12 Hypothalamic Hormones

GnRH, TRH, GHRH, SRIF, CRH, and Dopamine

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CONTENTS

1. INTRODUCTION

Alcmaeon, a sixth-century BC physiologist philosopher, introduced the brain as the center of human thinking, organizer of the senses, and coordinator for survival. However, the need for a visible connection between the brain and the rest of the body to explain a rapid and effective way of communication that would maintain homeostasis led Aristotle to the erroneous conclusion that the heart was the central coordinating organ and blood the means of information transmission. In contemporary medicine, the two ancient concepts are integrated in the exciting field of neuroendocrinology. The traditional distinctions between neural (brain) and hormonal (blood) control have become blurred. Endocrine secretions are influenced directly or indirectly by the central nervous system (CNS), and many hormones influence brain function. The hypothalamic-pituitary unit is the mainstay of this nonstop, interactive, and highly efficient connection between the two systems. Its function is mediated by hypothalamic-releasing or hypothalamic-inhibiting hormones, including gonadotropin-releasing hormone (GnRH), thyrotropin-releasing hormone (TRH), growth hormone–releasing hormone (GHRH), somatostatin (SRIF), corticotropin-releasing hormone (CRH), and the neurotransmitter dopamine.

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2. GnRH

2.1. GnRH Protein and Its Structure

The existence of GnRH as a hypothalamic factor was demonstrated in 1960. Systemic injection of acid hypothalamic extracts released LH from rat anterior pituitaries. The structure of GnRH was elucidated in 1971. The decapeptide pyroGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-amide was named luteinizing hormone-releasing hormone (LHRH). The term has been supplanted by GnRH, since this peptide not only releases LH from the gonadotropes, but also follicle-stimulating hormone (FSH). An FSH-specific hypothalamic-releasing hormone, however, may also exist and be similar to the LHRH/GnRH protein, explaining the difficulty researchers have met with its purification.

GnRH plays a pivotal role in reproduction. Phylogenetically, this protein has been a releasing factor for pituitary gonadotropins, since the appearance of vertebrates. The structures of its gene and encoded protein have been highly preserved. Only one form of GnRH has been identified in most placental mammals, but six additional highly homologous GnRH forms have been found in other more primitive vertebrates. Only three amino acids vary in these six molecules, which together with the mammalian protein (mGnRH) form a family of molecules with diversity of function, including stimulation of gonadotropin release; regulation of sexual behavior and placental secretion; immunostimulation; and, possibly, mediation of olfactory stimuli. In the human brain, placenta, and other tissues, where the gene is expressed, GnRH protein is the same. In other species, however, several GnRH forms are expressed in the various tissues and have different functions. In amphibians, mGnRH releases gonadotropins from the pituitary, but another, nonmammalian GnRH is responsible for slow neurotransmission in sympathetic ganglia.

Marked diversification of function exists within the relatively small GnRH peptide. The residues at the amino (N)- and corboxy (C)-termini appear to be primarily responsible for binding to the GnRH receptor, whereas release of LH and FSH depends on the presence of residues 1–4. These critical residues are conserved in evolution. In addition, residues 5, 7, and 8 form a structural unit, which is important for the biologic activity of GnRH receptors. Thus, the functional unit formed by the side chains of His^2, Tyr^5, and Arg^8 is necessary for full biologic activity of mGnRH. Substitution of the Arg residue reduces potency in releasing both LH and FSH, whereas replacement of the Leu^7 increases the potency for LH release, but does not alter that for FSH. Similar structure-function specificity is present in the remaining GnRH family members. The secondary structure of all GnRH peptides is highly conserved, too. A β-turn, formed by residues 5–8, creates a hairpin loop, which aligns the N- and C-termini of the GnRH molecule and provides the active domain of the hormone.

2.2. GnRH Gene and Its Expression

GnRH is synthesized as part of a larger peptide, the prepro-GnRH precursor. The latter contains a signal sequence, immediately followed by the GnRH decapeptide; a processing sequence (Gly-Lys-Arg) necessary for amidation; and a 56-amino-acid-long fragment, called GnRH-associated peptide, or GAP. Thus, the structure of prepro-GnRH is similar to that of many secreted proteins, in which the active sequence is coded along with a signal and processing sequences, and an "associated" peptide that is cleaved prior to secretion. GAP appears to coexist with GnRH in hypothalamic neurons, but its function remains elusive. Its sequence is considerably less preserved among species, and it does not appear to bind to specific receptors. GAP was initially thought to inhibit the secretion of prolactin (PRL), but this was not confirmed in vivo.

The human GnRH gene is located on the short arm of chromosome 8 (Table 1) and in all mammals consists of four exons. The first exon encodes the 5′-untranslated region (UTR). The second exon encodes prepro-GnRH up to the first 11 amino acids of GAP. The third and fourth exons encode the remaining sequence of the GAP and the 3′-UTR. Interestingly, the opposite strand of DNA is also transcribed in the hypothalamus and the heart. The function of this transcript, named SH, is unknown and may be involved in GnRH gene regulation. Despite the presence of many sequence changes among the GnRH genes of different species, the intro/exon boundaries have been preserved through evolution. The presence of highly homologous other GnRH forms in nonmammalian vertebrates suggests a common evolutionary process, that of the duplication of one common ancestor gene.

Expression of the GnRH gene is subject to significant species- and tissue-specific regulation. One example is the alternative splicing of the first GnRH gene exon in the mammalian brain and placenta. The promoter region of the rat GnRH gene has been sequenced and studied extensively. Sequences that can bind transcription factors, such as Pit-1, Oct-1, and Tst-1, as well as estrogen and other steroid hormone response elements exist in the 5′-flanking region of the rat GnRH gene, suggesting a quite complex and extensive hormonal regulation of its expression.

2.3. GnRH Receptor

The first step in GnRH action is recognition of the hormone by a specific cell membrane receptor (GnRH-R). The latter was recently cloned from several species, including human. It is a member of the seven-transmembrane segment class, characteristic of G protein–linked receptors. Several differences exist, however, between the GnRH-R and the other members of this superfamily of membrane proteins. The highly conserved Asp-Glu, which is essential for function and is found in the second seven-transmembrane segment of many receptors, is replaced in the GnRH-R with Asp. In addition, the GnRH-R lacks a polar cytoplasmic C-terminal region and has a novel phosphorylation site adjacent to the third seven-transmembrane segment.

The concentration of GnRH-Rs in the pituitary gland is tightly regulated and changes with the physiologic

Table 1
Genes, Pathophysiology, and Clinical Use of Hypothalamic Hormones

Hormone	*Chromosome*	*Receptor*	*Associated disorders*	*Clinical Use*
GnRH	8p	GnRH-R	Kallmann syndrome, precocious puberty, *hpg* mouse.	GnRH test, GnRH superagonists and antagonists
TRH	3	TRH-R	"Hypothalamic" hypothyroidism	TRH test
GHRH	20p	GHRH-R	*lit*–, *dw*–, and *dwj*– mice, "hypothalamic" GH deficiency	GHRH test, GHRH analogs and antagonists
SRIF	3q	SSTR-1–5		SRIF analogs
CRH	8q	CRH-R 1α, 1β CRH-R2	"Hypothalamic" adrenal insufficiency, chronic fatigue, fibromyalgia, atypical and melancholic depression, stress, autoimmune states	CRH test, CRH analogs and antagonists
Dopamine		D-1R–D-5R (pituitary: D2-R)	Nonadenomatous hyperprolactinemia	D-2R agonists

state of the organism. During the estrous cycle of rats, hamsters, ewes, and cows, the maximum number of receptors is observed just prior to the preovulatory surge of LH; thereafter, the number decreases and may require several days to achieve proestrous levels. Ovariectomy increases the number decreases significantly after exposure to androgens and during pregnancy and lactation. Several in vitro models employing pituitary cell cultures have indicated a biphasic response of GnRH-R to physiologic concentrations of GnRH. An initial desensitization of gonadotropes to GnRH is associated with downregulation of the receptor. This phase followed by an upregulation of the receptor number, which, however, is not associated with increased sensitivity to GnRH, since gonadotropes respond with near-maximal LH release, when only 20% of available GnRH-Rs are occupied.

The regulation of GnRH-R gene expression and protein function by GnRH provides the basis for the effects of constant GnRH infusion of GnRh superagonists on LH and FSH secretion. Whereas low or physiologic concentrations of GnRH stimulate the synthesis of GnRH-R, constantly high concentrations of this hormone downregulate the receptor in a process that involves physical internalization of agonist-occupied receptors. This is accompanied by loss of a functional calcium channel and other mechanisms. Indeed, GnRH regulates pituitary LH and FSH synthesis and release by a Ca^{2+}-dependent mechanism involving GnRH-R-mediated phosphoinositide hydrolysis and protein kinase C (PKC) activation. A G protein or multiple G proteins coupled to GnRH-R also play(s) and intermediatory role. This protein appears to be different from G_s or G_i, and similar to that hypothesized to be involved in TRH mediation of action. Following GnRH stimulation, an increase in phospholipid metabolism and intracellular Ca^{2+} and accumulation of inositol phosphates occur in pituitary gonadotropes. Calmodulin and its dependent protein system are important intracellular mediators of the Ca^{2+} signal in the gonadotropes.

In addition to its action on the gonadotropes, GnRH exerts a variety of effects in the CNS. Lordosis and mounting behaviors are facilitated by intraventricular and subarachnoid administration of GnRH, or local infusion of this peptide in the rat hypothalamic ventromedial nucleus (VMN) and central gray. GnRh can change the firing patterns of many neurons and is present in presynaptic nerve terminals. These actions are mediated through GnRH-R. The latter has been found to be widely distributed in the rat brain, in areas such as the hypothalamic VMN and arcuate nucleus (but not the preoptic region), the olfactory bulb and the nucleus olfactorius, the septum, and the amygdala and hippocampus. With few exceptions, CNS GnRH-R binds to GnRH analogs with the same affinity as the pituitary GnRH-R does. However, the former may not share the same second-messenger system(s) with the latter, since it is unclear whether Ca^{2+} is needed for hippocampal GnRh action. Aside from the CNS, GnRH-R is present in the gonads (rat and human ovary, rat testis) and rat immune system. GnRH has also been demonstrated to stimulate the production of ovarian steroidogenesis from isolated rat ovaries. The physiologic significance of these actions, however, remains unclear.

2.4. GnRH-Secreting Neurons: Embryology and Expression

Almost all the GnRH in mammalian brains is present in the hypothalamus and regions of the limbic system, hippocampus, cingulate cortex, and olfactory bulb. GnRH-expressing neurons migrate during development from their original place on the medial side of the olfactory placode into the forebrain. The GnRH neurons, which are generated by cells of the medial olfactory pit, do not have a GnRH secretory function before they attain their target sites in the basal forebrain. They do, however, express the GnRH gene, a feature that allowed their detection by *in situ* hybridization. In mice, these cells are first noted in the olfactory epithelium by d 11 of embryonic life. By d 12 and 13, they are seen migrating across the nasal septum toward the forebrain, arriving at the preoptic area (POA) of the developing hypothalamus by d 16–20. GnRh neuron migration is dependent on a neural cell adhesion molecule, a cell-surface protein that mediates sell-to-cell adhesion, is expressed by cells surrounding the GnRH neurons, and appears to be a "guide" for their migration.

By immunocytochemistry, GnRH cell bodies are found scattered in their final destination, the POA, among the fibers of the diagonal band of Broca and in the septum, with fibers projecting not only to the median eminence, but also through the hypothalamus and midbrain. In primates, more anteriorly placed cell bodies in the POA and septum are connected with dorsally projecting fibers that enter extrahypothalamic pathways presumably involved in reproductive behavior, whereas more posteriorly placed cell bodies in the medial hypothalamus itself give rise to axons that terminate in the median eminence. The two types of GnRH neurons are also morphologically different; the former have a smooth cytoplasmic contour, whereas the latter have "spiny" protrusions. Similar anatomic and functional plasticity has been documented at the level of the GnRH neuronal terminal.

GnRH may be present in other areas of the nervous system. In frogs, a GnRH-like peptide in sympathetic ganglia is thought to be an important neurotransmitter. GnRH can enhance or suppress the electrical activity of certain neurons in vitro. GnRH is also present in the placenta, where its mRNA was first isolated. Interestingly, GnRH, like TRH, is secreted into milk.

