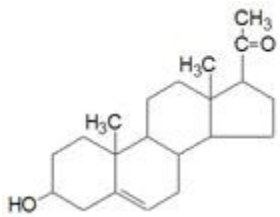
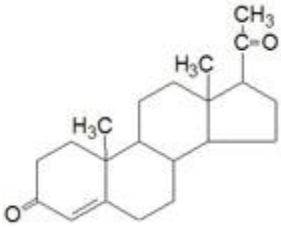
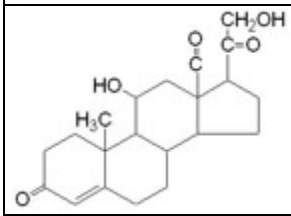
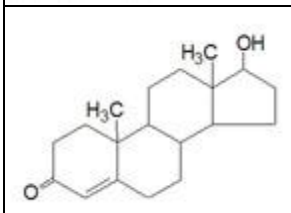
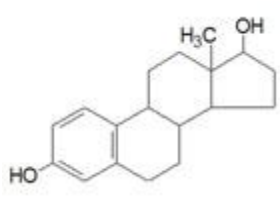
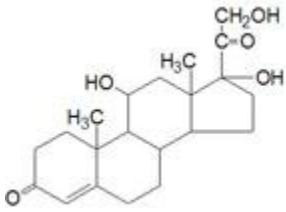
Introduction to the Steroid Hormones

The steroid hormones are all derived from cholesterol. Moreover, with the exception of vitamin D, they all contain the same cyclopentanophenanthrene ring and atomic numbering system as cholesterol. The conversion of C_{27} cholesterol to the 18-, 19-, and 21-carbon steroid hormones (designated by the nomenclature C with a subscript number indicating the number of carbon atoms, e.g. C_{19} for androstanes) involves the rate-limiting, irreversible cleavage of a 6-carbon residue from cholesterol, producing pregnenolone (C_{21}) plus isocaproaldehyde. Common names of the steroid hormones are widely recognized, but systematic nomenclature is gaining acceptance and familiarity with both nomenclatures is increasingly important. Steroids with 21 carbon atoms are known systematically as pregnanes, whereas those containing 19 and 18 carbon atoms are known as androstanes and estranes, respectively. The important mammalian steroid hormones are shown below along with the structure of the precursor, pregnenolone. Retinoic acid and vitamin D are not derived from pregnenolone, but from vitamin A and cholesterol respectively.

	Pregnenolone: produced directly from cholesterol, the precursor molecule for all C_{18}, C_{19} and C_{21} steroids
	Progesterone: a progestestin, produced directly from pregnenolone and secreted from the *corpus luteum*, responsible for changes associated with luteal phase of the menstrual cycle, differentiation factor for mammary glands
	Aldosterone: the principal mineralocorticoid, produced from progesterone in the *zona glomerulosa* of adrenal cortex, raises blood pressure and fluid volume, increases Na^+ uptake via the kidney
	Testosterone: an androgen, male sex hormone synthesized in the testes, responsible for secondary male sex characteristics, produced from progesterone
	Estradiol: an estrogen, principal female sex hormone, produced in the ovary, responsible for secondary female sex characteristics

	Cortisol: dominant glucocorticoid in humans, synthesized from progesterone in the zona fasciculata of the adrenal cortex, involved in stress adaptation, increases gluconeogenesis in liver, elevates blood pressure, increases Na+ uptake by the kidney, numerous effects on the immune system

All the steroid hormones exert their action by passing through the plasma membrane and binding to intracellular receptors (also referred to as nuclear receptors, NR). The mechanism of action of the thyroid hormones is similar in that they also interact with intracellular nuclear receptors. Both the steroid and thyroid hormone-receptor complexes exert their action by binding to specific nucleotide sequences in the DNA of responsive genes. These DNA sequences are identified as hormone response elements, HRE. The interaction of steroid-receptor complexes with DNA leads to altered rates of transcription of the associated genes.

Steroid Hormone Biosynthesis Reactions

The particular steroid hormone class synthesized by a given cell type depends upon its complement of peptide hormone receptors, its response to peptide hormone stimulation and its genetically expressed complement of enzymes. The following indicates which peptide hormone is responsible for stimulating the synthesis of which steroid hormone:

Luteinizing Hormone (LH): progesterone and testosterone
Adrenocorticotropic hormone (ACTH): cortisol
Follicle Stimulating Hormone (FSH): estradiol
Angiotensin II/III: aldosterone

The first reaction in converting cholesterol to C_{18}, C_{19} and C_{21} steroids involves the cleavage of a 6-carbon group from cholesterol and is the principal committing, regulated, and rate-limiting step in steroid biosynthesis. The enzyme system that catalyzes the cleavage reaction is known as P450-linked side chain cleaving enzyme (P450ssc) or 20,22-desmolase, or cholesterol desmolase, and is found in the inner mitochondrial membrane of steroid-producing cells, but not in significant quantities in other cells.

In order for cholesterol to be used for steroid hormone biosynthesis it must be transported from the outer mitochondrial membrane to the inner membrane. This transport process is mediated by steroidogenic acute regulatory protein (StAR) and this transport process represents the rate-limiting step in steroidogenesis..

Mitochondrial 20,22-desmolase (P450ssc) is a complex enzyme system consisting of a cytochrome P450 family member enzyme and the proteins ferredoxin reductase (also known as adrenodoxin reductase) and ferredoxin 1 (also known as adrenodoxin). Ferrodoxin reductase and adrenodoxin reductase are components of numerous cytochrome P450 enzyme complexes in addition to P450ssc. The cytochrome P450 enzyme of P450ssc is encoded by the CYP11A1 gene. The CYP11A1 gene is located on chromosome 15q24.1 and is composed of 10 exons that generate two alternatively spliced mRNAs. The CYP11A1 isoform a protein is 521 amino acids and represents the functional enzyme. The CYP11A1 isoform b

protein lacks a mitochondrial targeting sequences and thus, likely plays no role in the P450ssc. The ferrodoxin reductase gene (FDXR) is located on chromosome 17q25.1 and is composed of 14 exons that generate seven alternatively spliced mRNAs. The ferredoxin 1 gene (FDX1) is located on chromosome 11q22.3 and is composed of 4 exons that encode a 184 amino acid precursor protein.

The overall cholesterol side-chain cleavage occurs through a series of three reactions all catalyzed by the desmolase complex. The activity of each of these components is increased by two principal cAMP- and PKA-dependent processes. First, principally in response to the actions of ACTH in adrenal cortical cells, the level of cAMP rises. Secondly the increased cAMP results in the stimulated activity of PKA. Activated PKA phosphorylates a number of proteins involved in steroid hormone biosynthesis such as the steroidogenic acute regulatory protein (StAR) and lysosomal acid lipase (encoded by the LIPA gene; also called cholesterol ester hydrolase). The activation of lysosomal acid lipase results in the removal of the fatty acid esterification at the C-3 position of cholesterol leading to increased concentrations of free cholesterol. It is free cholesterol that is the substrate for mitochondrial 20,22-desmolase (P450ssc).

