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HYPOTHALAMIC HYPOPHYSIOTROPIC HORMONES AND NEUROTRANSMITTER REGULATION: CURRENT VIEWS

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INTRODUCTION

The discovery of the hypothalamic hormones, and their direct and indirect control of most of the endocrine system, constitutes a remarkable chapter in the history of endocrinology, physiology, pharmacology, biochemistry, and medicine. The existence of hormones in the brain of mammals was first postulated about 40–45 years ago (Friedgood, Brooks), and reports on their presence in the nervous tissues of nonmammalian species first appeared in the late 1920s (Ernst Sharrer and Berta Scharrer). However, it remained for the anatomist, Geoffrey Harris, of England to provide the necessary anatomical evidence on the vascular linkage of the hypothalamus to the pituitary that enabled him to postulate the "chemotransmitter hypothesis." This, in effect, stated that the hypothalamic portion of the brain produced and released hormones into the portal vessels emanating in the median eminence and coursing down the pituitary stalk to the pituitary gland. It remained for others, notably Schally and Guillemin, to do the tedious and ingenious labor necessary to isolate and chemically characterize at least three of the hypothalamic hormones, thyrotropin releasing hormone, luteinizing hormone–releasing hormone, and somatostatin. It is awesome to

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consider that without these brain peptides, thyroid function would cease, reproduction and all its related activities could not occur, and body growth and many related metabolic functions would be severely altered. The other hypothalamic hormones, which remain to be chemically defined and synthesized [corticotropin releasing factor (CRF), growth hormone releasing factor (GHRF), prolactin releasing factor (PRF), prolactin release–inhibiting factor (PIF), melanocyte stimulating hormone–releasing factor (MRF), and release-inhibiting factor (MIF)], also are of vital importance for many body functions.

Work on the influence of hypothalamic neurotransmitters on secretion of pituitary hormones began about 15 years ago. Much of the progress in this field has depended on the advances made by pharmacologists in developing methods for identifying and measuring these compounds in the brain. Most has been learned about the roles of hypothalamic dopamine (DA), norepinephrine (NE), and serotonin (5-HT) on secretion of pituitary hormones. Considerable evidence has accumulated in the last few years that the brain and possibly pituitary opiates also modulate secretion of pituitary hormones. These neurotransmitters are believed to act mainly by modulating the release of the hypothalamic hormones from their nerve terminals into the portal vessels. Several of these neurotransmitters have proven to be useful for treating clinical problems in endocrinology. The realization of the importance of these brain neurotransmitters in endocrine functions has led to a considerable degree of interaction and collaboration between endocrinologists and pharmacologists, and this trend undoubtedly will continue to grow in the future.

A number of reviews have appeared on the chemistry, pharmacology, and physiology of hypothalamic hormones and the neurotransmitters that influence their release (1–4). We attempt here to assess some of the more recent work in this field, and emphasize the physiology and pharmacology of these compounds in their relation to control of anterior pituitary function.

HYPOTHALAMIC HYPOPHYSIOTROPIC HORMONES

Luteinizing Hormone–Releasing Hormone

PHYSIOLOGY OF LHRH A large volume of information has accumulated on the effects of luteinizing hormone–releasing hormone (LHRH) on reproductive physiology and behavior. However, factors that regulate its synthesis in the hypothalamus and its release from the median eminence into the portal circulation are poorly understood. LHRH is a decapeptide that stimulates the release of luteinizing hormone (LH) and follicle-stimulating

hormone (FSH) from the gonadotropic cells of the anterior pituitary in a number of animal species, including man (5). Administration of synthetic LHRH to females increases release of LH and FSH, stimulates follicular maturation, and results in ovulation in many species tested. In males, LHRH increases the release of LH and FSH and stimulates spermatogenesis and androgen secretion. The action of LHRH on the gonadotropic cells of the pituitary is presumably mediated by binding to specific receptors with subsequent activation of cAMP (6). Antisera to LHRH decrease LH and FSH levels in a number of species, produce gonadal atrophy in rabbits, delay vaginal opening in immature female rats, and decrease sperm production in male rats (5). However, a majority of investigators continue to infer changes in LHRH synthesis and release based on measurements of concomitant changes in hypothalamic LHRH content and serum LH levels, reflecting the paucity of direct evidence for the role of LHRH in important physiological events. This topic has become more germane because of recent findings that in primates, LHRH may have only a permissive role in the LH surge, with the major influence produced by steroid hormone alterations of pituitary sensitivity to LHRH action (7). Thus, a complete understanding of the mechanisms of reproductive events must include those factors affecting LHRH synthesis and release.

LOCALIZATION OF LHRH

There is substantial agreement between bioassay and radioimmunoassay (RIA) techniques on the localization of LHRH. Bioassay techniques have localized LHRH activity in the hypothalamus of rodents in the medial basal region (MBH), as well as in more rostral regions, i.e. the suprachiasmatic nucleus, ventral portions of the medial preoptic area (MPOA), and anterior hypothalamus (8). RIAs of hypothalamic extracts have confirmed the bioassay data, and by using serial sections of the hypothalamus in different planes (9, 10) or utilizing a hypothalamic nuclear punch technique (11, 12) LHRH was localized in the retrochiasmatic, arcuate, and median eminence region, and in a prechiasmatic area that included the preoptic, anterior hypothalamic regions and the organum vasculosum of the lamina terminalis (OVLT).