2.5. GnRH Secretion and Action

Secretion of hypothalamic GnRH is required for reproductive function in all species of mammals studied. Its secretion is subject to regulation by many hormones and neurotransmitters that act on the endogenous GnRH secretory rhythm, the "GnRH pulse generator." The latter provides a GnRH pulse into the hypophyseal-portal vessels at approx 90 intervals, which can be slowed down or accelerated by gonadal hormones. Testosterone and progesterone in physiologic concentrations and hyperprolactinemia slow the discharge rate of the generator, whereas estrogens have no effect on the frequency of the GnRH pulses. Females of all species respond to estrogens with an acute increase in LH and, to a lesser degree, FSH, a phenomenon that explains the "ovulatory LH surge" via positive estrogen feedback on the pituitary.

The mechanism of the estrogen-induced LH release has yet to be elucidated. The presence of testicular tissue prevents the estrogen-stimulatory effect on GnRH and LH secretion, but testosterone, although it slows down the GnRH pacemaker, does not completely abolish the estrogen effect. Since estrogen releases LH in castrated male monkeys, a nontestosterone testicular hormone other than inhibin may be responsible for this blocking effect in males.

GnRH secretion responds to emotional stress, changes in light-dark cycle, and sexual stimuli through the inputs that GnRH neurons receive from the rest of the CNS. Norepinephrine stimulates LH release through the activation of α-adrenergic receptors, and administration of α-antagonists blocks ovulation. A population of β-adrenergic neurons, which are inhibitory of GnRH secretion, has also been identified. Dopamine has inhibitory effects, but the role of epinephrine, G-aminobutyric acid (GABA), and serotonin is less clear. Acetylcholine may increase GnRH secretion, because it can induce estrus in the rat that is blocked by atropine. Glutamate stimulates GnRH secretion via the *N*-methyl-D-aspartate (NMDA) receptor. Naloxone can stimulate LH secretion in humans, but this effect is modulated by the hormonal milieu. Thus, administration of naloxone increases LH levels in the late follicular and luteal phases, but not in the early follicular phase or in postmenopausal women. It has been postulated that endogenous opioids may mediate the effects of gonadal steroids on GnRH secretion, since β-endorphin levels are markedly increased by administration of estrogen and progesterone.

Disruption of reproductive function in mammals is a well-known consequence of stress. This effect is thought to be mediated through activation of both the central and peripheral stress system. CRH directly inhibits hypothalamic GnRH secretion via synaptic contacts between CRH axon terminals and dendrites of GnRH neurons in the medial POA. The role of CRH regulation of GnRH secretion may be species specific with important differences noted between rodents and primates. Endogenous opioids mediate some of these effects of CRH, but

their importance varies with species, as well as with the period of the cycle and the gender of the animals. CNS cytokines also regulate GnRH secretion and function. Central injection of interleukin-1 (IL-1) inhibits GnRH neuronal activity and reduces GnRH synthesis and release. These effects are in part mediated through endogenous opioids and CNS prostaglandins (PGs). IL-1 and possibly other central cytokines may act as endogenous mediators of the inflammatory stress-induced inhibition of reproductive function.

2.6. Gonadotropin Deficiency: Kallmann Syndrome

In 1943, Kallmann and associates described a clinical syndrome of hypogonadism and anosmia affecting both men and women. The pathologic documentation of the characteristic neuroanatomic defects of the syndrome led to the term *olfactory-genital dysplasia* for what is now known as *Kallmann syndrome*. With the discovery of GnRH in 1971, the defect was determined to be hypothalamic in all patients with the syndrome, who subsequently were shown to resume normal gonadotropin secretion after repeated and/or pulsatile administration of GnRH.

The genetic basis of Kallmann syndrome, which has in most cases an X-linked inheritance, was recently elucidated at the molecular level. The earlier evidence that GnRH-secreting neurons migrate to the hypothalamus from the olfactory placode during development, combined with the observation that many patients with the X-linked form of ichthyosis caused by steroid sulfatase deficiency also had deafness and hypogonadotropic hypogonadism, led to identification of the *KAL* gene. The latter maps at chromosomes Xp22.3, is contiguous to the steroid sulfatase gene, and codes for a protein that is homologous to the fibronectins, with an important role in neural chemotaxis and cell adhesion.

Since the identification of the *KAL* gene, several defects have been described in patients with Kallman syndrome. Contiguous gene deletions have been found in patients with other genetic defects, such as ichthyosis, blindness, and/or deafness, whereas smaller deletions of the *KAL* gene are found in patients with anosmia and GnRH deficiency. These patients also demonstrate cerebellar dysfunction, oculomotor abnormalities, and mirror movements. Mutations of the gene that cause only anosmia in some affected patients have been described, and recently, *KAL* gene defects were reported in few patients with isolated gonadotropin deficiency.

Selective, idiopathic GnRH deficiency (IGD) is thought to be caused by various genetic defects that may include the GnRH gene itself. Patients with IGD and hereditary spherocytosis were recently described and are believed to have contiguous gene deletions involving the 8p11-p21.1 locus. In a murine model of hypogonadotropic hypogonadism (the mouse), the defect was found to be caused by a deletion of the GnRH gene and was recently repaired by gene replacement therapy.

2.7. Clinical Uses of GnRH

GnRH and its long-acting agonist analogs are, respectively, used in the treatment of GnRH deficiency, including menstrual and fertility disorders in women and hypothalamic hypogonadism in both sexes, and the treatment of central precocious puberty (CPP) in both boys and girls. Soon after the pulsatile nature of gonadotropin secretion was characterized, the requirement for intermittent stimulation by GnRH to elicit physiologic pituitary responses was determined. This led to the development of long-acting GnRH analogs, which provide the means of medical castration not only in CPP, but in a variety of disorders, ranging from endometriosis to uterine leiomyomas and prostate cancer. GnRH antagonists are currently being developed for the treatment of hormone-dependent cancers, such as prostate cancer, and for potential use of a male contraceptive in combination with testosterone.

GnRH is also used in clinical testing for the identification of CPP in children and the diagnosis of GnRH deficiency in all age groups. The gonadotropin response to 100 μg GnRH (intravenously [iv]) changes from an FSH-predominant response during the prepubertal years to an LH-predominant response during puberty. Significant gender differences exist in the peak hormonal values attained following GnRH stimulation, and the test is used in combination with other criteria for establishment of the diagnosis of precocious puberty. The same test is used in adults with suspected central hypogonadism. The lack of LH and FSH response to 100 μg GnRH iv is compatible with GnRH deficiency or pituitary hypogonadism, and repeated stimulation with GnRH may be needed to distinguish patients with Kallmann syndrome or selective IGD. The GnRH stimulation test is particularly useful in testing the efficacy of medical castration by GnRH agonists.

3. TRH

3.1. Prepro-TRH and Its Structure

TRH was the first hypothalamic-releasing factor to be isolated in 1969. Its discovery was followed by the description of GnRH, somatostatin, CRH, and GHRH, all in the early 1070s. TRH is a tripeptideamide (pGlu-His-Pro-NH_2), synthesized as part of a large prohormone termed *prepro-TRH*. The latter contains repeating sequences (Gln-His-Pro-Gly), the number of which

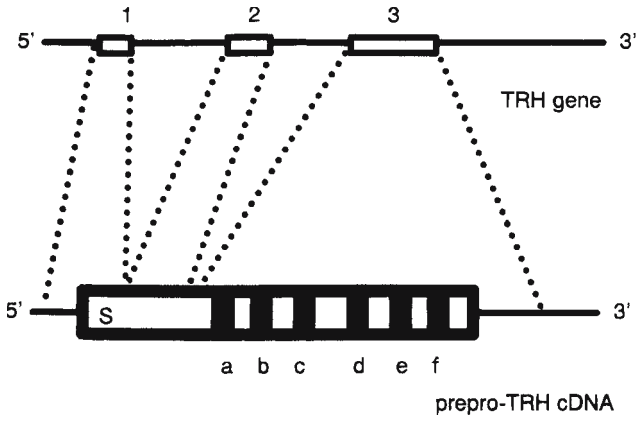

Fig. 1. Schematic representation of human TRH gene and its encoded cDNA. Three exons (1, 2, and 3) code for a transcript that contains a single peptide (S) and six potential copies (a–f) of the TRH tripeptide. This structure is highly preserved in evolution and is considered a model mechanism by which multiple copies of small peptides are produced from a single transcript.

varies from species to species. There are five of these repeats in the rat and six in the human preprohormone, and each can give rise to a TRH molecule after extensive posttranslational processing, which includes enzymatic cleavage of the prepro-TRH transcript, cyclization of the amino-terminal glutamic acid, and exchange of an amide for the carboxy-terminal glycine (Fig. 1). This structure, highly conserved in the mammalian genome, is considered a model of large production of small molecules from a single gene. copy.

The human prepro-TRH gene is on chromosome 3, has three exons, and encodes a cDNA that extends 3.7 kb. Exon 1 encodes the 5´ UTR of the mRNA, exon 2 encodes the signal sequence and part of the amino-terminal peptide, and exon 3 codes for the six potential copies of RH and the C-terminal peptide (Fig. 1). The rat prepro-TRH gene has a similar structure and size, but exon 3 codes for only five potential copies of TRH. The human prepro-TRH protein is smaller than that of the rat (242 amino acids long compared with 255 in the rat) and has a 60% homology to the latter.

Analysis of the rat 5´-flanking sequences has revealed the presence of many regulatory sequences that underline the complex regulation and determine the tissue-specific expression of the gene. A glucocorticoid-responsive element and an SP-1 transcription factor-binding sequence are located 100–200 bp upstream, whereas closer to the start site are sequences that are imperfect copies of the cyclic adenosine monophosphate (cAMP) regulatory element (CRE), and those that bind the triidothyronine (T_3) receptor (c-*erb* A) and the activating protein-1 (AP-1) transcription factor. As is the case in other pluripotential prohormone proteins, the connecting sequences between the repeat TRH units in the prepro-TRH transcript have the potential to modulate the biologic activity of TRH and are involved in long-term storage of the uncleaved molecule.

3.2. TRH Receptor

The pituitary TRH receptor (TRH-R) is a member of the seven-transmembrane segment–G protein–coupled receptor (GPCR) family. The gene that codes for the human TRH-R is located on chromosome 8p23. It consists of two exons, and its coded peptide has 398 amino acids. Although highly homologous to the rat and mouse TRH-Rs, the human transcript has a distinct C-terminal. Arg-283 and Arg-306, in transmembrane helices 6 and 7, respectively, appear to be important for binding and activation. A binding pocket formed by the third transmembrane segment domain is also important for binding with TRH. Recently, two TRH-R cDNAs encoding for a long and a short isoform have been identified in the rat. Their regulation of expression and second-messenger systems appears to be cell specific. The exact pattern of their distribution in the brain and elsewhere has not been determined.

Evidence supports a central role for the phosphoinositol/Ca^{2+} system mediating TRH actions. Following binding to TRH, TRH-R stimulates hydrolysis of the membrane lipid phosphatidylinositol 4,5-biphosphate to yield inositol 1,4,5-triphosphate and diacylglycerol. Both function as second messengers of the TRH-R and stimulate pKC. The response is Ca^{2+} dependent and involves a G protein as an intermediary. TRH stimulates a rapid, biphasic elevation of intracellular Ca^{2+}. The early phase is believed to come from intracellular Ca^{2+} stores and the sustained second phase from the influx of extracellular Ca^{2+} through voltage-dependent Ca^{2+} channels. A rapid translocation of pKC to the membrane has also been reported in response to TRH. As a result of TRH-R activation, a series of proteins is phosphorylated.

TRH does not appear to have a primary action on adenylate cyclase activity, despite the unequivocal evidence that cAMP stimulates thyroid-stimulating hormone (TSH) secretion from pituitary thyrotropes. However, cAMP-induced TSH secretion may not e TRH dependent. TRH action is exerted on the membrane and does not depend on internalization of TRH-R, although the latter does take place. The TRH-R C-terminus is important for receptor-mediated endocytosis, a process that is clathrin mediated and acidic pH dependent.

The receptor is specific for TRH and does not bind to any other known peptides. Several TRH analogs have been designed that bind to TRH-R with high affinity and

mimic TRH action. The receptor is widely distributed in the CNS and many nonneuronal tissues, but its second-messenger systems in tissues other than the pituitary have not been elucidated. Rat TRH-R mRNA, indistinguishable from that of the pituitary thyrotropes, is found in the hypothalamus, cerebrum, cerebellum, brain stem, spinal cord, and retina. Extraneuronal sites include the immune system and the gonads.