Long-term regulation of steroid synthesis is also effected at the level of the gene encoding desmolase (CYP11A1). This gene contains a cAMP response element (CRE) that binds the transcription factor identified as cAMP-response element-binding protein, CREB (encoded by the CREB1 gene). Humans express seven genes in the CREB subfamily of the basic leucine zipper (bZIP) family of transcription factors. The seven CREB gene are identified as CREB1, CREB3, CREB5, and CREB3-like 1, 2, 3, and 4 (CREB3L1, CREB3L2, CREB3L3, and CREB3L4). The CREB3L3 encoded protein is commonly identified as CREBH. The CREB1 encoded proteins are most closely related in structure and function to two additional transcriptional factors called cAMP response element modulator (CREM) and activating transcription factor 1 (ATF-1). Another member of the activating transcription factor (ATF) family, ATF-4, was originally identified as CREB2. CREB is phosphorylated by PKA in the cytosol and then migrates to the nucleus where it binds to CREs in target genes such as CYP11A1. The consequences of CREB binding to CYP11A1 are increased rates of desmolase RNA transcription, thereby leading to increased levels of the enzyme.

Finally, cholesterol is a negative feedback regulator of HMG-CoA reductase (HMGR) activity. Thus, when cytosolic cholesterol is depleted, *de novo* cholesterol synthesis is stimulated by freeing HMGR of its feedback constraints. Subsequent to desmolase activity, pregnenolone moves to the cytosol, where further processing depends on the cell (tissue) under consideration.

The various hydroxylases involved in the synthesis of the steroid hormones have a nomenclature that indicates the site of hydroxylation (e.g. 17α-hydroxylase introduces a hydroxyl group to carbon 17). These hydroxylase enzymes are members of the cytochrome P450 class of enzymes and as such also have a nomenclature indicative of the site of hydroxylation in addition to being identified as P450 class enzymes (e.g. the 17α-hydroxylase is also identified as P450c17). The officially preferred nomenclature for the cytochrome P450 class of enzymes is

to use the prefix **CYP**. Thus, 17α-hydroxylase is identified as CYP17A1. There are currently 57 identified CYP genes in the human genome.

Primary Enzyme Activities of Steroid Hormone Biosynthesis

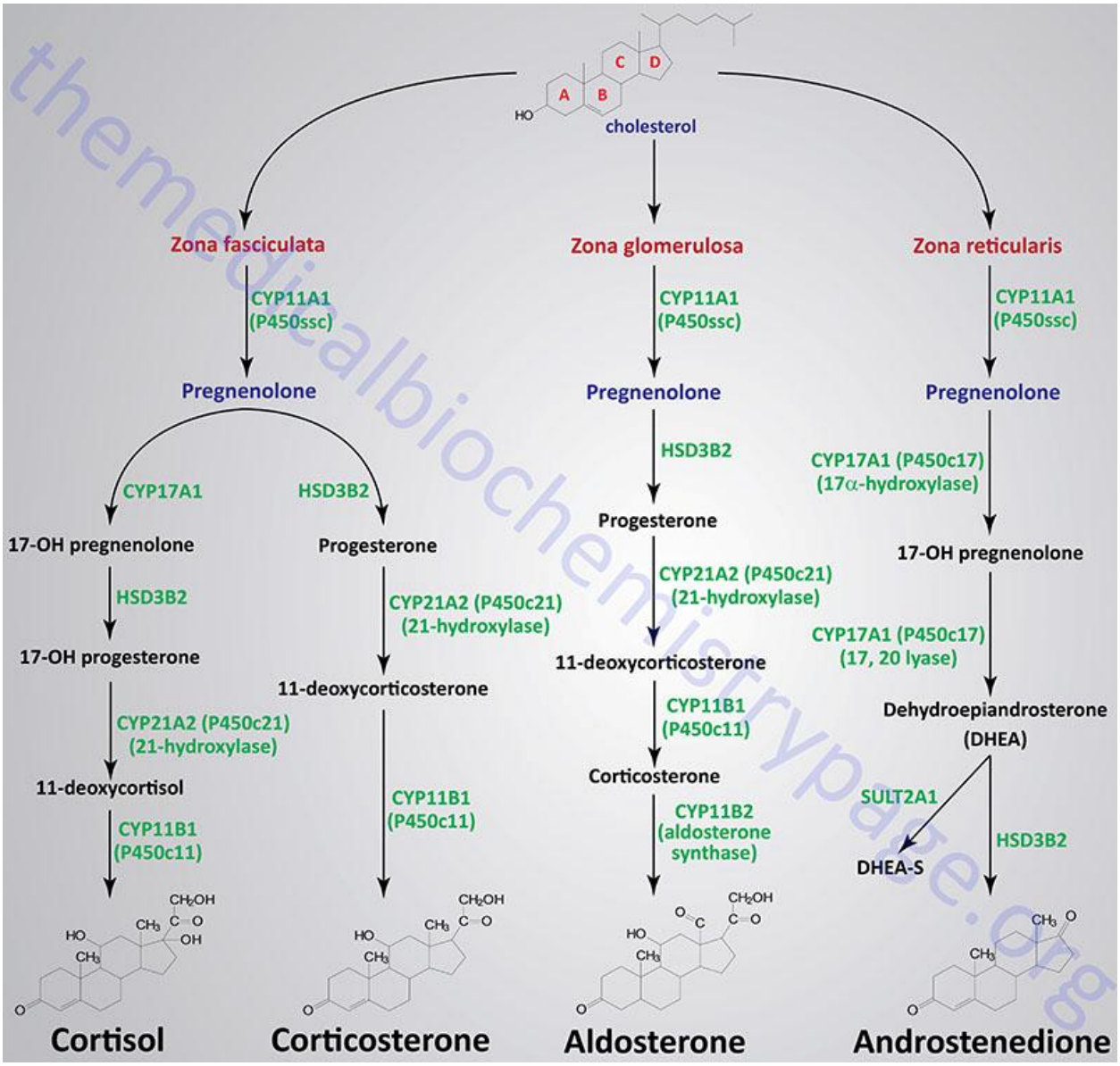
Common Name(s)	Gene ID	Activities	Primary Site of Expression
steroidogenic acute regulatory protein	STAR	mediates transport of cholesterol from outer mitochondrial membrane to the inner membrane	all steroidogenic tissues except placenta and brain
desmolase, P450ssc	CYP11A1	cholesterol-20,22-desmolase	steroidogenic tissues
3β-hydroxysteroid dehydrogenase type 1	HSD3B2	3β-hydroxysteroid dehydrogenase	steroidogenic tissues
P450c11	CYP11B1	11β-hydroxylase	only in zona fasciculata and zona reticularis of adrenal cortex
P450c17	CYP17A1	two activities: 17α-hydroxylase and 17,20-lyase	steroidogenic tissues
P450c21	CYP21A2	21-hydroxylase	not expressed in the zona reticularis
aldosterone synthase	CYP11B2	18α-hydroxylase	exclusive to zona glomerulosa of adrenal cortex
estrogen synthetase	CYP19A1	aromatase	gonads, brain, adrenals, adipose tissue, bone
17β-hydroxysteroid dehydrogenase type 3	HSD17B3	17-ketoreductase	steroidogenic tissues
sulfotransferase	SULT2A1	sulfotransferase	liver, adrenals
5α-reductase type 2	SRD5A2	5α-reductase	steroidogenic tissues

Steroids of the Adrenal Cortex

The adrenal cortex is responsible for production of three major classes of steroid hormones: **glucocorticoids**, which regulate carbohydrate metabolism; **mineralocorticoids**, which regulate the body levels of sodium and potassium; and **androgens**, whose actions are similar to that of steroids produced by the male gonads. Cholesterol, acquired from the diet or from LDL, or

produced de novo in adrenal cortical cells, serves as the precursor for all of the adrenal steroid hormones. Cholesterol uptake from the blood occurs through the binding of LDL to the LDL receptor. Chronic stimulation of the adrenal cortex by ACTH leads to increased LDL receptor gene expression resulting in increased receptor density.