Localization of LHRH by RIA has generally produced consistent results. However, immunocytochemical (ICC) techniques employed to find perikarya responsible for the synthesis of the decapeptide have been more controversial. Early attempts to identify LHRH perikarya in the arcuate nucleus of untreated animals produced neither valid nor consistent results, and it is now generally accepted that LHRH perikarya are found in the preoptic and septal areas (13). The difficulty in localizing LHRH in perikarya may

result not only from differences in ICC technique or low concentrations of LHRH in perikarya, but also from the use of antisera that have different antigenic properties. Hoffman et al (14) used five different antisera against LHRH, which were directed against different positions of the LHRH molecule. Two general neuronal fields were identified in mice: Field 1 included the retrochiasmatic area, tuberal area, and arcuate nucleus. Fibers from this area course rostrally to the anterior commissure and dorsally to the thalamus. Since this area has been identified only by the Sorrentino F antiserum (conjugated at the 2-histidyl position of the decapeptide), it was hypothesized that LHRH may be bound in a prohormone state at the N terminus. The recent demonstration of ACTH cell bodies in this area necessitates further experimentation to determine whether these cell bodies also are recognized by the Sorrentino F antiserum. Field 2 perikarya, which have been confirmed by others (13), were found in the medial preoptic, preoptic periventricular, and medial septal areas and in fibers projecting to the OVLT, median eminence, and thalamic regions. These cell bodies have been identified with antisera against LHRH conjugated at the N or C terminals of the decapeptide; the immunoreactivity and subsequent binding of LHRH to these antisera are not suppressed by alterations at either of these positions. King et al (15) studied the differences in LHRH content in four brain areas, using an antiserum against LHRH adsorbed on polyvinylpyrrolidone (PVP) that fails to recognize LHRH fragments with modified C or N terminals (No. 422), and an antiserum against LHRH conjugated to BSA that recognizes LHRH with modified C or N terminals (No. 743). The arcuate and MPOA areas had higher concentrations of LHRH immunoreactivity, as determined by antiserum No. 743, whereas median eminence and OVLT regions exhibited similar levels of LHRH as determined by both antisera. These results indicate that immunoreactive LHRH in perikarya may be bound at the N or C terminus, whereas that found in fiber regions is the mature decapeptide.

There is additional evidence that immunoreactive LHRH in perikarya may represent prohormone species of LHRH. Chromatography of hypothalamic extracts on Sephadex G-25® demonstrated three different molecular weight species of immunoreactive LHRH (16). These putative prohormone species were not affected by acid hydrolysis or boiling in 8 M urea, suggesting that they do not represent aggregated or protein-bound LHRH. Furthermore, estradiol implants for two weeks into ovariectomized female rats increased both the mature and high molecular weight forms of immunoreactive LHRH (17). Together, these data provide strong evidence for the existence of a prohormone for LHRH that may be enzymatically cleaved into the mature form of LHRH as it is transported down the axon terminal.

Although all LHRH antisera cross-react with the mature decapeptide, few antisera against LHRH are classified by their cross-reaction with LHRH fragments. Even less information is available on the capacity of LHRH antisera to detect prohormone species of LHRH. The classification of LHRH antisera becomes especially important since LHRH immunoreactivity has been detected in human milk (18), and in rat testis extracts (19). In rat testis, for example, some LHRH antisera have different abilities to detect substances similar to LHRH. That these substances are not the native hormone is demonstrated by the fact that antisera which require intact N and C terminals for binding do not cross-react with the substance. Thus, caution must be exercised in interpreting results using unclassified antisera.

More recently, LHRH that possesses both immunological and biological activity has been isolated from human placental extracts (20). Studies of incorporation of tritiated amino acids into LHRH further demonstrated that LHRH can be synthesized by the placenta in vitro. These results, if confirmed, suggest that LHRH of placental origin may regulate human chorionic gonadotropin (hCG) production and steroid hormone levels during pregnancy.

HYPOTHALAMIC LHRH CONTENT

Much information has accumulated on changes in hypothalamic LHRH content under various physiological and nonphysiological conditions. However, interpretation of these data is limited even with simultaneous determination of serum LH and FSH concentrations. LHRH content in the MBH of intact female rats fluctuates throughout the estrous cycle, with a major decrease occurring on the day of proestrus (21). Although the decrease in LHRH content is highly correlated with the surge of serum LH on the evening of proestrus, changes in LHRH content during estrus or diestrus show no clear relationship to serum LH. In rostral hypothalamic areas, including the MPOA and OVLT, there are temporal increases in LHRH content on diestrous day II, with a smaller increase on proestrus (22). The increases in rostral hypothalamic LHRH were hypothesized to result from increases in synthesis and transport of LHRH to caudal areas responsible for the release of the hormone into the portal vasculature. Despite the low correlation of hypothalamic LHRH and serum LH throughout the estrous cycle, it is clear that the content of LHRH in the arcuate-median eminence region of the hypothalamus in male and female rats is influenced by the negative feedback effects of gonadal steroids under experimental conditions. Ovariectomy decreases hypothalamic LHRH content and increases pituitary secretion of gonadotropins, whereas estrogen reverses this effect. Progesterone acted synergistically with estrogen in increasing LHRH content

(23). Although changes in LHRH content in the MBH occur in response to circulating steroids, basal levels of LHRH in rostral areas are not affected by castration or estrogen replacement (24).

Changes in hypothalamic LHRH content reflect concomitant alterations in LHRH synthesis, metabolism, or release. It is doubtful that any stimulus can affect any one of these systems independently. The situation is made more complex by investigators using different antisera against LHRH, only a limited number of which are characterized by binding to LHRH fragments. It is preferable to assess hypothalamic function by measuring changes in LHRH synthesis and release, but totally satisfactory methods for accomplishing these have not yet been developed. Sampling of portal blood from animals has proven to be very difficult and few laboratories are qualified to use this technique. Also, the animal preparation is unphysiological, since it is necessary to use anesthetics and radical surgical procedures to collect portal blood. Results also may be difficult to interpret. For example, some investigators fail to observe increases in LHRH in portal blood after castration when LH and FSH in the blood show marked increases, whereas others report increases in LHRH preceding the LH surge in female rats which others cannot confirm.

DEGRADATION OF LHRH

The activity of LHRH in the hypothalamus can be altered by stimuli that affect LHRH degradation. The hypothalamus contains specific peptidases which metabolize LHRH, and their activity and/or concentration is increased by estradiol (25), LH (26), and prostaglandins (27). Conversely, progesterone or gonadectomy have been shown to decrease degradative enzyme activity (25, 28). There are few studies of the effects of pharmacological stimuli on LHRH metabolism, but recent evidence indicates that dopamine, as well as peptide hormones, can influence LHRH degradation, suggesting a possible mechanism whereby neurotransmitters or peptides can affect hypothalamic LHRH activity (29, 30).

