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HYPOTHALAMIC HORMONES: SUBCELLULAR DISTRIBUTION AND MECHANISMS OF RELEASE

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INTRODUCTION

The regulation of anterior pituitary hormone secretion depends on the release of hypothalamic substances into the capillaries of the median eminence (ME). Three such peptides, thyrotropin-releasing hormone (TRH), luteinizing hormone-releasing hormone (LHRH), and somatostatin (SRIF) have been isolated and structurally characterized (1, 2). TRH stimulates thyrotropin (TSH) and prolactin secretion, LHRH releases luteinizing hormone (LH) and follicle-stimulating hormone (FSH), and SRIF inhibits growth hormone (GH) and TSH release. Evidence also exists for corticotropin (ACTH)—releasing factor (CRF), GH-releasing factor (GRF), and another prolactin-releasing factor (PRF) separate from TRH, but their structures are at present unknown. Pituitary prolactin secretion is predominantly inhibited by the hypothalamus under physiologic conditions and there is growing evidence that dopamine acts as the principal prolactin-inhibiting factor (PIF) (3).[2]

Evidence based on morphologic, electrophysiologic, and pharmacologic experiments indicates that the actions of the identified hypothalamic hormones are not restricted to regulation of anterior pituitary hormone secretion (4–13). The detection of TRH, LHRH, and SRIF in extrahypothalamic brain regions, their modification of neuronal activity when applied by microiontophoresis to individual neurons

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[2]The hypothalamic regulation of melanocyte stimulating hormone (MSH) is not considered in this review. The discovery of the structural identities of the opioid peptides and β-MSH within a pituitary hormone, β-lipotropin (see chapter by Simon & Hiller, this volume), has greatly complicated the issue of whether there are specific hypothalamic hormones for regulation of α- and β-MSH secretion. Neurohypophysial hypothalamic hormones (vasopressin, oxytocin, neurophysin) are also excluded from consideration.

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in several brain regions, and numerous studies of behavioral effects have led to the suggestion that they may act as synaptic transmitters or neuromodulators in a manner analogous to that proposed for other CNS peptides such as substance P and the endorphins.

The secretion of the hypothalamic peptides is thought to be modified by the actions of CNS neurotransmitters. This evidence is derived largely from in vivo studies of the effects of systemic or intraventricular administration of drugs that affect synthesis, re-uptake, or degradation of neurotransmitters (14–17). In particular, the monoamines dopamine (DA), norepinephrine (NE), epinephrine (EP), serotonin (5-HT), and histamine (HIS) have been shown to influence anterior pituitary secretion by effects mediated at hypothalamic or other CNS sites. Acetylcholine (ACh) and γ-aminobutyric acid (GABA) also affect hypothalamic regulation of pituitary secretion. With the exception of DA, which acts at the pituitary level to suppress prolactin secretion (3), and 5-HT, which may inhibit LH (18), there is no evidence to implicate direct pituitary effects of the neurotransmitters.

The purpose of this review is to present evidence on brain localization and subcellular distribution of the hypothalamic hormones and to summarize pharmacologic data on mechanisms of their release from brain tissue. Detailed consideration is not given to the issue of peptide biosynthesis, which has been extensively reviewed by Reichlin et al (1), Vale and co-workers (2), and Gainer et al (19). The mechanisms of degradation of peptides in CNS is briefly considered.

LUTEINIZING HORMONE-RELEASING HORMONE

Localization in the Central Nervous System

Biochemical and immunocytochemical studies show that LHRH is present in highest concentration in the ME where it is localized in nerve terminals that end on capillary loops of pituitary portal vessels (20–25). Electronmicroscopic immunohistochemical studies in the guinea pig suggest that LHRH–reactive material in ME nerve terminals is concentrated in granular vesicles measuring 400–800 Å (23).

