ADRENOCORTICOTROPIC HORMONE (ACTH; CORTICOTROPIN)

As summarized in Figure 42–1, ACTH is synthesized as part of a larger precursor protein, pro-opiomelanocortin (POMC), and is liberated from the precursor through proteolytic cleavage at dibasic residues by the serine endoprotease, prohormone convertase 1 (also known as prohormone convertase 3). The importance of this enzyme in POMC processing is best illustrated in a rare group of patients with prohormone convertase 1 mutations who present with impaired POMC processing, secondary hypocortisolism, childhood obesity, hypogonadotropic hypogonadism, diabetes, and neonatal onset enteropathy (Farooqi et al., 2007). A number of other biologically important peptides, including endorphins, lipotropins, and the melanocyte-stimulating hormones (MSH), also are produced by proteolytic processing of the same POMC precursor (Chapter 18).

**Figure 42–1.**

*Processing of pro-opiomelanocortin to adrenocorticotropic hormone and the sequence of adrenocorticotropic hormone.* The pathway by which pro-opiomelanocortin (POMC) is converted to adrenocorticotropic hormone (ACTH) and other peptides in the anterior pituitary is depicted. The amino acid sequence of human ACTH is shown. The light blue boxes behind the ACTH structure indicate regions identified as important for steroidogenic activity (residues 6-10) and binding to the ACTH receptor (15-18). α-Melanocyte-stimulating hormone also derives from the POMC precursor and contains the first 13 residues of ACTH. LPH, lipotropin; MSH, melanocyte-stimulating hormone. Human ACTH is a peptide of 39 amino acids (Figure 42–1). Whereas removal of a single amino acid at the amino terminus considerably impairs biological activity, a number of amino acids can be removed from the carboxyl-terminal end without a marked effect. The structure–activity relationships of ACTH have been studied extensively, and it is believed that a stretch of four basic amino acids at positions 15-18 is an important determinant of high-affinity binding to the ACTH receptor, whereas amino acids 6-10 are important for receptor activation.

The actions of ACTH and the other melanocortins liberated from POMC are mediated by their specific interactions with five melanocortin receptor (MC1-5R) subtypes comprising a distinct subfamily of G protein-coupled receptors. The well-known effects of MSH on pigmentation result from interactions with the MC1R on melanocytes. MC1Rs also are found on cells of the immune system and are thought to mediate the anti-inflammatory effects of α-MSH in experimental models of inflammation. ACTH, which is identical to α-MSH in its first 13 amino acids (Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val), exerts its effects on the adrenal cortex through the MC2R. The affinity of ACTH for the MC1R is much lower than for the MC2R; however, under pathological conditions in which ACTH levels are persistently elevated, such as primary adrenal insufficiency, ACTH also can signal through the MC1R and cause hyperpigmentation. Recent studies have defined key roles for β-MSH (Lee et al., 2006) and possibly other melanocortins acting via the MC4R (Farooqi et al., 2003) and MC3R (Mencarelli et al., 2008) in the hypothalamic regulation of appetite and body weight, and they therefore are the subject of considerable investigation as possible targets for drugs that affect appetite. The role of MC5R is less well defined, but studies in rodents suggest that MSH triggers aggressive, pheromone-related behavior via the MC5R (Morgan and Cone, 2006).
ACTIONS ON THE ADRENAL CORTEX

Acting via MC2R, ACTH stimulates the adrenal cortex to secrete glucocorticoids, mineralocorticoids, and the androgen precursor dehydroepiandrosterone (DHEA) that can be converted peripherally into more potent androgens. The adrenal cortex histologically and functionally can be separated into three zones that produce different steroid products under different regulatory influences. The outer zona glomerulosa secretes the mineralocorticoid aldosterone, the middle zona fasciculata secretes the glucocorticoid cortisol, and the inner zona reticularis secretes DHEA (Figure 42–2) and its sulfated derivative DHEAS, which circulates at concentrations 1000 times greater than DHEA. DHEAS can be converted to DHEA in the periphery by DHEA sulfatase.