The adrenal cortex is composed of three main tissue regions: zona glomerulosa, zona fasciculata, and zona reticularis. Although the pathway to pregnenolone synthesis is the same in all zones of the cortex, the zones are histologically and enzymatically distinct, with the exact steroid hormone product dependent on the enzymes present in the cells of each zone. Many of the enzymes of adrenal steroid hormone synthesis are of the class called cytochrome P450 enzymes. These enzymes all have a common nomenclature and a standardized nomenclature. The standardized nomenclature for the P450 class of enzymes is to use the abbreviation CYP. For example the P450ssc enzyme (also called 20,22-desmolase or cholesterol desmolase) is identified as CYP11A1. As indicated earlier, in order for cholesterol to be converted to pregnenolone in the adrenal cortex it must be transported from the outer mitochondrial membrane to the inner where CYP11A1 resides and this transport process is mediated by the transport protein StAR.



Synthesis of the various adrenal steroid hormones from cholesterol. Only the terminal hormone structures are included. 3β-DH and $\Delta^{4,5}$-isomerase are the two activities of 3β-hydroxysteroid dehydrogenase type 1 (gene symbol HSD3B2), P450c11 is 11β-hydroxylase (CYP11B1), P450c17 is CYP17A1. CYP17A1 is a single microsomal enzyme that has two steroid biosynthetic activities: 17α-hydroxylase which converts pregnenolone to 17-hydroxypregnenolone (17-OH pregnenolone) and 17,20-lyase which converts 17-OH pregnenolone to DHEA (dehydroepiandrosterone). P450c21 is 21-hydroxylase (CYP21A2, also identified as CYP21 or CYP21B). Aldosterone synthase is also known as 18α-hydroxylase (CYP11B2). The gene symbol for sulfotransferase is SULT2A1.
Conversion of pregnenolone to progesterone requires the two enzyme activities of HSD3B2: the 3β-hydroxysteroid dehydrogenase and $\Delta^{4,5}$-isomerase activities. Zona glomerulosa cells lack the CYP17A1 that converts pregnenolone and progesterone to their C_{17} hydroxylated analogs. Thus, the pathways to the glucocorticoids (deoxycortisol and cortisol) and the androgens (DHEA and androstenedione) are blocked in these cells. Zona glomerulosa cells are unique in the adrenal cortex in that they are the only cells expressing the enzyme responsible

for converting corticosterone to aldosterone, the principal and most potent mineralocorticoid. This enzyme is P450c18 (or 18α-hydroxylase, CYP11B2), also more commonly called aldosterone synthase. The result is that the zona glomerulosa is mainly responsible for the conversion of cholesterol to the weak mineralocorticoid, corticosterone and the principal mineralocorticoid, aldosterone. Cells of the zona fasciculata and zona reticularis lack aldosterone synthase (CYP11B2) that converts corticosterone to aldosterone, and thus these tissues produce only the weak mineralocorticoid corticosterone. However, both these zones do contain the CYP17A1 missing in zona glomerulosa and thus produce the major glucocorticoid, cortisol. Zona fasciculata and zona reticularis cells also contain CYP17A1, whose 17,20-lyase activity is responsible for producing the androgens, dehydroepiandrosterone (DHEA) and androstenedione. Thus, fasciculata and reticularis cells can make corticosteroids and the adrenal androgens, but not aldosterone.

As noted earlier, P450ssc (CYP11A1) is a mitochondrial activity. Its product, pregnenolone, moves to the cytosol, where it is converted either to androgens or to 11-deoxycortisol and 11-deoxycorticosterone by enzymes of the endoplasmic reticulum. The latter two compounds then re-enter the mitochondrion, where the enzymes are located for tissue-specific conversion to glucocorticoids (cortisol) or mineralocorticoids (aldosterone), respectively.

Overview of Adrenal Steroid Hormone Functions

The predominant adrenal steroid hormones are the glucocorticoids and the mineralocorticoids. The androgenic hormones produced by the adrenal cortex, although exerting important functions, are most significant in the context of adrenal dysfunction. The glucocorticoids are a class of hormone so called because they are primarily responsible for modulating the metabolism of carbohydrates. In a broad sense the glucocorticoids regulate energy homeostasis, embryonic development and postnatal life, and the responses to stress, thereby directly affecting survival and reproduction. The mineralocorticoids control the excretion of electrolytes (minerals), hence the derivation of the name of this class of hormone. The glucocorticoids can exert mineralocorticoid effects through their ability to bind and activate the mineralocorticoid receptor. The adrenal androgenic hormones, androstenedione and DHEA, circulate bound primarily to sex hormone-binding globulin (SHBG). Although some of the circulating androgen is metabolized in the liver, the majority of interconversion occurs in the gonads , skin, and adipose tissue. DHEA is rapidly converted to the sulfated form, DHEA-S, in the liver and adrenal cortex. The primary biologically active metabolites of the androgens are testosterone and dihydrotestosterone which function by binding intracellular receptors, thereby effecting changes in gene expression leading to the manifestation of the secondary male sex characteristics.

Glucocorticoid Functions

The glucocorticoids represent a group of steroid hormones whose major function is the modulation of carbohydrate metabolism, hence the derivation of the term, glucocorticoid. The primary glucocorticoid in humans is cortisol. Although

modulation of glucose homeostasis is a major glucocorticoid-induced effect, while simultaneously inhibiting all other metabolic pathways not directly involved in glucose production, the glucocorticoids do have other physiologically and biochemically significant functions. Glucocorticoids also regulate overall energy homeostasis, they modulate embryonic and post-natal development, and they modulate stress responses that affect survival and reproduction. Indeed, physiological increases in the production of glucocorticoids is an anticipatory response designed to meet the increased energy demands associated with stress as well as those of major developmental changes occurring during the normal life cycle.

Cortisol inhibits uptake and utilization of glucose resulting in elevations in blood glucose levels. Cortisol acts as an insulin antagonist and also suppresses the release of insulin, both effects leading to reduced glucose uptake and enhanced hepatic gluconeogenesis. The effect of cortisol on blood glucose levels is further enhanced through the increased breakdown of skeletal muscle protein and adipose tissue triglycerides which provides energy and substrates for gluconeogenesis. Cortisol also increases the synthesis of gluconeogenic enzymes, in particular phosphoenolpyruvate carboxykinase (PEPCK, encoded by the PCK1 gene) and glucose-6-phosphatase (G6P, encoded by the G6PC gene). The increased rate of protein metabolism leads to increased urinary nitrogen excretion and the induction of urea cycle enzymes. In order to effectively accomplish these metabolic changes glucocorticoids inhibit several energy-consuming processes such as digestion, and reproduction, as well as inflammatory and other immune responses.