3.3. TRH-Secreting Cells

In addition to anticipated regions of immunostaining for pro-TRH in the hypothalamus, immunoreactivity for this prohormone is detected in many other regions of the rat brain. These include the reticular nucleus of the thalamus, pyramidal cells of the hippocampus, cerebral cortex, external plexiform layers of the olfactory bulb, sexually bimorphic nucleus of the POA, anterior commissural nucleus, caudate-putamen nucleus, supraoptic nucleus, substania nigra, pontine nuclei, external cuneate nucleus, and dorsal motor nucleus of the vagus. TRH is also present in the pineal gland and the spinal cord. The extensive extrahypothalamic distribution of TRH, its localization in nerve endings, and the presence of TRH receptors in brain tissue suggest the TRH serves as a neurotransmitter or neuromodulator in many areas of the brain. There is also evidence that posttranslational processing of the prepro-TRH transcript is not identical throughout the CNS. In many areas of the rat brain, C- but not N-terminal extensions of the TRH are found, indicating that the dibasic residues of the latter are subject to enhanced cleavage compared to the former. Differential processing of the prepro-TRH transcript amplifies the biologic significance of its gene product and is similar to that of other potent propeptides with wide distribution and array of action in the mammalian brain, such as the preproenkephalins (-A and -B) and propiomelanocortin (POMC).

In extraneuronal tissues, prepro-TRH mRNA that is identical to that of the hypothalamus is found in mammalian pancreas, normal thyroid tissue, and medullary thyroid carcinoma cell lines. In the rabbit prostate, a TRH-related peptide was found that is believed to be derived from a precursor distinct from the hypothalamic TRH prohormone. In nonmammals and as the phylogenetic scale is descended, TRH concentration in nonhypothalamic areas of the brain and extraneural tissues increases. TRH is present and functions solely as a neurotransmitter in primitive vertebrates that do not synthesize TSH. The peptide is also found in the skin of some species of frogs, which provides testimony to the common embryologic origin of the brain and skin from the neuroectoderm.

3.4. Regulation of TRH Synthesis and Secretion

TSH secretion by the anterior pituitary thyrotropes is characterized by a circadian rhythm with a maximum around midnight and a minimum in the later afternoon hours. Superimposed to the basic rhythm are smaller, ultradian TSH peaks occurring every 2–4 h. TRH appears to be responsible for the ultradian TSH release that is also regulated by somatostatin. Imput from the suprochiasmatic nucleus and potentially other circadian pacemakers is required for this part of hypothalamic TRH secretion. Several other brain regions have been implicated in the regulation of TRH secretion, including the limbic system, the pineal gland, and CNS areas involved in the stress response.

Hypothyroidism, induced either pharmacologically or by thyroidectomy, increases the concentration of prepro-TRH mRNA at least twofold in the medial and periventricular parvocellular neurons of experimental animals. This response occurs shortly after levorotatory thyroxine (T_4) falls to undetectable levels, and parallels the gradual rise in serum TSH. This response is not TSH mediated, because hyphysectomy has not effect, whereas the administration of T_4 completely prevents it and supraphysiologic doses of T_4 cause an even further decline. Interestingly, the increase in prepro-TRH mRNA levels in hypothyroid animals occurs over several weeks, whereas its decline following administration of T_4 is faster, occurring within 24 h. Because of the absence of Type II deiodinase in the paraventricular nucleus (PVN), the feedback regulation of prepro-TRH gene expression is mediated by circulating levels of free T_3 rather than by intracellular conversion of T_4 into T_3. This serves to increase the sensitivity of TRH neurons to declining levels of thyroid hormone. The hypothalamic TRH neuron thus determines the set point of the thyroid hormone feedback control.

The dramatic feedback effects of thyroid hormone on TRH synthesis appear to be limited to the TRH-synthesizing neurons of the hypothalamic PVN. In contrast to the medial and periventricular parvocellular PVN neurons, no increase in prepro-TRH mRNA was observed in the anterior parvocellular subdivision cells of hypothyroid animals, a hypothalamic region that is functionally diverse. Similarly, no change was detectable in any other TRH neuronal population in the hypothalamus or the thalamus. Thus, the nonhypophysiotropic TRH neurons of the CNS may not be subject to thyroid hormone control. Their function is regulated via a variety of neurotransmitters, including catecholamines, other neuropeptides, and perhaps excitatory amino acids.

Catecholamines have an important regulatory role in the secretion of hypothalamic TRH. The stimulation of ascending α_1-adrenergic neurons from the brain stem causes activation of hypothalamic TRH neurons, and norepinephrine induces TRH secretion in vitro. Dopamine inhibits TSH release and the administration of α-methyl-*p*-tyrosine, a tyrosine hydroxylase inhibitor, diminishes the cold-induced TSH release. The action of serotonin is unclear, because both stimulatory and inhibitory responses have been found.

Endogenous opioids inhibit TRH release and so does somatostatin, which inhibits TSH secretion as well. Glucocorticoids decrease hypothalamic prepro-TRH mRNA synthesis both directly and indirectly via somatostatin. However, in vitro studies have shown upregulation of the prepro-TRH transcript by dexamethasone in several cell lines. This discrepancy may be explained by the in vivo complexity of prepro-TRH gene regulation vs the deafferentiated in vitro system. Thus, even though the direct effect of glucocorticoids on hypothalamic TRH synthesis is stimulatory, the in vivo effect is normally overridden by inhibitory neuronal influences, such as those emanating from the hippocampus via the fornix.

3.5. Endocrine and Nonendocrine Action of TRH

The iv administration of TRH in humans if followed by a robust increase in serum TSH and PRL levels. TRH is the primary determinant of TSH secretion by the pituitary thyrotropes, but its physiologic role in PRL secretion is unclear. PRL, but not TSH, is elevated in nursing women. The administration of anti-TRH antibody does not block the physiologic PRL rise during pregnancy or suckling. On the other hand, the PRL response to TRH is dose dependent and suppressible by thyroid hormone pretreatment. Hyperprolactinemia and galactorrhea have been observed in primary hypothyroidism.

Normally, TRH does not stimulate secretion of other pituitary hormones. However, GH release is stimulated by administration of TRH in many subjects with acromegaly, occasionally in midpuberty, and in patients with renal failure, anorexia nervosa, and depression. TRH can also stimulate adrenocorticotrophic hormone (ACTH) release by corticotropinomas in Cushing disease and Nelson syndrome, and FSH and α-subunit by pituitary gonadotropinomas and clinically nonfunctioning adenomas.

As a neurotransmitter, TRH has a general stimulant activity, with its most significant roles being thermoregulation and potentiation of noradrenergic and dopaminergic actions. Directly, TRH regulates temperature homeostasis, by stimulating the hypothalamic preoptic region, which is responsible for raising body temperature in response to signals received from the skin and elsewhere in the brain. Indirectly, TRH elevates body temperature by activating thyroid gland function and regulation sympathetic nerve activity in the brain stem and spinal cord. TRH participates in regulation of the animal stress response by increasing blood pressure and spontaneous motor activity. Other TRH actions include potentiation of NMDA receptor activation, by changing the electrical properties of NMDA neurons, and alteration of human sleep patterns.

TRH appears to function as a neurotrophic factor in addition to being a neurotransmitter. Its administration in animals decreases the severity of spinal shock and increases muscle tone and the intensity of spinal reflexes. Recently, TRH was found to play an important role in fetal extrathymic immune cell differentiation and, thus, appears to be involved in the neuroendocrine regulation of the immune system.

In the CNS, a TRH-degrading ectoenzyme (TRH-DE) degrades TRH to acid TRH and cyclic dipeptide (cycled His-Pro). The former has some of the TRH actions, but the latter may function as a separate neurotransmitter with its own distinct actions, such as increase in stereotypical and inhibition of eating behaviors. TRH-DE is regulated in a manner that is the mirror image of that of TRH-R; thus, its mRNA levels are increased by thyroid hormone and decreased by antithyroid agents.

3.6. Clinical Uses of TRH

Oral, im, or iv administration of TRH stimulates the immediate secretion of TSH and PRL from the anterior pituitary. The maximal response is obtained after a 400 μg iv injection of TRH, but the most frequently administered dose is 200–550 μg. The peak serum TSH concentration is achieved 20–30 min after the iv bolus of TRH, but in individuals with central (hypothalamic) hypothyroidism, this response is delayed and prolonged. In primary hypothyroidism, the TSH response to TRH stimulation is accentuated, and in patients with isolated TSH deficiency, TRH fails to elicit an increase in serum TSH, whereas the PRL response is normal. In thyrotoxicosis, because even minute amounts of supraphysiologic thyroid hormone suppress the hypothalamic-pituitary-thyroid axis, TSH response to TRH are blunted. However, owing to the wide variation in TRH-induced increases in serum TSH levels in normal individuals, interpretation of the test is difficult, and the latter is seldom necessary in clinical practice.

The most frequent use of TRH testing, prior to the advent of third-generation TSH assays, was in patients with mild or borderline thyrotoxicosis and equivocal

levels of thyroid hormone. Another application of the TRH test was in the diagnosis of central hypothyroidism, caused by lesions of the hypothalamic-pituitary area. However, the loss of circadian TSH variation is a far more sensitive test than TRH stimulation for the diagnosis of secondary (central) hypothyroidism and has replaced the latter in clinical practice. Currently, the TRH stimulation test is mot useful in the differential diagnosis of TSH-secreting adenomas and thyroid resistance with determination of the plasma α-subunit vs intact TSH concentration ratio. A ratio > 1 suggests the presence of a TSH-secreting adenoma. The test is also useful in the identification of gonadotropinomas and clinically nonfunctioning pituitary adenomas, which respond to TRH with an FSH and/or a glycoprotein α-subunit predominant gonadotropin response, whereas healthy individuals do not have a gonadotropin or an α-subunit response to TRH. The observation that patients with acromegaly respond to TRH with an increase in their GH levels has been in clinical use of a diagnostic provocative test and as a way to monitor the therapeutic response of patients with acromegaly to transsphenoidal surgery, pituitary radiation, or somatostatin analog treatment.

4. GHRH

4.1. Prepro-GHRH Gene and Its Product

In contrast to GNRH and TRH, a deca- and tripeptide, respectively, GHRH is larger and exists in more than one isoform in the human hypothalamus. The first evidence for a hypothalamic substance with GH-releasing action because available in 1960, when it was shown that rat hypothalamic extracts could release GH from pituitary cells in vitro. It was not until 1980 that part of the peptide was purified from a nonhypothalamic tumor in a patient with acromegaly. Subsequently, three isoforms of the peptide were identified and sequenced from pancreatic islet cell adenomas with ectopic GHRH production. Two of the three isoforms were also present in human hypothalamus (GHRH-[1–44]NH_2 and GHRH[1–40]OH) and differ only by four amino acids at the C-terminus. GHRH-(1–44)NH_2 is the most abundant form and homologous to the GHRH of other species, but the shorter, 40-amino-acid isoform has equipotent bioactivity and is physiologically important. The third form, HGRH(1–37)OH, has only been found in neuroendocrine tumors from patients with acromegaly and is less potent in releasing GH. The shortest prepro-GHRH sequence with GH-releasing activity consists of the first 29 amino acids of the intact GHRH, whereas the GHRH(1–27) form has no biologic activity.

The human *GHRH* gene is on chromosome 20p12 (Table 1). It is 10 kb long and consists of five exons. The mRNA transcript is 750 bp long and generates on GHRH molecule but exhibits heterogeneity owing to an alternative splice site present in the fifth exon. Like the other hypothalamic peptides, GHRH is coded in a larger prohormone molecule. Prepro-GHRH contains a 30-residue signal peptide and the GHRH(1–44) sequence, followed by an amidation signal and a 30- or 31-residue C-terminus peptide (GCTP). The prepro-GHRH peptide undergoes extensive posttranslational processing during which the signal peptide is removed and the rest of the molecule is cleaved by endopeptidases to GHRH(1–45)-glycine and GCTP. GHRH(1–45) is then converted into GHRH(1–44)NH_2 by peptidylglycine α-amidating monooxygenase. In the human hypothalamus, pituitary, extrahypothalamic brain, and several other normal and tumor tissues, endopeptidases convert GHRH(1–44)NH_2 into GHRH(1–40)OH, a form that is absent in other species studied to date.