**Figure 42–2.**

The adrenal cortex contains three anatomically and functionally distinct compartments. The major functional compartments of the adrenal cortex are shown, along with the steroidogenic enzymes that determine the unique profiles of corticosteroid products. Also shown are the predominant physiological regulators of steroid production: angiotensin II (Ang II) and K⁺ for the zona glomerulosa and ACTH for the zona fasciculata. The physiological regulator(s) of dehydroepiandrosterone (DHEA) production by the zona reticularis are not known, although ACTH acutely increases DHEA biosynthesis.

Cells of the outer zone have receptors for angiotensin II and express aldosterone synthase (CYP11B2), an enzyme that catalyzes the terminal reactions in mineralocorticoid biosynthesis. Although ACTH acutely stimulates mineralocorticoid production by the zona glomerulosa, this zone is regulated predominantly by angiotensin II and extracellular K⁺ (Chapter 25) and does not undergo atrophy in the absence of ongoing stimulation by the pituitary gland. In the setting of persistently elevated ACTH, mineralocorticoid levels initially increase and then...
In contrast, cells of the zona fasciculata have fewer receptors for angiotensin II and express two enzymes, steroid 17α-hydroxylase (CYP17) and 11β-hydroxylase (CYP11B1), that catalyze the production of glucocorticoids. In the zona reticularis, CYP17 carries out an additional C17-20 lyase reaction that converts C21 corticosteroids to C19 androgen precursors.

In the absence of the anterior pituitary, the inner zones of the cortex atrophy, and the production of glucocorticoids and adrenal androgens is markedly impaired.

Persistently elevated levels of ACTH, due either to repeated administration of large doses of ACTH or to excessive endogenous production, induce hypertrophy and hyperplasia of the inner zones of the adrenal cortex, with overproduction of cortisol and adrenal androgens. Adrenal hyperplasia is most marked in congenital disorders of steroidogenesis, in which ACTH levels are continuously elevated as a secondary response to impaired cortisol biosynthesis. There is some debate regarding the relative roles of ACTH versus other POMC-derived peptides in stimulating adrenal growth, but the essential role of the anterior pituitary in maintaining the integrity of the zona fasciculata is indisputable.

**MECHANISM OF ACTION**

ACTH stimulates the synthesis and release of adrenocortical hormones. Because specific mechanisms for steroid hormone secretion have not been defined and steroids do not accumulate appreciably in the gland, it is believed that the actions of ACTH to increase steroid hormone production are mediated predominantly at the level of de novo biosynthesis.

ACTH, binding to MC2R (a GPCR), activates the Gs-adenyl cyclase-cyclic AMP-PKA pathway. Cyclic AMP is an obligatory second messenger for most, if not all, effects of ACTH on steroidogenesis. Mutations in MC2R account for ~25% of the cases of familial glucocorticoid deficiency, a rare syndrome of familial resistance to ACTH (Clark et al., 2005).

Temporally, the response of adrenocortical cells to ACTH has two phases. The acute phase, which occurs within seconds to minutes, largely reflects increased supply of cholesterol substrate to the steroidogenic enzymes. The chronic phase, which occurs over hours to days, results largely from increased transcription of the steroidogenic enzymes. A summary of the pathways of adrenal steroid biosynthesis and the structures of the major steroid intermediates and products of the human adrenal cortex are shown in Figure 42–3. The rate-limiting step in steroid hormone production is the conversion of cholesterol to pregnenolone, a reaction catalyzed by CYP11A1, the cholesterol side-chain cleavage enzyme. Most of the enzymes required for steroid hormone biosynthesis, including CYP11A1, are members of the cytochrome P450 superfamily of mixed-function oxidases that play important roles in the metabolism of xenobiotics such as drugs and environmental pollutants, as well as in the biosynthesis of such endogenous compounds as steroid hormones, vitamin D, bile acids, fatty acids, prostaglandins, and biogenic amines (Chapter 6). The rate-limiting components in this reaction regulate the mobilization of substrate cholesterol and its delivery to CYP11A1 in the inner mitochondrial matrix.
Pathways of corticosteroid biosynthesis. The steroidogenic pathways used in the biosynthesis of the corticosteroids are shown, along with the structures of the intermediates and products. The pathways unique to the zona glomerulosa are shown in orange box, whereas those that occur in the inner zona fasciculata and zona reticularis are shown in gray box. The zona reticularis does not express 3β-HSD and thus preferentially synthesizes DHEA. CYP11A1, cholesterol side-chain cleavage enzyme; 3β-HSD, 3β-hydroxysteroid dehydrogenase; CYP17, steroid 17β-hydroxylase; CYP21, steroid 21-hydroxylase; CYP11B2, aldosterone synthase; CYP11B1, steroid 11β-hydroxylase.