The anti-inflammatory activity of the glucocorticoids is exerted, in part, through inhibition of phospholipase A_2 (PLA_2) activity with a consequent reduction in the release of arachidonic acid from membrane phospholipids. Arachidonic acid serves as the precursor for the synthesis of various eicosanoids. The immune modulating effects of glucocorticoids are exploited pharmacologically and is the basis for the anti-inflammatory effects of drugs such as prednisone (an intermediate-acting steroid) and dexamethasone (a long-acting steroid). Synthetic glucocorticoids exhibit high affinity for the glucocorticoid receptor and mimic the effects of long-term exposure to high levels of natural glucocorticoids such as cortisol. Thus, the use of synthetic glucocorticoids, such as dexamethasone and prednisone, can lead to severe side effects including hypertension, diabetes, osteoporosis, and glaucoma.

Cortisol is the most important naturally occurring glucocorticoid in humans. As indicated in the Figure above, cortisol is synthesized in the zona fasciculata of the adrenal cortex. When released to the circulation, cortisol is almost entirely bound to protein. A small portion of circulating cortisol is bound to albumin with more than 80% being bound by a specific glycosylated α-globulin called transcortin or corticosteroid-binding globulin (CBG). Transcortin is encoded by the serpin family A member 6 (SERPINA6) gene. Between 5% and 10% of circulating cortisol is free and biologically active. The function of CBG is not only transport of cortisol in the blood but regulated distribution of the hormone into tissues. Following uptake into tissues cortisol bioavailability is regulated by two enzymes that function in opposition to one another. One enzyme, 11β-hydroxysteroid

dehydrogenase 2 (encoded by the HSD11B2 gene) oxidizes cortisol to its inactive metabolite, cortisone. The other enzyme, 11β-hydroxysteroid dehydrogenase 1 (encoded by the HSD11B1 gene) reduces the 11-oxo group (11-oxoreductase activity) in cortisone and 11-dehydrocorticosterone generating the active glucocorticoids, cortisol and corticosterone.

Once inside the cell, glucocorticoids exert their effects on by direct binding to an intracellular receptor that is a zinc-finger transcription factor belonging to the nuclear hormone receptor superfamily. Typical of the structure of nuclear hormone receptors, the glucocorticoid receptor (GR or GCCR) is composed of three functional domains. The N-terminal domain (NTD) harbors the potent transcriptional regulatory function, most often referred to as the activation function 1 (AF1) domain. The NTD comprises amino acid residues 1–420. The AF1 domain interacts with numerous transcriptional coregulators and the basal transcription factors that are involved in control of expression of glucocorticoid-responsive genes. In addition to harboring the AF1 domain, the NTD is the site of the major post-translational regulation of GR function. There are at least seven sites of Ser phosphorylation in the NTD of the GR. The DNA-binding domain (DBD), which encompasses the amino acids that form the two zinc-finger domains, is located from amino acids 421–486. The ligand-binding domain (LBD) resides in the C-terminus of the protein and encompasses amino acids 528–777. There is a domain between the DBD and the LBD termed the hinge region that harbors two nuclear localization signals identified as NL1 and NL2. The second activation function domain (AF2) is found in the extreme C-terminal region of the GR. The AF2 domain, like the AF1 domain, also binds to transcriptional coregulators in a ligand-dependent manner.

The GR is encoded by the NR3C1 (nuclear receptor subfamily 3 group C member 1) gene. The NR3C1 gene is located on chromosome 5q31.3 and is composed of 8 protein coding exons (exons 2-9) and 13 variants of exon 1 that differ as a result of upstream promoter elements. The NR3C1 gene also generates at least 15 alternatively spliced mRNAs. Several of these alternative mRNAs are translated from alternative in-frame translation initiation codons. The predominant GR species are identified as GRα (777 amino acids) and GRβ (742 amino acids). The NR3C1 gene is constitutively expressed in virtually every cell type, but tissue-specific expression patterns of the alternative GR isoforms result in tissue-specific transcriptional outcomes. It is also important to note that most glucocorticoids bind to the mineralocorticoid receptor (MR encoded by the NR3C2 gene) as well, and as such, can exhibit mineralocorticoid-like activities.

Major Target Tissues for Glucocorticoid Regulation of Metabolism

The major organs that are targets for the metabolic regulatory actions of the glucocorticoids are the liver, adipose tissue, and skeletal muscle. Since the liver is the major organ tasked with the global regulation of glucose homeostasis it is not surprising that this organ is a major cortisol target. Similar to the role of glucagon in the liver, glucocorticoids are essential for the hepatic role of maintaining blood glucose levels during fasted states. In addition, glucocorticoids exert effects on the liver during periods of stress to ensure adequate glucose is

released. Within the liver, two primary target genes for cortisol are the gluconeogenic genes, PCK1 (cytoplasmic phosphoenolpyruvate carboxykinase) and G6PC (glucose-6-phosphatase). Maximal expression of both the PCK1 and G6PC genes requires additional factors including the nuclear receptor, PPARα. Within the liver the PPARA gene, which encodes PPARα, is also a target for cortisol activation.

The energy required for the liver to carry out gluconeogenesis during periods of fasting, or stress, is derived by the oxidation of fatty acids. The principal source of the fatty acids used by the liver is adipose tissue. Glucocorticoid effects on lipid homeostasis in adipose tissue are complex encompassing both increased lipogenesis through adipocyte differentiation and increased lipolysis. Glucocorticoids increase adipose tissue fatty acid release by increasing the expression of the hormone-sensitive lipase gene (symbol: LIPE) and the gene encoding monoglyceride lipase (symbol: MGLL). Under normal physiological conditions glucocorticoids promote preadipocyte differentiation into mature adipocytes and increase dietary fat intake. During glucocorticoid-induced adipocyte differentiation a lipolytic transcriptional program is activated and includes numerous genes involved in triglyceride synthesis, lipid transport, and lipid storage. Triglyceride synthesis genes turned on by cortisol include several AGPAT genes (acylglycerol-3-phosphate acyltransferases) that encode enzymes that incorporate a fatty acid into lysophosphatidic acid and the LPIN1 gene that encodes phosphatidic acid phosphatase that removes the phosphate from phosphatidic acid generating a diglyceride.

In skeletal muscle, glucocorticoids regulate protein and glucose metabolism. Following the consumption of food, insulin release promotes glucose uptake by skeletal muscle where it is stored as glycogen. During periods of fasting, stress, or during exercise, catecholamines (epinephrine) and glucocorticoids stimulate glycogen breakdown. When glucocorticoid levels are elevated, such as during pharmacological treatment with corticosteroids, or under conditions of hypercortisolemia (e.g. Cushing syndrome) there is a resultant insulin resistance, inhibited protein synthesis, and enhanced proteolysis within skeletal muscle. The enhanced proteolysis occurs as a means to provide amino acid carbon skeletons to the liver for gluconeogenesis, a pathway that is highly activated in response to excess glucocorticoid levels. These pathophysiological consequences within skeletal muscle result in muscle weakness and atrophy. Many of the genes that are activated in skeletal muscle by glucocorticoids encode proteins that interfere with insulin signaling in skeletal muscle resulting in the observed decreases in protein synthesis and increases in protein degradation.

