The integration of body functions in humans and other higher organisms is carried out by the nervous system, the immune system, and the endocrine system. The endocrine system is composed of a number of tissues that secrete their products, **endocrine hormones**, into the circulatory system; from there they are disseminated throughout the body, regulating the function of distant tissues and maintaining homeostasis. In a separate but related system, **exocrine** tissues secrete their products into ducts and then to the outside of the body or to the intestinal tract. Classically, endocrine hormones are considered to be derived from amino acids, peptides, or sterols and to act at sites distant from their tissue of origin. However, the latter definition has begun to blur as it is found that some secreted substances act at a distance (classical endocrines), close to the cells that secrete them (**paracrines**), or directly on the cell that secreted them (**autocrines**). **Insulin-like growth factor-I (IGF-I)**, which behaves as an endocrine, paracrine, and autocrine, provides a prime example of this difficulty.

Hormones are normally present in the plasma and interstitial tissue at concentrations in the range of 10^{-7}M to 10^{-10}M. Because of these very low physiological concentrations, sensitive protein receptors have evolved in target tissues to sense the presence of very weak signals. In addition, systemic feedback mechanisms have evolved to regulate the production of endocrine hormones.

Once a hormone is secreted by an endocrine tissue, it generally binds to a specific plasma protein carrier, with the complex being disseminated to distant tissues. Plasma carrier proteins exist for all classes of endocrine hormones. Carrier proteins for peptide hormones prevent hormone destruction by plasma proteases. Carriers for steroid and thyroid hormones allow these very hydrophobic substances to be present in the plasma at concentrations several hundred-fold greater than their solubility in water would permit. Carriers for small, hydrophilic amino acid-derived hormones prevent their filtration through the renal glomerulus, greatly prolonging their circulating half-life.

Tissues capable of responding to endocrines have 2 properties in common: they posses a receptor having very high affinity for hormone, and the receptor is coupled to a process that regulates metabolism of the target cells. Receptors for most amino acid-derived hormones and all peptide hormones are located on the plasma membrane. Activation of these receptors by hormones (the first messenger) leads to the intracellular production of a second messenger, such as cAMP, which is responsible for initiating the intracellular biological response. Steroid and thyroid hormones are hydrophobic and diffuse from their binding proteins in the plasma, across the plasma membrane to intracellularly localized receptors. The resultant complex of steroid and receptor bind to response elements of nuclear DNA, regulating the production of mRNA for specific proteins.

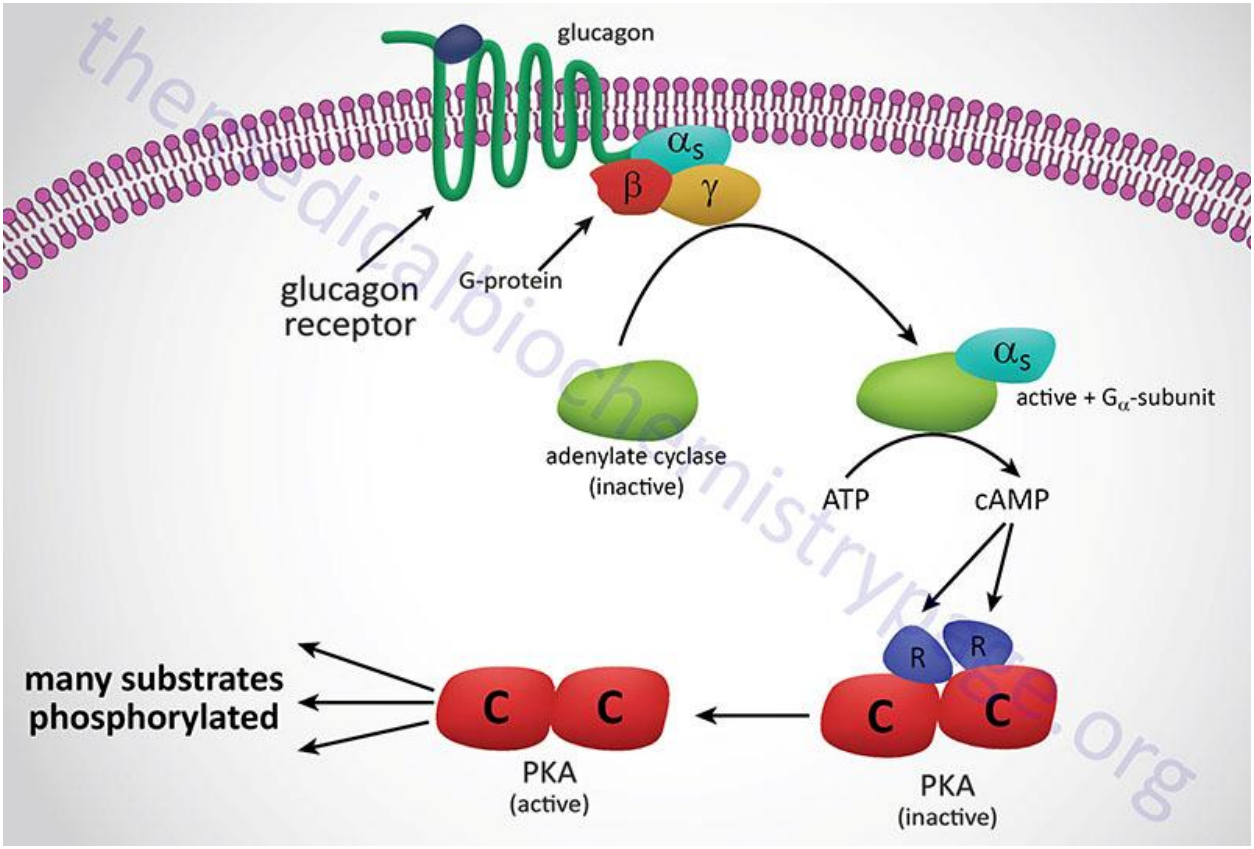
Receptors for Peptide Hormones

With the exception of the thyroid hormone receptor, the receptors for amino acid-derived and peptide hormones are located in the plasma membrane.

Receptor structure is varied: some receptors consist of a single polypeptide chain with a domain on either side of the membrane, connected by a membrane-spanning domain. Some receptors are comprised of a single polypeptide chain that is passed back and forth in serpentine fashion across the membrane, giving multiple intracellular, transmembrane, and extracellular domains. Other receptors are composed of multiple polypeptides. For example, the insulin receptor is a disulfide-linked tetramer with the β-subunits spanning the membrane and the α-subunits located on the exterior surface.

Subsequent to hormone binding, a signal is transduced to the interior of the cell, where second messengers and phosphorylated proteins generate appropriate metabolic responses. The main second messengers are cAMP, Ca^{2+}, inositol-1,4,5-triphosphate (IP_3), and diacylglycerol (DAG). The generation of cAMP occurs via activation of G-protein coupled receptors (GPCRs) whose associated G-proteins activated adenylate cyclase. Adenylate cyclase then converts ATP to cAMP and the subsequent increases in cAMP lead to activation of cAMP-dependent protein kinase (PKA) as shown in the Figure below. GPCRs also couple to G-protein activation of phospholipase C-β (PLCβ). Activated PLCβ hydrolyzes membrane phospholipids (as described below) resulting in increased levels of IP_3 and DAG. Downstream signaling proteins are phosphorylated on serine and threonine by PKA and DAG-activated protein kinase C (PKC) leading to alterations in their activities. Additionally, a series of membrane-associated and intracellular tyrosine kinases phosphorylate specific tyrosine residues on target enzymes and other regulatory proteins.

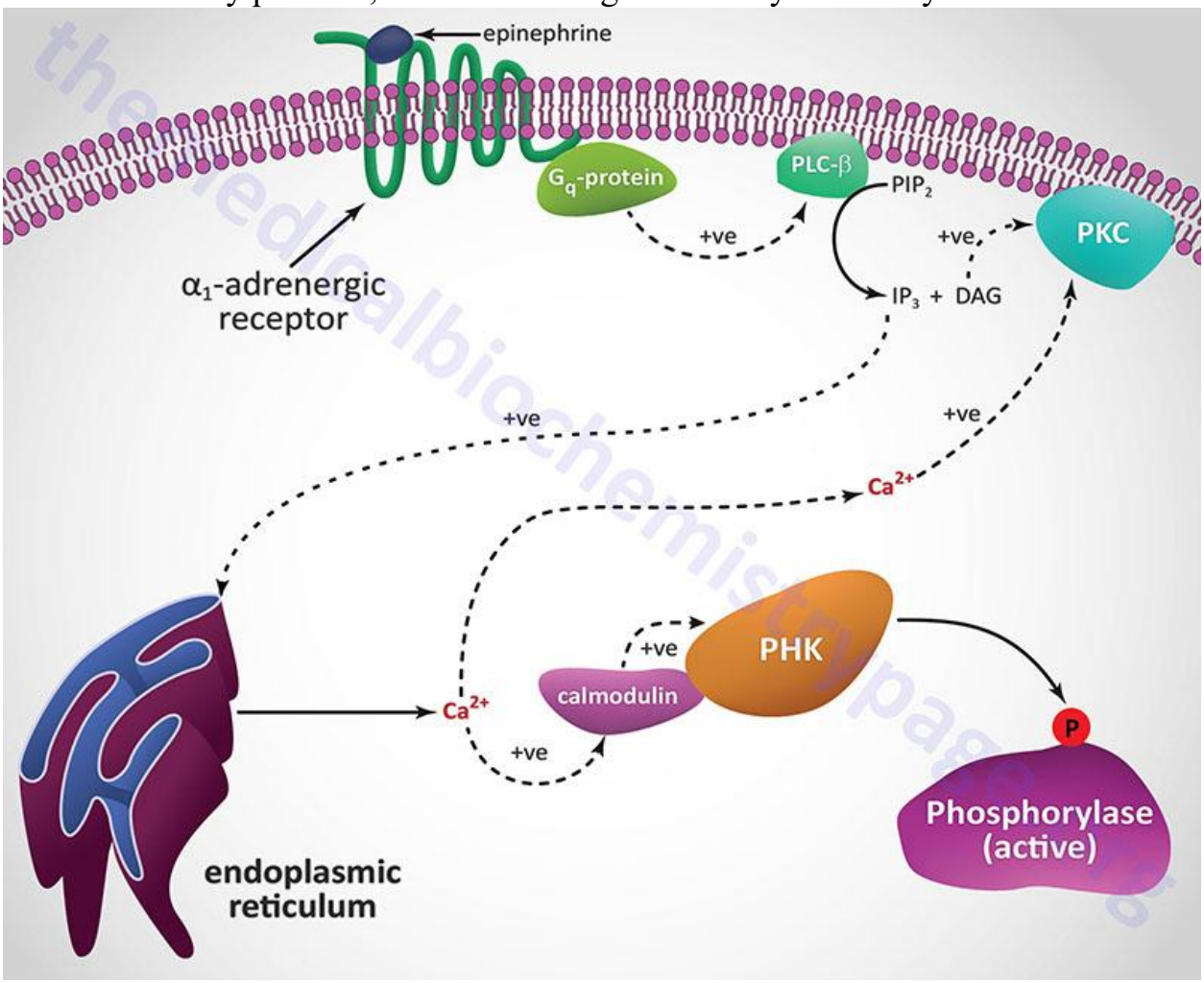
The hormone-binding signal of most, but not all, plasma membrane receptors is transduced to the interior of cells by the binding of receptor-ligand complexes to a series of membrane-localized GDP/GTP binding proteins known as G-proteins. The classic interactions between receptors, G-protein transducer, and membrane-localized adenylate cyclase are illustrated below using the pancreatic hormone glucagon as an example. When G-proteins bind to receptors, GTP exchanges with GDP bound to the α subunit of the G-protein. The G_α-GTP complex binds adenylate cyclase, activating the enzyme. The activation of adenylate cyclase leads to cAMP production in the cytosol and to the activation of PKA, followed by regulatory phosphorylation of numerous enzymes. Stimulatory G-proteins are designated G_s, inhibitory G-proteins are designated G_i.