The human prepro-GHRH transcript has been identified in hypothalamus, nonhypothalamic areas of the brain, testicular germ cells, and a variety of neuroendocrine tissues and tumors. The hypothalamic expression of the gene is primarily under the control of GH. Deficieincy of the latter, caused by hypophysectomy or defects in the GH gene, is associated with increased GHRH mRNA steady-state levels. Conversely, GH treatment decrease the synthesis of GHRH. These effects are exerted directly on the GHRH-secreting neurons, since GH receptor mRNA has been colocalized with prepro-GHRH mRNA in many areas of the brain, including the hypothalamus and thalamus, septal region, hippocampus, dentate gyrus, and amygdala. Preliminary results also indicate an inhibitory effect of insulin-like growth factor-1 (IGF-1) on prepro-GHRH mRNA.

Baseline GHRH mRNA levels are greater in hypothalami of male rats compared with hypothalami of female rats. This sexually bimorphic expression of the prepro-GHRH gene in the rat is significantly regulated by gonadal steroids. Administration of dihydrotestosterone to ovariectomized rates masculinizes their GH-secretion pattern and increases hypothalamic prepro-GHRH mRNA content. Conversely, administration of estrogens to male rats decreases GHRH synthesis, although this is not a consistent finding. In addition, GH-feedback inhibition of GHRH synthesis appears to be sex specific. Furthermore, after caloric deprivation of genetically obese and/or diabetic animal models, GHRH synthesis is decreased in a GH-independent fashion.

Tissue-specific regulation is exhibited by the prepro-GHRH gene in the mouse placenta. The transcript in this tissue contains a first exon that is approx 8–12 kb upstream from the mouse hypothalamic first exon,

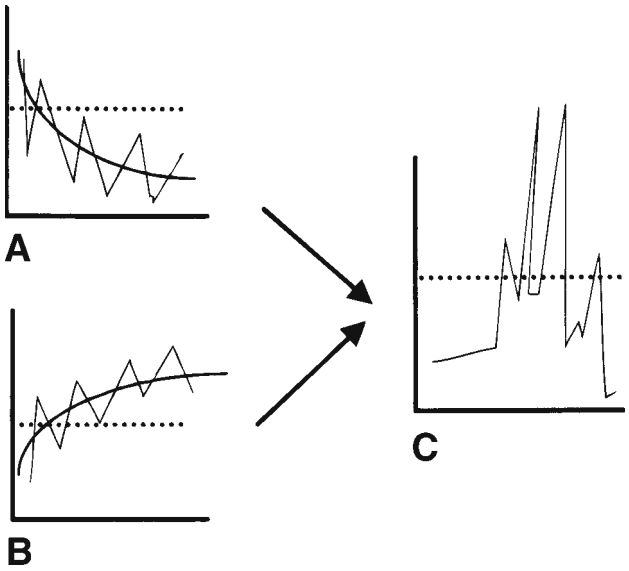

Fig. 2. Regulation of GH secretion. The theory proposed by Tannenbaum and Ling suggests that every secretory pulse of GH (**C**) is the product of a GHRH pulse (**B**) and an SRIF trough (**A**).

indicating a different transcription start site. The human placenta does not contain the prepro-GHRH transcript. A GHRH-like mRNA and peptide have been detected in rat and human testes.

4.3. GHRH Secretion

GHRH-containing nerve fibers arise from neurons of the ventromedial and arcuate nuclei of the hypothalamus. These neurons receive a variety of inputs from diverse areas of the CNS. Signals from sleep centers are excitatory and linked to the sleep cycle, whereas signals from the amygdala and ascending noradrenergic neurons from the brain stem are linked to activation of the stress system and responsible for stress-induced GH release. The VMN integrates the secretion of glucoregulatory hormones and also influences GHRH release in response to hypoglycemia.

The secretion of GH is regulated by the excitatory GHRH and the inhibitory somatostatin (SRIF) (Fig. 2). Functional and anatomic reciprocal interactions exist between GHRH and SRIF neurons, in the ventromedial/arcuate and periventricular nuclei, respectively. Endogenous SRIF blocks GHRH release from the median eminence, whereas intracerebral administration of SRIF stimulates GHRH secretion from the specific neurons. The importance of SRIF in the regulation of GHRH secretion is demonstrated by the presence of high-affinity SRIF receptors in the GHRH neurons of the ventrolateral portion of the arcuate nucleus. Regulation of SRIF and the endogenous zeitgeber in the suprachiasmatic nucleus and elsewhere are responsible for the ultradian GHRH secretion. The latter, along with the tonic pulses of SRIF, defines the GH-circadian release, which is synchronized with the sleep cycle.

Neuronal inputs to the GHRH-secreting neurons are transmitted via a variety of neurotransmitters. Sleep-induced GH release is mediated mainly by serotoninergic and cholinergic fibers. The spontaneous ultradian pulses of GH, caused by GHRH or transient inhibition of SRIF, can be blocked by α-antagonists or drugs that inhibit catecholamine biosynthesis. β_2-Agonists stimulate GH secretion, presumably by inhibiting SRIF release. Anticholinergic substances block all GH-stimulatory responses, with the exception of that of hypoglycemia. L-dopa and dopamine stimulate GH release in humans, though in vitro dopamine inhibits GH secretion by normal pituitary or somatotropinomas. It has been postulated that the in vivo stimulatory effect of L-dopa and dopamine is owing to their local conversion into norepinephrine.

In addition to SRIF, many other CNS peptides interact with GHRH and affect GH secretion. Endogenous opiates, particularly β-endorphin, stimulate the GHRH neuron and induce GH release. Vasoactive intestinal peptide (VIP) and peptide histidine isoleucine (PHI) stimulate rat GH and PRL secretion. Since VIP and PHI do not bind to GHRH-R, it is not clear whether these effects of GH secretion are mediated at the hypothalamic or the pituitary level, or both. In humans, VIP-induced GH secretion has been observed only in acromegaly. PACAP has been shown to stimulate GH release in rats in vitro; however, this action may not be specific, since it also enhances the secretion of PRL, ACTH, and LH. Central administration of TRH induces GH release by Ca^{2+}-dependent, cAMP-independent mechanism that is modified by the presence of GHRH and is species specific. In humans, TRH-induced GH secretion is observed only in acromegaly. Galanin, motilin, and neuropeptide (NPY) enhance GHRH-induced GH release from rat pituitary cells. NPY and a structurally similar hormone, the pancreatic polypeptide, have opposite effects on GH secretion, depending on the dose and the route of administration. A subset of GHRH neurons contains NYP, which appears to enhance GH secretion in vitro. After intracerebroventricular (ICV) administration, however, NPY inhibits GH release, demonstrating additional function at the level of the GHRH or SRIF neuron. This may be via inhibition of ascending noradrenergic neurons from the brain stem, which normally stimulates GH secretion via GHRH.

4.4. Pathophysiology of GHRH Action

GHRH secretion and GHRH-R binding to its ligand in rodents are decreased with aging. The GH response to GHRH stimulation is similarly decreased in elderly humans. Studies in children with short stature have failed to demonstrate deficiency in either GHRH synthesis or action, although GHRH-induced GH secretion may be augmented in young adults with idiopathic tall stature. The human prepro-GHRH gene was recently excluded as a cause for short stature in familial GH deficiency by linkage and single-strand conformation analysis. Nevertheless, mutations in this gene and those of the GHRH-R and its second messengers are still candidates for familial disorders of human growth. In support of the latter is a well-studied rodent model of GHRH deficiency. GHRH-R of the *lit* mouse contains a missense mutation in the extracellular domain that disrupts receptor function. Another animal model, the *dw* rat, demonstrates a defect in the ability of GHRH-activated $G_s\alpha$ to stimulate adenylate cyclase, which results in low or undetectable GH levels. In contrast to the *dw* (Snell) and *dwJ* (Jackson) dwarf mice with similarly low GH levels, in which mutations are present in the Pit-1 pituitary transcription factor, the *dw* rat defect has not been elucidated. Recent studies have shown normal Pit-1 and GHRH mRNA levels, and a normal $G_s\alpha$ sequence, indicating that another or other proteins are responsible for this phenotype.

Hypersecretion of GHRH causes sustained GH secretion, somatotrope hyperplasia, and adenoma formation. A transgenic mouse expressing the human GHRH gene exhibits GH hypersecretion associated with somatrotrope and lactotrope hyperplasia that eventually leads to adenoma formation. Indeed, approximately half of human GH-secreting tumors contain point mutations of the $G_s\alpha$ gene that interfere with the intrinsic guanosine triphosphate activity of G_s and lead to constitutive activation. A similar pathophysiologic mechanism explains the presence of somatotropinomas in patients with McCune-Albright syndrome.

4.5. Clinical Uses of GHRH and Its Analogs

The GHRH stimulation test is rarely used in clinical practice because of the wide variability of GH responses in healthy individuals. In the diagnosis of GH deficiency, pharmacologic agents, such as clonidine, arginine, and l-dopa, provide more sensitive and specific GH stimulation tests.

GH-releasing peptides (GHRPs) are oligopeptides with GH-releasing effects that bind to receptors different from the GHRH-R in the hypothalamus and elsewhere in the CNS. The original GHRP was a synthetic, met-enkephalin-derived hexapeptide (His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2), which was a much more potent GH secretagogue than GHRH both in vivo and in vitro. When administered in large doses, GHRPs enhance ACTH and PRL release from the pituitary, whereas in smaller doses and/or after prolonged oral administration, only GH is secreted. Recently, a peptide analog (hexarelin) has been shown to be a relatively specific and potent GH secetagogue after oral administration in GH-deficient adults and children. Nonpeptide, equipotent analogs were subsequently synthesized that could be administered orally. Their use is still investigational.

5. SRIF

5.1. Somatostatin Gene and Protein

The first evidence for the existence of SRIF was provided in 1968, when hypothalamic extracts were shown to inhibit GH secretion from pituitary cells in vitro. A tetradecapeptide was isolated a few years later in parallel to the discovery of a factor in pancreatic islet extracts that inhibited insulin secretion. The term *somatostatin* was applied to the originally described cyclic peptide (S-14), but today it is used for other members of this family of proteins, which in mammals include the 28-amino-acid form (S-28) and a fragment corresponding to the first 12 amino acids of S-28 (S-28[1–12]). S-14 contains two cysteine residues connected by a disulfide bond that is essential for biologic activity, as are residues 6–9, which are contained within its ring structure.

The mammalian SRIF gene is located on chromosome 3q28 (Table 1), spans a region of 1.2 kb, and contains two exons. The SRIF mRNA is 600 nucleotides long and codes for a 116-amino-acid precursor, preprosomatostatin. Unlike GHRH, the sequence of the SRIF gene is highly conserved in evolution. Single-cell protozoan organisms have a somatostatin-like peptide, whereas the mammalian and one of the two anglerfish somatostatins are identical. A total of seven genes coding for the somatostatin family of peptides have been described in the animal kingdom. Posttranslational processing of preprosomatostatin by a number of peptidases/convertases is also conserved and results in various molecular forms with some degree of functional specificity. S-14 is the predominant form in the brain, whereas S-28 predominates in the gastrointestinal (GI) tract, especially the colon. Specificity of somatostatin form appears to be determined by the presence of different convertases in the various tissues and cell lines examined.

The 5´-UTR of the SRIF gene contains several cAMP and other nuclear transcription factor–responsive elements. Administration of GH increases SRIF mRNA levels in the hypothalamus, whereas GH deficiency does not always cause a decrease in the level of

SRIF gene expression. Glucocorticoids enhance hypothalamic somatostatin expression, but the effect may be indirect through the activation of β-adrenergic neurons. T_3 also regulates brain somatostatin mRNA levels in vitro. Extensive SRIF gene tissue-specific regulation has been described, a necessary phenomenon for a gene that is so widely expressed and has so many functions.

5.2. Somatostatin Receptors

In 1992, five different somatostatin receptor genes (SSTR- 1–5) were identified, which belong to the seven-transmembrane segment domain receptor family. The tissue expression of these receptors matches with the distribution of the classic binding sites of somatostatin in the brain, pituitary, islet cells, and adrenals. The pituitary SRIF receptor appears to be SSTR-2, but other actions of the different forms of somatostatin have not yet been attributed to a single receptor subtype. The clinically useful somatostatin agonists (octreotide, lanreotide, and vapreotide) bind specifically to SSTR-2 and less to SSTR-3 and are inactive for SSTR-1 and SSTR-4.