Mineralocorticoid Functions

As the name of this class of hormone implies, the mineralocorticoids control the excretion of electrolytes (minerals). The major circulating mineralocorticoid is aldosterone. Aldosterone exerts its primary effects through actions on the kidneys but also functions in the colon and sweat glands. The principle effect of aldosterone is to enhance sodium (Na^+) reabsorption in the connecting tubule (CNT) and cortical collecting duct of the nephrons in the kidneys. Within these

regions of the nephron aldosterone induces the expression of the Na+,K+-ATPase subunit genes (ATP1A1 and ATP1B1), the genes encoding the subunits (SCNN1A, SCNN1B, and SCNN1C) of the epithelial sodium channel (ENaC), and the SLC12A3 gene (encoding the Na+-Cl– cotransporter, NCC). The net effect of the induction of these transporter genes, by aldosterone, is enhanced Na^+ reabsorption as a function of the apical membrane localized ENaC and NCC transporters and delivery to the blood via the action of the basolateral membrane localized Na^+,K^+-ATPase. Secondary to the Na^+ uptake is efflux of potassium (K^+) to the tubular lumen. In addition to K^+ excretion, aldosterone enhances the excretion of hydrogen (H^+) ions from the collecting duct which is a compensating action to counter the accumulation of the positive charge imparted by increased Na^+ reabsorption. However, the action of aldosterone is also exerted on sweat glands, stomach, and salivary glands to the same effect, i.e. sodium reabsorption. This action is accompanied by the retention of chloride (Cl^-) and water resulting in the expansion of extracellular volume leading to increased vascular pressure.

Aldosterone, like all steroid hormones, functions as a ligand activating the transcriptional activity of the mineralocorticoid receptor, MR. The MR, being a member of the nuclear receptor superfamily, is encoded by the NR3C2 (nuclear receptor subfamily 3 group C member 2) gene. The NR3C2 gene is located on chromosome 4q31.23 and is composed of two alternative non-coding exons and eight coding exons (exons 2–9). Alternative splicing yields three mRNAs the encode two distinct protein isoforms. The primary MR protein is composed of 984 amino acids. An additional MR protein, encoded by two of the alternatively spliced mRNAs, is composed of 867 amino acids.

Like all nuclear receptors the MR is composed of an N-terminal domain (NTD), a central DNA-binding domain (DBD), and a C-terminal ligand-binding domain (LBD). The activation function domain 1 (AF-1) is located in the NTD and a second activation function domain (AF-2) is located at the C-terminal end of the LBD. The AF-1 domain in the MR is actually comprised of two distinct domains identified as AF-1a and AF-1b. The AF-1a domain is located in amino acids 1–167 and AF-1b is located in amino acids 445–602. The AF-1a and AF-1b domains are responsible for the recruitment of various transcriptional coactivators to the MR. The NTD also contains an inhibitory domain that resides between the two AF-1 domains. The DBD of the MR is encoded by exons 3 and 4 and contains two zinc-finger domains that facilitate receptor dimerization and DNA binding. The LBD of the MR is highly homologous to the LBD of the GR which explains the ability of glucocorticoids to bind to and activate the MR. In addition, the close homology between the AF-2 domain of the MR, present in the LBD, and that of the GR AF-2 domain explains why these two receptors recruit a nearly identical set of transcriptional coactivators. The interaction of transcriptional coactivators with the AF-2 domain occurs in a ligand-binding dependent manner.

In addition to aldosterone, the glucocorticoids, cortisol and corticosterone, bind to and activate the MR. In aldosterone target tissues the expression of the 11β-hydroxysteroid dehydrogenase 2 (HSD11B2) gene ensures that cortisol is deactivated by conversion to cortisone and corticosterone is deactivated by

conversion to 11-dehydrocorticosterone. Deoxycorticosterone (DOC; 11-deoxycorticosterone) exhibits some mineralocorticoid action but only about 3% of that of aldosterone.

In the absence of ligand the mineralocorticoid receptor (similarly to the glucocorticoid receptor) remains in the cytosol contained within multiprotein chaperone complexes. The cytosolic MR-containing complex is highly similar to that of the cytosolic GR complexes in that it includes heat-shock protein 90 (hsp90), hsp70, the co-chaperone identified as p23 (encoded by the prostaglandin E synthase 3, PTGES3 gene), and various immunophilins (e.g., FKBP51 and FKBP52), all of which prevent its degradation and assist in its maturation. Upon ligand binding, the MR complex changes its conformation leading to release of the ligand-bound receptor and exposure of the two nuclear localization signals in the MR allowing for rapid transport into the nucleus. Within the nucleus the ligand bound MR binds to the same type of hormone response element (HRE) to which the GR, progesterone receptor, and androgen receptor binds. As indicated above, this HRE is an imperfect palindrome composed of two 6 bp half sites separated by a 3 bp spacer with the sequences AGAACAnnnTGTTCT. Within the nucleus the MR recruits and interacts with numerous transcriptional coregulators that assist the transcriptional regulatory actions of the MR. The major transcriptional coactivators that interact with the MR include p300/CBP, SRC-1 (steroid receptor coactivator 1), PGC-1α, and ELL (RNA polymerase II elongation factor). The interaction of ELL with the MR represents a selective transcriptional coactivator. Transcriptional corepressors also interact with the MR including nuclear receptor corepressor 1 (NCoR1: encoded by the NCOR1 gene) and silencing mediator of retinoic and thyroid receptors (SMRT: encoded by the NCOR2 gene).

Initially expression of the aldosterone receptor gene was thought to be restricted to polarized tight epithelial cells such as those present in the nephron of the kidney. However, more recent data demonstrates that the NR3C2 gene is quite ubiquitously expressed and is also expressed in numerous non-epithelial cell types. In additional to the kidneys, the NR3C2 gene is expressed at high levels in the gastrointestinal system, and at moderate levels in the endocrine, reproductive, skeletal, and cardiovascular systems, as well as being expressed at moderate levels in numerous metabolic tissues. Within the central nervous system both the NR3C1 (GR) and NR3C2 (MR) genes are expressed with the NR3C2 expression level being higher than that of NR3C1.

Regulation of Adrenal Steroid Synthesis

Adrenocorticotropic hormone (ACTH), synthesized by corticotropic cells of the anterior pituitary, regulates steroid hormone production in the adrenal cortex, primarily within cells of the zona fasciculata and zona reticularis. ACTH binds to the ACTH receptor present in the plasma membrane of adrenal cortical cells. The ACTH receptor is identified as MC2R for melanocortin-2 receptor. The ACTH receptor is a Gs-type G-protein coupled receptor (GPCR) and ACTH binding triggers activation of adenylate cyclase, elevation of cAMP, and increased PKA. Activation of PKA leads to phosphorylation and activation of cholesterol ester

esterase leading to increased concentrations of free cholesterol, the substrate for steroid hormone synthesis. The effect of ACTH on the production of cortisol is particularly important, with the result that a classic feedback loop results in cortisol inhibiting ACTH release from the pituitary. The ACTH-cortisol regulatory loop is required to the regulation of the circulating levels of corticotropin releasing hormone (CRH), ACTH, and cortisol.