Representative pathway for the activation of cAMP-dependent protein kinase, PKA. In this example glucagon binds to its' cell-surface receptor, there by activating the receptor. Activation of the receptor is coupled to the activation of a receptor-coupled G-protein (GTP-binding and hydrolyzing protein) composed of three subunits. Upon activation the α-subunit dissociates and binds to and activates adenylate cyclase through the energy of GTP hydrolysis. Adenylate cyclase then converts ATP to cyclic-AMP (cAMP). The cAMP thus produced then binds to the regulatory subunits of PKA leading to dissociation of the associated catalytic subunits. The catalytic subunits are inactive until dissociated from the regulatory subunits. Once released the catalytic subunits of PKA phosphorylate numerous substrates using ATP as the phosphate donor.

A second class of peptide hormones induces the transduction of two second messengers, DAG and IP_3 (diagrammed below for α_1-adrenergic stimulation by epinephrine). Hormone binding to receptor is followed by interaction with a stimulatory G-protein which is followed in turn by G-protein activation of membrane-localized PLCβ. G-proteins that are coupled to receptor-activation of PLCβ are termed G_q-proteins. PLCβ hydrolyzes phosphatidylinositol-4,5-bisphosphate (PIP_2) to produce two messengers: IP_3, which is soluble in the cytosol, and DAG, which remains in the membrane phase. Cytosolic IP_3 binds to specific receptors in the endoplasmic reticulum (ER) membranes, which are ligand-gated Ca2+ channels allowing stored Ca^{2+} to be released to the cytosol. The released ER Ca^{2+} activates numerous enzymes, many by activating their calmodulin or calmodulin-like subunits. DAG has two roles: it binds and activates protein kinase C (PKC), and it opens Ca^{2+} channels in the plasma membrane,

reinforcing the effect of IP_3. Like PKA, PKC phosphorylates serine and threonine residues of many proteins, thus modulating their catalytic activity.



Pathways involved in the regulation of glycogen phosphorylase by epinephrine activation of α_1-adrenergic receptors. Epinephrine (and norepinephrine) activation of α_1-adrenergic receptors involves the subsequent activation of the associated G_q-type G-protein followed by activation of phospholipase Cβ, PLCβ. Active PLCβ hydrolyzes membrane-associated phosphatidylinositol-4,5-bisphosphate (PIP_2) into diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (IP_3). The released IP_3 binds to receptors in ER/SR membranes which results in release of stored Ca^{2+} to the cytosol. In the case of glycogen phosphorylase, the Ca^{2+} binds to, and activates the calmodulin subunit of PHK, thereby activating this enzyme. PHK is phosphorylase kinase. PHK is sometimes referred to as glycogen synthase-glycogen phosphorylase kinase. Active PHK phosphorylates and activates glycogen phosphorylase resulting in increased glucose release from glycogen.

Only one receptor class, that for the natriuretic peptides (e.g. atrial natriuretic peptide, ANP: also sometimes called atrial natriuretic factor, ANF), has been shown to be coupled to the production of intracellular cGMP. ANP, a peptide secreted by cardiac atrial tissue, is much like other peptide hormones in that it is secreted into the circulatory system and has effects on distant tissues. The principal sites of ANP action are within vascular smooth muscle cells leading to vasodilation

and in the kidney glomerulus, where it modulates the rate of filtration, increasing Na+ excretion in the urine. The receptors for the natriuretic factors are integral plasm membrane proteins, whose intracellular domains catalyze the formation of cGMP following natriuretic factor-binding. Intracellular cGMP itself exerts effects in vascular smooth muscle and in addition it activates cGMP-dependent protein kinase (PKG), which phosphorylates and modulates enzyme activity, leading to additional biological effects of the natriuretic factors.

Many amino acid and peptide hormones are elaborated by neural tissue, with ultimate impact on the entire system. When their composition was still unknown, hypothalamic secretory products were known as releasing factors, since their effect was to release endocrine hormones from the pituitary. More recently the releasing factors have been renamed releasing hormones. Currently, both names are in common use.

Releasing hormones are synthesized in neural cell bodies of the hypothalamus and secreted at the axon terminals into the portal hypophyseal circulation, which directly bathes the anterior pituitary. These peptides initiate a cascade of biochemical reactions that culminate in hormone-regulated, whole-body biological end points. Cells of the anterior pituitary, with specific receptors for individual releasing hormones, generally respond through a Ca^{2+}, IP_3, PKC-linked pathway that stimulates exocytosis of preexisting vesicles containing the various anterior pituitary hormones. The pituitary hormones are carried via the systemic circulation to target tissues throughout the body. At the target tissues they generate unique biological activities.

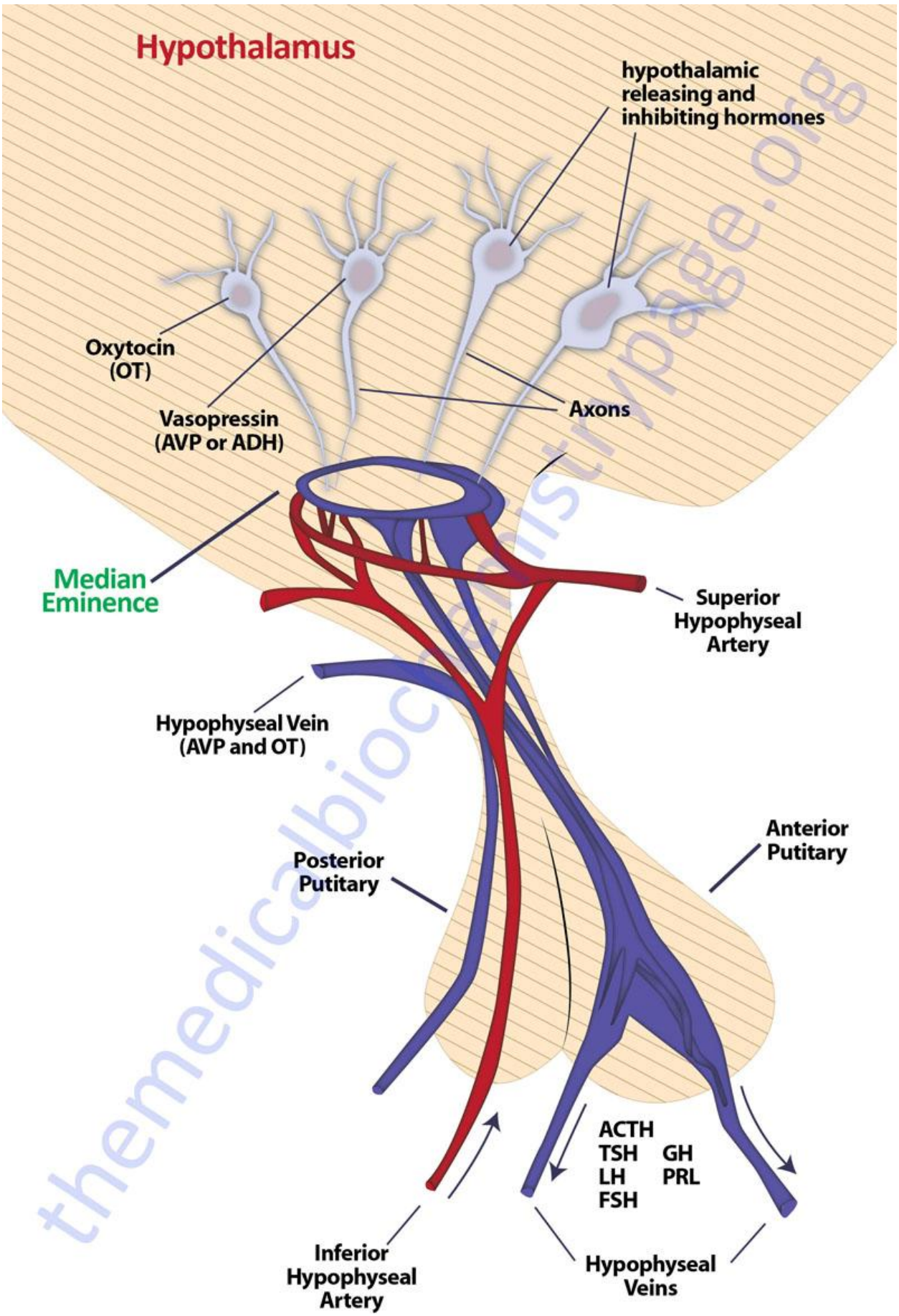
The secretion of hypothalamic, pituitary, and target tissue hormones is under tight regulatory control by a series of feedback and feed- forward loops. This complexity can be demonstrated using the growth hormone (GH) regulatory system as an example. The stimulatory substance growth hormone releasing hormone (GHRH) and the inhibitory substance somatostatin (SS) both products of the hypothalamus, control pituitary GH secretion. Somatostatin is also called growth hormone-inhibiting hormone (GHIH). Under the influence of GHRH, growth hormone is released into the systemic circulation, causing the target tissue to secrete insulin-like growth factor-1, IGF-1. Growth hormone also has other more direct metabolic effects; it is both hyperglycemic and lipolytic. The principal source of systemic IGF-1 is the liver, although most other tissues secrete and contribute to systemic IGF-1. Liver IGF-1 is considered to be the principal regulator of tissue growth. In particular, the IGF-1 secreted by the liver is believed to synchronize growth throughout the body, resulting in a homeostatic balance of tissue size and mass. IGF-1 secreted by peripheral tissues is generally considered to be autocrine or paracrine in its biological action.

Systemic IGF-1 also has hypothalamic and pituitary regulatory targets. The negative feedback loops cause down-regulation of GH secretion directly at the pituitary. The longer positive feedback loop, involving IGF-1 regulation at the hypothalamus, stimulates the secretion of GHIH, which in turn inhibits the secretion of growth hormone by the pituitary. The latter is a relatively unusual

negative feed-forward regulatory process. In addition, a shorter negative feedback loop is shown that involves direct IGF-1 action on the pituitary, leading to down-regulation of GH secretion. Similar feedback loops exist for all the major endocrine hormones, and many subtle nuances modulate each regulatory loop.

The Hypothalamic-Pituitary Axis

The hypothalamus is located below the thalamus and just above the brain stem and is composed of several domains (nuclei) that perform a variety of functions. The hypothalamus forms the ventral portion of the region of the brain called the diencephalon. Anatomically the hypothalamus is divided into three broad domains termed the posterior, tuberal, and anterior regions. Each of these three regions is further subdivided into medial and lateral areas. The various nuclei of the hypothalamus constitute the functional domains of the various hypothalamic areas. A few of the specific nuclei of the hypothalamus include the paraventricular nucleus (PVN) which is located in the anterior medial area and is involved in oxytocin and vasopressin release from the pituitary and the arcuate nucleus of the hypothalamus (ARC, also abbreviated ARH), the dorsomedial hypothalamic nucleus (DMH), and the ventromedial nucleus (VMN) all of which are located in the tuberal medial area. The ARC is involved in control of feeding behavior as well as secretion of various pituitary releasing hormones, the DMH is involved in stimulating gastrointestinal activity, and the VMN is involved in satiety (sensation of being full). The most important overall function of the hypothalamus is to link the central nervous system to the endocrine system via the pituitary gland (also termed the hypophysis). The hypothalamus is involved in the control of certain metabolic processes as well as other functions of the autonomic nervous system. With respect to this discussion the hypothalamus synthesizes and secretes a variety of neurohormones, referred to as hypothalamic-releasing factors, that act upon the pituitary to direct the release of the various pituitary hormones.