To ensure an adequate supply of substrate for steroidogenesis, the adrenal cortex uses multiple sources of cholesterol (Kraemer, 2007), including:
- circulating cholesterol and cholesterol esters taken up via the low-density lipoprotein and high-density lipoprotein receptor pathways
- endogenous cholesterol liberated from cholesterol ester stores via activation of cholesterol esterase
- endogenous cholesterol from de novo biosynthesis.

The mechanisms by which ACTH stimulates the translocation of cholesterol to the inner mitochondrial matrix are not fully defined. A 37,000-Da phosphoprotein—designated the steroidogenic acute regulatory protein—clearly plays essential roles in cholesterol delivery. Mutations in the gene encoding this phosphoprotein are found in patients with congenital lipoid adrenal hyperplasia, a rare congenital disorder in which adrenal cells become engorged with cholesterol deposits secondary to an inability to synthesize any steroid hormones (Stocco, 2002). An important component of the trophic effect of ACTH is the enhanced transcription of genes that encode the individual steroidogenic enzymes, with associated increases in the steroidogenic capacity of the gland. Myriad transcriptional regulators participate in the induction of steroid hydroxylases by ACTH. Among these is the nuclear receptor NR5A1 (steroidogenic factor 1), a transcription factor required for the development of the adrenal cortex and for the expression of most of the steroidogenic enzymes (Parker et al., 2002).

**EXTRA-ADRENAL EFFECTS OF ACTH**

In large doses, ACTH causes a number of metabolic changes in adrenalectomized animals, including ketosis, lipolysis, hypoglycemia (immediately after treatment), and resistance to insulin (later after treatment). Given the large doses of ACTH required, the physiological significance of these extra-adrenal effects is questionable. ACTH also reportedly improves learning in experimental animals, an effect postulated to be mediated via distinct receptors in the CNS.

**REGULATION OF ACTH SECRETION**

Hypothalamic-Pituitary-Adrenal Axis
The rate of glucocorticoid secretion is determined by fluctuations in the release of ACTH by the pituitary corticotropes. These corticotropes are regulated by corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP), peptide hormones released by specialized neurons of the endocrine hypothalamus, which, in turn, are regulated by several neurotransmitters from the CNS. These three organs collectively are referred to as the hypothalamic-pituitary-adrenal (HPA) axis, an integrated system that maintains appropriate levels of glucocorticoids (Figure 42–4). The three characteristic modes of regulation of the HPA axis are diurnal rhythm in basal steroidogenesis, negative feedback regulation by adrenal corticosteroids, and marked increases in steroidogenesis in response to stress. The diurnal rhythm is entrained by higher neuronal centers in response to sleep-wake cycles, such that levels of ACTH peak in the early morning hours, causing the circulating glucocorticoid levels to peak at 8 A.M. As discussed later, negative feedback regulation occurs at multiple levels of the HPA axis and is the major mechanism that maintains circulating glucocorticoid levels in the appropriate range. Stress can override the normal negative feedback control mechanisms, leading to marked increases in plasma concentrations of glucocorticoids.

**Figure 42–4.**

Overview of the hypothalamic-pituitary-adrenal (HPA) axis and the immune inflammatory network. Also shown are inputs from higher neuronal centers that regulate CRH secretion. + indicates a positive regulator, – indicates a negative regulator, + and – indicates a mixed effect, as for NE (norepinephrine). In addition, arginine vasopressin stimulates release of ACTH from corticotropes.