Aldosterone secretion from the zona glomerulosa is stimulated by an entirely different mechanism. Angiotensin II, and to a lesser extent angiotensin III, stimulate zona glomerulosa cells by binding a plasma membrane G-protein coupled receptor (angiotensin receptor 1; AT_1) that is coupled to a G_q-type G-protein that activates phospholipase Cβ (PLCβ). Angiotensin II is derived from the liver synthesized precursor protein, angiotensinogen, via the concerted actions of kidney-derived renin and the membrane-bound Zn^{2+}-dependent protease, angiotensin-converting enzyme (ACE). Angiotensin III is derived from angiotensin II via the action of another membrane-bound Zn^{2+}-dependent protease, glutamyl aminopeptidase. Upon angiotensin II binding to AT_1 there is a resultant increase in PKC activity and an elevation in intracellular Ca^{2+} levels. These events lead to increased CYP11A1 (P450ssc) activity and increased production of aldosterone. In the kidney, aldosterone regulates sodium (Na^+) retention by stimulating the expression of the mRNA for the Na^+/K^+–ATPase responsible for the re-accumulation of sodium from the urine.

The interplay between renin from the kidney and plasma angiotensinogen is important in regulating plasma aldosterone levels, sodium and potassium levels, and ultimately blood pressure. This hormonal regulatory process is referred to as the renin-angiotensin-aldosterone system, RAAS. Among the drugs most widely employed to lower blood pressure are the angiotensin converting enzyme (ACE) inhibitors and the angiotensin receptor blockers (ARBs). All drugs that are ACE inhibitors end with the suffix **–pril** and all drugs that are ARBs end with the suffix **–sartan**. The ACE inhibitors are potent competitive inhibitors of the enzyme that converts angiotensin I to the physiologically active angiotensin II. This feedback loop is closed by potassium, which is a potent stimulator of aldosterone secretion. Changes in plasma potassium of as little as 0.1mM can cause wide fluctuations (±50%) in plasma levels of aldosterone. Potassium increases aldosterone secretion by depolarizing the plasma membrane of zona glomerulosa cells and opening a voltage-gated calcium channel, with a resultant increase in cytoplasmic calcium and the stimulation of calcium-dependent secretory processes.

Although fasciculata and reticularis cells each have the capability of synthesizing androgens and glucocorticoids, the main pathway normally followed is that leading to glucocorticoid production. However, when genetic defects occur in the three enzyme complexes leading to glucocorticoid production, large amounts of the most important androgen, dehydroepiandrosterone (DHEA), are produced. These mutations lead to hirsutism and other masculinizing changes in secondary sex characteristics in females as is seen in several of the congenital adrenal hyperplasias, CAH.

Glucocorticoid Synthesis in the Liver, Adipose Tissue, & Skeletal Muscle

Liver, adipose tissue, and skeletal muscle convert the inactive glucocorticoids, cortisone and 11-dehydrocorticosterone, to the active hormones cortisol and corticosterone via a pathway that is directly controlled by metabolic reactions taking place within the endoplasmic reticulum, ER. The ER has been recognized for many years as a key organelle responding to changes in nutrient levels. Of particular significance to the role of the ER in nutrient responsiveness are cells of liver (hepatocytes), adipose tissue (adipocytes), and the pancreas (β-cells). Extreme metabolic conditions that include both over feeding and prolonged fasting/starvation result in the activation of ER stress response pathways. One major ER stress induced response to aberrant levels of nutrition is the unfolded protein response (UPR). Activation of the UPR can eventually result in insulin resistance, as is typical in type 2 diabetes, apoptosis, and excess inflammatory responses. The consumption of excess calories is also associated with the stimulation of the ER-localized pathways of intracellular glucocorticoid activation in many different cells, but particularly in the liver and adipose tissue. The over feeding induced increases in intracellular glucocorticoids plays an important role in the pathology of obesity, the metabolic syndrome, and type 2 diabetes.

With respect to the ER and intracellular glucocorticoid activation the critical components are glucose-6-phosphate and the ER-localized glucose-6-phosphate dehydrogenase activity, which is referred to as the H form of the glucose-6-phosphate dehydrogenase. The G form of glucose-6-phosphate dehydrogenase is the cytoplasmic enzyme that serves a critical function in the oxidative reactions of the pentose phosphate pathway. The H form of glucose-6-phosphate dehydrogenase activity is identified as hexose-6-phosphate dehydrogenase (encoded by the H6PD gene) and also as glucose 1-dehydrogenase. Whereas the G6PD encoded enzyme resides in the cytosol, the H6PD encoded enzyme resides within the ER and the sarcoplasmic reticulum (SR). The H6PD gene is located on chromosome 1p36.22 and is composed of 7 exons that generate two alternatively spliced mRNAs encoding precursor proteins of 802 amino acids and 791 amino acids. The H6PD gene is not expressed in erythrocytes. Within the ER, hexose-6-phosphate dehydrogenase converts glucose-6-phosphate and $NADP^+$ to 6-phosphogluconate and NADPH in a single step, whereas this process in the cytosol requires two separate enzymes. In addition to glucose-6-phosphate, H6PD can metabolize other hexose-6-phosphates, glucose-6-sulfate, and glucose. One of the primary functions of the ER- and SR-localized NADPH is to maintain redox homeostasis within these organelles. Loss of ER redox homeostasis can lead to ER stress and induction of the unfolded protein response (UPR) which, if severe enough will trigger cell death via the apoptotic pathway.

Another principal function of the NADPH produced by ER-localized hexose-6-phosphate dehydrogenase is to provide the reducing energy to ER-localized reductases, specifically those involved in steroid hormone metabolism, with 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1; encoded by the HSD11B1 gene) being particularly important. The HSD11B1 gene is located on chromosome 1q32.1 and is composed of 7 exons that generate three alternatively spliced mRNAs all of which encode the same 292 amino acid protein. The primary

function of the HSD11B1 encoded enzyme is to reduce the 11-oxo groups (11-oxoreductase activity) in cortisone and 11-dehydrocorticosterone which generates the active glucocorticoids, cortisol and corticosterone, respectively. Although this enzyme can also inactivate (11β-dehydrogenase activity) cortisol and corticosterone by catalyzing the oxidation reactions converting cortisol to cortisone and corticosterone to 11-dehydrocorticosterone, these inactivating reactions are of minimal significance to intracellular glucocorticoid regulation. The primary determinant of the oxo-reductase activity of HSD11B1 is the ratio of NADPH to $NADP^+$ in the ER. Of clinical significance to the role of ER-localized NADPH is that mutations in the H6PD gene are associated with glucocorticoid deficiency.
Humans express a second 11β-hydroxysteroid dehydrogenase encoding gene identified as HSD11B2. Unlike the enzyme encoded by the HSD11B1 gene, the enzyme encoded by the HSD11B2 gene possesses only the 11β-dehydrogenase activity. The HSD11B2 gene is located on chromosome 16q22.1 and is composed of 5 exons that encode a 405 amino acid protein. The HSD11B2 gene is expressed primarily in aldosterone-responsive tissues, such as those of the distal tubules of the nephrons of the kidneys. In these tissues the HSD11B2 encoded enzyme is responsible for inactivating cortisol in order to prevent inappropriate activation of the mineralocorticoid receptor (MR). Although the normal receptor for cortisol is the glucocorticoid receptor (GR), the mineralocorticoid receptor has nearly identical affinities for aldosterone (the mineralocorticoid) and cortisol. Mutations in the HSD11B2 gene are associated with an apparent mineralocorticoid excess-induced hypertension due to the lack of ability to inactivate cortisol. As opposed to the use of $NADP^+$ by the HSD11B1 enzyme in the direction of cortisol and corticosterone oxidation, the HSD11B2 enzyme utilizes NAD^+ as its cofactor. The expression of the HSD11B2 gene is also found in cells that do not also express the MR such as the placenta. In cells that do not express the MR the function of the HSD11B2 enzyme is to protect those cells from the growth-inhibiting effects of cortisone.
Within adipose tissue and skeletal muscle, both major insulin responsive tissues, the intracellular concentration of glucose-6-phosphate is a direct function of the blood levels of both glucose and insulin. In these tissues, insulin action, through its receptor, results in mobilization of GLUT4 transporters to the plasma membrane leading to enhanced glucose uptake from the blood. In the liver, the uptake of glucose, via the GLUT2 transporter, is solely dependent on glucose levels in the blood and given that the K_m of GLUT2 for glucose is high (on the order of 15mM), glucose uptake by the liver only occurs, to a significant degree, during the post-feeding period. As the levels of glucose-6-phosphate rise in these tissues there is a concomitant increase in the activities of the ER-localized proteins that are involved in intracellular glucocorticoid activation. These ER-localized proteins include the H6PD and HSD11B1 encoded proteins, as well as the ER membrane-localized glucose-6-phosphate transporter encoded by the G6PT1 gene. The increase in G6PT1, H6PD, and HSD11B1 activities leads to increased conversion of inactive glucocorticoids to their active forms (cortisol and corticosterone).