The localization of LHRH cell bodies is more controversial as most workers have failed to find satisfactory numbers of LHRH–staining neuronal perikarya. Barry and collaborators (26) described scattered LHRH–immunoreactive cells in the preoptic and suprachiasmatic regions in several species. Zimmerman (27, 28) and Naik (29, 30) found reactive neurons in the arcuate (infundibular) nucleus in the mouse and rat. Recently, Hoffman et al (31) have suggested that different antigenic sites on LHRH antibodies may determine the observed distribution of immunoreactive perikarya.

LHRH pathways in extrahypothalamic brain have also been identified. Silverman et al (32) examined the distribution of LHRH in the rhesus monkey. LHRH–containing cell bodies were scattered throughout the entire hypothalamus and LHRH positive axons were distributed to the ME, hypothalamic-septal region, the lamina terminalis, the diagonal band of Broca, and to the vicinity of the anterior commissure. Elde, Hokfelt, and collaborators (20, 21) described LHRH axons in para-olfactory cortex, amygdala, and other extrahypothalamic regions. LHRH

nerve terminals are prominent in the organum vasculosum of the lamina terminalis, a circumventricular organ located at the anterior extent of the third ventricle.

Subcellular Distribution and Release

Brain subcellular fractionation techniques, pioneered by Whittaker (33) and De Robertis (34), have provided the basis for isolation of pinched-off nerve terminals which spontaneously seal to form particles known as synaptosomes. This method has been widely used to study the localization of putative neurotransmitters and peptides in brain tissue. Subsequent rupture of the synaptosomes permits analysis of its constituent particles and presumptive localization of substances to synaptic granules and vesicles.

A synaptosomal localization of LHRH activity, determined by bioassay and radioimmunoassay, has been proposed by several groups (35–37). Shin et al (38) reported that LHRH activity was localized to the subcellular fraction composed largely of mitochondria, mitochondrial condensation debris, dense core granules, and other unidentified particulate matter. A clear analysis of the various particulate fractions by ultrastructural and enzymatic markers was not provided. Recovery of radioimmunoassayable LHRH in ultrastructurally identified synaptosomes with close correlation to lactic dehydrogenase (LDH) distribution led Ramirez et al (39) to conclude that most, if not all, hypothalamic LHRH is present in nerve endings. Using similar methods, Barnea et al (40) reported a population of LHRH-containing particles with sedimentation characteristics similar to those of synaptosomes containing NE and DA. However, in these initial studies, hypo-osmotic shock failed to alter the sedimentation characteristics of some of the LHRH-containing particles. Since synaptosomes are ruptured by hypo-osmotic shock (33, 34), the possibility arose that some of the LHRH was contained in a nonsynaptosome component. In a subsequent study Barnea et al (41) reported two distinct populations of LHRH contained in small and large particles which could be separated by nonequilibrium density gradient centrifugation. The majority of the LHRH was in the large particle fraction. Hypo-osmotic shock or deoxycholate (a membrane-solubilizing agent) released LHRH only from the large particles, which Barnea et al interpreted to be synaptosomes. They postulated that the small particles may have been derived from other cellular or subcellular structures (42, 43).

Ontogenetic studies of subcellular LHRH compartmentalization have demonstrated that the peptide is segregated in different particles during development (44). LHRH is detectable only in trace amounts in the hypothalami of 22-day-old fetuses but is readily demonstrable in 2-day-old neonates. At this age, the peptide is predominantly in large particles that fulfill criteria for synaptosomes. LHRH appeared in small particles by the fourteenth and twenty-first postnatal days in males and females respectively. These studies provide evidence that hypothalamic LHRH is primarily localized to synaptosomes and support the hypothesis that the peptide is released into the portal vessels on depolarization of ME nerve terminals.

