than 5 α R2 (Melcangi *et al.* 1993, 1998, Poletti *et al.* 1997a,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, 5 α R may affect central GC feedback and regulation.

With such a wide distribution of 5 α -reductase expression, it follows that 5 α -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 5 α reduction may yield metabolites exerting effects in many organ systems.

Substrates

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

5 α R 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 5 α R2 (Wigley *et al.* 1994, Wang *et al.* 1999). 5 α -Reduced dihydrosteroids are rapidly reduced at the 3 keto position by 3 α or 3 β -hydroxysteroid dehydrogenase, enzymes of the aldo-keto reductase 1C family. The ratio of 3 α vs 3 β metabolites varies greatly in a tissue-dependent manner but, in the liver, 3 α -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 5 α R1 having a pH optimum of 6–8.5 and

5 α R2 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 5 α -R isozymes accept a range of 3-oxo-4-ene steroids as substrates (Andersson & Russell 1990, Thigpen *et al.* 1993a), 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 9 α 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 5 α R2 than 5 α R1, with low K_m s of <0.1 μ M (Normington & Russell 1992). Testosterone is also the most researched substrate of 5 α R1, 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 μ M 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 μ M, respectively, for type 1 and 0.376 μ M for type 2 (Normington & Russell 1992). The K_m for 5 α -reduction of cortisol is slightly lower than that for 5 β -reduction measured in human hepatic cytosol (27 μ M; Iyer *et al.* 1990), but these routes appear to make balanced contributions to metabolic clearance of steroids by first pass metabolism. 3 α ,5 α -Reduced cortisol metabolites represent just under half of urinary tetrahydro-metabolites of cortisol synthesised in the liver (Fukushima *et al.* 1960), with 3 α ,5 β -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 5 α R (McGuire & Tomkins 1960, Gold & Crigler 1972) and, while it is a better substrate than cortisol (McGuire & Tomkins 1960), its 5 α -reduced metabolites are not readily found in urine (Bush & Mahesh 1959). 5 α -Tetrahydrocortisone, if present, is rapidly converted into 5 α -tetrahydrocortisol (Bush & Mahesh 1959), possibly by 11 β -hydroxysteroid dehydrogenase 1. This contrasts with the more skewed 5 β -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 5 α R, 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 5 α -reductases in the adrenal gland plays part of a mechanism to regulate steroidogenesis (Carsia *et al.* 1984).

Actions of substrate vs product

Modification by 5 α or 5 β reduction results in distinct orientations of the A-ring, with the 5 α conformation adopting a planar 'trans' structure, whereas the 5 β 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 5 α -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 5 α -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, 5 α -dihydroprogesterone is a ligand for the progesterone receptor (Pasqualini & Nguyen 1980) and is the principle progestogen in some species (Meyer *et al.* 1997a). Its subsequent 3 α -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). 5 α -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 5 α R has generally been thought to inactivate these steroids prior to excretion and, throughout the years, there have only been sporadic reports of 5 α -reduced GCs exerting effects through GR.

A few studies have suggested that the 5 α -reduced metabolites of cortisol or corticosterone can bind GR, first demonstrated by Baxter & Tomkins (1971), who showed that 5 α -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 5 α -dihydrocorticosterone (5 α DHB) was demonstrated by Carlstedt-Duke *et al.* (1977). While McInnes *et al.* (2004) concurred that 5 α -DHB was a weak GR ligand, they also demonstrated that 5 α -tetrahydrocorticosterone (5 α THB) could displace dexamethasone with a similar K_d as corticosterone (Fig. 3A). Recently, 5 α -DHB has also been suggested as a ligand for a putative, alternative DHB receptor (Sheppard *et al.* 1998).

The potency and efficacy of 5 α -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). 5 α THB 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 5 α -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); 5 α THB induces PEPCK weakly (McInnes *et al.* 2004), 5 α -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 5 α THB 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 5 α THB was administered instead (Fig. 4A). This lack of metabolic disruption has also been demonstrated in rats, where administration of 5 α -reduced dihydroglucocorticoids (5 α -dihydrocortisol and 5 α -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), 5 α THB suppresses the HPA axis. *Ex vivo*, 5 α -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 5 α -reduced steroids possess anti-inflammatory abilities, *in vitro* and *in vivo*: 5 α THB 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 5 α -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 5 α -reduced metabolites, has been crystallised with human GR. Nonetheless, insights into

Figure 3 5 α -Reduced glucocorticoid binds and selectively activates GR. (A) 5 α -Tetrahydrocorticosterone (5 α THB) and 5 α -dihydrocorticosterone (5 α DHB) (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 5 α THB (268 nM) and 5 α DHB (336 nM) were similar to that of corticosterone (153 nM) and higher than dexamethasone (38 nM; $n=6$ /treatment). (B) 5 α THB, but not 5 α DHB, 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. * $P<0.05$ vs vehicle (veh), # $P<0.05$ vs B. Following chronic infusion of steroids (50 μ g/day, 2 weeks) to mice ($n=10$ –12/group): (C) corticosterone, but not 5 α THB, impaired glucose tolerance, as demonstrated by increased insulin concentrations ($P<0.001$ 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) 5 α THB caused immune suppression to a similar extent to corticosterone, assessed by a significant reduction ($P<0.01$) 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 $\pm$ 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 5 α -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 5 α -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 5 α -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 5 α DHT with AR.

Testosterone is a less potent androgen than 5 α DHT, 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 5 α DHT 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 5 α DHT, 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 5 α DHT. 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). 5 α DHT 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 5 α -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 5 α -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 5 α -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 5 α -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 5 α -dihydrotestosterone (5 α DHT) in the androgen receptor ligand-binding domain (AR-LBD). When bound with AR, testosterone (A) and 5 α DHT (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 5 α DHT (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. 5 α DHT 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 2Q71) and 5 α DHT with AR-LBD (Protein Data Bank Code 1T63).

tissues where 5 α -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 5 α -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 5 α - and 5 β -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 5 α -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 5 α -reduction is less (Westerbacka *et al.* 2003) and mice lacking 5 α -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 5 α -reduction of GCs.

Genetic variants in 5 α Rs have not been described in polygenic obesity or type 2 diabetes, and in particular, regulation of 5 α R1 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 5 α R1, whereas 5 α R2 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 5 α -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

5 α -Reduced GCs have received little attention in terms of their biological activity, despite other 5 α -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 5 α -reduced GC has not been investigated in bone.

A further avenue of interest in 5 α -reduced GCs relates to the common pharmacological inhibition of 5 α Rs in clinical practice. 5 α -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 5 α -reduced metabolites, leaving them susceptible to adverse metabolic effects and inflammatory changes, effects more marked with dutasteride, a new dual 5 α R 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 5 α -reduced GCs in health, disease and therapeutics will provide important

insights into the effects in patients on 5 α -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.

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Author contribution statement

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

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