Diagrammatic representation of the interactions between the hypothalamus and the pituitary. The hypothalamic releasing and inhibiting hormones exert their effects on the release of anterior pituitary hormones. Oxytocin and vasopressin (antidiuretic hormone, ADH) are released directly from hypothalamic axons that terminate in the posterior pituitary, and the hormones are secreted from there directly into the systemic circulation. AVP: arginine vasopressin. ADH: antidiuretic hormone. ACTH: adrenocorticotropic hormone. TSH: thyroid stimulating hormone. LH: luteinizing hormone. FSH: follicle stimulating hormone. GH: growth hormone. PRL: prolactin.

The pituitary gland has two lobes called the posterior and anterior lobes. Each lobe secretes peptide hormones that exert numerous effects on the body. Each of the pituitary hormones is described in detail in the following sections. It is the aim of this discussion to provide the background for understanding what pituitary hormones are released and what are the triggers for their release. The posterior pituitary excretes the two hormones, oxytocin and vasopressin. The anterior pituitary secretes six hormones: adrenocortiocotropic hormone (ACTH, also called corticotropin), thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), growth hormone (GH), and prolactin (PRL). The hormone ACTH is derived from a large precursor protein identified as pro-opiomelanocortin (POMC) as described below. The secretion of the anterior pituitary hormones is under control of the hypothalamus, hence the description of the system as the hypothalamic-pituitary axis. The secretion of the hormones ACTH, TSH, FSH , LH, and GH are stimulated by signals from the hypothalamus, whereas, PRL secretion is inhibited by hypothalamic signals.

The secretion of anterior pituitary hormones results in response to hypophysiotropic hormones that are carried in the portal hypophysial vessels from the hypothalamus to the pituitary. These hypothalamic hormones are commonly referred to as releasing or inhibiting hormones. There are six hypothalamic releasing and inhibiting hormones: corticotropin-releasing hormone (CRH), thyrotropin-releasing hormone (TRH), gonadotropin-releasing hormone (GnRH), luteinizing hormone-releasing hormone (LHRH), growth hormone-releasing hormone (GHRH), growth hormone release-inhibiting hormone (GHIH, more commonly called somatostatin), and prolactin release-inhibiting hormone (PIH or PIF). Hypothalamic extracts also contain a prolactin-releasing substance (sometimes referred to as prolactin-releasing hormone, PRH). Several peptides found in the hypothalamus (e.g. TRH) can stimulate prolactin secretion so it is as yet unclear whether PRH is the physiologic prolactin-releasing substance. GnRH has been shown to stimulate the release of both FSH and LH and as a consequence the term GnRH is more appropriately used than LHRH.

The hypothalamic releasing and inhibiting hormones are secreted from the median eminence of the hypothalamus. The GnRH-secreting neurons are primarily in the medial preoptic area of the hypothalamus. The somatostatin-

secreting neurons reside in the periventricular nuclei. The TRH-secreting and CRH-secreting neurons are found in the medial parts of the periventricular nuclei. The GHRH-secreting neurons reside in the arcuate nuclei which is the same region that contains dopamine-secreting neurons. Most of the receptors for the hypophysiotropic hormones are GPCRs.

The Gonadotropins

The glycoprotein hormones are the most chemically complex family of the peptide hormones. All members of the family are highly glycosylated. Each of the glycoprotein hormones is an (α:β) heterodimer, with the α-subunit being identical in all members of the family. The biological activity of the hormone is determined by the β-subunit, which is not active in the absence of the α-subunit.

The molecular weight of the gonadotropins (follicle stimulating hormone, FSH; luteinizing hormone, LH, and human chorionic gonadotropin, hCG) is about 25,000 Daltons, whereas that of the thyroid tropic hormone, thyroid stimulating hormone (TSH) is about 30,000. Synthesis of FSH and LH occurs in the same cells of the anterior pituitary and secretion of both is controlled by the hypothalamic decapeptide hormone GnRH. All members of the glycoprotein family transduce their intracellular effects via their respective receptors and the associated G-protein, adenylate cyclase, second-messenger systems. The gonadotropins (LH, FSH and hCG) bind to cells in the ovaries and testes, stimulating the production of the steroid sex hormones estrogen, testosterone (T), and dihydrotestosterone (DHT).

FSH and LH

The synthesis and release of FSH and LH is controlled by the action of the hypothalamic releasing factor GnRH. The function of GnRH is to induce an episodic release of both FSH and LH that determines the onset of puberty and ovulation in females. GnRH binds to its receptor on gonadotropes and initiates a signaling cascade that results in release of FSH and LH. The control of the hypothalamic-pituitary axis at the level of FSH and LH is controlled by several additional proteins including follistatin, activin, and leptin. Follistatin is a protein that binds to and inhibits proteins of the transforming growth factor-β family (TGFβ) of which activin is a member. Therefore, follistatin inhibits the activity of activin on promoting FSH synthesis and release.

In females FSH stimulates follicular development and estrogen synthesis by granulosa cells of the ovary. In males FSH promotes testicular growth and within the Sertoli cells of the seminiferous tubules of the testis FSH enhances the synthesis of androgen-binding proteins, ABP. The function of ABP is to bind testosterone (T) and dihydrotestosterone (DHT), as well as 17β-estradiol, resulting in the concentration of the male sex hormones within these cells. The concentration of T and DHT leads to the enhancement of spermatogenesis. In females, LH induces thecal cells of the ovary to synthesize estrogens and progesterone and promotes estradiol secretion. The surge in LH release that occurs in mid-menstrual

cycle is the responsible signal for ovulation. Continuous LH secretion stimulates the corpus luteum to produce progesterone. In males, LH binds to Leydig cells of the testis resulting in the induction of the steroidogenic acute regulatory (StAR) protein. The function of StAR is to transport cholesterol from the outer mitochondrial membrane to the inner membrane where steroid hormone biosynthesis is initiated, therefore, the result of the induction of StAR synthesis is increased synthesis and secretion of T.

The predominant form of the FSH receptor is a 678 amino acid glycosylated protein that is a member of the GPCR family of receptors. Binding of FSH to its receptors results in activation of adenylate cyclase leading to increased PKA activity.

The LH receptor is referred to as the LH-choriogonadotropin receptor (LHCGR). The LHCGR contains a large extracellular domain that includes several leucine-rich repeats (LRR). There are other members of the GPCR family that contain LRR in their extracellular domains and this subfamily of receptors is referred to as the LRR-containing GPCR (LRG) family. The LHCGR is coupled a G-protein that activates adenylate cyclase resulting in increased PKA activity. The LHCGR is expressed in the ovary, thecal cells, stromal cells, luteinizing granulosa, and luteal cells of the ovary and in Leydig cells of the testes.

Thyroid Stimulating Hormone (TSH)

As indicated above, TSH (also called thyrotropin) is a member of the glycoprotein hormone family and as such is composed of a common α-subunit encoded by the CGA gene and a unique β-chain. The β-chain of TSH is encoded by the TSHB gene (thyroid-stimulating hormone, β-chain) which is on chromosome 1p13.2 and contains 3 exons, the first of which is non-coding.

Secretion of TSH is stimulated by thyrotropin-releasing hormone (TRH) from the hypothalamus. TRH, a tripeptide, is synthesized by neurons in the supraoptic and supraventricular nuclei of the hypothalamus and stored in the median eminence. TRH is transported to the anterior pituitary via the pituitary portal circulation and binds to a specific receptor located on TSH- and prolactin-secreting cells. There are two TRH receptors, identified as TRH-R1 and TRH-R2, both of which are G-protein coupled receptors (GPCRs). Both TRH receptors are coupled to G_q-type G-proteins. Binding of TRH to its receptor activates a typical PLCβ-mediated signaling cascade. The TRH-induced signaling leads to TSH secretion as well as increased TSH transcription and post-translational glycosylation. Although both receptors are expressed differentially in the brain and in peripheral tissues, they exhibit indistinguishable TRH-binding affinities. However, only TRH-R1 is expressed at functional levels in the anterior pituitary. The TRH-mediated release of TSH is pulsatile with peak secretion being exerted between midnight and 4am.

The synthesis and release of TSH is controlled by two pathways. The first is exerted by the level of T3 within thyrotropic cells which regulates TSH

expression, translation and release. The second regulation is of course exerted by TRH as described above. While in the circulation TSH binds to receptors on the basal membrane of thyroid follicles. The receptors are coupled through G-protein activation of adenylate cyclase as well as PLCβ. The TSH receptor gene (symbol: TSHR) is on chromosome 14q31.1 and is composed of 12 exons that generate three alternatively spliced mRNAs. The major TSHR encoded protein is a 764 amino acid glycosylated member of the GPCR family of receptors. The TSHR and the LHCGR proteins share a significant degree of homology. TSH binding to its receptor triggers a signaling cascade that results in increased thyrocyte cAMP, PKA, IP_3, and DAG leading to, in the short term, increased secretion of the thyroid hormones, thyroxin (T4) and triiodothyronine (T3). TSH-binding to its receptor also results in increased TSH synthesis and thyroid cell growth.

Chronic stimulation of the TSH receptor causes an increase in the synthesis of a major thyroid hormone precursor, thyroglobulin. Thyroglobulin produced on rough endoplasmic reticulum has a molecular weight of 660,000. It is glycosylated and contains more than 100 tyrosine residues, which become iodinated and are used to synthesize T3 and T4. Thyroglobulin is exocytosed through the apical membrane into the closed lumen of thyroid follicles, where it accumulates as the major protein of the thyroid and where maturation takes place. Mature, iodinated thyroglobulin is taken up in vesicles by thyrocytes and fuses with lysosomes. Lysosomal proteases degrade thyroglobulin releasing amino acids and T3 and T4, which are secreted into the circulation. These compounds are very hydrophobic and require a carrier protein for delivery to target tissues. In the plasma, T3 and T4 are bound to a carrier glycoprotein known as thyroxin-binding globulin and are disseminated throughout the body in this form. The feedback loop that regulates T3 and T4 production is a single short negative loop, with the T3 and T4 being responsible for down-regulating pituitary TSH secretion. Meanwhile, continuously secreted hypothalamic TRH is responsible for up-regulating TSH production. The TSH actually secreted by thyrotropes is the net result of the negative effects of T3 and T4 and the positive effect of TRH.

Thyroid hormones act by binding to cytosolic receptors very similar to steroid hormone receptors, and for this reason T3 and T4 are often classified along with the hydrophobic steroid hormones. The principal role of thyroid hormones is also like that of steroid hormones. In adults, the ligand receptor combination binds to thyroid hormone response elements in nuclear DNA and is responsible for up-regulating general protein synthesis and inducing a state of positive nitrogen balance.

Numerous congenital and acquired forms of hypothyroidism and hyperthyroidism are the result of alterations in the expression, processing, and function of the TSHR. The most common TSHR disorder resulting in hyperthyroidism (thyrotoxicosis) is Graves disease. Graves disease is caused by thyroid-stimulating autoantibodies (TSAb, also called thyroid-stimulating immunoglobulins, TSIs) which bind to and activate the human TSH receptor, leading to the thyrotoxicosis characteristic of this disease. TSAbs bind to the TSH receptor and mimic the TSH

stimulation of the gland by increasing intracellular cAMP. The hyperactivated thyroid then secretes excessive T3 and T4. Graves disease is classified as a form of thyrotoxicosis, the name for the clinical syndrome resulting from tissues exposed to high levels of thyroid hormones. One theory proposed for the development of the TSAb is that there is a defect in suppressor T cells that allows helper T cells to stimulate B cells to produce thyroid autoantibodies. The clinical features of Graves disease are thyrotoxicosis, goiter (enlarged thyroid gland), an ophthalmopathy in the form of exophthalmos (eyes bulge out), and dermopathy in the form of pretibial myxedema (localized lesions of the skin, primarily in the lower legs, resulting from the deposition of hyaluronic acid).