Direct experimental evidence of release of LHRH from nerve tissue has been derived from in vitro experiments. Bennett, Edwardson and co-workers (45–47) exposed synaptosome preparations of whole hypothalamus, ME, and medial-basal periventricular hypothalamus to DA, NE, 5-HT, ACh, and EP, and determined the

release of immunoreactive LHRH. DA stimulated LHRH release in synaptosomes obtained from either whole hypothalamus or ME; 5-HT, NE, ACH, and EP had no effect. Rotzstejn et al (48, 49) argued that in order to investigate the physiological significance of neurotransmitter effects on LHRH release, the following criteria must be met: (*a*) enzymatic degradation of the peptide in the media must be inhibited in order to yield an accurate evaluation of the material released by drugs; (*b*) membrane depolarization produced by excess K^+ or electrical stimulation ought to be effective in releasing the peptide; and (*c*) both basal and depolarization-induced peptide release must be temperature- and Ca^{2+}-dependent. With these requirements fulfilled, Rotzstejn et al showed that DA induced LHRH release from incubations of medial-based hypothalamic fragments whereas NE had no effect. DA failed to release LHRH from caudal hypothalamic or preoptic area tissue, where endogenous DA concentrations are known to be much less. The stimulatory effect of DA on LHRH release was blocked by pimozide, a dopamine receptor antagonist. Although these workers were unable to show LHRH release from synaptosomal preparations using high K^+, Warberg et al (50) subsequently demonstrated such an effect.

These observations on effects of DA on LHRH release are, in general, consistent with earlier in vivo studies which showed that intraventricular administration of DA stimulated LH and FSH release, whereas direct injection into the portal vessels was ineffective (51–55). These observations suggest that DA acts as a facilitatory neurotransmitter in eliciting LHRH release but do not provide information with respect to the site involved. The close association of DA and LHRH terminals in the outer zone of the ME allows the postulation that these effects may occur by axoaxonic connections as proposed by Fuxe et al (56, 57), although the latter proposed that DA was inhibitory to LHRH release.

THYROTROPIN-RELEASING HORMONE

Localization

The highest concentration of TRH is in the ME. Measurements based on radioimmunoassay indicate that TRH is distributed throughout the entire hypothalamus and preoptic area. Extrahypothalamic TRH is found in cerebral cortex, basal ganglia, brain stem, and spinal cord and accounts for 75–80% of total brain TRH (58–62). The peptide is also present in the pineal gland (63) and in high concentration in frog skin (64). Immunohistochemical studies demonstrate that axon terminals reactive for TRH are concentrated predominantly in the medial portion of the external zone of the ME (20). Other nerve terminals were visualized by Elde & Hokfelt (20, 21) in the dorsomedial, paraventricular, and perifornical region of the hypothalamus. TRH terminals were found outside the hypothalamus in the nucleus accumbens, lateral septal nuclei, several brain stem nuclei, and in the ventral horn and intermediolateral cell column of the spinal cord. Cell bodies for TRH were present in several brain areas; those that project to the ME appeared to be concentrated in the periventricular region of the anterior hypothalamus (20). Lesions of this region result in depletion of ME TRH terminals (20) and a reduction in TSH secretion (65).

Subcellular Distribution and Release

Barnea et al (40, 41, 66) found immunoreactive TRH in two subcellular populations, consisting of large and small particles, which had sedimentation characteristics similar to those of synaptosomes containing NE and DA. The TRH particles differed in certain physical characteristics from those containing LHRH. After hypo-osmotic treatment or exposure to solubilizing agents, most of the TRH recovered was near the top of the gradient, presumably in nonparticulate form. The remaining fraction of TRH, which was associated with small particles, had a sedimentation coefficient similar to that of synaptic vesicles containing ACh (33). These physical properties strongly supported the conclusion that both of the TRH-containing subcellular particles were in synaptosomes.

The two populations of TRH-containing particles were characterized ontogenetically (44). In 22-day-old rat fetuses, TRH was associated almost entirely with the subpopulation of small particles. In neonates an age-dependent increase in the fractional amount of TRH confined to the subpopulation of large particles was observed so that by the seventh day of age the peptide was equally distributed in the two subpopulations. Comparing LHRH and TRH, it was concluded that the subcellular compartmentalization of the two peptides was age-dependent and developed separately and asynchronously.