Following release into the hypophyseal plexus, CRH is transported via this portal system to the anterior pituitary, where it binds to specific membrane receptors on corticotropes. Upon CRH binding, the CRH receptor activates the $G_\alpha$–adenyl cyclase–cyclic AMP pathway within corticotropes, ultimately stimulating both ACTH biosynthesis and secretion. CRH and CRH-related peptides called...
arginine vasopressin (AVP) also acts as a secretagogue for corticotropes, significantly potentiating the effects of CRH. Animal studies suggest that the potentiation of CRH action by AVP probably contributes to the full magnitude of the stress response in vivo. Like CRH, AVP is produced in the parvocellular neurons of the paraventricular nucleus and secreted into the pituitary plexus from the median eminence. After binding to V1b receptors, AVP activates the Gq-PLC-IP3-Ca2+ pathway to enhance the release of ACTH. In contrast to CRH, AVP apparently does not increase ACTH synthesis (Surget and Belzung, 2008).

negative feedback of glucocorticoids

Glucocorticoids inhibit ACTH secretion via direct and indirect actions on CRH neurons to decrease CRH mRNA levels and CRH release and via direct effects on corticotropes. The indirect inhibitory effects on CRH neurons appear to be mediated by specific corticosteroid receptors in the hippocampus. At lower cortisol levels, the mineralocorticoid receptor (MR), which has a higher affinity for glucocorticoids than classical glucocorticoid receptors (GR), is the major receptor species occupied. As glucocorticoid concentrations rise and saturate the MR, the GR becomes increasingly occupied. Both the MR and GR apparently control the basal activity of the HPA axis, whereas feedback inhibition by glucocorticoids predominantly involves the GR.

In the pituitary, glucocorticoids act through the GR to inhibit the release of ACTH from corticotropes and the expression of POMC. These effects are both rapid (occurring within seconds to minutes) and delayed (requiring hours and involving changes in gene transcription mediated through the GR).

the stress response

Stress overcomes negative feedback regulation of the HPA axis, leading to a marked rise in corticosteroid production. Examples of stress signals include injury, hemorrhage, severe infection, major surgery, hypoglycemia, cold, pain, and fear. Although the precise mechanisms that underlie this stress response and the essential actions played by corticosteroids are not fully defined, it is clear that their increased secretion is vital to maintain homeostasis in these stressful settings. As discussed later, complex interactions between the HPA axis and the immune system may be a fundamental physiological component of this stress response (Elenkov and Chrousos, 2006).

assays for ACTH

Initially, ACTH levels were assessed by bioassays that measured induced steroid production or the depletion of adrenal ascorbic acid. Immunochemiluminescent assays that use two separate antibodies directed at distinct epitopes on the ACTH molecule now are widely available. These assays increase considerably the ability to differentiate patients with primary hypoadrenalism due to intrinsic adrenal disease, who have high ACTH levels due to the loss of normal glucocorticoid feedback inhibition, from those with secondary forms of hypoadrenalism, due to low ACTH levels resulting from hypothalamic or pituitary disorders. The immunochemiluminescent ACTH assays also are useful in differentiating between ACTH-dependent and ACTH-independent forms of hypercorticism: High ACTH levels are seen when the hypercorticism results from pituitary adenomas (e.g., Cushing's disease) or nonpituitary tumors that secrete ACTH (e.g., the syndrome of ectopic ACTH), whereas low ACTH levels are seen in patients with excessive glucocorticoid production due to primary adrenal disorders. Despite their considerable utility, one problem with the immunoassays for ACTH is that their specificity for intact ACTH can lead to falsely low values in patients with ectopic ACTH secretion; these tumors can secrete aberrantly processed forms of ACTH that have biological activity but do not react in the antibody assays.

therapeutic uses and diagnostic applications of ACTH

There are anecdotal reports that selected conditions respond better to ACTH than to corticosteroids (e.g., multiple sclerosis), and some clinicians continue to advocate therapy with ACTH. Despite this, ACTH currently has only limited utility as a therapeutic agent. Therapy with ACTH is less predictable and less convenient than therapy with corticosteroids. In addition, ACTH stimulates mineralocorticoid and adrenal androgen secretion and may therefore cause acute retention of salt and water, as well as virilization. Although ACTH and the corticosteroids are not pharmacologically equivalent, all proven therapeutic effects of ACTH can be achieved with appropriate doses of corticosteroids with a lower risk of side effects.