All five SRIF receptors are expressed in rat brain and pituitary, whereas the exact distribution of the receptor subtypes is not known for the periphery. In the fetal pituitary, SSTR-4 is not expressed. SSTR-4 is coexpressed with SSTR-3 in cells of the rat brain, in the hippocampus, in the subiculum, and in layer IV of the cortex. SSTR-3 alone is expressed in the olfactory bulb, dentate gyrus, several metencephalic nuclei, and cerebellum, whereas SSTR-4 is primarily in the amygdala, pyramidal hippocampus, and anterior olfactory nuclei. Human pituitary adenomas express multiple SSTR transcripts from all five genes, although SSTR-2 predominates. SSTR-5 mRNA, which has not been reported in other human tumors, is expressed in neoplastic pituitary tissues, including GH-secreting adenomas.

The main pituitary SRIF receptor, SSTR-2, demonstrates heterogeneity by alternative splicing. Two isoforms (SSTR-2A and SSTR-SB) have been identified, and their expression is subject to tissue-specific regulation. In human tumors, the predominant form is SSTR-2A. In the mouse brain, SSTR-2A was mainly present in cortex, but both mRNAs were found in hippocampus, hypothalamus, striatum, mesencephalon, cerebellum, pituitary, and testis. The promoter region of the human SSTR-2 gene shares many characteristics with the promoters of other GPCR-encoding genes, including a number of GC-rich regions, binding sites for several transcription factors, and the absence of coupled TATAA and CAAT sequences.

SRIF inhibits adenylate cyclase activity on binding to the SSTRs. The latter are coupled to the adenylate cyclase–inhibitory G protein, G_i, which is activated in a manner similar to that for G_s. Additionally, SRIF induces a dose-dependent reduction in the basal intracellular Ca^{2+} levels. Ca^{2+} channel agonists abolish this effect, indicating that SRIF acts by reducing Ca^{2+} influx through voltage-sensitive channels. Voltage on either side of the cell membrane is altered via K^+ channels that are stimulated by SRIF, resulting in hyperpolarization of the cell and a decrease in the open Ca^{2+} channels. The role of the inositol phosphate–diacylglycerol–pKC and arachidonic acid–eicosanoid pathways in mediating SRIF action is uncertain.

Recently, evidence was presented that the widespread inhibitory actions of somatostatin may be mediated by its ability to inhibit the expression of the *c-fos* and *c-jun* genes. Interference with in effects of AP-1 results in inhibition of cellular proliferation, but this could be important for the control of tumor growth. It is not clear how the SSTRs mediate this action of somatostatin, but one way may be the stimulation of several protein phosphatases that inhibit AP- 1 binding and transcriptional activity.

5.3. SRIF Secretion

Somatostatin-secreting cells, in contrast to GHRH-secreting cells, are widely dispersed throughout the CNS, peripheral nervous system, tissues of neuroectodermal origin, placenta, GI tract, and immune system. Those neurons secreting SRIF and involved in GH regulation are present in the periventricular nuclei of the anterior hypothalamus. The-axonal fibers-sweep laterally and inferiorly to terminate in the outer layer of the median eminence. SRIF neurons are also present in the ventromedial and arcuate nuclei, where they contact GHRH containing perikarya providing the anatomic basis for the concerted action of the two hormones on the pituitary somatotropes.

The secretory pattern of GH is dependent on the interaction between GHRH and SRIF at the level of the somatotrope (Fig. 2). Both hormones are required for pulsatile secretion of GH, since GHRH and/or SRIF antibodies can abolish spontaneous GH pulses in vivo. The manner by which the two proteins maintain GH secretion has been the subject of intense investigation for more than two decades. The prevailing theory is that proposed by Tannenbaum and Ling, who suggested that GH pulses are the consequence of GHRH pulses together with troughs of SRIF release (Fig. 2). Additional factors, however, appear to contribute to this basic model of GH secretion, such as the regulation of the SSTRs, the IGFs (particularly IGF- 1), other

hypothalamic hormones (CRH and perhaps TRH), the glucocorticoids, and gonadal steroids.

GH stimulates SRIF secretion, and SRIF mRNA levels are increased by GH and/or IGF- 1. Hypothalamic SRIF mRNA levels are decreased by gonadectomy in both male and female rats, whereas estradiol (E_2) and testosterone reverse these changes in female and male rats, respectively. In humans, GH-pulse frequency does not appear to be different in the two genders, but GH trough levels are higher and peaks lower in women than men. Pulsatile GH secretion in the rat is diminished in states of altered nutrition (diabetes, obesity, deprivation). In vivo administration of SRIF antiserum restores GH secretion in food-deprived rats. During stress, CRH-mediated SRIF secretion provides the basis for inhibition of GH secretion observed in this state. TRH appears to stimulate SRIF release, whereas galanin increases hypothalamic SRIF secretion. Acetylcholine inhibits SRIF release and induces GHRH secretion. Similarly, the other neurotransmitter-mediated regulation of hypothalamic SRIF secretion mirrors that of the GHRH, although studying SRIF neurons has been proven to be a task of considerable difficulty, because of their multiple connections and widespread presence.

In the pituitary, SRIF inhibits GH and TSH secretion and occasionally that of ACTH and PRL. In the GI tract, pancreas, and genitourinary tract, somatostatin inhibits gastrin, secretin, gastric inhibitory peptide, VIP, motilin, insulin, glucagon, and renin. These actions are the result of a combined endocrine, autocrine, and paracrine function of somatostatin, which is supported by its widespread gene expression and receptor distribution.

5.4. SRIF Analogs

In view of its ability to affect so many physiologic regulations, SRIF was expected to be of therapeutic value in clinical conditions associated with hyperactivity of endocrine and other systems. The finding that many tumors from neuroendocrine and other tissues expressed the SSTR subtypes raised these expectations, which, however, were hampered by the short half-life need for iv administration and nonspecific activity of the native peptide. These problems were overcome with the introduction of a number of SRIF analogs, which are more potent, have longer action and different activities than somatostatin, and do not require iv administration. The best-studied among these analogs is octreotide (D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr[ol]), which is currently used extensively in neuroendocrine tumor chemotherapy, the treatment of acromegaly, and for radioisotopic detection of these and other neoplasms.

6. CRH

6.1. CRH Gene and Prepro-CRH

The idea that the hypothalamus controlled pituitary corticotropin (ACTH) secretion was first suggested in the late 1940s, whereas experimental support for the existence of a hypothalamic CRH that regulates the hypothalamic-pituitary-adrenal (HPA) axis was obtained in 1955. In 1981, the sequence of a 41-amino-acid peptide from ovine hypothalami, designated CRH, was reported. This peptide showed greater ACTH-releasing potency in vitro and in vivo than any other previously identified endogenous or synthetic peptide.

CRH is synthesized as part of a prohormone. It is processed enzymatically and undergoes enzymatic modification to the amidated form (CRH[1–41]NH_2). Mammalian CRH has homologies with nonmammalian vertebrate peptides xCRH and sauvagine in amphibia (from frog brain/spleen and skin, respectively), and urotensin-I in teleost fish. It also has homologies with the two diuretic peptides Mas-DPI and Mas-DPII from the tobacco homworm *Manduca sexta*. The vertebrate homologs have been tested and found to possess potent mammalian and fish pituitary ACTH–releasing activity. In addition, they decrease peripheral vascular resistance and cause hypotension when injected into mammals.

The N-terminal of CRH is not essential for binding to the receptor, whereas absence of the C-terminal amide abolishes specific CRH binding to its target cells. Oxidation of a methionine residue abolishes the biologic activity of CRH, and this may be a mechanism for neutralization of the peptide in vivo. CRH bioavailability is also regulated by binding to CRH-binding protein (CRHBP), with which it partially colocalizes in the rat CNS and other tissues. CRHBP is present in the circulation, where it determines the bioavailability of CRH. In the CNS, CRHBP plays a role analogous to that of enzymes and transporters that decrease the synaptic concentration of neurotransmitters either by breaking it down (acetylcholinesterase) or by taking it up at the presynaptic site (dopamine, serotonin).

The CRH gene is expressed widely in mammalian tissues, including the hypothalamus, brain and peripheral nervous system, lung, liver, GI tract, immune cells and organs, gonads, and placenta. The biologic roles of extraneural CRH have not yet been fully elucidated, although it is likely that it might participate in the auto/paracrine regulation of opioid production and analgesia, and that it may modulate immune/inflammatory responses and gonadal function.

The human *CRH* gene has been mapped to chromosome 8 (8q13) (Table 1). It consists of two exons. The

3´-untranslated region of the hCRH gene contains several polyadenylation sites, which may be utilized differentially in a potentially tissue-specific manner. CRH mRNA polyA-tail length is regulated by phorbol esters in the human hepatoma CRH-expressing cell line NPLC, and this may have potential relevance for differential stability of CRH mRNA in various tissues in vivo. Alignment of the human, rat, and ovine CRH (oCRH) gene sequences has allowed comparison of the relative degree of evolutionary conservation of their various segments. These comparisons revealed that the 330-bp-long proximal segment of the 5´-flanking region of the hCRH gene had the highest degree of homology (94%), suggesting that it may play a very important role in CRH gene regulation throughout phylogeny. A conserved polypurine sequence feature of unknown biologic significance is present at –829 of hCRH (–801 of the oCRH gene) as well as in the –400-bp 5´-flanking region of POMC, rat GH, and other hormone genes. A segment at position 2213–2580 of the 5´-flanking region of the hCRH gene has >80% homology to members of the type-O family of repetitive elements, and another at –2835 to –2972 has homology to the 3´-terminal half of the Alu I family of repetitive elements.

CRH regulation by the PKA pathway is well documented. Administration of cAMP increases CRH secretion from perfused rat hypothalami, and forskolin, an activator of adenylate cyclase, increases CRH secretion and CRH mRNA levels in primary cultures of rat hypothalamic cells. Regulation of the hCRH gene by cAMP has also been demonstrated in the mouse tumorous anterior pituitary cell line AtT-20, stably or transiently transfected with the hCRH gene. The hCRH 5´-flanking sequence contains a perfect consensus CRE element that is conserved in the rat and sheep.

TPA, an activator of pKC and ligand of the TPA-response element that mediates epidermal growth factor (EGF) function and binds AP-l, stimulates CRH mRNA levels and peptide secretion in vitro. TPA also increases CRH mRNA levels by almost 16-fold and CRH mRNA poly-A tall length by about 100 nucleotides in the human hepatoma cell line NPLC. The proximal 0.9 kb 5´-flanking the hCRH gene confers TPA inducibility to a CAT reporter in transient expression assays. In the absence of a clearly discernible perfect TRE in this region, it has been suggested that the CRE of the CRH promoter may, under certain conditions, elicit TRE-like responses, thus conferring TPA responsivity to the CRE site. Further upstream into the 5´-flanking region of the hCRH gene, eight perfect consensus AP-1-binding sites have been detected. Their ability to mediate TPA-directed enhancement of hCRH gene expression has not yet been tested by conventional reporter gene assays. EGF, however, has been shown to stimulate ACTH secretion in the primate and to stimulate directly CRH secretion by rat hypothalami in vitro.

Glucocorticoids play a key regulatory role in the biosynthesis and release of CRH. They downregulate rat and ovine hypothalamic CRH content. However, adrenalectomy and administration of dexamethasone in the rat elicit differential CRH mRNA responses in the PVN and the cerebral cortex, respectively, stimulating and suppressing it in the former, but not influencing it in the latter. Glucocorticoids can also stimulate hCRH gene expression in other tissues, such as the human placenta and the central nucleus of the amygdala. A construct containing the proximal 900 bp of the 5´-flanking region of the hCRH gene was found to confer negative and positive glucocorticoid effects, depending on the coexpression of a glucocorticoid receptor (GR)–containing plasmid. The molecular mechanism by which glucocorticoids regulate IICRH gene expression is somewhat obscure. Suppression might be mediated by the inhibitory interaction of the activated GR with the *c-jun* component of the AP- 1 complex. On the other hand, glucocorticoid enhancement of hCRH gene expression might be mediated by the potentially active half-perfect glucocorticoid-responsive elements (GREs) present in the 5´-flanking region of the gene, since half-GREs have been shown to confer delayed secondary glucocorticoid responses in other genes.