At the other end of the spectrum are disorders that lead to hypothyroidism. Deficiency in iodine is the most common cause of hypothyroidism worldwide. Indeed the practice of producing iodized table salt was to stem the occurrence of hypothyroidism. When hypothyroidism is evident in conjunction with sufficient iodine intake it is either autoimmune disease (Hashimoto thyroiditis) or the consequences of treatments for hyperthyroidism that are the cause. In the embryo, thyroid hormone is necessary for normal development and hypothyroidism in the embryo is responsible for cretinism, which is characterized by multiple congenital defects and mental retardation. Because the neurological consequences of congenital hypothyroidism are severe neonatal screening for thyroid hormone levels at birth is routine. Most infants born with congenital hypothyroidism appear normal at birth. However, if left untreated the symptoms will include a thick protruding tongue, poor feeding, prolonged jaundice (which exacerbates the neurological impairment), hypotonia (recognized as "floppy baby syndrome"), episodes of choking, and delayed bone maturation resulting in short stature.

The Pro-Opiomelanocortin (POMC) Family

POMC is expressed in both the anterior and intermediate lobes of the pituitary gland. The primary protein product of the POMC gene is a 285 amino acid precursor that can undergo differential processing to yield at least 8 peptides, dependent upon the location of synthesis and the stimulus leading to their production. POMC is produced in the pituitary, the ARC of the hypothalamus, the nucleus of the solitary tract (NTS for Latin term *nucleus tractus solitarii*; specialized cells within the medulla responsible for sensations of taste and visceral sensations of stretch), as well as in several peripheral tissues such as the skin and reproductive organs. Within the brain neurons that respond to POMC-derived peptides (termed POMCm neurons) are critical in the regulation of overall energy balance via the melanocortin peptides (primarily α-MSH; this is N-terminally acetylated MSH).

The processing of POMC involves glycosylations, acetylations, and extensive proteolytic cleavage at sites shown to contain regions of basic protein sequences.

The proteases that recognize these cleavage sites are tissue-specific; thus, the physiologically active product of the anterior pituitary is ACTH .

Many of the other POMC products are synthesized in other neural tissues that contain proteases with appropriate specificity. In human embryos and in pregnant women, the intermediate lobe is active and leads to the production of endorphins and enkephalins. These same endorphin-producing pathways are active in other neural tissues, and since they bind to the opioid receptors in other parts of the brain they are assumed to represent natural opioid-like analgesic compounds.

Adrenocorticotropic Hormone, ACTH

ACTH is a 39 amino acid peptide that is derived by post-translational modification from the 241 amino acid preproprotein, POMC. ACTH is the main physiologically active product of the actions of the hypothalamic releasing hormone, CRH, on the anterior pituitary. Although CRH is the primary stimulus for ACTH release, other hormones also exert effects on ACTH release. CRH stimulates a pulsatile secretion of ACTH with peak levels seen before waking and declining as the day progresses. Negative feedback on ACTH secretion is exerted by cortisol at both the hypothalamic and anterior pituitary levels. Thus, the primary product of the systemic actions of ACTH regulates the further actions of this corticotropic hormone. Additional factors that influence ACTH secretion include physical, emotional, and chemical stresses. These stressors include pain, cold exposure, acute hypoglycemia, trauma, depression, and surgery. The stress-mediated increases in ACTH secretion are the result of the actions of vasopressin and CRH.

The biological role of ACTH is to stimulate the production of adrenal cortex steroids, principally the glucocorticoids cortisol and corticosterone. ACTH also stimulates the adrenal cortex to produce the mineralocorticoid, aldosterone as well as the androgen, androstenedione. ACTH exerts its effects on the adrenal cortex by binding to a specific receptor that is a member of the melanocortin receptor family. 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 activity of adrenal cortex tissue. The main effect of these events is to increase the activity of CYP11A1 (also called P450-linked side chain-cleaving enzyme, P450ssc, 20,22-desmolase, or cholesterol desmolase), which converts cholesterol to pregnenolone during steroid hormone synthesis. Because of the distribution of enzymes in the various adrenal cortex subdivisions, the principal physiological effect of ACTH is production of the glucocorticoids.

Secondary adrenal insufficiency occurs in patients with deficiencies in pituitary ACTH production or secretion. Whereas, primary adrenal insufficiency (adrenal hypoplasia) is characteristic of Addison disease which was originally diagnosed as the result of lesions in the adrenal glands caused by tuberculosis. Secondary adrenal insufficiency is characterized by weakness, fatigue, nausea, vomiting, and

anorexia. On the opposite side of the abnormal ACTH spectrum are the adrenal hyperplasias. These include the congenital adrenal hyperplasias (CAH) and Cushing syndrome. The CAH are a family of inherited disorders that result from loss-of-function mutations in one of several genes involved in adrenal steroid hormone synthesis. Endogenous causes of Cushing syndrome are pituitary corticotrope adenomas resulting in excess ACTH production and secretion. The characteristic features of Cushing syndrome are psychiatric disturbances (depression, mania, and psychoses), central obesity, hypertension, diabetes, moon-shaped face, thin fragile skin, easy bruising, and purple striae (stretch marks). In addition, Cushing syndrome patients manifest with gonadal dysfunction that is characteristic of hyperandrogenism with excess body and facial hair (hirsutism) and acne.

Vasopressin and Oxytocin

The principal hormones of the posterior pituitary are the nonapeptides oxytocin and vasopressin (mammalian form is called arginine vasopressin, AVP). Vasopressin is also known as antidiuretic hormone (ADH). The amino acid sequences of vasopressin and oxytocin differ by only two amino acids. Both of these hormones are synthesized as prohormones in neural cell bodies of the hypothalamus and mature as they pass down axons in association with carrier proteins termed **neurophysins**. The axons terminate in the posterior pituitary, and the hormones are secreted directly into the systemic circulation. The neurophysins themselves are derived from the oxytocin and vasopressin preproproteins. The oxytocin preproprotein contains neurophysin 1 and the vasopressin preproprotein contains neurophysin 2.

Vasopressin is known as antidiuretic hormone (ADH), because it is the main regulator of body fluid osmolarity through induced renal reabsorption of water. The designation arginine vasopressin (AVP) is used when discussing vasopressins from different mammals. Marsupials and pigs produce a vasopressin peptide where the arginine is replaced by a lysine and is thus, referred to as lysine vasopressin. The secretion of vasopressin is regulated in the hypothalamus by osmoreceptors which sense water and Na^+ concentration and stimulate increased vasopressin secretion when plasma osmolarity increases. The secreted vasopressin increases the reabsorption rate of water in principal cells of the collecting ducts of the kidney tubule, causing the excretion of urine that is concentrated in Na^+ and thus yielding a net drop in osmolarity of body fluids. Vasopressin deficiency leads to production of large volumes of watery urine and to polydipsia (increased desire for fluid intake). These symptoms are diagnostic of a condition known as diabetes insipidus. Diabetes insipidus has numerous causes that include effects on the hypothalamus and/or pituitary (central diabetes insipidus) or the kidneys (nephrogenic diabetes insipidus).

Vasopressin binds plasma membrane receptors are that G-protein coupled receptors (GPCR) that activate signaling events through associated G-proteins

that are coupled to the cAMP second messenger system or through the PLCβ pathway. There are three kinds of vasopressin receptors designated V_{1A} (V1A or just V1), V_{1B} (V1B: also known as the V3 receptor), and V_2 (V2).

The V_{1A} and V_{1B} receptors are GPCR that activate G_q-type G-proteins leading to hydrolysis of PIP_2 resulting in increased intracellular Ca^{2+} concentration and the activation of PKC. The V_2 receptor is a GPCR that activates G_s-type G-proteins leading to the activation of adenylate cyclase resulting in increased cAMP levels and activation of PKA. The V_{1A} (V1) receptors are found in vascular smooth muscle cells and vasopressin binding to these receptors triggers vascular contraction resulting in increased blood pressure. The V_{1A} receptor is also expressed in smooth muscle cells of the uterus (myometrium), in hepatocytes, and in platelets. The V_2 receptor is found primarily in the principal cells of the collecting ducts of the kidney tubules where its activation is responsible for triggering vasopressin-mediated water retention, thereby, affecting osmolarity. Activation of the V_2 receptor triggers the mobilization of vesicles containing the water transporter, aquaporin 2 (AQP2), to the apical membranes of principal cells. Mutations in the gene encoding the V_2 receptor are responsible for X-linked nephrogenic diabetes insipidus. The V_2 receptor is also expressed in vascular endothelial and vascular smooth muscle cells. The V_{1B} (V_3) receptor is expressed in the anterior pituitary.

Oxytocin is produced in the magnocellular neurosecretory cells of the hypothalamus and is then stored in axon terminals of the anterior pituitary. While stored in the pituitary, oxytocin is bound to neurophysin I in Herring bodies. Secretion of oxytocin is stimulated by electrical activity of the oxytocin cells of the hypothalamus. The actions of oxytocin are elicited via the interaction of the hormone with high affinity receptors. The oxytocin receptor is a GPCR that is coupled to a G_q type G-protein leading to activation of PLCβ and thus the hydrolysis of PIP_2 resulting in increased intracellular Ca^{2+} concentration and the activation of PKC. The affinity for the oxytocin receptor for oxytocin is dependent upon Mg^{2+} and cholesterol, both of which act as positive allosteric regulators.

Oxytocin secretion in nursing women is stimulated by direct neural feedback obtained by stimulation of the nipple during suckling. This response to oxytocin is referred to as the "let-down response". Its physiological effects include the contraction of mammary gland myoepithelial cells, which induces the ejection of milk from mammary glands. The other primary action of oxytocin is the stimulation of uterine smooth muscle contraction leading to childbirth. The uterine effect of oxytocin is due, in part, to increased production and release of the prostaglandin $PGF_{2\alpha}$ from the myometrium and to a lesser extent from the decidua. In males the circulating levels of oxytocin increase at the time of ejaculation. It is believed that the increase in oxytocin levels causes increased contraction of the smooth muscle cells of the vas deferens thereby propelling the sperm toward the urethra.

Growth Hormone

Growth hormone (GH), human chorionic somatomammotropin, hCS (also called human placental lactogen, hPL), and prolactin (PRL) comprise the growth hormone family. All have about 200 amino acids, 2 disulfide bonds, and no glycosylation. Although each has special receptors and unique characteristics to their activity, they all possess growth-promoting and lactogenic activity. Mature GH (22,000 daltons) is synthesized in acidophilic pituitary somatotropes as a single polypeptide chain. Because of alternate RNA splicing, a small amount of a somewhat smaller molecular form is also secreted.

Humans respond to natural or recombinant human or primate growth hormone with appropriate secretion of IGF-1, but growth hormone of other species has no normal biological effect in man. The latter is puzzling because interspecies GH homologies are quite high in many cases, and most other species respond well to human growth hormone. In humans, growth hormone promotes gluconeogenesis and is consequently hyperglycemic. It promotes amino acid uptake by cells, with the result that GH therapy puts an organism into positive nitrogen balance, similar to that seen in growing children. Finally, growth hormone is lipolytic, inducing the breakdown of tissue lipids and thus providing energy supplies that are used to support the stimulated protein synthesis induced by increased amino acid uptake.