SYNTHESIS OF LHRH

The synthesis of LHRH has been studied in whole and sliced hypothalami, using incorporation of tritiated amino acids into LHRH (31, 32). However, the purification and identification of LHRH in the hypothalamic extract by chromatography or bioassay has proven very difficult, in part, because of the low concentrations of newly synthesized hormone. More recently, the use of specific antiserum against LHRH to precipitate the tritiated decapeptide has proven to be useful, since it avoids some of the problems of purifica-

tion (33). Others have measured in vitro production of LHRH by comparing changes in LHRH content between incubated and nonincubated hypothalami; this technique may be deceiving because it is unable to differentiate the conversion of prohormone species to LHRH from true de novo synthesis of LHRH (34).

LHRH DETERMINATIONS IN PERIPHERAL BLOOD

There have been many reports describing LHRH levels in serum of several species. However, the wide range of LHRH concentrations (less than 1 pg/ml to greater than 16 ng/ml) and the lack of correlation between serum LHRH and pituitary LH release suggest that many of these data are artifactual (35). Early studies indicated that macromolecular components of serum that were unrelated to LHRH could inhibit binding of radioiodinated LHRH to its specific antibody in RIA, thus producing artifically high concentrations of LHRH in serum (36, 37). More recent studies indicate that antiserum specificity, affinity, and method of radioiodination can influence the levels of LHRH detected in plasma (35).

Several attempts have been made to reduce or eliminate nonspecific interference in plasma LHRH determinations by extracting LHRH with methanol (36) or florisil (37), and there has been some success in measuring plasma LHRH with these methods. However, the rapid degradation of LHRH in plasma and the exceedingly small quantities of this hormone in plasma require a most sensitive assay (38, 39). The recent demonstration of immunologically and biologically active LHRH in human milk (18) and placental extracts (20) suggests that LHRH concentrations in peripheral plasma may have little physiological relationship to LHRH release from the hypothalamus.

EVIDENCE FOR A DIRECT EFFECT OF LHRH ON THE GONADS

LHRH and its agonists stimulate release of both LH and FSH from the pituitary of many species. However, large doses of LHRH and its agonists result in such paradoxical effects as inhibition of ova implantation (40, 41) and gestation (42, 43), delay in sexual maturation (44–46), reduced levels of plasma progesterone (41, 43), testicular atrophy, and suppression of spermatogenesis (46). Recently, these effects of LHRH and its agonists were found to be associated with a reduction of hCG/LH receptors in testicular and ovarian tissues (47, 48). Injection of large doses of LH or hCG also has been shown to decrease both ovarian adenylate cyclase activity and the number of LH receptors in the ovaries (49, 50). It was suggested that the

reduction of hCG/LH receptors by LHRH and its agonists was accounted for by "down regulation" resulting from an excess of circulating gonadotropins (47–48, 51). The mode of interference with implantation of fertilized ova in rats treated with the LHRH agonist, [D-Trp^6]-LH-RH, appears to be entirely different from that induced by administering large doses of LH or hCG. More recent data using in vitro incubation of ovarian or testicular homogenates, or gonadal extracts from hypophysectomized rats, indicate that LHRH and its agonists exert a direct effect on estrogen, progesterone, or testosterone production by these tissues (52, 53). The doses of LHRH used in these studies were not physiological, and it is unknown whether the normally low circulating levels of LHRH can substantially affect steroid hormone production by these tissues.

Somatostatin

PHYSIOLOGY OF SOMATOSTATIN Krulich et al (54) were the first to report the existence of a hypothalamic substance that inhibits the secretion of GH from the pituitary. Since that report, the structure of growth hormone release–inhibiting factor, or somatostatin has been characterized as a tetradecapeptide (55) and demonstrated to affect many pituitary hormones. Somatostatin depresses basal levels of GH in a number of species, including man, and antagonizes the increase in GH release produced by a number of physiological and pharmacological stimuli (56). Chronic administration of somatostatin decreases pituitary GH content and inhibits synthesis and release of TSH from cultured rat pituitary cells in vitro, and in vivo in hypothyroid humans. There is also some evidence that somatostatin may influence prolactin secretion in acromegaly (57), although it has not been shown to alter prolactin secretion in normal individuals. Passive immunization with antiserum against somatostatin has been reported to increase GH and TSH release (58), to enhance the release of TSH in response to TRH administration and to cold exposure (59), and to antagonize the decrease in GH release in response to electric shock (60) or to starvation stress (61). Paradoxically, somatostatin also may increase GH release from the anterior pituitary under some circumstances. Intraventicular injection of somatostatin into rats increased serum GH levels within 15 min, and it was hypothesized that this may represent an ultrashort feedback loop effect on somatostatin secretion (62).

Tannenbaum et al (63) have shown that the secretion of GH in rats is pulsatile in nature, with a period of approximately 3.3 hr, and evidence suggests that somatostatin has an important role in establishing this rhythm. Lesions of the medial preoptic area, which decrease hypothalamic somatostatin content and increase basal GH concentrations in serum, also

result in more frequent surges of GH. Administration of antiserum against somatostatin increased trough GH levels, but did not influence the mean peak levels of GH (64). Martin has suggested that somatostatin may be secreted intermittently and interact with a putative GHRF to establish the characteristic rhythm of GH secretion (64).

LOCALIZATION OF SOMATOSTATIN Somatostatin has been localized by RIA and bioassay in the median eminence, arcuate nucleus, medial preoptic area, and periventricular nucleus of the rat hypothalamus (65). Somatostatin immunoreactivity also was demonstrated in the ventromedial nucleus (VMN), amygdala, mammillary bodies, and olfactory tubercle (66). The failure to detect somatostatin activity by bioassay techniques in the VMN was hypothesized to result from the high concentrations of GHRF (65, 66) in this area.