Although glucose consumption directly results in increased intracellular glucocorticoid activation, the consumption of fatty acids will indirectly activate this pathway. Free fatty acids are known to interfere with glucose oxidation via the mechanism first proposed by Philip Randle and coworkers in 1963 and now referred to as the glucose-fatty acid cycle. Briefly, the oxidation of fatty acids leads to increased mitochondrial NADH levels which impair the movement of carbon through the TCA cycle resulting in citrate transport to the cytosol which in turn leads to inhibition of the 6-phosphofructo-1-kinase (PFK1) activity of glycolysis. The increased mitochondrial NADH level also inhibits the PDHc reaction further impairing the oxidation of glucose. Thus, one of the effects of over eating, either carbohydrate or lipids, particularly within adipose tissue and skeletal muscle, is enhanced glucocorticoid activation.

When the H6PD gene was knocked out in mice the pathology that resulted included fasting hypoglycemia, low hepatic glycogen content, increased sensitivity to insulin, and decreased negative feedback on the hypothalamic-pituitary-adrenal axis. These results strongly implicate an important role for the triad of G6PT1, H6PD, and HSD11B1 in the metabolic modifications that result in response to feeding. Excess nutrient intake, either in the form of carbohydrate or lipid, can result in increased intracellular glucocorticoid activation, especially in adipose tissue and skeletal muscle, both of which are critical insulin-responsive tissues. Glucocorticoids have been known for quite some time to induce a state of insulin resistance in both adipose tissue and skeletal muscle. Glucocorticoids interfere with insulin signaling in these tissues resulting in impaired GLUT4 mobilization to the plasma membrane, impaired glucose oxidation, and impaired glycogen synthesis. Within visceral adipose tissue, glucocorticoids stimulate preadipocyte differentiation and triglyceride synthesis. In the liver, glucocorticoids stimulate gluconeogenesis which leads to an exacerbation of the hyperglycemia that is the result of insulin resistance in skeletal muscle and adipose tissue. All of these metabolic disturbances contribute to the development of the metabolic syndrome and the onset of type 2 diabetes

Gonadal Steroid Hormones

Although many steroids are produced by the testes and the ovaries, the two most important are testosterone and estradiol. These compounds are under tight biosynthetic control, with short and long negative feedback loops that regulate the secretion of follicle stimulating hormone (FSH) and luteinizing hormone (LH) by the pituitary and gonadotropin releasing hormone (GnRH) by the hypothalamus. Low levels of circulating sex hormone reduce feedback inhibition on GnRH synthesis (the long loop), leading to elevated FSH and LH. The latter peptide hormones bind to gonadal tissue and stimulate P450ssc activity, resulting in sex hormone production via cAMP and PKA mediated pathways. The roles of cAMP and PKA in gonadal tissue are the same as that described for glucocorticoid production in the adrenals, but in this case adenylate cyclase activation is coupled to the binding of LH to plasma membrane receptors.

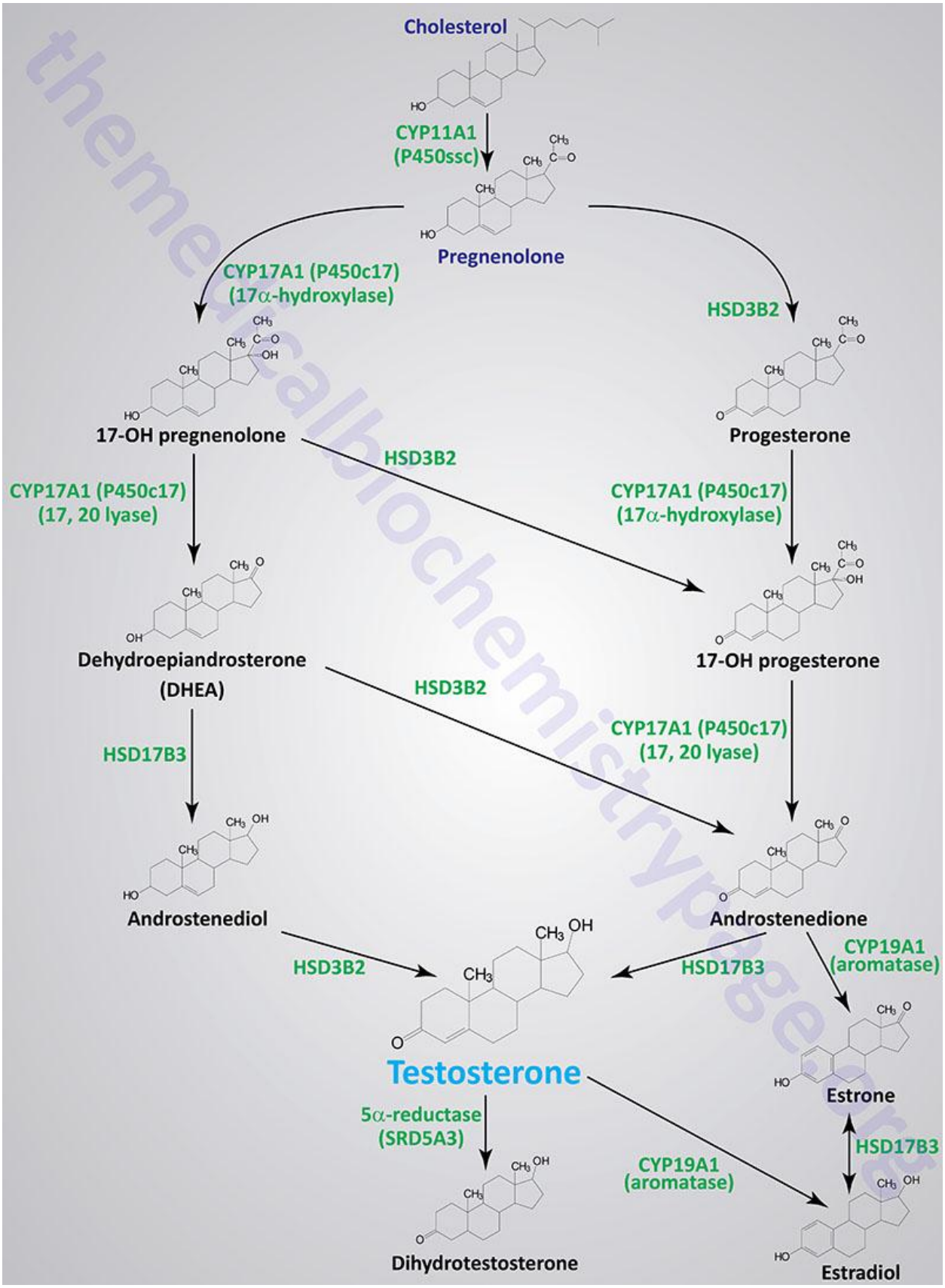
The biosynthetic pathway to sex hormones in male and female gonadal tissue includes the production of the androgens, androstenedione and

dehydroepiandrosterone. Testes and ovaries contain an additional enzyme, a 17β-hydroxysteroid dehydrogenase, that enables androgens to be converted to testosterone.