There are a number of genetic deficiencies associated with GH. GH-deficient dwarfs lack the ability to synthesize or secrete GH, and these short-statured individuals respond well to GH therapy. Pygmies lack the IGF-1 response to GH but not its metabolic effects; thus in pygmies the deficiency is post-receptor in nature. Finally, Laron dwarfs have normal or excess plasma GH, but lack liver GH receptors and have low levels of circulating IGF-1. The defect in these individuals is clearly related to an inability to respond to GH by the production of IGF-1. The production of excessive amounts of GH before epiphyseal closure of the long bones leads to gigantism, and when GH becomes excessive after epiphyseal closure, sacral bone growth leads to the characteristic features of acromegaly.

Human Chorionic Somatomammotropin (hCS)

Human chorionic somatomammotropin is produced by the placenta late in gestation. This hormone has also been called human placental lactogen (hPL) and chorionic growth hormone-prolactin (CGP). The amino acid composition of hCS is similar to human growth hormone. Evidence suggests that due to the similarities between growth hormone, hCS and prolactin they likely evolved from a single progenitor hormone gene. At its height the hormone is secreted at a rate of about 1 g/day, the highest secretory rate of any known human hormone. However, little hCS reaches the fetal circulation. The amount of hCS that is

secreted is proportional to the size of the placenta. Low levels of hCS during pregnancy are a sign of placental insufficiency. The biological actions of hCS are similar to those of growth hormone suggesting that it functions as a maternal growth hormone of pregnancy. The hormone induces the retention of potassium, calcium, and nitrogen, decreases glucose utilization and increases lipolysis.

Prolactin (PRL)

Prolactin is produced by acidophilic pituitary lactotropes. The prolactin protein is 198 amino acids in length with a molecular weight of 22,000 Daltons. Prolactin is known to bind zinc (Zn^{2+}) and the binding of this metal stabilizes prolactin in the secretory pathway. Prolactin is the lone trophic hormone of the pituitary that is routinely under negative control by prolactin release-inhibiting hormone (PIH or PIF), which is, in fact, the catecholamine neurotransmitter, dopamine. Decreased hypophyseal dopamine production, or damage to the hypophyseal stalk, leads to rapid up-regulation of PRL secretion. A number of other hypothalamic releasing hormones induce increased prolactin secretion; as a result, it is unclear whether a specific prolactin-releasing hormone (PRH) exists for up-regulating PRL secretion. Prolactin does not appear to play a role in normal gonadal function yet hyperprolactinemia in humans results in hypogonadism.

Prolactin secretion increases during pregnancy and promotes breast development in preparation for the production of milk and lactation. Although prolactin serves this important function in breast development during pregnancy there is no evidence to indicate that it functions during normal breast tissue development before or during puberty. During pregnancy the increased production of estrogen enhances breast development but it also suppresses the effects of prolactin on lactation. Following parturition (birth) estrogen (as well as progesterone) levels fall allowing lactation to occur. This is to ensure that lactation is not induced until the baby is born.

The Pancreatic Polypeptide Family

The pancreatic polypeptide (PP) family of hormones comprises two gut hormones, pancreatic polypeptide (PP) and peptide tyrosine-tyrosine (PYY), as well as the central nervous system hormone neuropeptide Y (NPY). Each of these peptide hormones contains 36 amino acids consisting of numerous tyrosines (hence the Y peptides nomenclature) and an α-amidation at the C-terminus. The three-dimensional structure of these hormones includes a hairpin-like motif referred to as the pancreatic polypeptide fold (PP-fold). The PP-fold is required for interaction of the hormones with specific G-protein coupled receptors (GPCRs).

Gastrointestinal Hormones and Peptides

There are more than 30 peptides currently identified as being expressed within the digestive tract, making the gut the largest endocrine organ in the body. The regulatory peptides synthesized by the gut include hormones, peptide neurotransmitters and growth factors. Indeed, several hormones and neurotransmitters first identified in the central nervous system and other endocrine organs have subsequently been found in endocrine cells and/or neurons of the gut. Visit the Table of Vertebrate Hormones page to see a more complete list of gastrointestinal peptides and hormones.

Hormone	Location	Major Action
Cholecystokinin (CCK)	enteroendocrine I cells predominantly of the duodenum, jejunum	stimulates gallbladder contraction and bile flow; increases secretion of digestive enzymes from pancreas; vagal nerves in the gut express CCK1 (CCK_A) receptors
Enterostatin	derived from N-terminal end of pancreatic colipase; pentapeptide human enterostatin contains the sequence: APGPR	regulates fat intake; peripheral or central administration inhibits consumption of a high-fat diet but not a low-fat diet
FGF19	gallbladder, duodenum, ileum	member of the large FGF family of growth factors; expression of FGF19 gene activated by transcription factor FXR, FXR is activated when ileal enterocytes absorb bile acids, when released to the portal circulation FGF19 stimulates hepatic glycogen and protein synthesis while inhibiting glucose production; reduces the expression and activity of CYP7A1 which is the rate-limiting enzyme in bile acid synthesis; acts in the gallbladder to induce relaxation and refilling with bile acids
Gastrin	made in enteroendocrine G cells of the gastric antrum and duodenum	gastric acid and pepsin secretion; exists in two major forms: little gastrin (17 amino acids) and big gastrin (34 amino acids), both result from a single

		precursor protein of 101 amino acid; both species contain a Y residue in the C-terminal portion of the protein that may be either sulfated (gastrin II) or nonsulfated (gastrin I); both forms bind to the CCK2 (CCK_B) receptor on stomach and gut parietal cells with an affinity equal to that of CCK; the C-terminal tetrapeptide of both gastrins and CCK are identical and possess all the biological activities of both gastrin and CCK
Gastrin-releasing peptide (GRP), is a bombesin-related peptide; also called neuromedin B	stomach, duodenum	released from vagal nerve; stimulates release of gastrin from G cells of the stomach and CCK from small intestinal enteroendocrine I cells
Ghrelin	primary site is A (X-like) enteroendocrine cells of the stomach oxyntic (acid secreting) glands, minor synthesis in intestine, pancreas and hypothalamus	regulation of appetite (increases desire for food intake); energy homeostasis; glucose metabolism; gastric secretion and emptying, insulin secretion
Glucagon-like peptide 1 (GLP-1)	enteroendocrine L cells predominantly in the ileum and colon	potentiates glucose-dependent insulin secretion; inhibits glucagon secretion; inhibits gastric emptying
Glucagon-like peptide 2 (GLP-2)	enteroendocrine L cells predominantly in the ileum and colon	enhances digestion and food absorption; inhibits gastric secretions; promotes intestinal mucosal growth
Glucose-dependent insulinotropic polypeptide (GIP), originally called gastric inhibitory polypeptide	enteroendocrine K cells of the duodenum and proximal jejunum	inhibits secretion of gastric acid; enhances insulin secretion
Motilin	proximal small intestine	initiates inter-digestive intestinal motility; stimulates release of PP; stimulates gallbladder contractions

Nesfatin-1	primarily expressed in enteroendocrine X/A-like cells in stomach and in white adipose tissue	proteolytic product of the 420 amino acid precursor protein encoded by the NUCB2 (nucleobindin 2) gene; following removal of the 24 amino acid signal peptide the 396 amino acid peptide is cleaved by proprotein convertases 1/3 (PC 1/3) and PC2; nesfatin-1 consists of amino acids 1–82; proteolytic processing also generates nesfatin-2 (amino acids 85–163) and nesfatin-3 (amino acids 166–396); stimulates reduced feeding via actions in the hypothalamus
Neurotensin	enteroendocrine N cells along the small intestine and proximal large intestine; also in hypothalamus	a 13-amino acid peptide derived from a precursor that also produces neuromedin; involved in satiety responses and slows gastric emptying; also involved in nociception (sensation of pain)
Obestatin	primary site is stomach, minor synthesis in intestine	derived from pro-ghrelin protein; acts in opposition to ghrelin action on appetite
Oxyntomodulin	enteroendocrine L cells predominantly in the ileum and colon	contains all of the amino acids of glucagon (see Figure below); inhibits meal-stimulated gastric acid secretion similar to GLP-1 and GLP-2 action; induces satiety, decreases weight gain, and increases energy consumption; has weak affinity for GLP-1 receptor as well as the glucagon receptor, may mimic glucagon actions in liver and pancreas
Pancreatic polypeptide: PP	pancreas F cells, colon and rectum	inhibits pancreatic bicarbonate and protein secretion
Peptide tyrosine tyrosine (PYY)	enteroendocrine L cells predominantly in the ileum and colon	reduced gut motility; delays in gastric emptying, and an inhibition of gallbladder contraction; exerts effects on

		satiety via actions in the hypothalamus
Secretin	enteroendocrine S cells of the duodenum and to a lesser extent the jejunum	pancreatic bicarbonate secretion; inhibits gastric secretions; stimulates PP secretion
Somatostatin	made by D cells of the gut and δ-cells of the pancreas, also produced in hypothalamus and other organ systems	inhibits release and action of numerous gut peptides, e.g. CKK, PP, gastrin, secretin, motilin, GIP; also inhibits insulin and glucagon secretion from pancreas
Substance P, a member of the tachykinin family that includes neurokinin A (NKA) and neurokinin B (NKB)	entire gastrointestinal tract	CNS function in pain (nociception); involved in vomit reflex; stimulates salivary secretions; induces vasodilation antagonists; have anti-depressant properties
Vasoactive intestinal peptide (VIP)	pancreas	smooth muscle relaxation; stimulates pancreatic bicarbonate secretion

Natriuretic Hormones

Natriuresis refers to enhanced urinary excretion of sodium. This can occur in response to specific hormonal signals, in certain disease, states and through the action of diuretic drugs. At least three natriuretic hormones have been identified and are referred to as ANP. Atrial natriuretic peptide (ANP: also called atrial natriuretic factor, ANF) was the first cardiac natriuretic hormone identified. This hormone is secreted by cardiac myocytes when sodium chloride intake is increased and when the volume of the extracellular fluid expands. Specifically, ANP is released from myocytes in the wall of the right atrium of the heart in response to increased venous volume returning to the heart via the inferior and superior vena cavae. Active ANP is a 28-amino acid peptide containing a 17-amino acid ring formed by intrachain disulfide bonding. Two smaller forms of ANP have also been isolated from the brain. A second natriuretic peptide (originally called BNP since it was first isolated from porcine brain) has been identified and found in human heart and blood (but not human brain). BNP is a 32-amino acid peptide and has different amino acids in its 17-amino acid ring as well as being encoded for by a different gene. Although the acronym BNP is still commonly used, the protein is now known as B-type natriuretic peptide or also ventricular natriuretic peptide (but still with the BNP acronym) since it is secreted by cardiac ventricular myocytes. In humans, a third natriuretic peptide (CNP) is present in the brain but not in the heart.

The action of ANP is to cause natriuresis presumably by increasing glomerular filtration rate (its exact mechanism of action remains unclear). ANP induces relaxation of the mesangial cells of the glomeruli and thus may increase the surface area of these cells so that filtration is increased. Alternatively, ANP might act on tubule cells to increase sodium excretion. Other effects of ANP include reducing blood pressure, decreasing the responsiveness of adrenal glomerulosa cells to stimuli that result in aldosterone production and secretion, inhibit secretion of vasopressin, and decreasing vascular smooth muscle cell responses to vasoconstrictive agents. These latter actions of ANP are counter to the effects of angiotensin II. In fact, ANP also lowers renin secretion by the kidneys thus, lowering circulating angiotensin II levels.