Somatostatin-containing fibers have been localized by immunocytochemistry in the external zone of the median eminence and ventromedial nucleus of guinea pigs (67). More recent studies, using the peroxidase-labeled antibody method, demonstrated somatostatin-containing nerve fibers in the median eminence and in part of the tuberoinfundibular tract (68). No cell bodies were found in these studies, but the somatostatin peptide was generally localized in the arcuate and ventromedial nuclei, and medial basal area of the hypothalamus. Within the tuberoinfundibular tract, somatostatin-containing nerve fibers were generally caudal, dorsal, and medial to LHRH fibers, demonstrating that these peptides are localized in separate subsystems of this tract. More recently, both LHRH and somatostatin have been localized in nerve endings of the OVLT region of the rat hypothalamus, in close proximity to the capillary network. Recent experiments also demonstrated the presence of somatostatin perikarya in the periventricular and preoptic regions of the rat hypothalamus (69, 70). This is further supported by evidence indicating that lesions of the preoptic-anterior hypothalamic area increase serum levels of GH, whereas stimulation of this area increased somatostatin levels in portal blood and decreased serum GH levels.

HYPOTHALAMIC SOMATOSTATIN CONTENT

Few data are available on the effects of physiological stimuli on somatostatin content or precursors. It has been demonstrated that hypophysectomy decreases somatostatin content in the median eminence, possibly suggesting a negative feedback relationship between pituitary GH and hypothalamic somatostatin (71), but the specific nature of this feedback is unclear. During development, hypothalamic somatostatin concentration increases steadily until about day 28 in rats, and there is a high correlation between somato-

statin content and serum growth hormone (72). We have recently found that aged male rats have reduced levels of somatostatin in hypothalamic extracts and decreased GH in serum when compared with these parameters in young rats (73). Moreover, acute injection of somatostatin antiserum increased GH levels more in old than in young rats, suggesting a role for somatostatin in the reduced secretion of GH with age.

EVIDENCE FOR SOMATOSTATIN PRECURSORS

As with other hypothalamic hormones, there is increasing evidence that somatostatin is synthesized as a prohormone and is enzymatically cleaved to the mature peptide with axonal transport to the median eminence (17). Several investigators report that two higher molecular weight forms of somatostatin are present in hypothalamic extracts (74). Acid hydrolysis or treatment with 8 M urea did not affect their elution pattern, whereas gentle trypsinization converted higher molecular weight forms to lower molecular weight forms without affecting immunoreactivity. There are also indications that these higher molecular weight forms may be released into portal blood. Purification of portal blood extracts by gel or cationic exchange chromatography revealed a higher molecular weight immunoreactive form of somatostatin and somatostatin species that have different ionic charges, but were otherwise indistinguishable from synthetic somatostatin (75). Although higher molecular weight forms of somatostatin in hypothalamic tissue may represent a prohormone, the biological significance of their release into portal blood remains to be determined.

Thyrotropin Releasing Hormone

PHYSIOLOGY OF THYROTROPIN RELEASING HORMONE The release of thyrotropin stimulating hormone (TSH) from the anterior pituitary is regulated by the two thyroid hormones, thyroxine and triiodothyronine, and the tripeptide, TRH, which is released from the median eminence into the portal circulation. Early reports indicated that TRH released TSH from the pituitary both in vivo and in vitro in a number of species, including man (76). Furthermore, hypothalamic lesions, hypothalamic deafferentation, or passive immunization with antisera against TRH decreased TSH release and resulted in hypothyroidism. Although thyroidectomy was reported to increase the capacity of hypothalamic extracts to release pituitary TSH in a bioassay system (77), others have found no effect of thyroidectomy on hypothalamic TRH content (78). Since somatostatin can inhibit TRH-induced release of TSH from the anterior pituitary (79), the presence of this hormone could alter bioassays of TRH in the hypothalamus.

LOCALIZATION OF TRH TRH is found throughout the brain, but is more concentrated in the hypothalamus, particularly in the median eminence (80). Other areas of the hypothalamus that have TRH activity include the dorsomedial, ventromedial, paraventricular, arcuate, and preoptic nuclei, and the septal area (81). There is a marked discrepancy between bioassay and RIA determinations of TRH content in the hypothalamus, and this may be due, in part, to somatostatin, which is present in the same hypothalamic areas as TRH, and can inhibit TRH-induced TSH release. More recent attempts to identify perikarya responsible for TRH synthesis, using immunocytochemical techniques, have found immunoreactive perikarya in the dorsomedial and perifornical areas.

HYPOTHALAMIC TRH CONTENT Measurements of hypothalamic TRH content have not produced consistent results, and it is doubtful that such assays are meaningful since they are not necessarily indicative of release rate. Although TRH is important for the maintenance of TSH and thyroid function, thyroidectomy or T_4 replacement was reported to have no consistent effect on immunoreactive hypothalamic TRH (78). In response to cold stress, TSH and T_3/T_4 release were reported to increase, but TRH concentrations in portal blood and hypothalamic extracts were found to be variable (82). It is generally accepted that hypothalamic deafferentation and lesions of the preoptic area block the cold-induced TSH rise, indicating that TRH is an important mediator of cold stress. The inability to find consistent changes in TRH content may be related both to the specificity of the antiserum used and the method of extraction. Additionally, the rapid metabolism of TRH in plasma and tissue (83) may contribute to the variations in TRH concentrations in portal blood and hypothalamus.

SYNTHESIS OF TRH Several reports suggest that TRH may have higher molecular weight forms (82), but this has not been confirmed nor has the exact nature of the putative precursor been demonstrated. Some investigators have suggested that TRH may be synthesized by nonribosomal methods (84), in contrast to LHRH and somatostatin, but methodological difficulties in isolating TRH activity after hypothalamic incubation with tritiated amino acids make these interpretations premature.

