Pituitary Hormones in Brain: Where, How, and Why?

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Summary. Peptide and protein hormones usually considered as being of pituitary origin have been detected within the central nervous system by means of radioimmunoassay, bioassay, and immunocytochemical techniques. Intracerebral administration of some of these hormones or fragments thereof elicit behavioral responses, suggesting that they may have a physiological role similar to that described for other peptidergic neurotransmitter or neuromodulator substances. Evidence available for some of these hormones indicates that they are synthesized within the central nervous system and that their regulation may differ from that of their pituitary counterparts.

Numerous investigators have reported that the brain contains several peptides that are similar to the hormones in the anterior and intermediate lobes of the pituitary (*1-18*). These observations are of interest because behavioral changes have been observed in experimental animals injected intracranially with some of these hormone-like materials or their fragments (*19*). Such findings have raised many questions concerning the distribution, synthesis, regulation, and function of these substances. The purpose of this article is to summarize and critically review the data available on such questions.

Distribution of Pituitary Hormones in the Central Nervous System

Adrenocorticotropic hormone (ACTH), α-melanocyte stimulating hormone (α-MSH), β-lipotropic hormone (β-LPH), and β-endorphin. These peptides are considered together because in studies (*20-24*) of their synthesis in normal mouse pituitary gland and in a mouse pituitary tumor cell line it has been found that they are contained within a common precursor glycosylated molecule (see Fig. 1). This molecule has been variably referred to as 31K ACTH/endorphin, or "pro-opiocortin," its molecular weight being approximately 31,000. This finding explains earlier studies demonstrating that ACTH and β-LPH occur within the same anterior pituitary cell (*25*), possibly within the same secretory granule (*26*). Both ACTH and β-LPH can potentially be further processed to other smaller component peptides. In the anterior pituitary it is believed that the naturally occurring hormone, ACTH (*1-39*), is secreted without further processing (*27*); but there is some question concerning the extent and circumstances under which β-LPH undergoes further processing to β-endorphin (*28-31*). In the intermediate lobe it is believed that ACTH (*1-39*) serves as a precursor molecule that is enzymatically cleaved and processed to α-MSH (melanocyte stimulating hormone) and CLIP (corticotropin-like intermediate lobe peptide) [ACTH(18-39)] (*27*), while β-LPH is cleaved to γ-LPH [β-LPH(1-58)] and β-endorphin [β-LPH(61-91)] (*27, 32*). Therefore, to assess the significance of the presence of this group of peptides in brain and to obtain insights as to their origin and processing within the central nervous system, it becomes necessary to study their relative anatomic distribution and concentration.

Guillemin *et al.* (*33*) in 1962 speculated that MSH's or peptides related to ACTH "may have . . . a diencephalic origin." This idea was based on the demonstration of ACTH, α-MSH, and β-MSH-like activities in extracts of hog hypothalamus; similar findings in dog hypothalamus were simultaneously reported by Schally *et al.* (*34*). These peptides were identified on the basis of their biological activity and by chromatographic characterization.

More recently, these peptides have been studied by radioimmunoassay and bioassay, molecular sieve chromatography, and immunocytochemistry. Immunoassayable ACTH (*5-7, 11, 18, 35*), α-MSH (*3, 4, 15*), β-LPH (*11, 36, 37*), and β-endorphin (*11, 12, 16, 38*) have been found in the brains of several species, including rat, cow, monkey, and human. The concentrations of these peptides are either unchanged or may even increase in the brain after hypophysectomy (*4, 6, 7, 16*). Biological activity of the identified peptide has been verified only for ACTH, which is the only one for which a specific bioassay is available (*6*). In all instances the highest concentrations of these peptides have been detected in the hypothalamus. Significant concentrations of these peptides are also present in the limbic system, with lesser concentrations in midbrain, pons, medulla, striatum, cortex, and cerebellum.

Immunocytochemical studies in both intact (*8, 15, 17, 39-44*) and hypophysectomized (*8*) animals of several species have shown that the only cell bodies thus far detected within the central nervous system containing all of these peptides are within and lateral to the arcuate (infundibular) nucleus. Although identical sections have not been stained with antiserums for all of these peptides, the distribution of these peptides appears similar, with the fibers containing them being most dense in the hypothalamus and projecting to amygdala, preoptic area, dorsal and ventromedial nuclei, paraventricular nucleus, periaqueductal gray, reticular formation, thalamus, and stria terminalis. Where sequential staining of the same section or staining of adjacent sections has been performed for ACTH and β-LPH, save for one study (*40*), there is general agreement that there are no cells or fibers that contain ACTH which do not contain β-LPH (*39, 43, 45*).