Three different natriuretic peptide receptors have been identified. These receptors are ANPR-A, ANPR-B, and ANPR-C. The natriuretic peptide receptors are members of the larger family of single transmembrane spanning guanylate cyclase enzymes. ANP and BNP are the natural ligands for the ANPR-A receptor and bind with relatively equivalent affinity and elicit the same responses. CNP is the ligand for the ANPR-B receptor. The function of the ANPR-C protein is to serve as a clearance receptor removing natriuretic peptides from the blood via receptor-mediated endocytosis.

When ANP or BNP bind to receptor, an increase in the intrinsic guanylate cyclase activity results leading to production of cyclic GMP (cGMP). Within vascular smooth muscle cells the increased cGMP exerts numerous effects resulting in smooth muscle relaxation and vasodilation. Activation of cGMP-dependent protein kinase (specifically PKGIα) leads to phosphorylation of regulatory subunits of myosin light chain phosphatases leading to enhanced removal of phosphates from myosin light chains and reduced contractile activity. The activity of cGMP is also direct in that it inhibits smooth muscle plasma membrane L-type Ca^{2+} channels resulting in reduced intake of Ca^{2+} and, as a consequence, reduced activation of mysosin light chain kinases furthering vasodilation.

Renin-Angiotensin System

The renin-angiotensin system (RAS; also commonly called the renin-angiotensin-aldosterone system, RAAS) is responsible for regulation of blood pressure. Bioactive angiotensin (angiotensin II) is derived from the precursor protein, angiotensinogen, predominantly produced by the liver. Angiotensinogen is derived from the AGT gene which is located on chromosome 1q42.2 and is composed of 5 exons that encode a 485 amino acid preproprotein. Renin is a protease/hormone produced by the kidneys and is responsible for cleavage of angiotensinogen initiating the production of bioactive angiotensin II. Following removal of the leader peptide from preprorenin, functional renin is released from prorenin by an as yet unidentifed renal protease. Given that the circulating levels of angiotensinogen and angiotensin converting enzyme are in excess, the release of renin from the kidney represents the rate limiting step in the RAAS.

The intra-renal baroreceptor system is a key mechanism for regulating renin secretion. A drop in blood pressure results in the release of renin from juxtaglomerular cells (JG cells; also called granular cells) which are specialized smooth muscle cells in the wall of the afferent arterioles at the base of the glomerulus in the juxtaglomerular apparatus. These are the only cells in the human body that synthesize and secrete renin. Renin secretion is also regulated by the rate of Na^+ and Cl^- transport across the apical (tubular lumen side) membrane of epithelial cells of the macula densa. The kidney macula densa is a cluster of specialized epithelial cells found in the nephron just distal to the loop of Henle. The transporter responsible for the sensing of Na^+ and Cl^- flux is the apical (tubular lumen side) membrane-localized NKCC2 (Na^+-K^+-Cl^- cotransporter 2) transporter. The higher the rate of transport of these ions the lower the rate of renin secretion. The only enzymatic function for renin (an aspartyl protease) is to cleave a 10-amino acid peptide from the N-terminal end of angiotensinogen. This cleaved decapeptide is called angiotensin I. Recent evidence has demonstrated that prorenin and renin can exert effects, unrelated to the enzymatic activity of renin, through binding to a specific receptor expressed within the vasculature and the glomerulus of the kidney (see below).

Angiotensin I is then cleaved by the action of angiotensin-converting enzyme (ACE: a membrane-bound Zn^{2+}-dependent dicarboxypeptidase) generating the bioactive octapeptide hormone, angiotensin II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe). The function of ACE is to remove two amino acids from the C-terminal end of angiotensin I. Angiotensin-converting enzyme is highly expressed by vascular endothelial cells, renal proximal tubular epithelial cells, ciliated intestinal epithelial cells, lung epithelia, and developing male germ cells. Expression is also found in several areas of the brain and in the choroid plexus. When monocytes differentiate to macrophages, and when dendritic cells are immunologically activated, expression of ACE is induced. Unlike renin which has a single substrate, ACE exhibits activity to a wide range of substrates ranging in size from tripeptides to proteins of 42 amino acids. The major substrates for ACE are angiotensin I and bradykinin, both of which are involved in the regulation of blood pressure.

Angiotensin II can be cleaved by another membrane-bound Zn^{2+}-dependent peptidase (glutamyl aminopeptidase, also known as aminopeptidase A), which removes one amino acid from the N-terminal end generating the heptapeptide hormone angiotensin III (Arg-Val-Tyr-Ile-His-Pro-Phe). The vasopressive activity of angiotensin III is similar, but less potent, than angiotensin II and is exerted by binding to the same receptors to which angiotensin II binds. Like angiotensin II, angiotensin III exerts effects leading to increased blood pressure. Whereas, angiotensin II exerts its effects equivalently on several target tissues, angiotensin III is most potent via actions within the central nervous system. Indeed, evidence indicates that angiotensin III is the primary effector of the renin-angiotensin system in the control of blood pressure via the central sympathetic nervous system.

As indicated above, the renin gene encodes a 406 amino acid preproprotein. Removal of the signal peptide (20 amino acids) and the pro portion (46 amino acids) of prorenin results in the production of the 340 amino acid active form of the enzyme. Several alternative mRNAs are generated from the REN gene via alternative promoter usage and via alternative splicing. However, the function of these alternative mRNAs is not fully defined. The angiotensin-converting enzyme gene (symbol: ACE) is located on chromosome 17q23.3 and is composed of 26 exons that generate three alternatively spliced mRNAs, each of which generate a distinct ACE isoform. ACE isoform 1 is referred to as the endothelial or somatic form and is a 1306 amino acid precursor. The ACE isoform 2 is referred to as the testicular form and is a 732 amino acid precursor.

Angiotensin II was originally identified as hypertensin and angiotonin. It is one of the most potent naturally occurring vasoconstrictors. In addition to its effects on the vasculature, angiotensin II (and its derivative angiotensin III) acts on the kidneys to increase renal tubular Na^+ and Cl^- reabsorption, water retention, and K^+ excretion, on the posterior pituitary to induce the release of vasopressin (anti-diuretic hormone, ADH), stimulates the zona glomerulosa cells of the adrenal cortex to secrete aldosterone, and exerts effects in the central nervous system resulting in increased sympathetic outflow from the rostral ventrolateral medulla of the brain stem. The vasoconstrictive action of angiotensin II is primarily exerted on the arterioles and leads to a rise in both systolic and diastolic blood pressure. It is this action of angiotensin II on blood pressure that led to the development of a class of drugs called the ACE inhibitors for use as anti-hypertensive drugs. As the name implies, ACE inhibitors prevent ACE from converting angiotensin I to angiotensin II. All of the drugs that inhibit the activity of ACE contain the suffix "**–pril**", e.g. captopril. One side-effect of the use of ACE inhibitors is a persistent dry cough leading to lack of compliance with the drug in a number of patients. The cause of the dry cough is due to reduced degradation of bradykinin, a known substrate for ACE. Although bradykinin is primarily involved in the contractile activity of vascular smooth muscle, it also induces constriction of the bronchioles in the lungs and it is this latter activity that contributes to the dry cough with the use of ACE inhibitors.

In individuals that are depleted of sodium or who have liver disease (e.g. cirrhosis), the pressive actions of angiotensin II are greatly reduced. These conditions lead to increased circulating levels of angiotensin II which in turn leads to a down-regulation in the numbers of angiotensin II receptors on smooth muscle cells. As a consequence, administration of exogenous angiotensin II to these individuals has little effect. In addition to the other physiological responses to angiotensin II indicated, angiotensin II affects the contractility of the mesangial cells of the kidney leading to decreased glomerular filtration rate. One additional effect of angiotensin II is to potentiate the release of norepinephrine from adrenal medullary chromaffin cells.

Two distinct types of angiotensin II receptors have been identified, AT_1R and AT_2R. The AT_1R is a classical serpentine G-protein coupled receptor (GPCR). The

AT_1R is coupled to both a G_q-protein that leads to activation of PLCβ, and a G_i-protein that inhibits adenylate cyclase. The AT_1R is expressed in the heart, blood vessels, kidney, adrenal cortex, lung and brain. The AT_1R protein is encoded by the AGTR1 gene located on chromosome 3q24. The AT_2 receptor is also a GPCR and is coupled to activation of G_i-proteins. Expression of the AT_2R is limited, in the adult, to the brain (predominantly the cerebellum), adrenal gland, and myometrium. Within the brain the primary AT_2R ligand is angiotensin III.

Due primarily to the development of a dry hacking cough with the use of ACE inhibitors, drugs that block the activity of the AT_1R were developed for the treatment of hypertension. This class of drug is called the angiotensin receptor blocker (ARB) class. All of the ARB drugs are AT_1R antagonists and thus, exert their anti-hypertensive effects at the level of the receptor itself. All drugs in the ARB class contain the suffix "**–sartan**". In addition to their use in the treatment of hypertension the ARBs are used to treat diabetic nephropathy and congestive heart failure.

Parathyroid Hormone (PTH)

Parathyroid hormone (PTH) is synthesized and secreted by chief cells of the parathyroid glands in response to systemic Ca^{2+} levels. There are four parathyroid glands that lie adjacent to the thyroid gland and consist of two superior glands and two inferior glands. As PTH is synthesized it is processed through the ER and Golgi apparatus where first the signal peptide is removed and then the propeptide, with the active PTH molecule, is stored in dense neuroendocrine-type granules.

There exists a related protein identified as PTH-related protein (PTHrP). PTHrP was identified originally as a protein causing severe hypercalcemia in patients with pheochromocytoma (PCC) which is a rare malignancy of the chromaffin cells of the adrenal medulla. The normal functions of PTHrP include roles in fetal bone development where it suppresses the maturation of chondrocytes so that the onset of hypertrophic differentiation during endochondral bone growth is delayed. In addition PTHrP exhibits antiproliferative effects in adults by regulating epidermal and hair follicle cell growth as well as inhibiting angiogenesis.

Secretion of PTH from the parathyroid gland is controlled via the activity of the Ca^{2+} sensing receptor (CaSR). The CaSR is expressed in the parathyroid glands, renal tubule cells, bone marrow, thyroid gland C-cells, gastrin-secreting cells in the stomach, several areas of the brain, as well as in several other tissues. The CaSR is a G-protein coupled receptor (GPCR) that responds to small changes in circulating Ca2+ concentrations. When initially characterized, the CaSR was the first receptor shown to be activated by a ligand that was an ion. The CaSR belongs to the class C GPCR family. The CaSR is coupled to the activation of G_i, G_q, and G_{12}-type G-proteins resulting in decreased production of cAMP and increased production of DAG and and IP_3.

The rate of PTH secretion is controlled by the interaction of Ca^{2+} with the CASR. The synthesis and secretion of PTH from chief cells is constitutive, but Ca^{2+} regulates the level of PTH in chief cells (and thus its secretion) by increasing the rate of PTH proteolysis when plasma Ca^{2+} levels rise and by decreasing the proteolysis of PTH when Ca^{2+} levels fall. The role of PTH is to regulate Ca^{2+} concentration in extracellular fluids. The feedback loop that regulates PTH secretion therefore involves the parathyroid gland, extracellular Ca^{2+}, and the PTH target tissues.

PTH acts by binding to cAMP- and PLCβ-activating plasma membrane receptors, initiating a cascade of reactions that culminates in the biological response. There are two receptors that recognize PTH identified as the PTH-1 and PTH-2 receptors (PTH1R and PTH2R). Both receptors are related to a small sub-family of peptide hormone receptors that includes the receptors for ACTH, calcitonin, vasoactive intestinal peptide (VIP), and secretin.