EFFECTS OF TRH ON PROLACTIN RELEASE In addition to stimulating TSH release, TRH has been shown to release prolactin in vivo in many species including man (4) and in vitro from pituitary tumor cells (85) and nontumorous pituitary cells (86, 87). It has been suggested that TRH may be a physiological stimulator of prolactin release. We and other investiga-

tors have not been able to confirm a stimulatory effect of TRH on prolactin release by the rat hemipituitary in vitro (88), and some doubt remains that TRH directly influences pituitary prolactin release. A recent report indicates that a metabolic product of TRH, histidyl-proline-diketopiperazine, can inhibit the secretion of prolactin from GH_3-cultured pituitary tumor cells and depress prolactin release in proestrous rats in vivo (89). We have been unable to confirm the inhibitory effect of this compound in vitro on pituitaries from estrogen-primed female rats. The unequivocal stimulatory action of TRH on prolactin release in vivo may be mediated via hypothalamic mechanisms. There is considerable evidence that TRH can alter the activity of several hypothalamic neurotransmitters (90).

Growth Hormone–Releasing Factor, Corticotropin Releasing Factor, Prolactin Releasing Factor, and Prolactin Release–Inhibiting Factor

GHRF Our laboratory was the first to demonstrate that rat hypothalamic extracts can stimulate release of GH (91) from the anterior pituitary in vitro. Although the structure of GHRF has not been determined, there is much physiological evidence for its existence: (*a*) many centrally acting drugs increase the release of GH from the pituitary (2, 3); (*b*) electrical stimulation of the VMN increases GH release, whereas lesions of this area decrease GH release (64); (*c*) hypothalamic deafferentation produced with a Halasz knife or passive immunization with somatostatin antiserum does not affect the pulsatile nature of GH release (64). More recently, it has been demonstrated that acromegalic patients have increased GHRF activity in the serum and cerebrospinal fluid (92, 93). This may indicate an important role for GHRF in acromegaly.

CRF Although CRF was purified from hypothalamic extracts as early as 1955, its isolation and identification have still not been accomplished. The existence of CRF is supported by the finding that (*a*) electrical stimulation of the hypothalamus increases ACTH release, whereas lesions of this area decrease ACTH release (94, 95); (*b*) hypothalamic extracts release pituitary ACTH in vitro (96, 97); and (*c*) centrally acting drugs can increase ACTH release from the pituitary and cortisol release from the adrenal gland of many species. Several tentative amino acid sequences have been proposed for CRF and some investigators have hypothesized that vasopressin may be a physiological CRF because of its ability to release ACTH. More recent data, however, suggest that CRF and vasopressin are separate hormones since much more vasopressin is required to release ACTH (98).

PRF Although prolactin release from the anterior pituitary is primarily under inhibitory control by the hypothalamus, there is some evidence for the presence of a hypothalamic prolactin releasing factor (99). TRH releases prolactin in vivo in many species and in vitro from dispersed pituitary cancer cells and was proposed as a physiological PRF. More recent evidence suggests that PRF and TRH activity in hypothalamic extracts can be chromatographically separated, and this provides strong evidence for the existence of a separate PRF (see section on effects of TRH on prolactin release).

PIF It is generally accepted that the release of prolactin from the anterior pituitary is regulated by inhibitory factor(s) released from the hypothalamus. Hypothalamic lesions or transplantation of the pituitary to a site distal to the hypothalamus increases synthesis and release of prolactin from the pituitary (100). Dopamine, which is highly concentrated in the median eminence, is a potent inhibitor of prolactin release both in vivo and in vitro, and is a physiological regulator of prolactin secretion. Somatostatin also has been reported to inhibit the release of prolactin in vitro (79), but has not been shown to influence prolactin release in vivo. Other investigators reported isolation of a PIF from hypothalamic extracts that is chromatographically unrelated to catecholamines or somatostatin (101).

EFFECTS OF HYPOTHALAMIC NEUROTRANSMITTERS ON RELEASE OF HYPOTHALAMIC AND ANTERIOR PITUITARY HORMONES

A number of recognized and putative hypothalamic neurotransmitters have been demonstrated to influence the release of anterior pituitary hormones. Several recent reviews have appeared on this subject (2, 3). Most is known about the roles of dopamine, norepinephrine, and serotonin (5-hydroxytryptamine, 5-HT), each of which is highly concentrated in the hypothalamus. Histamine, acetylcholine, brain opiates, GABA, substance P, neurotensin, bombesin, vasoactive intestinal peptide, and other putative neurotransmitters also are present in the hypothalamus and have been reported to alter release of pituitary hormones. These neurotransmitters are believed to act on the receptors of neurons carrying the hypothalamic peptidergic hormones to promote or inhibit their release into the portal vessels through which they reach the anterior pituitary. There also is evidence that at least one neurotransmitter, dopamine, is released directly into the portal vessels and acts on the pituitary to inhibit prolactin release. There

is no evidence that it directly influences the release of any other pituitary hormone.

Many drugs that either increase or decrease brain catecholamine or 5-HT metabolic activity (turnover) have been used to study their effects on pituitary function, but since none of these drugs are entirely specific in their actions, and may affect more than one neurotransmitter, caution must be used in interpreting conclusions based on their reported actions. L-DOPA, the precursor of the catecholamines, may enter and displace 5-HT in neurons; 5-hydroxytryptophan, and to a lesser extent, tryptophan, may enter and displace catecholamines in neurons; acetylcholine and the brain opiates may act on pituitary hormones via catecholaminergic and serotonergic pathways, etc. Studies involving placement of electrolytic lesions or electrical stimulation of particular areas in the hypothalamus also must be viewed with caution, since the neurons lesioned or stimulated may contain more than one amine or induce release of amines from adjacent neurons.

Fluorometric, immunohistochemical, and radioenzymatic methods have been used to measure and to localize the biogenic amines and other neurotransmitters, as well as the peptidergic hormones, in the hypothalamus and other areas of the brain. Changes in the concentrations and metabolism of these substances in discrete areas of the hypothalamus during different endocrine states have yielded considerable knowledge of their role in pituitary function. Here too, caution must be exercised in interpreting published reports, since the results depend on the accuracy and specificity of the reagents and methods used, e.g. specificity of antisera, immunohistological staining method, quantitativeness of methods. The contradictions in the literature on the role of neurotransmitters on release of AP hormones undoubtedly are related to the limitations of the procedures used. Despite these problems, much has been learned during recent years about the role of hypothalamic neurotransmitters on anterior pituitary function.