It is of interest to compare reported concentrations of these peptides in brain with those in the pituitary. Concentrations of ACTH and LPH in brain (depending upon region) are approximately two to three orders of magnitude less than in anterior pituitary. Similar considerations apply when comparing α-MSH and β-endorphin concentrations to those

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0036-8075/79/0727-0366$01.75/0

in intermediate lobe. Hypothalamic β-endorphin concentrations, however, vary from only half (*16, 46*) to two orders of magnitude (*29, 47*) less than those in anterior pituitary. [Such variation may in part be due to the use of different extraction methodologies (*29*) in some of which β-endorphin may arise from enzymatic conversion of β-LPH.]

The foregoing comparisons, however, may be misleading. Substantial differences may exist between the pituitary and brain forms as to their rates of synthesis, biological half-lives, types of precursor present, and posttranslational processing. Such processing may give rise to fragments (such as those proposed to have biologically significant effects on central nervous system function) that are not recognized by the immunological and biological assays currently used to detect pituitary forms of these peptides. In addition, the brain has a much larger mass than the pituitary, within which the ACTH-related peptides are not symmetrically distributed, so that high local concentrations may exist in discrete areas. Last, such peptides in brain are presumably restricted to a much smaller distribution volume than is pituitary-derived material that is released into the peripheral circulation.

One other question to consider is whether these peptides in brain arise from the same high-molecular-weight glycoprotein precursor as in pituitary. Since the processing of this precursor differs in anterior and intermediate pituitary lobes, giving rise to different relative molar ratios of ACTH, β-LPH, α-MSH, and β-endorphin, it is of interest to compare the molar ratios of these peptides in various brain regions with those in anterior or intermediate pituitary lobe.

It is difficult to interpret the limited data available. This is mainly because of methodological problems related to the use by different investigators of antibodies of different specificities and insufficient cross-reactivity characterization, and to the different methods of handling the tissue to be assayed. These problems include the following. (i) Available ACTH, β-LPH, and β-endorphin antibodies possess dissimilar cross-reactivity with the precursor molecule; likewise, different ACTH antibodies have dissimilar cross-reactivity with various COOH- and NH_2-terminal ACTH fragments. Therefore, if processing of the high-molecular-weight precursor material varies in different brain areas (secondary to the varying distribution of specific cleaving enzymes), the amount detected representing a given molecular form will vary with the antibody employed. (ii) β-LPH antibodies are species-specific, in contrast to the lack of such specificity of ACTH antibodies; since there is no available antiserum for rat β-LPH, comparative studies have been conducted in larger species which are killed under nonbasal conditions. (iii) All available β-endorphin antiserums exhibit some degree of cross-reactivity with β-lipotropin (necessitating the more laborious characterization by gel filtration of material detected with this antibody). (iv) Frozen tissue has usually been used for these studies. In earlier studies from our laboratory we reported that relative concentrations of immunoreactive ACTH, β-LPH, and β-endorphin varied in different brain regions. These studies were performed on tissues that had been immediately frozen and subsequently homogenized in 0.2*M* HCl. We have since observed (*29*) that thawing is associated with significant conversion of β-LPH to β-endorphin, this perhaps being secondary to the liberation of proteases contained in subcellular particles, which are lysed during freezing. Therefore, reported differences in relative peptide concentrations (*11*) in various brain regions may have been a consequence of the use of such frozen tissue, coupled with the possibility of differential distribution of such degradative enzymes.

Despite these caveats, our finding that on a molar basis lower β-LPH than ACTH concentrations are present in hypothalamus, hippocampus, and amygdala (*11*), and higher β-LPH than ACTH concentrations are present in midbrain, pons, medulla, and spinal cord, supports the suggestion of differential precursor processing in various brain areas. More detailed kinetic studies of different areas with radioactively labeled compounds, as well as isolation and characterization of specific proteases, will be necessary before a full understanding of this problem can be achieved.