Winokur et al (67) reported that both hypothalamic and extrahypothalamic TRH is localized in subcellular fractions consisting predominantly of synaptosomes. After osmotic shock, they found the highest TRH concentration in the 96,000 X g (M_2) fraction which was previously shown to be NE-enriched by De Robertis (68). These data suggest that TRH is associated with small particulate material, i.e. synaptic vesicles, comparable in size to those that contain NE.

These conclusions are of interest when compared with in vitro evidence for a role of neurotransmitters in TRH release. Grimm & Reichlin (69) reported that hypothalamic fragments preincubated with tritiated amino acids released labeled TRH after exposure to DA or NE. They interpreted this effect to be due to NE, since addition of disulfiram, an inhibitor of dopamine-β-hydroxylase, abolished the effect. 5-HT inhibited TRH release in this system.

Studies with synaptosomal preparations have yielded slightly different results. Bennett et al (46) found that ME synaptosomes release TRH when stimulated with DA, whereas other monoamines were ineffective. Stimulation of synaptosomes from other hypothalamic regions was unaffected. Serotonin was inhibitory to TRH release as was the case in the studies of Grimm & Reichlin (69). Warberg et al (50) showed that TRH release from synaptosomes is Ca^{2+}-dependent, but they did not investigate the role of monoamines. Kardon and co-workers (70) reported that TRH concentration in brain was unaltered by various in vivo manipulations. Hypophysectomy, T4 treatment, and depletion of brain catecholamines and serotonin with α-methyl-p-tyrosine, p-chlorophenylalanine, and reserpine had no effect on TRH concentration.

A different approach to the interactions of NE and TRH was studied by Tsang & Martin (71). These workers examined the effects of several peptides on the β-adrenergic stimulation of cyclic AMP in the pineal gland incubated in vitro.

NE-stimulated cyclic AMP was blocked by addition of TRH at concentrations of 0.1–1.0 μg/ml, whereas inactive analogues of TRH had no effect and LHRH and SRIF required higher concentrations to be effective. These workers postulated that TRH may act to interfere with β-receptor stimulation. These findings may have physiologic relevance as TRH is present in considerable concentration in the pineal gland (60, 63) and in the frog shows variations with season and environmental illumination (63).

SOMATOSTATIN

Localization

The tetradecapeptide SRIF was isolated from ovine hypothalamic extracts by Guillemin and associates (72). Studies by bioassay and radioimmunoassay have shown highest concentrations of SRIF in the ME (73), but like TRH, substantial amounts have been found in extrahypothalmic brain areas including the preoptic area, amygdala, cerebral cortex, brain stem, and spinal cord (20). Concentrations are low or undetectable in cerebellum and pineal gland in the rat (74). Immunocytochemical studies have confirmed widespread localization of SRIF in the external zone of the ME (20). Neuronal cell bodies are localized mainly in the anterior periventricular area (75); lesions here cause depletion of ME and preoptic area SRIF without affecting amygdala, cortex, brain stem, or spinal cord (58, 76). Somatostatin-staining nerve terminals have been identified in the hypothalamic ventromedial and suprachiasmatic nuclei and outside the hypothalamus in the stria terminalis, nucleus accumbens, medial caudate nucleus, amygdala, and in the dorsal horn of the spinal cord (20, 21). In the latter location, SRIF is in close juxtaposition to fibers containing substance P (20). Dorsal root spinal ganglia cells also have shown somatostatin immunoreactivity in separate cells, and it has been postulated that the peptide may function in relay of sensory inputs to the CNS (22); its function in relation to substance P is currently under investigation in several laboratories (9, 13). SRIF has also been localized outside the CNS in the stomach, duodenum, pancreatic islets, and in nerve fibers of the gut (20).