Prolactin

It was demonstrated many years ago that the hypothalamus chronically depressed prolactin release, and that removal of hypothalamic connections to the pituitary resulted in enhanced prolactin release (99). Thus, pituitary stalk section (and prevention of portal vessel regeneration), placement of a lesion in the median eminence (final common pathway to pituitary), or transplantation of the pituitary to an extrapituitary site produced a rise in prolactin release. Crude extracts of hypothalamic tissue were shown to inhibit prolactin release in vitro and in vivo, and the name prolactin release–inhibiting factor was given to the putative peptide that inhibited prolactin release. However, there is considerable evidence now that the major sub-

stance in the hypothalamus that inhibits prolactin release is dopamine (100, 101). Administration of drugs that increase dopaminergic activity in the hypothalamus (and brain generally), such as L-DOPA or MAO inhibitors; dopamine receptor stimulators, such as some ergot drugs, apomorphine, or piribedil; or dopamine reuptake inhibitors, such as amineptine, depress serum prolactin levels in man and animals. Contrariwise, drugs that reduce dopamine activity, such as α-methylparatyrosine, reserpine, phenothiazines, haloperidol, sulpiride, or α-methyldopa, elicit an increase in blood prolactin levels.

Effective levels of dopamine have been found to be released directly into the hypothalamopituitary portal vessels (102, 103), and to act on the pituitary to inhibit prolactin release. However, there is some evidence that not all of the PIF activity in the hypothalamus is due solely to dopamine. Several investigators have reported that when they removed all catecholamines from hypothalamic extracts, they could still find PIF activity in the extracts (101). It is possible that dopamine releases a peptide PIF from neurons in the hypothalamus.

Cholinergic drugs also have been shown to inhibit prolactin release, and their mode of action appears to be exerted via the dopaminergic system. Administration of acetylcholine directly into the lateral ventricles of the hypothalamus, or systemic injection of pilocarpine or physostigmine, resulted in a reduction in serum prolactin concentration in rats (104). Atropine, a cholinergic blocking agent, prevented the inhibitory action of pilocarpine on prolactin release. Pilocarpine was found to block stress or suckling-induced release of prolactin, suggesting that the cholinergic system probably has a physiological role in regulating prolactin secretion. Prior administration of drugs, such as reserpine, chlorpromazine, or haloperidol, prevented pilocarpine from inhibiting prolactin release, indicating that cholinergic drugs act by increasing dopamine activity.

In 1960, we reported that hypothalamic extracts could release prolactin, and suggested that the hypothalamus contained a prolactin releasing factor (PRF). The finding that TRH released prolactin in animals and man (85) has led some to suggest that TRH accounts for the PRF activity of the hypothalamus. However, it was reported that TRH can be separated chromatographically from PRF in hypothalamic extracts (105). There is very little evidence that TRH acts physiologically to release prolactin. Under most conditions, when prolactin release is increased, TSH release is decreased or unchanged. Serotonin (5-HT) has been demonstrated to be an important promoter of prolactin release in rats (106), although its reported effects on prolactin in man are contradictory.

We reported in 1963 that 5-HT could release prolactin in rats and rabbits as indicated by its ability to initiate milk secretion (107). Later, Kamberi

et al (106) induced prolactin release by injecting 5-HT into the third ventricle of rats. Systemic injection of tryptophan or 5-hydroxytryptophan also increased serum prolactin in the rat. Quipazine, a 5-HT reuptake inhibitor, elevated serum prolactin values in rats, (108, 109), whereas *para*-chloroamphetamine, *para*-chlorophenylalanine, and methysergide inhibited prolactin release. Electrical stimulation of the raphe nucleus, the major source of serotonergic neurons in the hypothalamus, increased serum prolactin levels in rats, whereas electrolytic lesions placed in this area decreased serum prolactin levels (3). In male rats subjected to restraint stress, serum prolactin values and 5-HT concentration and turnover in the hypothalamus were increased (110), suggesting that stress acted via serotonin to promote prolactin release. Hypothalamic 5-HT activity also was found to increase during suckling in postpartum lactating rats (111), suggesting that this may be the mechanism whereby the suckling stimulus increases prolactin release. Dopamine activity was not altered by the suckling stimulus, although prior administration of L-DOPA was shown to inhibit suckling-induced prolactin release. There is some evidence that serotonin elevates serum prolactin levels by increasing release of PRF (112).

In 1962 (107), our laboratory reported that administration of morphine induced prolactin release in rats as indicated by initiation of lactation. More recently, we and others demonstrated that endogenous brain opiates such as methionine-enkephalin and β-endorphin released prolactin in rats. In addition, the surprising finding was made that naloxone alone reduced basal serum prolactin values in normal rats. This suggested that the endogenous opiates in the hypothalamus tonically elevated prolactin release. Naloxone also was shown to inhibit stress-induced prolactin release (113), and to depress growth of prolactin-dependent mammary cancers in rats (114). The ability of brain opiates and morphine to increase prolactin release has been shown to be associated with their action in reducing dopamine and increasing 5-HT activity in the hypothalamus (115, 116). These observations provide cogent evidence that the brain opiates have a physiological role in regulating prolactin secretion.