Gonadal steroids may modulate hGRH gene expression. Human female hypothalami have higher CRH content than the male ones. E_2 stimulates rat PVN CRH mRNA levels. A bidirectional interaction between the HPA and gonadal axes has been suggested on the basis of hCRH gene responsiveness to gonadal hormones. A direct E_2 enhancement of the CAT reporter was found by using two overlapping hCRH 5´-flanking region-driven constructs. Furthermore, the two perfect half-palindromic estrogen-response elements (EREs) present in the common area of both CRH constructs bound specifically to a synthetic peptide spanning the DNA-binding domain of the human estrogen receptor, suggesting that hCRH gene is under direct E_2 regulation.

Tissue-specific regulation of hCRH gene expression has been suggested for the human decidua and placenta. In rodents, such regulation was absent, which probably accounts for the differences in placental CRH expression between these species and primates. Differential distribution of short and long hCRH mRNA transcripts has been detected in several tissues and under varying physiological conditions. Tissue-specific and/or stress-

dependent differential utilization of the two hCRH promoters may explain these observations. Differential mRNA stability would then be a particularly important feature in CRH homeostasis, primarily in conditions of chronic stress, since in the latter case, sustained production of CRH would be required, and the long stable mRNAs produced by activation of the distal promoter would be beneficial to the organism.

6.2. CRH Receptors

In the pituitary, CRH acts by binding to membrane receptors (CRH-Rs) on corticotropes, which couple to guanine nucleotide–binding proteins and stimulate the release of ACTH in the presence of Ca^{2+} by a cAMP-dependent mechanism. CRH stimulation of cAMP production increases in parallel with the secretion of ACTH in rat pituitary corticotropes and human corticotrope cells. In addition to enhancing the secretion of ACTH, CRH stimulates the *de novo* biosynthesis of POMC. CRH regulation of POMC gene expression in mouse AtT-20 cells involves the induction of *c-fos* expression by cAMP- and Ca^{2+}-dependent mechanisms.

Sequence analysis of hCRH-R cDNAs isolated from cDNA libraries prepared from human corticotropinoma or total human brain mRNA revealed homology to the GPCR superfamily. The hCRH-R cDNA sequences of the tumor and normal brain were aligned and found to be identical. The hCRH-R gene has been assigned to 17q12-qter. Human/rodent CRH-R protein sequences differ primarily in their extracellular domains. In particular, positively charged arginine amino acids are present in the third and fourth positions of the extracellular amino-terminal domain sequences of the rodent, but not the hCRH-R peptide. This might be responsible for the differential activity of the α-helical 9–41 CRH antagonist between rodents and primates.

Central sites of CRH-R expression include the hypothalamus, the cerebral cortex, the limbic system, the cerebellum, and the spinal cord, consistent with the broad range of neural effects of CRH administered intracerebroventricularly, including arousal, increase in sympathetic system activity, elevations in systemic blood pressure, tachycardia, suppression of the hypothalamic component of gonadotropin regulation (GnRH), suppression of growth, and inhibition of feeding and sexual behaviors characteristic of emotional and physical stress.

A splice variant of the hypothalamic hCRH-R, referred to as hCRH-$R1A_2$, was identified in a human Cushing disease tumor cDNA library, in which 29 amino acids were inserted into the first intracellular loop. This protein has a pattern of distribution similar to that of the hypothalamic hCRH-R (hCRH-R1A). A different CRH-R, designated CRH-R2, was recently cloned from a mouse heart cDNA library. It is expressed in the heart, epididymis, brain, and GI tract and has its own splice variant expressed in the hypothalamus. The pattern of expression of the CRH-R2 protein differs from that of CRH-R1A, but its functional significance is currently unknown. Apparently, both rodents and humans express the CRH-R2 type.

6.3. CRH Neurons: Regulation and the Central Stress System

CRH is the primary hormonal regulator of the body's stress response. Exciting information collected from anatomic, pharmacologic, and behavioral studies in the past decades has suggested a broader role for CRH in coordinating the stress response than had been suspected previously (Fig. 3). The presence of CRH-R in many extrahypothalamic sites of the brain, including parts of the limbic system and the central arousal-sympathetic systems in the brain stem and spinal cord, provides the basis for this role. Central administration of CRH was shown to set into motion a coordinated series of physiologic and behavioral responses, which included activation of the pituitary–adrenal axis and the sympathetic nervous system, enhanced arousal, suppression of feeding and sexual behaviors, hypothalamic hypogonadism, and changes in motor activity, all characteristics of stress behaviors. Factors other than CRH also exert major regulatory influences on the corticotropes.

It appears that there is a reciprocal positive interaction between CRH and arginine vasopression (AVP) at the level of the hypothalamic-pituitary unit. Thus, AVP stimulates CRH secretion, whereas CRH causes AVP secretion in vitro. In nonstressful situations, both CRH and AVP are secreted in the portal system in a pulsatile fashion, with approx 80% concordancy of the pulses. During stress, the amplitude of the pulsation increases, whereas if the magnocellular AVP-secreting neurons are involved, continuous elevations of plasma AVP concentrations are seen.

Both CRH and AVP are released following stimulation with catecholamines. Indeed, the two components of the stress system in the brain, the CRH/AVP and the locus cerulus/noradrenergic (LC/NE) neurons, are tightly connected and are regulated in parallel by mostly the same factors. Reciprocal neural connections exist between the CRH and noradrenergic neurons, and there are autoregulatory ultrashort neg\ative-feedback loops on the CRH neurons exerted by CRH and on the catecholaminergic neurons exerted by NE via collateral fibers and presynaptic receptors. Both CRH and noradrenergic neurons are stimulated by serotonin and acetylcholine and inhibited by glucocorticoids, by the GABA/

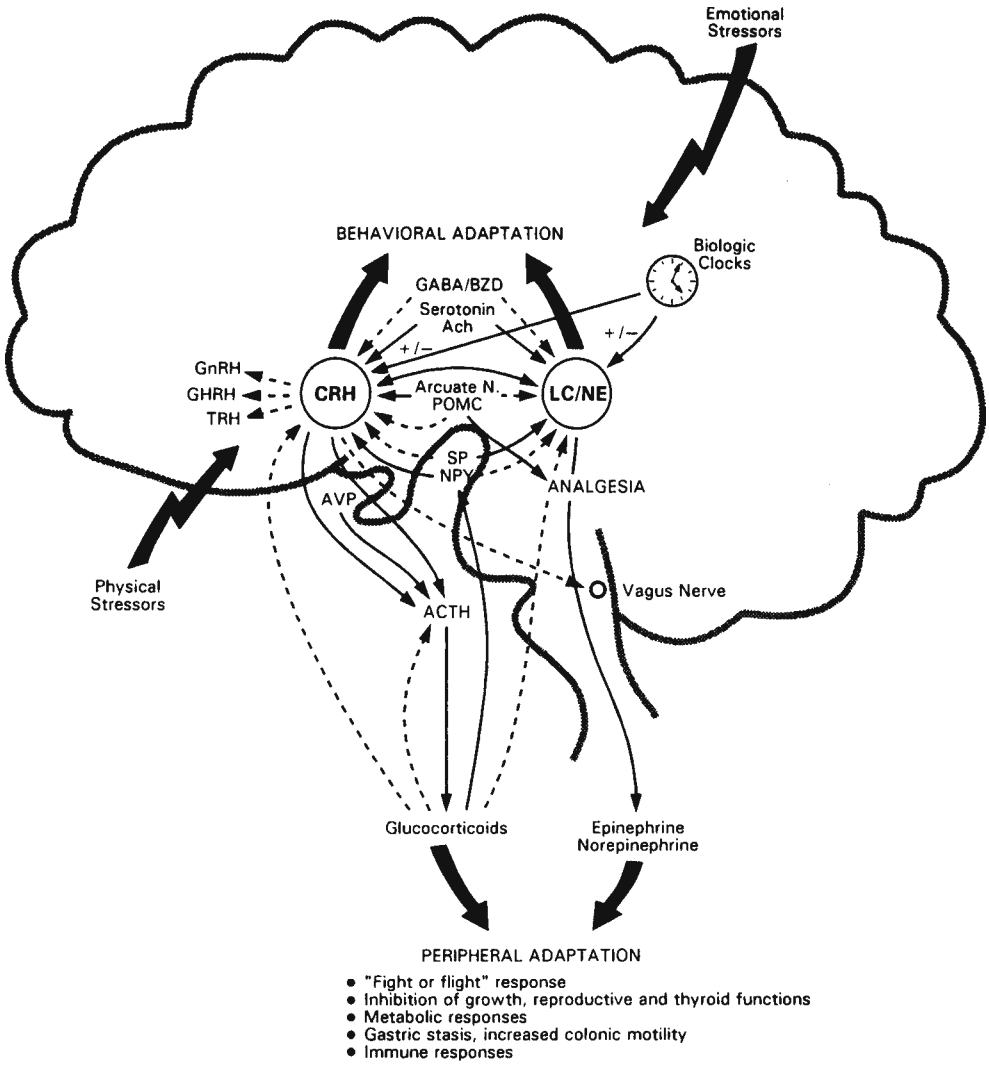

Fig. 3. Simplified representation of central and peripheral components of stress system, their functional interrelations, and their relations to other CNS systems involved in stress response. Solid lines represent direct or indirect activation, and dashed lines represent direct or indirect inhibition. Ach acetylcholine; ACTH = corticotropin; Arcuate N = arcuate nucleus; AVP = vasopressin; GABAIBZD = γ-aminobutyric acid/benzodiazepine receptor system; GHRH = growth hormone–releasing hormone; GnRH = gonadotropin-releasing hormone; LC = locus cerulus; NE = norepinephrine; NPY neuropeptide Y; PAF = platelet-activating factor; POMC = proopiomelanocortin; RH = corticotropin-releasing hormone; SP substance P; TRH = thyrotropin-releasing hormone.

benzodiazepine receptor system and by POMC-derived peptides (ACTH, α-melanocyte-stimulating hormone, β-endorphin) or other opioid peptides, such as dynorphin. Intracerebroventricular administration of NE acutely increases CR11, AVP, and ACTH concentrations, whereas NE does not affect pituitary ACTH secretion. Thus, catecholamines act mainly on suprahypophyseal brain sites and increase CR11 and AVP release.

Activation of the stress system stimulates hypothalamic POMC-peptide secretion, which reciprocally inhibits the activity of the stress system, and, in addition, through projections to the hindbrain and spinal cord, produces analgesia. CR11 and AVP neurons cosecrete dynorphin, a potent endogenous opioid derived from the cleavage of prodynorphin, which acts oppositely at the target cells. NPY- and substance P (SP)–secreting neurons also participate in the regulation of the central stress system by resetting the activity of the CRH and AVP neurons. Activation of the central NPY system overrides the glucocorticoid negative feedback exercised at hypothalamic and other suprahypophyseal areas, since icy administration of NPY causes sustained hypersecretion of CRH and AVP, despite high plasma

cortisol levels. NPY, on the other hand, suppresses the LCINE sympathetic system through central actions on these neurons. The importance of NPY lies in the fact that it is the most potent appetite stimulant known in the organism and may be involved in the regulation of the HPA axis in malnutrition, anorexia nervosa, and obesity. SP is an 11-amino-acid peptide that belongs to the tachykinin family, together with neurokinins A and B. SP is present in the median eminence and elsewhere in the central and peripheral nervous systems. In the hypothalamus, it exerts negative effects on the CRH neurons, whereas it regulates positively the LC/NE neurons of the brainstem. SP plays a major role in the neurotransmission of pain and may be involved in the regulation of the HPA axis in chronic inflammatory or infectious states. NPY, somatostatin, and galanin are colocalized in noradrenergic vasoconstrictive neurons, whereas VIP and SP are colocalized in cholinergic neurons.

CRH neurons may be affected during stress by other factors, such as angiotensin II, the inflammatory cytokines, and lipid mediators of inflammation. The latter two are particularly important, because they may account for the activation of the HPA axis observed during the stress of inflammation. In the human, interleukin-6 (IL-6) is an extremely potent stimulus of the HPA axis. The elevations of ACTH and cortisol attained by IL-6 are well above those observed with maximal stimulatory doses of CRH, suggesting that parvocellular AVP and other ACTH secretagogues are also stimulated by this cytokine. In a dose response, maximal levels of ACTH are seen at doses at which no peripheral AVP levels are increased. At higher doses, however, IL-6 stimulates peripheral elevations of AVP, indicating that this cytokine is also able to activate magnocellular AVP-secreting neurons. The route of access of the inflammatory cytokines to the central CRH and AVP-secreting neurons is not clear, given that the cellular bodies of both are protected by the blood-brain barrier. It has been suggested that they may act on nerve terminals of these neurons at the median eminence through the fenestrated endothelia of this circumventricular organ. Other possibilities include stimulation of intermediate neurons located in the organum vasculosum of the lamina terminalis, another circumventricular organ. In addition, crossing the blood-brain barrier with the help of a specific transport system has not been excluded. Furthermore, and quite likely, each of these cytokines might initiate a cascade of paracrine and autocrine events with sequential secretion of local mediators of inflammation by nonfenestrated endothelial cells, glial cells, andlor cytokinergic neurons, finally causing activation of CR11 and AVP-secreting neurons.