Subcellular Distribution

Subcellular preparations of medial-basal hypothalamus, preoptic area, and amygdala indicated that over 70% of SRIF immunoreactivity was localized to the synaptosome fraction (74). It was hypothesized that SRIF, in addition to being released into blood vessels of the ME, may also have been liberated from nerve terminals in other brain regions.

The CNS actions of SRIF are currently under study. Microiontophoretic application to single neurons in the hypothalamus, cerebral cortex, cerebellum, and spinal cord results in depression of firing rates in the rat (9, 10, 12). Studies with synaptosomes obtained from guinea pig cortex suggest that SRIF inhibits uptake or efflux of Ca^{2+} from nerve terminals, an effect not observed with TRH or LHRH (77). This effect on calcium transport is similar to that reported in pancreas (78–80), where it has been proposed that SRIF causes inhibition of insulin secretion by interference

with calcium transport. No studies have been reported to indicate the influence of neurotransmitters on release of SRIF from hypothalamic fragments or synaptosomes.

CORTICOTROPIN-RELEASING FACTOR

Release

The structure of CRF has not been determined so that studies describing its distribution are based on in vitro and in vivo techniques that measure ACTH release from the pituitary or corticosteroid release from the adrenal. Jones and co-workers (81–83) have extensively investigated the effects of drugs on release of CRF from incubated rat hypothalami. Both ACh and 5-HT stimulated CRF release in this system. The ACh effect was blocked by atropine but also by several other drugs, including GABA, NE, and melatonin. Similarly, 5-HT stimulation was blocked, not only by the serotonin antagonist, methysergide but also by hexamethonium, atropine, NE, and melatonin. It was postulated that 5-HT acts via excitation of a cholinergic interneuron and that NE, GABA, and melatonin affect other inhibitory receptors. These workers showed that ME-pituitary stalk fragments (which contain no neuronal perikarya) failed to release CRF after exposure to ACh which they interpreted to mean that ACh acted on dendrites or perikarya of CRF neurons in the medial-basal hypothalamus.

Bennett, Edwardson & Bradford (84–86) have examined CRF release from synaptosomal preparations of rat and sheep hypothalamus. Both electrical and excess K^+ (55 mmol) resulted in CRF release; the effect was Ca^{2+} dependent. Release of CRF occurred after addition of ACh, an effect that was blocked by atropine. ACh-stimulated CRF release was partially inhibited by DA and NE, but not by 5-HT.

These preliminary results make difficult any conclusive interpretation of the role of neurotransmitters in CRF release. In general, however, the findings agree with in vivo observations that ACh and 5-HT act to enhance ACTH secretion (14, 15).

GROWTH HORMONE–RELEASING FACTOR

Physiologic and biochemical studies have asserted the existence of a separate GRF, but its structure has thus far eluded characterization (1, 2, 87–90). Hypothalamic extracts contain a substance that releases GH both in vivo and in vitro, and the material is separate from the other identified peptides (1, 2). Pituitary secretion of GH is episodic and intermittent rather than continuous, and this rhythm is dependent upon the medial-basal hypothalamus, specifically the ventromedial-arcuate region (88). Lesions of these nuclei abolish physiologic GH surges without affecting SRIF content in the ME (91). Krulich and co-workers (92) using in vitro bioassays suggested that GRF was localized in the lateral region of the ventromedial (VM) nucleus.

The surges of GH are dependent upon catecholamines, in particular NE (93, 94). Administration of α-MT abolished the pulses; however, they could be restored by administration of clonidine, an α-adrenergic agonist, but not by apomorphine, a dopamine agonist (93). Phenoxybenzamine, an α-adrenergic blocking agent, also

completely abolished the surges, whereas dopamine blockers had much less marked effects (95). Serotonin antagonists partially inhibited GH secretion (95). These studies implicate monoamines in the mediation of the GH surges (and presumably of GRF release) although direct studies are lacking.