Gonadotropins

Early work by Sawyer and colleagues (117, 118) showed that norepinephrine (NE) and epinephrine (E) induced ovulation in rats and rabbits, whereas dibenamine inhibited ovulation. More recent work has confirmed these observations and revealed that NE and E can induce LH release as measured by radioimmunoassay. E has not been demonstrated to be physiologically important for the release of LH, and is present in only very minute amounts in the hypothalamus. On the other hand, there is ample evidence for a role by NE in producing LH release and ovulation. Intraventricular

infusion of NE was observed to induce increased release of LHRH in the hypophysial portal circulation of the rat, and this is believed to be its mode of action. An increase in NE turnover was observed in the preoptic hypothalamus on the afternoon of proestrus during the estrous cycle of the rat, which leads to an LH and FSH surge on the evening of proestrus and ovulation early on the following morning. Castration of the male or female rat has been demonstrated to increase NE turnover in the hypothalamus and to increase LH and FSH release, whereas administration of gonadal steroids to castrated rats decreases NE turnover and reduces LH and FSH release (119). Although the reports on positive stimulation by NE on LH and FSH release in several animal species appear to be in agreement, a recent article by Clifton & Sawyer (120) indicates that depletion of hypothalamic NE by transecting the ascending noradrenergic pathway in the midbrain does not interfere with the LH response to ovarian steroid feedback. They suggest that NE modulates, but is not indispensable for feedback control of LH release. There also is little or no evidence as yet that NE is essential for gonadotropin release in primates.

The role of dopamine (DA) in gonadotropin release is controversial. Early work by Schneider & McCann (121) indicated that DA promoted LH and FSH release from rat pituitary tissue incubated with hypothalamic fragments, and Kamberi (122) reported that DA injected into the third ventricle of rats increased LHRH levels in the portal blood. In other experiments, DA was shown to be effective in producing LH release in ovariectomized rats after they had been primed with estrogen and progesterone, suggesting that the steroid medium is important for the LH releasing action of DA (123). Other workers, notably Fuxé and co-workers (124), have claimed that DA inhibits LH release. They reported that DA turnover in the hypothalamus was decreased on the day of proestrus, when LH release is increased, and that DA turnover was increased during pregnancy and lactation when LH release is low. Intraventricular injection of DA failed to produce LH release in estrogen-primed rats, and did not induce ovulation in normal or constant estrous rats (125). Sawyer et al (126) observed that DA did not induce ovulation in rabbits, and blocked the action of NE in producing ovulation. LeBlanc et al (127) found that i.v. infusion of DA into normal women reduced blood levels of both LH and prolactin. Dopamine agonists such as piribedil and apomorphine also were found to reduce serum LH levels in normal male rats (128). Although most of the recent work shows that DA inhibits LH release, it is important to consider that DA can be converted to NE in the brain, and that NE is stimulatory to LH release.

An inverse relationship has been demonstrated between prolactin and gonadotropin secretion. In general, when blood levels of prolactin in animals and man are high, gonadotropins are low. This state prevails during

early postpartum lactation, after ovariectomy, when high doses of estrogen are administered, and also in the galactorrhea-amenorrhea syndrome in women. It has been demonstrated that administration of high doses of prolactin to rats increases DA turnover in the median eminence (129), and this may be one of the mechanisms by which LH release is inhibited. However, administration of dopaminergic drugs (e.g. L-DOPA, ergot drugs, apomorphine, piribedil) to women with the galactorrhea-amenorrhea syndrome inhibits prolactin secretion and lactation, and results in resumption of menstrual cycles. Therefore, other mechanisms than DA must be involved in return of normal gonadotropin secretion in these women.

Under most conditions 5-HT inhibits gonadotropin release. Systemic injections of 5-HT or direct infusion of 5-HT into the third ventricle depressed LH release (122). Serotonin blocked ovulation produced by administration of pregnant mare serum (PMS) in immature rats, and inhibited superovulation induced by PMS in such rats (130). Serotonin also inhibited LH release in ovariectomized rats (131). Inhibitory effects on release of LH also have been reported in immature rats after administration of melatonin, which is mainly present in the pineal gland (132). Melatonin is present only in minute amounts in the hypothalamus, and it is not established that it acts physiologically to depress LH release. In contrast to the inhibitory effects reported for 5-HT under many conditions, it also has been observed to stimulate LH release during the early preovulatory period in rats. Héry et al (133) reported a positive effect of 5-HT on cyclic gonadotropin release in female rats, and this has been confirmed by others. The dual effects that 5-HT can exert on gonadotropin release remain to be clarified.

Recent reports indicate that the brain opiates may have a role in regulating gonadotropin release (134). Morphine and several endogenous brain opiates have been shown to inhibit LH release, ovulation, and to depress testosterone secretions in a variety of species, including man. These effects of the opiates can be counteracted by administering naloxone, a specific receptor antagonist of opiates. Our laboratory made the surprising observation that when naloxone alone was injected into normal male rats not previously treated with opiates, this resulted in a significant rise in serum LH and FSH values (135). This observation has been widely confirmed in mature male and female rats, in sexually immature female rats (136, 137), and in other species, including primates. This suggests that the endogenous brain opiates tonically depress gonadotropin release. Naloxone was observed to elevate serum LH levels in castrated male rats (138) and to block testosterone and estrogen (D. A. Van Vugt and J. Meites, unpublished) inhibition of LH release. Preliminary observations in rats by our laboratory have shown that the brain opiates inhibit NE and stimulate 5-HT activity

in the hypothalamus, whereas naloxone administration results in enhanced NE turnover (D. A. Van Vugt and J. Meites, unpublished).

Growth Hormone

DA, NE, and 5-HT have been reported to increase growth hormone (GH) release in animals and man (3). Dopaminergic drugs such as L-DOPA, several ergot drugs, apomorphine, and piribedil, each increased GH release, whereas inhibitors of dopamine activity such as methyldopa, haloperidol, or reserpine inhibited GH release. NE and its agonists such as clonidine also promoted GH release. α-Adrenergic receptors appear to be involved, since administration of an α-adrenergic blocker such as phentolamine inhibited GH release, whereas propranolol, a β-blocker, had little effect (139, 140).

The role of 5-HT on GH release is more controversial, since both stimulatory and inhibitory effects have been reported (3). Administration of 5-HTP, the precursor of 5-HT, increased plasma GH levels in rats, but this effect may be due to uptake of 5-HTP by catecholaminergic neurons and release of DA and NE. Several investigators reported that 5-HT inhibits GH release in rats (141, 142). Whether the effects of DA, NE, and 5-HT are exerted on the GH releasing factor (GRF) or on somatostatin has not been definitely determined.