Male Sex Steroid Hormones

In males, LH binds to Leydig cells, stimulating production of the principal Leydig cell hormone, testosterone. Testosterone is secreted to the plasma and also carried to Sertoli cells by androgen binding protein (ABP). In Sertoli cells the Δ4 double bond of testosterone is reduced, by the action of steroid 5α-reductase, producing dihydrotestosterone (DHT). Testosterone and DHT are carried in the plasma, and delivered to target tissue, by a specific gonadal-steroid binding globulin (GBG). In a number of target tissues, testosterone can be converted to DHT. Dihydrotestosterone is the most potent of the male steroid hormones, with an activity that is 10 times that of testosterone. Because of its relatively lower potency, testosterone is sometimes considered to be a prohormone.

Humans express three different steroid 5α-reductase genes, SRD5A1, SRD5A2, and SRD5A3 where SRD5A3 is the primary enzyme carrying out the testosterone to DHT conversion. The SRD5A1 gene is located on chromosome 5p15.31 and is composed of 7 exons that generate three alternatively spliced mRNAs encoding three distinct isoforms of the enzyme. The SRD5A2 gene is located on chromosome 2p23.1 and is composed of 10 exons that encode a protein of 254 amino acids. Mutations in the SRD5A2 gene are the cause of a form of male pseudohermaphroditism. The SRD5A3 gene is located on chromosome 4q12 and is composed of 6 exons that encode a protein of 318 amino acids. In addition to DHT formation, the SRD5A3 encoded enzyme (also called polyprenol reductase) is required for the conversion of polyprenol to dolichol which is necessary for the synthesis of N-linked glycoproteins. Mutations in the SRD5A3 gene are associated with the development of a particular form of congenital disorder of glycosylation (CDG) identified as CDG-Iq.



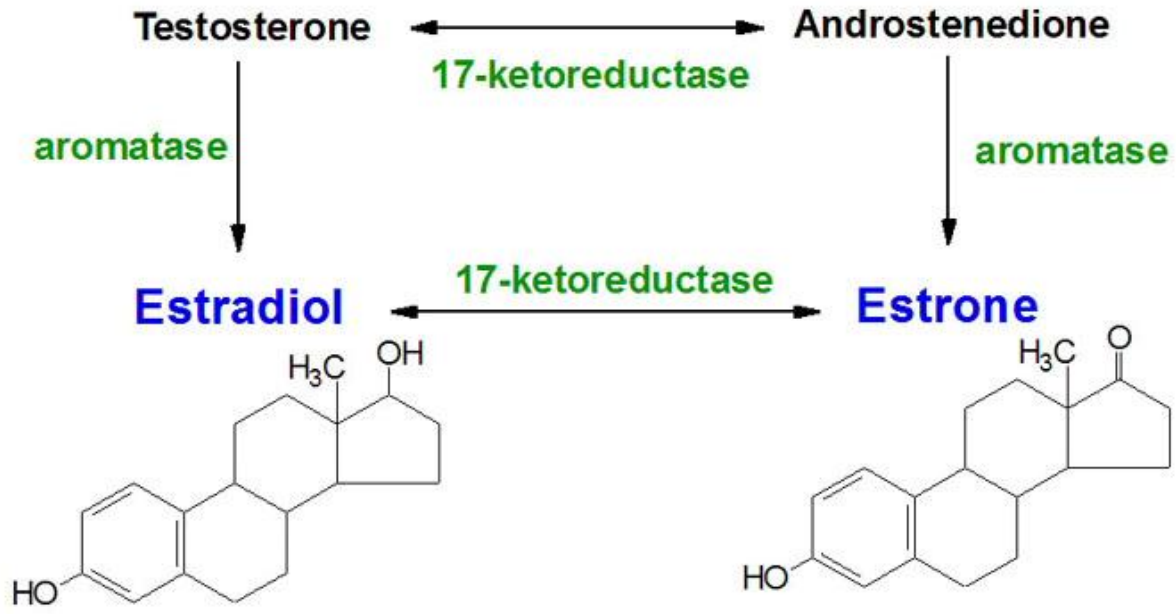
Synthesis of the male sex hormones in Leydig cells of the testis. P450ssc (CYP11A1), 3β-DH (HSD3B2), and P450c17 (CYP17A1) are the same enzymes as those needed for adrenal steroid hormone synthesis. 17,20-lyase is the same activity of CYP17A1 described above for adrenal hormone synthesis. Aromatase (also called estrogen synthetase) is CYP19A1. 17-ketoreductase is also called 17β-

hydroxysteroid dehydrogenase type 3 (gene symbol HSD17B3). The full name for the primary form of 5α-reductase is 5α-reductase type 3 (gene symbol SRD5A3). Humans express three 5α-reductase genes identified as SRD5A1, SRD5A2, and SRD5A3.

Testosterone is also produced by Sertoli cells but in these cells it is regulated by FSH, again acting through a cAMP- and PKA-regulatory pathway. In addition, FSH stimulates Sertoli cells to secrete androgen-binding protein (ABP), which transports testosterone and DHT from Leydig cells to sites of spermatogenesis. There, testosterone acts to stimulate protein synthesis and sperm development.

Female Sex Steroid Hormones

In females, LH binds to thecal cells of the ovary, where it stimulates the synthesis of androstenedione and testosterone by the usual cAMP- and PKA-regulated pathway. An additional enzyme complex known as aromatase is responsible for the final conversion of the latter 2 molecules into the estrogens. Aromatase is a complex endoplasmic reticulum enzyme found in the ovary and in numerous other tissues in both males and females. Its action involves hydroxylations and dehydrations that culminate in aromatization of the A ring of the androgens.



Synthesis of the major female sex hormones in the ovary. Synthesis of testosterone and androstenedione from cholesterol occurs by the same pathways as indicated for synthesis of the male sex hormones. Aromatase (also called estrogen synthetase) is CYP19A1.

Aromatase activity is also found in granulosa cells, but in these cells the activity is stimulated by FSH. Normally, thecal cell androgens produced in response to LH diffuse to granulosa cells, where granulosa cell aromatase converts these androgens to estrogens. As granulosa cells mature they develop competent large numbers of LH receptors in the plasma membrane and become increasingly responsive to LH, increasing the quantity of estrogen produced from these cells. Granulosa cell estrogens are largely, if not all, secreted into follicular fluid. Thecal cell estrogens

are secreted largely into the circulation, where they are delivered to target tissue by the same globulin (GBG) used to transport testosterone.