The PTH receptors are coupled to G_s-type G-proteins that activate adenylate cyclase resulting in increased cAMP and consequent activation of PKA, as well as G_q-type G-proteins that activate PLCβ resulting in hydrolysis of membrane PIP_2 releasing IP_3 and DAG. The IP_3 stimulates release of intracellular Ca^{2+} stores and DAG activates PKC. The PTH-1 receptor is activated by both PTH and PTHrP, whereas, the PTH-2 receptor is activated only by PTH. The PTH-1 receptor is found predominantly in bone and kidney. The body response to PTH is complex but is aimed in all tissues at increasing Ca^{2+} levels in extracellular fluids. PTH induces the dissolution of bone by indirectly stimulating osteoclast activity (through initial activation of osteoblasts which express the PTH-1 receptor), which leads to elevated plasma Ca^{2+} and phosphate. In the kidney, PTH reduces renal Ca^{2+} clearance by stimulating its reabsorption. At the same time, PTH reduces the reabsorption of phosphate and thereby increases its clearance. Finally, PTH acts on the liver, kidney, and intestine to stimulate the production of the steroid hormone 1,25-dihydroxycholecalciferol (calcitriol), which is responsible for enhancing Ca2+ absorption in the intestine.

With respect to the kidneys and overall calcium homeostasis, the major regulator is PTH. Within the nephron of the kidney calcium reabsorption from the glomerular filtrate occurs in several locations including the proximal tubule, the thick ascending limb (TAL) of the loop of Henle, the distal convoluted tubule (DCT), and in the connecting tubule (CNT). Within the proximal tubule and the TAL the reabsorption of calcium occurs via passive paracellular (between cells) transport mechanisms due to concentration gradients and electrochemical gradients established by various ion transporters. In these regions of the nephron PTH has no direct effects on calcium movement from the tubular lumen to the blood. However, hyperparathyroidism results in decreased sodium transport in the proximal tubule and TAL which exerts a secondary negative effect on calcium transport in these segments. The result of these effects of hyperparathyroidism is hypercalciuria and nephrolithiasis (kidney stones).

The primary sites for direct effects of PTH, within the kidney, are the DCT and the CNT. The ability of PTH to exert an increase in calcium reabsorption in these regions of the distal nephron is due to altered activity of the major calcium reabsorption transporter in these segments identified as transient receptor potential vanilloid 5, TRPV5. Reabsorption of calcium via TRPV5 leads to activation of the calcium-binding protein calmodulin. Calmodulin then binds to cytosolic sites in the C-terminus of TRPV5 which results in inactivation of the transporter. PTH activation of the PTH-1 receptor (PTH1R) in cells of the DCT and CNT results in activation of PKA through the associated G_s-type G-protein. PKA activation results in the phosphorylation of numerous sites on TRPV5 resulting in a loss of calmodulin binding and a consequent increase in calcium reabsorption by TRPV5. In addition, PTH action in the DCT and CNT leads to increased TRPV5 abundance in the apical membrane allowing for more calcium reabsorption. This latter effect of PTH is exerted through the action of PKC which is activated by the G_q-type G-protein associated with the PTHR1. One of the major intracellular calcium-binding proteins in the duodenum of humans that is involved in movement of calcium from the apical to the basolateral membrane for eventual transport to the blood is calbindin-D9K . A related protein, calbindin-D_{28K} is expressed in the DCT of the nephron. The action of PTH in the DCT results in increased levels of calbindin-D_{28K} allowing for more reabsorbed calcium to be transported to the basolateral membrane for efflux to the blood.

Within the kidneys PTH is also critical for the regulation of phosphate homeostasis. Phosphate reabsorption occurs exclusively in the proximal tubule where approximately 80% of the phosphate in the glomerular filtrate is reabsorbed. What is not reabsorbed appears in the urine indicating that the distal regions of the nephron do not contribute to phosphate reabsorption. The major phosphate transporters of the proximal tubule are encoded by the SLC34A1 and SLC34A3 genes which encode the Na^+-phosphate cotransporter 2a (NPT2a) and NPT2c proteins. Within the proximal tubule the PTH-1 receptor is expressed on both the apical and basolateral membranes of the epithelial cells of the tubule. The basolateral membrane PTH-1 receptor binds the PTH present in the blood circulating through the peritubular capillaries. The apical membrane PTH-1 receptor has been shown to bind small fragments of PTH, that are still biologically active, filtered in the glomerulus. The signal transduction cascade initiated by basolateral membrane PTH-1 receptors involves a G_s-type G-protein and the activation of PKA. The apical membrane PTH-1 receptor activates a signaling cascade involving a G_q-type G-protein and the activation of PKC. The ability of the PTH-1 receptor to activate both PKA and PKC pathways is controlled by its interaction with the proteins identified as Na^+-H^+exchanger regulator factor 1 (NHERF1) and NHERF2. When the PTH-1 receptor is associated with either of these proteins it activates the PKC pathway and when these factors are not associated with the receptor it activates the PKA pathway. Both NHERF1 and NHERF2 are exclusively localized to the apical membrane which explains the difference in PKC versus PKA activation at the different membranes. Regardless of basolateral or apical membrane localization, PTH-1 receptor activation, and the

resultant increase in kinase activity, results in inhibition of Na^+-dependent phosphate reabsorption in the proximal tubule.

The necessity for PTH to simultaneously activate Ca^{2+} reabsorption and inhibit phosphate (principally the HPO_4^{2-} form) reabsorption is to ensure that the Ca^{2+} remains in the ionized state. In the blood, calcium is distributed between the protein-bound form (35%-50%, primary protein is albumin), that complexed with phosphate and organic acids (10%), and the ionized (free Ca^{2+}) form. During hypocalcemic conditions the level of ionized calcium in the blood can drop significantly before the other bound forms. In the presence of adequate phosphate ion in the blood, any increased uptake of calcium into the blood would most likely form insoluble salts with the phosphate, thereby, restricting cellular access to the ionized Ca^{2+} that is needed in the hypocalcemic state. For this reason the PTH-mediated inhibition of renal reabsorption of phosphate, in conjunction with increased Ca^{2+} reabsorption, ensures that the absorbed calcium remains in the ionized state in the blood.

Although the primary function of PTH is to respond to reduced circulating Ca^{2+} levels and a portion of its action results in resorption of bone Ca^{2+}, the use of recombinant PTH has proven beneficial in the treatment of osteoporosis. PTH increases bone turnover (resorption) but it also increases the formation of new bone and the latter effect on bone is more pronounced than resorption. Intermittent infusion of recombinant PTH (Forteo®) results in new bone formation and has shown efficacy in the treatment of the bone loss associated with osteoporosis. This PTH-induced phenomenon occurs as a result of the laying down of protein matrix and mineralization that occurs not only in the previous existing matrix, but in the new matrix that is formed. Patients receiving Forteo demonstrate an increase in bone density as measured at the osteoporosis center(s) as well as a decrease in fractures compared to other forms of treatment. Whereas, continuous elevation of PTH levels, as occurs in humans due to abnormal secretion from the parathyroid glands results in loss of bone mass leading to osteoporosis, intermittent elevation of PTH that occurs with the daily injections of Forteo has the opposite effect of building bone.

Calcitonin Family

Calcitonin is a 32-amino acid peptide secreted by C cells of the thyroid gland. Calcitonin is a hypocalcemia-inducing peptide that exerts its effects in numerous species by antagonizing the effects of PTH. In humans, however, the role of calcitonin in calcium homeostasis is of limited physiological significance. The circulating levels of calcitonin are low and extreme variations in these levels have not been associated with disruptions in calcium or phosphate homeostasis in humans.

In non-human species, calcitonin exerts its hypocalcemia-inducing effects primarily through inhibition of osteoclast-mediated bone resorption. Calcitonin has been shown to reduce the synthesis of osteopontin (OPN, also referred to as

secreted phosphoprotein 1, SPP1), a protein made by osteoclasts and responsible for attaching osteoclasts to bone. Secondarily, calcitonin affects serum Ca^{2+} levels by the stimulation of renal Ca^{2+} clearance.

In humans the major benefits of calcitonin are its use in the treatment of osteoporosis and to suppress bone resorption in Paget disease. Paget disease (osteitis deformans) is a disorder of bone remodeling that results in accelerated rates of bone turnover and disruption of normal bone architecture. The naturally occurring calcitonins vary in amino acid sequence between species. The salmon calcitonin is 10–100 times more potent than mammalian calcitonins in lowering serum calcium levels and because of this activity it is used therapeutically such as in the treatment of Paget disease. The other medically significant fact related to calcitonin is its use as a biomarker for sporadic and inherited forms of thyroid medullary cancers in humans.

In addition to calcitonin, the calcitonin family of peptides includes amylin, adrenomedulin (AM) and adrenomedulin 2 (AM2, also known as intermedin). These peptides all interact with receptor complexes that contain the calcitonin receptor at their cores. The two G protein-coupled receptors (GPCRs) which are receptors for these peptides are the calcitonin receptor (CTR;) and the calcitonin receptor-like receptor (CLR,). These belong to the sub-family of GPCRs known as the secretin-like or class B family of GPCRs .CTR and CLR can form complexes with members of the membrane protein family called the receptor activity-modifying proteins (RAMPs), which consists of RAMP1, RAMP2 and RAMP3 in humans. RAMPs are type I transmembrane proteins composed of a large N-terminal extracellular domain, a single α-helical transmembrane domain and a short intracellular domain. These proteins regulate receptor pharmacology, receptor signaling, and receptor trafficking. RAMP association with CTR or with CLR generates multiple distinct receptor subtypes with different specificities for the calcitonin peptide family. CLR together with RAMP1 forms the CGRP receptor. In contrast, two AM receptors are formed by CLR and RAMP2 or RAMP3, respectively.

Insulin and Glucagon

The primary function of the pancreatic hormones is the regulation of whole-body energy metabolism, principally by regulating the concentration and activity of numerous enzymes involved in catabolism and anabolism of the major cell energy supplies.

Insulin

The earliest of these hormones recognized was insulin, whose major function is to counter the concerted action of a number of hyperglycemia-generating hormones and to maintain low blood glucose levels. Because there are numerous hyperglycemic hormones, untreated disorders associated with insulin generally lead to severe hyperglycemia and shortened life span. Insulin is a member of a family of structurally and functionally similar molecules that include the insulin-

like growth factors (IGF-1 and IGF-2), and relaxin. The tertiary structure of all 4 molecules is similar, and all have growth-promoting activities, but the dominant role of insulin is metabolic while the dominant roles of the IGFs and relaxin are in the regulation of cell growth and differentiation.

Insulin is synthesized as a preprohormone in the β-cells of the islets of Langerhans of the endocrine pancreas. The signal sequence of the preproinsulin protein is removed in the lumen of the endoplasmic reticulum and the proinsulin protein is packaged into secretory vesicles in the Golgi. Within these secretory vesicles to proprotein undergoes proteolysis to release the carboxy terminal A peptide, the amino terminal B peptide, and the C peptide which represents the central third of the proportein. The A and B peptides are then folded into the native structure of functional insulin by the formation of two disulfide bonds between these two peptides.

Insulin secretion from β-cells is principally regulated by plasma glucose levels and is referred to as glucose-stimulated insulin secretion, GSIS. The increased uptake of glucose by pancreatic β-cells leads to a concomitant increase in metabolism. The increase in metabolism leads to an elevation in the ATP/ADP ratio. This in turn leads to an inhibition of an ATP-sensitive K^+ channel. The net result is a depolarization of the cell leading to Ca^{2+} influx and insulin secretion.