testing the integrity of the HPA Axis

The major clinical use of ACTH is in testing the integrity of the HPA axis. Other tests used to assess the HPA axis include the insulin tolerance test (Chapter 38) and the metyrapone test (discussed later in this chapter). Cosyntropin (CORTROSYN, SYNACTHEN) is a synthetic peptide that corresponds to residues 1-24 of human ACTH. At the considerably supraphysiological dose of 250 μg, cosyntropin maximally stimulates adrenocortical steroidogenesis. In the standard cosyntropin stimulation test, 250 μg of cosyntropin is administered either intramuscularly or intravenously, with cortisol measured just before administration (baseline) and 30-60 minutes after cosyntropin administration. An increase in the circulating cortisol to a level greater than 18-20 μg/dL indicates a normal response. Some accept an increase of 9 μg/dL over the
baseline value as a positive response. In patients with pituitary or hypothalamic disease of recent onset or shortly after surgery for pituitary tumors, the standard cosyntropin stimulation test may be misleading because the duration of ACTH deficiency may have been insufficient to cause significant adrenal atrophy with frank loss of steroidogenic capacity. For these patients, some experts advocate a "low-dose" cosyntropin stimulation test, in which 1 μg of cosyntropin is administered intravenously, and cortisol is measured just before and 30 minutes after cosyntropin administration; the cutoff for a normal response is the same as that for the standard test. Care must be taken to avoid adsorption of the cosyntropin to plastic tubing and to measure the plasma cortisol precisely at 30 minutes after the cosyntropin injection. Although some studies indicate that the low-dose test is more sensitive than the standard 250-μg test, others report that this test also may fail to detect secondary adrenal insufficiency.

As already noted, primary adrenocortical insufficiency and secondary adrenocortical insufficiency are reliably distinguished by available sensitive assays for ACTH. More protracted ACTH stimulation tests rarely are used to differentiate between these disorders.

CRH Stimulation Test

Ovine CRH (corticorelin [ACTHREL]) and human CRH are available for diagnostic testing of the HPA axis, with the former used in the U.S. and the latter preferred in Europe. In patients with documented ACTH-dependent hypercorticism, CRH testing may help differentiate between a pituitary source (i.e., Cushing’s disease) and an ectopic source of ACTH. After two baseline blood samples are obtained 15 minutes apart, CRH (1 μg/kg) is administered intravenously over a 30- to 60-second interval, and peripheral blood samples are obtained at 15, 30, and 60 minutes for ACTH measurement. It is important that the blood samples be handled as recommended for the ACTH assay. At the recommended dose, CRH generally is well tolerated, although flushing may occur, particularly if the dose is administered as a bolus. Patients with Cushing’s disease respond to CRH with either a normal or an exaggerated increase in ACTH, whereas ACTH levels generally do not increase in patients with ectopic sources of ACTH. This test is not perfect: ACTH levels are induced by CRH in occasional patients with ectopic ACTH, and 5-10% of patients with Cushing’s disease fail to respond.

To improve the diagnostic accuracy of the CRH stimulation test, many authorities advocate sampling of blood from the inferior petrosal sinuses and the peripheral circulation after peripheral administration of CRH. In this test, an inferior petrosal/peripheral ratio of >2.5 supports a pituitary source of ACTH. When performed by a skilled neuroradiologist, this procedure increases diagnostic accuracy with a tolerable risk of complications from the catheterization procedure (Arnaldi et al., 2003).

Absorption and Fate

ACTH is readily absorbed from parenteral sites. The hormone rapidly disappears from the circulation after intravenous administration; in humans, the t1/2 in plasma is ~15 minutes, primarily due to rapid enzymatic hydrolysis.

Toxicity of ACTH

Aside from rare hypersensitivity reactions, the toxicity of ACTH is primarily attributable to the increased secretion of corticosteroids. Cosyntropin generally is less antigenic than native ACTH; thus, cosyntropin is the preferred agent for clinical use.