PROLACTIN-INHIBITING FACTOR AND PROLACTIN-RELEASING FACTOR

As extensively reviewed by others, there is evidence that DA inhibits prolactin secretion by direct effects on pituitary (1–3). DA has been found in high concentration in the external zone of the ME and has been measured in pituitary portal blood in concentrations of 1–20 ng/ml, levels that are 2–20-fold greater than in peripheral arterial blood (96). DA receptors can be demonstreated in pituitary homogenates but are lacking in ME (97). These observations taken together suggest that DA may be the primary PIF. However, there is also preliminary evidence for another hypothalamic PIF (1, 2, 98, 99) and for a PRF separate from TRH (100). Until the biochemical nature of these substances is clarified, studies of their distribution, localization, and mechanisms of release will be difficult to interpret.

DEGRADATION OF PEPTIDES

If hypothalamic peptides are released at synaptic sites in the CNS, it would be expected that mechanisms should exist for termination of their effects. We have been unable to find any evidence for re-uptake of peptides by nerve terminals. Parker and co-workers recently reported that tritiated TRH does not enter synaptosomes prepared from rat hypothalamus (101). Burt & Snyder (102) have reported that receptor sites for TRH can be demonstrated in several regions of the CNS, but these were interpreted to be effector rather than re-uptake sites. Intracellular binding sites have been proposed for SRIF in many tissues, including brain (103).

It is known that most peptides are rapidly inactivated when incubated with either CNS tissue or blood and this is the case with TRH, LHRH, and SRIF (1, 2, 104–106). There is increasing evidence that these inactivating effects are the result of specific peptidases for each peptide (104). The subcellular localization of the peptidases is currently under study. Over 90% of LHRH-inactivating enzymes are associated with the cytosol fraction (104). Griffiths et al (107, 108) investigated the subcellular localization of peptidase enzymes capable of inactivating SRIF using homogenates of hypothalamus and other brain regions from the rat. They found that both supernatant (soluble/cytoplasmic) and particulate (microsomal and mitochondrial) fractions rapidly inactivated SRIF.

ROLE OF OTHER PEPTIDES IN HYPOTHALAMIC-PITUITARY REGULATION

Evidence is rapidly accumulating that other brain peptides act at CNS sites to affect hypothalamic-pituitary control. The bulk of evidence pertains to GH and prolactin secretion. TRH stimulates GH secretion in certain pathologic conditions, including

acromegaly, renal failure, malnutrition, and thyroid deficiency (87). LHRH is also stimulatory to GH release in some cases of acromegaly (87). In urethane-anaesthetized rats systemic or intraventricular administration of TRH stimulates GH release (88). Several other peptides, including neurotensin, substance P, met-enkephalin, and β-endorphin (88), can elicit GH and prolactin secretion under certain circumstances. None are effective in directly causing GH secretion at the pituitary level or have been shown as yet to act under physiologic conditions.

CONCLUSIONS

The available current evidence indicates that the hypothalamic hormones, like other brain peptides, are distributed in specific hypothalamic and extrahypothalamic neuronal pathways and are concentrated in nerve terminals. Presumably, release of peptides occurs into the pituitary portal system after depolarization of axon terminals in ME. The localization of the peptides within synaptosomes of other brain regions suggests that they are also released at synaptic sites. The use of brain fragments and tissue slices should provide the methods for studies of peptide release mechanisms and interaction with neurotransmitters. The results obtained thus far from studies with synaptosomes suggest that interactions with other neurotransmitters may occur at axoaxonic sites both in ME and in other brain regions. A final definition of the CNS function of the hypothalamic hormones will require demonstration of their release from nerve terminals in brain, further delineation of their electrophysiologic effects, analysis of receptor sites, and elucidation of processes of inactivation. The observations that have already been made with respect to other peptides such as substance P (109) and the endorphins (9) provide guidelines along which to pursue such studies.

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