Chronic increases in numerous other hormones (including GH, hPL, estrogens, and progestins), up-regulate insulin secretion, probably by increasing the preproinsulin mRNA and enzymes involved in processing the increased preprohormone. The adrenergic hormone, norepinephrine, diminishes insulin secretion through its binding to α_2-adrenergic receptors of pancreatic β cells. Conversely, the adrenergic hormone epinephrine, by binding to β_2-adrenergic receptors on pancreatic β cells inhibits insulin secretion. Epinephrine counters the effect of insulin in liver where it binds to both α_1- and β_2-adrenergic receptors. Activation of α_1-adrenergic receptors increases release of stored intracellular Ca^{2+} which binds to the calmodulin subunit of phosphorylase kinase resulting in increased glycogenolysis and glucose release to the blood. The activation of hepatic β2-adrenergic receptors induces adenylate cyclase activity, increases cAMP, and activates PKA. These latter events induce both glycogenolysis and gluconeogenesis, both of which lead to increased serum glucose.

Insulin secreted by the pancreas is directly infused via the portal vein to the liver, where it exerts profound metabolic effects. In most other tissues insulin increases the number of plasma membrane glucose transporters, but in liver glucose uptake is dramatically increased because of increased activity of the enzymes glucokinase, phosphofructokinase-1 (PFK-1), and pyruvate kinase (PK), the key regulatory enzymes of glycolysis. The latter effects are induced by insulin-dependent activation of phosphodiesterase, with decreased PKA activity and diminished phosphorylation of the regulatory glycolytic enzymes. In addition, phosphatases specific for the phosphorylated forms of the glycolytic enzymes increase in activity under the influence of insulin. All these events lead to conversion of the glycolytic

enzymes to their active forms and consequently a significant increase in glycolysis. In addition, glucose-6-phosphatase activity is down-regulated. The net effect is an increase in the content of hepatocyte glucose and its phosphorylated derivatives, with diminished blood glucose.

In addition to the latter events, diminished cAMP and elevated phosphatase activity combine to convert glycogen phosphorylase to its inactive form and glycogen synthase to its active form, with the result that not only is glucose funneled to glycolytic products, but glycogen content is increased as well.

Insulin generates its intracellular effects by binding to a plasma membrane receptor, which is the same in all cells. The receptor is a disulfide-bonded glycoprotein. One function of insulin (aside from its role in signal transduction) is to increase glucose transport in extrahepatic tissue is by increasing the number of glucose transport molecules in the plasma membrane. Glucose transporters are in a continuous state of turnover. Increases in the plasma membrane content of transporters stem from an increase in the rate of recruitment of new transporters into the plasma membrane, deriving from a special pool of preformed transporters localized in the cytoplasm.

In addition to its role in regulating glucose metabolism, insulin stimulates lipogenesis, diminishes lipolysis, and increases amino acid transport into cells. Insulin also modulates transcription, altering the cell content of numerous mRNAs. It stimulates growth, DNA synthesis, and cell replication, effects that it holds in common with the IGF family of growth factors and relaxin.

Glucagon

Glucagon is a 29-amino acid hormone synthesized by the α-cells of the islets of Langerhans of the endocrine pancreas. The glucagon peptide is derived by proteolytic processing from the very much larger proglucagon protein. Like insulin, glucagon lacks a plasma carrier protein, and like insulin its circulating half life is also about five minutes. As a consequence of the latter trait, the principal effect of glucagon is on the liver, which is the first tissue perfused by blood containing pancreatic secretions.

The role of glucagon is well established. It binds to a plasma membrane G-protein coupled receptor (GPCR). The glucagon receptor is derived from the GCGR gene is composed of 15 exons that encode a protein of 477 amino acids. The major site of expression of the GCGR gene is the liver with the second highest level of expression seen in the kidney. Glucagon binding to its receptor results in activation of an associated G_s-type G-protein, which in turn activates adenylate cyclase causing increased production of cAMP. The resultant increases in cAMP lead to activation of the kinase, PKA, which in turn phosphorylates numerous substrates resulting in a reversal of most of the effects that insulin exerts upon the liver as described above. The increases in PKA activity also lead to a marked elevation of circulating glucose, with the glucose being derived from liver gluconeogenesis and

liver glycogenolysis. Activation of the glucagon receptor, in hepatocytes, has also been shown to be coupled to activation of phospholipase C-β (PLCβ) resulting in increased production of diacylglycerol (DAG) and inositol trisphosphate (IP_3) from membrane phosphatidylinositol-4.5-bisphosphate (PIP_2). The released IP_3 binds to specific receptors on the ER membrane which, when activated, leads to the mobilization of stored Ca^{2+} into the cytosol. Of significance to hepatic glucose homeostasis, the release of stored intracellular Ca^{2+} results in binding to the calmodulin subunits of several kinases such as calmodulin-dependent kinase II (CaMKII). Increased CaMKII activity leads to phosphorylation and consequent inhibition of the activity of glycogen synthase. In addition to direct changes in metabolic pathway activity, exerted primarily via PKA-mediated phosphorylations, the actions of PKA and CaMKII lead to changes in gene expression in these same cells. For example, increased hepatic PKA activity results in phosphorylation of cytoplasmic cAMP response element binding protein, CREB. When phosphorylated CREB migrates to the nucleus and binds to cAMP response elements (CRE) in target genes resulting in altered transcription. An important CREB target is the gene encoding the gluconeogenic enzyme, phosphoenolpyruvate carboxykinase, PEPCK.

As indicated, glucagon binds to its receptor in the liver and kidneys and to a lesser extent, in adipose tissue, heart, endocrine pancreas, adrenal glands, spleen, and cerebral cortex. The binding of glucagon to its receptors in adipose tissue results in increased activation of hormone-sensitive lipase, HSL. The actions of HSL lead to increased release of fatty acids stored in the triglycerides in adipose tissue. The released fatty acids enter the circulation, are bound by albumin and transported to various tissues for oxidation. In the liver the oxidation of fatty acids is necessary to provide the energy needed for gluconeogenesis which is activated in liver in response to glucagon. Within the endocrine pancreas, the glucagon receptor is found on the β-cells that secrete insulin. The effect of glucagon on these cells is to stimulate insulin release so that there results in a fine regulatory control over the overall level of circulating glucose.

Somatostatin

Somatostatin is a cyclic peptide hormone that is derived from the SST gene. The SST gene is composed of two exons that encode a 116 amino acid preproprotein. Somatostatin is produced and secreted by enteroendocrine D cells of the stomach and duodenum, δ-cells of the pancreas and is also secreted by the hypothalamus. There are two forms of somatostatin generated from the preproprotein and they are identified as SS-28 and SS-14. Both forms have identical C-terminal sequences. The SS-28 form is the predominant form within the gut and the SS-14 form predominates in the central nervous system. In neural tissue somatostatin inhibits GH secretion and, thus, has systemic effects. In the pancreas, somatostatin acts as a paracrine inhibitor of other pancreatic hormones and, thus, also has systemic effects. It has been speculated that somatostatin secretion responds principally to blood glucose levels, increasing as blood

glucose levels rise leading to down-regulation of glucagon secretion. In the gut, somatostatin is involved in the inhibition of gastric acid secretion.

Somatostatin has been shown to bind to six receptors encoded by five distinct genes. The somatostain receptor genes are identified as SSTR1–SSTR5, each of which encodes a GPCR-type receptor protein with the SSTR2 gene encoding two distinct receptor subtypes as a result of alternative mRNA splicing. The two SSTR2 encoded receptors are identifed as SSTR2A and SSTR2B, although the level of SSTR2B mRNA in humans is extremely low and not likely to be of any physiologic significance. All five SSTR genes are expressed throughout the central nervous system as well in several peripheral tissues such as the gut, pancreas, liver, kidney, lung, pituitary, thyroid, and cells of the immune system. The primary function of the somatostatin receptors is the suppression of secretory activities of numerous cell types. Somatostatin suppresses the secretion of growth hormone, prolactin, ACTH, cholecystokinin (CCK), gastrin, secretin, glucose-dependent insulinotropic peptide (GIP: also known as gastric inhibitory peptide), vasoactive intestinal peptide (VIP), glucagon, insulin, renin and aldosterone. Within the CNS, somatostatin functions as a neurotransmitter and neuromodulator.

Amylin

Amylin is a 37 amino acid peptide that is secreted from β-cells of the pancreas simultaneously with insulin in response to nutrient intake. Amylin was originally identified as a major component of diabetes-associated islet amyloid deposits, hence its original name of islet amyloid polypeptide preprotein, IAPP. The amylin protein is encoded by the IAPP gene. The IAPP gene is composed of 4 exons that generate two alternatively spliced mRNAs, both of which encode the same 89 amino acid preproprotein. The structurally active form of amylin exists with an intrachain disulfide bond and an amidated C-terminus. When assayed by immunohistochemical means approximately 60% of amylin peptide present in the plasma is glycosylated. The functional significance of the glycosylation is currently unknown and when assayed *in vitro* the glycosylated peptide is biologically inactive.

The primary actions attributable to amylin secretion are reduction in the rate of gastric emptying, suppression of food intake, and suppression of post-meal glucagon secretion. Collectively these three actions compliment the plasma glucose concentration regulating actions of insulin. The anorexigenic actions of amylin are most likely mediated within the CNS via neurons in the area postrema as evidenced by the fact that peripheral administration of amylin to animals results in neuronal activation in this region of the brain. The plasma half-life of amylin is quite short being less than 15 minutes. The clearance of amylin from the plasma occurs via the kidneys both through renal excretion and renal peptidases associated with the vascular supply. A stable analog of amylin called

pramlintide (Symlin ®) is used as an adjunct to insulin treatment for type 1 and type 2 diabetes. Patients who use pramlintide show a modest degree of weight loss. Current trials are being undertaken to establish the efficacy of pramlintide in the treatment of obesity in patients without diabetes.

Amylin exerts its effect via interaction with GPCR complexes of the secretin-like receptor family (GPCR class B receptors). There are three distinct receptor complexes that bind amylin. These complexes all contain the calcitonin receptor (CTR) as a core protein and either one of three receptor activity-modifying proteins (RAMPs), RAMP1, RAMP2 or RAMP3. The specific amylin receptors result from the dimerization of various splice variants of the calcitonin receptor (CTRa or CTRb) with either RAMP1, RAMP2 and RAMP3. These receptors are commonly referred to as AMY_1, AMY_2 and AMY_3 with either an "a" or "b" in the subscript designating which CTR splice variant of the calcitonin receptor is in the complex. Amylin receptors are expressed in the nucleus accumbens, the dorsal raphe and the area postrema in the hind brain. Studies in rats have demonstrated that AMY_{2a} and AMY_{3a} are the amylin receptor subtypes localized to the area postrema which indicates that the satiety inducing effects of amylin are the result of activation of these two receptor subtypes. Within the area postrema, the key second messenger system associated with the amylin receptors appears to be cGMP. The calcitonin receptor-like receptor (CRLR) and both RAMP1 and RAMP2 are expressed in the subfornical organ and are likely responsible for the involvement of amylin in drinking behaviors. RAMP1 and RAMP2 but not RAMP3 have been shown to be expressed in the rat nucleus accumbens suggesting that the amylin receptor in the nucleus accumbens is either AMY_1 or AMY_2. The precise role of these amylin receptors in the nucleus accumbens haven not been well-established but it has been proposed that they may link food intake behavior and motor activity to amylin function. Peripheral injection of amylin demonstrates that the peptide crosses the blood-brain barrier resulting in access to a number of brain regions such as the cerebellum, midbrain, frontal cortex, parietal cortex, and occipital cortex.