We recently reported that several cholinergic drugs, including pilocarpine and physostigmine, can increase GH release in rats (143). Acetylcholine also elevated serum GH levels when injected intraventricularly into rats. Cholinergic drugs also increased GH release in dogs (144), but others saw no effect on GH release by cholinergic drugs in rats. Morphine and endogenous brain opiates also can increase serum GH levels in rats, and other species, including man (134). In dogs, this effect was found to be mediated via the cholinergic system (144), but in rats the opiate effect on GH release was reported to be inhibited by cholinergic drugs. Administration of naloxone to rats was reported to reduce basal serum GH levels in male rats (134), but this has not been confirmed by other investigators (145). However, we have recently found that naloxone can inhibit the surge of GH that occurs during suckling in postpartum lactating rats (N. Miki and J. Meites, unpublished). Further work is necessary to determine the role of brain opiates on GH secretion.

Thyrotropin

There is evidence that NE stimulates release of TRH (146), which in turn promotes synthesis and release of TSH. Reserpine, a central catecholamine depleting drug, blocked cold-induced TSH release in rodents. Phentolamine also inhibited cold-induced TSH release (147). Inhibition of afferent adrenergic input to the median eminence by lesions placed in the anterior hypo-

thalamus, or rostral deafferentation of the medial basal hypothalamus, evoked a reduction in TSH release (148, 149). These observations provide further evidence that the noradrenergic system stimulates TSH release.

In contrast to NE, most reports indicate that the dopaminergic system inhibits TSH release in animals and man. Although results from studies utilizing L-DOPA have been equivocal, administration of dopaminergic drugs such as apomorphine and piribedil, significantly depressed serum TSH levels in normal male rats (128). Bromocriptine also reduced serum TSH release (150) in response to cold stress.

Both stimulatory and inhibitory effects have been observed for 5-HT on TSH release, although most of the evidence appears to favor an inhibitory role for 5-HT. Injection of 5-HT into the third ventricle of rats depressed TSH release (151). Incubation of hypothalamus in a 5-HT medium in vitro also inhibited TRH release (152). L-Tryptophan depressed serum TSH levels in rats (110), but 5-hydroxytryptophan increased TSH release (153). Chronic treatment with PCPA decreased serum TSH levels in rats (154). Mueller et al (110) found that immobilization stress in rats resulted in depressed serum TSH concentrations, and this was associated with increased concentration and turnover of 5-HT in the hypothalamus. There also is evidence that stress can increase NE turnover in the hypothalamus (155), but since NE stimulates TSH release, it cannot be responsible for the fall in TSH release observed during stress in rats.

Adrenocorticotropin

Ganong and his associates (2) have provided much evidence that catecholamines act centrally to inhibit adrenocorticotropin (ACTH) release. Although most of their work was done in dogs, catecholamines also were found to inhibit ACTH release in the rat, monkey, and man. L-DOPA administration also can inhibit ACTH release, but its effects appear to depend on conversion to NE rather than to DA. Injection of a dopaminergic drug such as apomorphine, or pimozide, a dopamine receptor blocker, had no effect on ACTH release. On the other hand, clonidine, a NE agonist, inhibited ACTH release. NE itself inhibited ACTH secretion when injected into the third ventricle. NE is believed to inhibit release of CRF from its neurons into the hypothalamopituitary portal vessels. Jones et al (156) showed by hypothalamopituitary co-incubation studies, that addition of NE to the medium depressed CRF and ACTH release, whereas addition of acetylcholine or 5-HT increased CRF and ACTH release. DA had no significant effect on release of either hormone.

The available evidence indicates that 5-HT stimulates ACTH release. Intraventricular administration of 5-HT into rats was effective, as was

systemic injection of 5-hydroxytryptophan (157). Infusions of 5-hydroxytryptophan also increased blood cortisol levels in human subjects. Cyproheptidine, a 5-HT receptor blocker (but also a DA receptor blocker), has been used to reduce the high ACTH secretion in Cushing's syndrome. Some workers reported that 5-HT inhibited ACTH release (157), but these investigators utilized nonspecific drugs and abnormal experimental models. The reported effects of stress on changes in 5-HT metabolism in the hypothalamus are in conflict, but in the study by Mueller et al in rats (110), hypothalamic 5-HT concentration and turnover were increased during immobilization stress, an observation consistent with the view that 5-HT increases ACTH release.

CONCLUSIONS

The past decade has been characterized by great advances in the purification, identification, and synthesis of hypothalamic hormones. Furthermore, there is a great deal of literature which unequivocally demonstrates that the regulation of anterior pituitary function is mediated by hypothalamic hormones. Yet, the majority of data on the control of hypothalamic hormones is inferred from concomitant changes in hypothalamic content and release of pituitary hormones, rather than by direct measurements of the hormones. Recent data indicate that hypothalamic hormones are regulated by neurotransmitters and hormones by altering peptide synthesis, degradation, and release. A complete understanding of the physiology of hypothalamic hormones must include each of these mechanisms.

Considerable progress has been made in the past ten years on the elucidation of the role of neurotransmitters in regulating pituitary function. As new neurotransmitters are discovered in the brain, they are tested for their endocrine effects. In a remarkably short period of time, the brain opiates have been involved in the regulation of pituitary hormone secretion and their mechanisms of action have been elucidated. The brain opiates may turn out to be as important as the biogenic amines in regulation of pituitary function.

The most critical methods of analyzing the effects of neurotransmitters on hypothalamic hormones involve measurements of synthesis, metabolism, or release of these hormones into the portal vasculature. No completely adequate methods have been developed for collecting portal blood, but recently a method has been developed for measuring catecholamines in the median eminence of rats using an in vivo voltametric technique (158–160). Hopefully, this will prove to be useful in measuring catecholamines under more physiological conditions.

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