In addition to setting the level of arousal and influencing the vital signs, the stress system interacts with two other major CNS elements; the mesocorticolimbic dopaminergic system and the amygdala/hippocampus. Both of these are activated during stress and, in turn, influence the activity of the stress system. Both the mesocortical and mesolimbic components of the dopaminergic system are innervated by the LC/NE sympathetic system and are activated during stress. The mesocortical system contains neurons whose bodies are in the ventral tegmentum, and whose projections terminate in the prefrontal cortex and are thought to be involved in anticipatory phenomena and cognitive functions. The mesolimbic system, which also consists of neurons of the ventral tegmentum that innervate the nucleus accumbens, is believed to play a principal role in motivational/reinforcement/reward phenomena.

The amygdala/hippocampus complex is activated during stress primarily by ascending catecholaminergic neurons originating in the brain stem or by inner emotional stressors, such as conditioned fear, possibly from cortical association areas. Activation of the amygdala is important for retrieval and emotional analysis of relevant information for any given stressor. In response to emotional stressors, the amygdala can directly stimulate both central components of the stress system and the mesocorticolimbic dopaminergic system. Interestingly, there are CRH peptidergic neurons in the central nucleus of the amygdala that respond positively to glucocorticoids and whose activation leads to anxiety. The hippocampus exerts important, primarily inhibitory influences on the activity of the amygdala, as well as on the PVN/CRH and LC/NE sympathetic systems.

6.4. CRH Secretion and Pathophysiology

ACTH, a 39-amino-acid peptide-proteolytic product of POMC, is the key effector of CRH action, as a regulator of glucocorticoid secretion by the adrenal cortex. The regulatory influence of CRH on pituitary ACTH secretion varies diurnally and changes during stress. The highest plasma ACTH concentrations are found at 6 AM to 8 PM, and the lowest concentrations are seen around midnight, with episodic bursts of secretion appearing throughout the day. The mechanisms responsible for the circadian release of CRH, AVP, and ACTH are not completely understood but appear to be controlled by one or more pacemakers, including the suprachiasmatic nucleus. The diurnal variation of ACTH secretion is disrupted if a stressor is imposed and/or changes occur in zeitgebers, e.g., lighting and activity. These changes affect CRH secretion, which, in turn, regulates ACTH responses.

Glucocorticoids are the final effectors of the HPA axis and participate in the control of whole-body homeostasis and the organism's response to stress. They play a key regulatory role in CRH secretion and the basal activity of the HPA axis, and in the termination of the stress response by exerting negative feedback at the CNS components of the stress system. The other component of the peripheral stress system is the systemic sympathetic and adrenomedullary divisions of the ANS. It widely innervates vascular smooth muscle cells, as well as the adipose tissue and the kidney, gut, and many other organs. In addition to acetylcholine, norepinephrine, and epinephrine, both the sympathetic and the parasympathetic divisions of the ANS secrete a variety of neuropeptides, including CRH itself.

Several states seem to represent dysregulation of the generalized stress response, normally regulated by the CRH neurons and the stress system. In melancholic depression, the cardinal symptoms are the hyperarousal (anxiety) and suppression of feeding and sexual behaviors (anorexia, loss of libido), and excessive and prolonged redirection of energy (tachycardia, hypertension), all of which are extremes of the classic manifestations of the stress reaction. Both the HPA axis and the sympathetic system are chronically activated in melancholic depression. In a postmortem study, individuals who have had depression were found to have had a three- to fourfold increase in the number of hypothalamic PVN CRH neurons, when compared with normal age-matched control subjects. This could be an inherent feature of melancholic depression or a result of the chronic, although intermittent, hyperactivity of the HPA axis that is known to occur in these patients.

Chronic activation of the HPA axis has been shown also in a host of other conditions, such as anorexia nervosa; panic anxiety; obsessive-compulsive disorder; chronic active alcoholism, alcohol and narcotic withdrawal, excessive exercising; malnuthtion; and, more recently, in sexually abused girls. Animal data are rather confirmatory of the association between chronic activation of the HPA axis and affective disorders. Traumatic separation of infant rhesus monkeys and laboratory rats from their mothers causes behavioral agitation and elevated plasma ACTH and cortisol responses to stress that are sustained later in life. Such activation of the CRH system was originally thought to be an epiphenomenon, as a result of stress. Administration of CRH to experimental animals, however, with its profound effect on totally reproducing the stress response suggested that CRH is a major participant in the initiation and/or propagation of a vicious cycle.

Interaction of CRH with the other hormone regulatory systems provides the basis for the various endocrine manifestations of CRH hypersecretion/ chronic hyperactivity of the stress system. CRH suppresses the secretion of GnRH by the arcuate neurons of the hypothalamus either directly or via the stimulation of arcuate POMC peptide-secreting neurons, whereas glucocorticoids exert inhibitory effects at all levels of the reproductive axis, including the gonads and the target tissues of sex steroids (Fig. 4A). Suppression of gonadal function caused by chronic HPA activation has been demonstrated in highly trained runners of both sexes and ballet dancers. These subjects have increased evening plasma cortisol and ACTH levels, increased urinary free cortisol excretion, and blunted ACTH responses to exogenous CRH; males have low LH and testosterone levels, and females have amenorrhea. Obligate athletes go through withdrawal symptoms and signs if, for any reason, they have to discontinue their exercise routine. This syndrome is possibly the result of withdrawal from the daily exercise-induced elevation of opioid peptides and from similarly induced stimulation of the mesocorticolimbic system. The interaction between CRH and the gonadal axis appears to be bidirectional. The presence of EREs in the promoter area of the human CRH gene and direct stimulatory estrogen effects on CRH gene expression implicate CRH, and, therefore, the HPA axis, as a potentially important target of ovarian steroids and a potential mediator of gender-related differences in the stress response.

In parallel to its effects on the gonadal axis, the stress system suppresses thyroid axis function via a number of known pathways, including SRIF-induced suppression of TRH and TSH secretion and glucocorticoid-mediated suppression of TSR secretion and the 5´-deiodinase enzyme (Fig. 4B). Thus, during stress, there is suppressed secretion of TRH and TSH and decreased conversion of T_4 into T_3 in peripheral tissues. This situation is similar to what is observed in the "euthyroid sick" syndrome, a phenomenon that serves to conserve energy during stress. The mediators of these changes in thyroid function include the CRH neurons, glucocorticoids, somatostatin, and cytokines. Accordingly, patients with melancholic depression; patients with anorexia; highly trained athletes; and patients with chronic, inflammatory diseases have significantly lower thyroid hormone concentrations than healthy control subjects.

Prolonged activation of the HPA axis leads to suppression of GH and inhibition of IGF-1 effects on target tissues (Fig. 4B). CRH-induced increases in somatostatinergic tone have been implicated as a potential mechanism of stress-induced suppression of GH secretion. In several stress system–related mood disorders, GH and/or IGF-1 levels are significantly decreased in

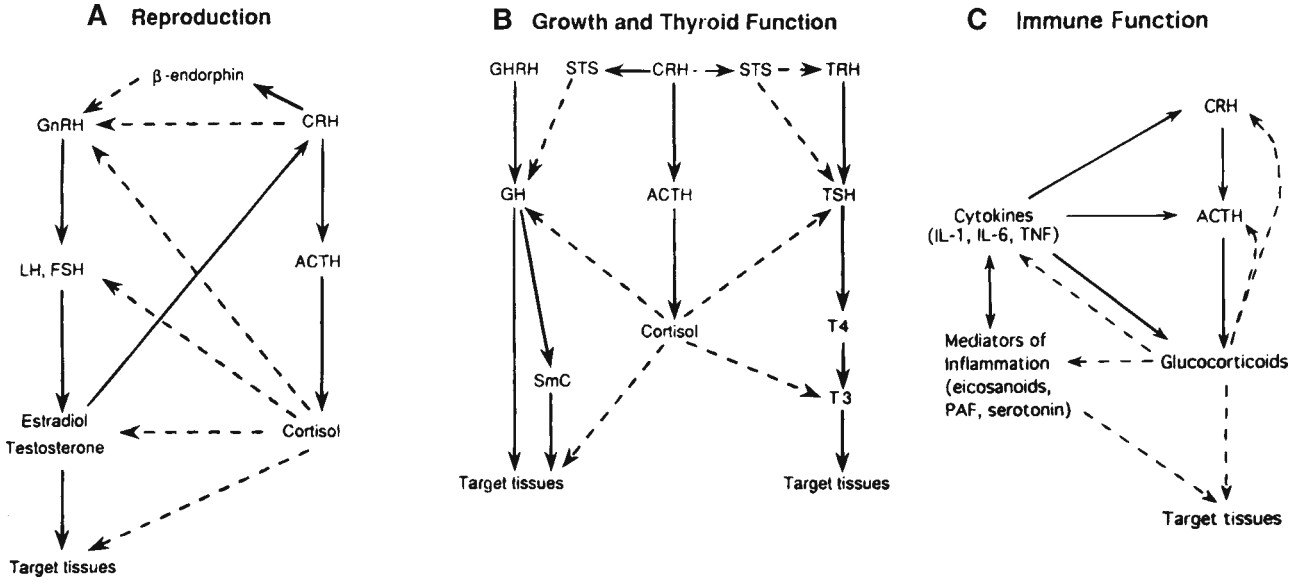

Fig. 4. Interactions of HPA axis and systems that subserve reproduction, growth, and metabolism. **(A)** Interactions between the HPA and reproductive axes; **(B)** interactions between HPA and and growth and thyroid axes; **(C)** interactions between HPA axis and immune system. Solid lines represent direct or indirect activation, and dashed lines, direct or indirect inhibition: Abbreviations are the same as those in Fig. 3; in addition, SmC = somatomedin C (insulin-like growth factor 1); STS = somatostatin (SRIF); TNF-α = tumor necrosis factor-α. (Modified with permission from Chrousos and Gold, 1992.)

animals and humans. Nervous pointer dogs, an animal model of anxiety with mixed panic and phobic features, were found to have low IGF- 1 levels and lower body growth than nonaffected animals. Patients with panic disorder, compared with healthy control subjects, had blunted GH responses to clonidine administered intravenously, and children with anxiety disorders can be short in stature.

The association between chronic, experimentally induced psychosocial stress and a hypercortisolism/metabolic syndrome-X–like state, with increased incidence of atherosclerosis, was recently reported in cynomolgus monkeys. In these animals, chronic psychosocial stress-induced activation of the HPA axis led to hypercortisolism, dexamethasone nonsuppression, visceral obesity, insulin resistance, hypertension, suppression of GH secretion, and osteoporosis.

GI function is also affected by chronic CRH hypersecretion. During stress, gastric emptying is delayed, whereas colonic motor activity increases in animals and humans. Innervations by the vagus nerve and the LCINE sympathetic system provide the network for the rapid responses of the GI system to stress. CRH microinjected into the PVN was shown to reproduce the stress responses of the GI system in an animal model, including inhibition of gastric emptying and stimulation of colonic transit and fecal excretion. This effect was abolished by the intrathecal administration of a CRH antagonist. CRH may be implicated in mediating the gastric stasis observed during the stress of surgery and/or anesthesia. IL- 1β, a potent cytokine that is found increased during surgery and in the immediate postoperative period, also inhibits gastric motility. Intrathecal administration of a CRH antagonist prevented surgery-induced rises in IL-1β in rats, thus suggesting that CRH may be an important mediator of IL-1β-induced gastric stasis. CRH hypersecretion could be the hidden link between the symptoms of chronic GI pain and history of abuse, since young victims of abuse demonstrate CRH hypersecretion.

A large infrastructure of anatomic, chemical, and molecular connections allows communication within and between the neuroendocrine and immune systems (Fig. 4C). In addition to the HPA axis, which via glucocorticoids exerts major immunosuppressive and anti-inflammatory effects, the efferent sympathetic/adrenomedullay system participates in the restraint immune/inflammatory reaction by transmitting neural signals to the immune system. This is mediated through a dense innervation of both primary and secondary lymphoid organs, and by reaching all sites of inflammation via postganglionic sympathetic neurons. The sympathetic system, when activated, causes systemic secretion of IL-6, which by inhibiting the other two inflammatory cytokines, tumor necrosis factor-α (TNF-α) and IL-1, and by activating the HPA axis, participates in the stress-

induced suppression of the immune inflammatory reactions. Stress-associated CRH hypersecretion, and the resultant glucocorticoid- , catecholamine-, and IL-6-mediated immunosuppression correlate well with such clinical observations as the suppression of the immune and inflammatory reaction during chronic psychologic and physical stress, the reactivation of autoimmune diseases during the postpartum period or following cure of Cushing syndrome, and the decreased ability of the stressed organism to fight viral infections and neoplasms.

In contrast to states with a hyperactive stress system, there is a host of different conditions, such as atypical or seasonal depression in the dark months of the year, the postpartum period, the period following the cessation of smoking, rheumatoid arthritis, and the chronic fatigue and fibromyalgia syndromes, that represent hypoarousal states. In these conditions, CRH secretion is decreased and symptoms, such as increase in appetite and weight gain, somnolence, and fatigue are seen.

6.5. Clinical Uses of CRH

The CRH stimulation test (1 µg/kg intravenously) is used clinically in the differential diagnosis of Cushing syndrome alone or in combination with inferior petrosal sinus sampling (IPSS). More than 80% of patients with CD respond to iv oCRH with an increase in ACTH and cortisol in the first 30–45 mm of the test. Most patients with ACTH-independent Cushing syndrome do not respond to this test, whereas ectopic ACTH-producing tumors occasionally respond. IPSS is the best available test for the diagnosis of CD, because >95% of the patients with CD respond to intravenously administered oCRH with a twofold increase in their petrosal sinus over peripheral ACTH levels in the first 3–10 min of the test, and 100% of the patients with Cushing syndrome from other causes do not respond. The administration of dexamethasone prior to the oCRH test has been suggested for the differential diagnosis of Cushing syndrome vs pgeudo-Cushing states. In primary adrenal insufficiency, patients respond to oCRH with markedly elevated ACTH levels, whereas two patterns have been described in patients with secondary arterial insufficiency: a pituitary pattern with absence of an ACTH response, and a hypothalamic pattern with a delayed and prolonged ACTH response to oCRH.

In the clinical investigation of depression and other disorders of the HPA axis, including anorexia nervosa, panic anxiety, abuse, malnutrition, addiction, and withdrawal syndromes and autoimmune diseases, the oCRH-stimulation test, as a sensitive indicator of corticotrope function, has been proven to be an invaluable tool. A variety of CRH analogs have been synthesized but not used in clinical trials. They bind specifically to the CRH-Rs, and in vitro studies have suggested that they might find therapeutic use in the treatment of disorders of the HPA axis.

Recently, two groups of substances were discovered that might be therapeutically useful. Nonpeptide CRH antagonists might prove useful in the treatment of melancholic depression, anorexia nervosa, panic anxiety, withdrawal from addiction agents, and other conditions associated with hyperactivation of the HPA axis. Conversely, CRFI-BP antagonists might provid~ a means of increasing levels of CRH in states characterized by low CRH, such as atypical depression, chronic fatigue/fibromyalgia syndromes, and autoimmune disorders.

7. DOPAMINE

7.1. Dopamine Synthesis and Dopaminergic Neurons

Dopamine is a catecholamine neurotransmitter and a hormone with a wide distribution and array of functions in the animal kingdom. It differs from the other catecholamines in that it is present in many nonneuronal tissues, but in relatively limited areas of the brain. It is a hypothalamic hormone directly involved in the regulation of PRL secretion from pituitary lactotropes, where, unlike other neurotransmitters, it forms its own short-feedback loop and is released in great quantities.

Dopamine is endogenously synthesized by hydroxylation of L-tyrosine (by tyrosine hydroxylase [TH]) and subsequent decarboxylation of the product (L-dopa) by the aromatic- L-amino acid decarboxylase.

The TH step is the rate-limiting step in the synthesis of dopamine. An increase in hydroxylation of tyrosine can be demonstrated rapidly after the stimulation of catecholaminergic neurons. Tetrahydrobiopterin is an important cofactor in the TH reaction, and its availability plays a regulatory role in the in vivo stimulation of TH activity. TH exhibits product inhibition by catecholamines and is stimulated by acetylcholine and by phosphorylation from a cAMP-dependent kinase. The TH gene is located on chromosome lip and codes for a cDNA that is approx 1900 bp long. Multiple mRNA species have been identified, indicating that tissue-specific regulation of TH gene expression is extensive. Unlike TH, which is only located in catecholamine-producing neurons and neuroendocrine cells, the L-dopa decarboxylase is expressed in many neuronal and nonneuronal tissues. It is not substrate specific and decarboxylates a variety of amino acids.

There are four major dopamine pathways in the mammalian forebrain. Nerve cell bodies of origin are

clustered in nuclei in the rostral midbrain of three of these pathways, with the borders between the nuclei not always well defined. These nuclei have been shown to contain dopamine neurons. Anatomically, the most distinctive nuclei are the paired substantia nigra neurons, whose axons ascend rostrally in the nigrostriatal pathway to provide dopaminergic innervation of the corpus striatum (caudate and putamen). The substantia nigra neurons selectively degenerate in Parkinson disease. A closely paired nucleus, the ventral tegmental area, lies medially and dorsally to the substantia nigra, and its dopamine neurons provide two ascending pathways: (1) the mesolimbic, which provides dopamine innervation to forebrain limbic structures, especially the nucleus accumbens in the ventral striatum; and (2) the mesocortical, which provides dopamine innervation to the frontal and cingulate cortex. The fourth dopamine nucleus, the arcuate, is in the hypothalamus, projects to the median eminence through the tuberoinfundibular pathway and the intermediate lobe (in species that have this structure) through the tuberohypophyseal pathway, and releases dopamine directly into the hypophyseal portal circulation.

Although all of these groups of neurons synthesize dopamine by identical mechanisms, they are not identical functionally. Alterations in pituitary function related to changes in dopamine secretion by tuberoinfundibular neurons do not necessarily reflect alterations in other central dopaminergic systems. Tuberoinfundibular neurons are components of the short-loop feedback control of PRL secretion by the pituitary lactotrophs, and they possess PRL receptors but not dopamine receptors. Thus, dopaminergic drugs and their antagonists act directly on the mesolimbic and nigrostriatal systems and on the pituitary, but not on the tuberoinfundibular system.

7.2. Dopamine Regulation of PRL Secretion

The synthesis and release of PRL from lactotropes have been extensively studied over the past two decades. Unlike other anterior pituitary cells, lactotropes release their hormone at a high rate in the absence of hypothalamic regulation. Lesioning the median eminence, transecting the pituitary stalk, and grafting the pituitary beneath the kidney capsule all result in hyperprolactinemia. The incubation of pituitary fragments or dispersed cells in vitro is also associated with a sustained release of PRL.

Dopamine is the long-sought hypothalamic PRL-release inhibiting factor and the main modulator of the pleiotropic regulation of PRL secretion. Concentrations of dopamine in portal blood are maintained at physiologically active levels, and lactotropes contain dopamine receptors. PRL levels increase after treatment with dopamine antagonists and when dopamine is removed from the perfusion medium of cultured pituitary cells. Neither dopamine nor hypothalamic function is necessary for the pulsatile release of PRL from the pituitary, but tonic inhibition by the former synchronizes PRL secretion.

Both TRH and VIP stimulate PRL release, although only the former appears to be affected by dopamine. Part of the suckling-induced release of PRL appears to be mediated by TRH, and this effect can be prevented by the administration of a dopamine agonist. A trough in vivo or removal of dopamine secretion in vitro appears to enhance PRL release by TRH. By contrast, the transient removal of dopamine has no effects on VIP-induced PRL release, and blockade of dopamine receptors does not potentiate VIP or oxytocin-stimulated PRL release. Significant reduction of the rat portal concentrations of dopamine is observed immediately before large releases of PRL, such as during the last day of pregnancy and in response to suckling and estradiol, the latter on the afternoon of proestruS.

7.3. Dopamine Receptors

Pituitary lactotrope regulation by dopamine is primarily through doparnine type-2 receptors (D2-R). Five DRs exist (D-1R–5R) and all their genes were cloned before 1991. They belong to the seven-transmembrane segment domain GPCR family and have common structural organization and some homology with serotoninergic and adrenergic receptors. D-IR and D-5R activate, whereas D-2R, D-3R, and D-4R inhibit adenylate cyclase. The third cytoplasmic loop is short in the former and long in the latter. It is generally believed that receptors with a short third cytoplasmic loop couple to stimulatory G proteins (G_s) and, thus, activate adenylate cyclase, whereas those with a long third cytoplasmic loop react with G_i and G_o, which inhibit adenylate cyclase, and G_q, which couples with PLC. Although the structures of the extra- and intracellular loops of the DRs vary with each receptor, the transmembrane domains are highly homologous. The genes for these receptors are located on different chromosomes in humans ($5q3^4$, 11q22, 3q13, 4pl6, and 4p16 for the D-1R, D-2R, D-3R, D-4R, and D-5R, respectively) and are intronless for the activating D-1R and D-5R but contain 6, 5, and 4 introns for the inhibitory D-2R, D-3R, and D-4R, respectively. Posttranslational processing is extensive for the latter three receptors, resulting in a greater number of receptor isoforms.

The action of dopamine on pituitary PRL release is mediated through D-2R, the first DR to be cloned, and a receptor that is abundant in the pituitary, striaturn,

nucleus accumbens, olfactory tubercle, and substantia nigra. There are two isoforms of the D-2R that differ in length by 29 amino acids owing to an insertion in the third cytoplasmic loop. Both forms are expressed in all the tissues in which D-2Rs are present, including the pituitary, and are equipotent in inhibiting adenylate cyclase and activating K^+ channels, the latter an action unique to D-2Rs among the dopamine receptors.

Administration of dopamine decreases cAMP concentration in pituitary cells in vitro. It also inhibits the Ca^{2+} second-messenger system and decreases intracellular Ca^{2+} concentration. PRL release is inhibited in Ca^{2+}-deficient medium and by Ca^{2+} channel blockers. The effects of dopamine on Ca^{2+} are mediated by a G protein–dependent mechanism or by direct coupling to Ca^{2+} channels. The effects of dopamine on PLC are less clear, and although PKC is involved in regulating PRL secretion, the evidence that dopamine regulates PKC activity is scant. Basal activity of PLC in the lactotropes is low, but dopamine dissociation from its receptor is associated with its activation. The latter is not dependent on adenylate cyclase activity, which is also significantly activated on dissociation of dopamine from the pituitary D-2R.

7.4. Hyperprolactinemia and the Use of D-2R Agonists

The anatomic (by surgery, or mass effects of a large pituitary or hypothalamic tumor) or functional (by the use of dopamine antagonists) uncoupling of the pituitary lactotropes from hypothalamic dopaminergic control results in hyperprolactinemia. The latter is a manifestation of a number of disorders of the hypothalamic-pituitary unit and leads to hypogonadism, decreased libido, and/or galactorrhea. It can also develop from the administration of neuroleptic drugs, such as reserpine (a catecholamine depletor), and phenothiazines, such as chlorpromazine and haloperidol. The PRL response to the latter is an excellent predictor of their antipsychotic effects.

Dopamine agonists have been developed and in clinical use for the management of hyperprolactinemia. Bromocriptine and, recently, pergolide and cabergoline are D-2R agonists that effectively restore PRL inhibition and are used in the medical management of pituitary prolactinomas. Only 10% of the latter are resistant to the action of bromocriptine; the rest respond with significant reduction of their size and resolution of hyperprolactinemia. Although dopamine agonists are useful for the reduction of PRL levels induced by disruption of hypothalamic function by other pituitary tumors, they are not effective in decreasing the size of non-PRL-secreting tumors.

The response to dopamine receptor stimulation and blockade is not specific for the central, pituitary, or peripheral actions of dopamine. Indeed, bromocriptine can induce schizophrenic psychosis in a small proportion of individuals with no prior history of mental disorders. In general, however, dopamine agonists have few side effects, and bromocriptine can be safely used during pregnancy, if needed.

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