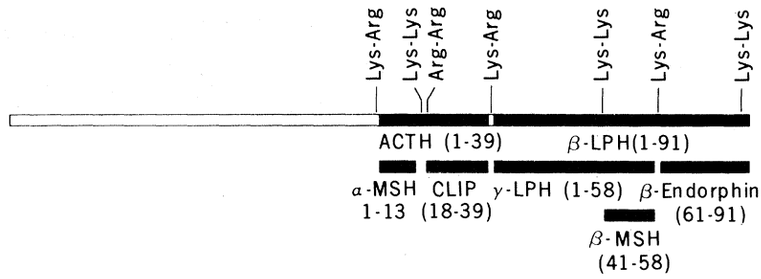

Fig. 1. Schematic representation of the bovine precursor molecule indicating an NH_2-terminal fragment, followed by ACTH(1-39), which is followed by the β-lipotropin (1-91) sequence. (A cloned complementary DNA of the messenger RNA of the precursor molecule has recently been sequenced.) ACTH(1-39) contains within it the peptide backbone of α-MSH and CLIP (corticotropin-like intermediate lobe peptide). β-Lipotropin contains within it the sequences of γ-LPH, β-MSH, and β-endorphin. Also indicated are the location of Lys-Arg or Lys-Lys groups that can be cleaved by proteolytic processing to yield the component peptides (*104*).

Other pituitary hormones. Significant amounts (250 picograms per milligram of tissue) of immunoreactive growth hormone (IRGH) have been detected in rat amygdala. In this area, decreased concentrations are present 7 days after hypophysectomy, whereas 30 days after hypophysectomy such concentrations are greater than those present in intact animals. In addition, IRGH has been found in amygdala cells maintained in tissue culture up to 16 days. IRGH-like material increased with time in the media of such cultures, whether derived from intact or hypophysectomized animals. Chromatography of this material with Sephadex G-100 revealed a profile identical to that of ^{125}I-labeled rat growth hormone. Immunohistochemical staining also demonstrated growth hormone-like material in numerous amygdala cells. Extracts of amygdaloid nucleus or anterior pituitary gland containing immunoequivalent amounts of extracted growth hormone had equivalent stimulation of growth of epiphyseal cartilage. Although on the basis of wet weight of tissue the amygdaloid nucleus contained the largest amount of growth hormone-like material, in a preliminary examination of other brain areas some workers have demonstrated similar material in cortex, hippocampus, and hypothalamus, with none present in caudate nucleus or cerebellum (*5*).

These same investigators noted the presence of immunoassayable and bioassayable thyroid-stimulating hormone (TSH) in extracts of rat amygdaloid nucleus (*5*); such extracts stimulated the growth of thyroid follicular epithelium. The concentrations of TSH are approximately 100 nanograms per milligram of tissue in intact rats, but 14 days after hypophysectomy these concentrations

quadruple, and 48 days after hypophysectomy they remain approximately twofold higher than those in intact animals (*5*). In another study (*48*), however, immunoreactive TSH concentrations of 2 ng/mg were reported in rat amygdala, as well as in preoptic area, thalamus, midbrain, pons, and medulla, whereas approximately fivefold greater concentrations were found in hypothalamus. When two human brains were assayed, immunoreactive TSH was found only in hypothalamus (*48*).

In a limited number of studies, prolactin has been found within the central nervous system. In the rat, immunohistochemical studies have provided evidence that prolactin-like material is present in fibers and nerve terminals in many hypothalamic areas, such as the anterior and posterior periventricular region, arcuate nucleus, dorsomedial hypothalamic nucleus, and preoptic area. Prolactin-containing fibers are also seen in the subependymal and inner layers of the median eminence, premammillary nuclei, and the paraventricular thalamic nucleus. Prolactin-like immunoreactive material was present in brain 1 month after hypophysectomy (*1*). Specific prolactin-binding sites have been demonstrated thus far only in the ependyma of the rat (*49*), sheep, pig, and rabbit choroid plexus; the technique used to locate these sites was injection of labeled prolactin in the presence or absence of excess unlabeled hormone in conjunction with light microscopic radioautography.

From the data discussed it appears that all hormones found in pituitary anterior and intermediate lobes (save for gonadotropins, which thus far have not been studied) are present within the central nervous system of several different species, including the human. Although there may be some anatomical specificity in the localization of these hormones (that is, growth hormone in the amygdala and the ACTH-related group of peptides in the hypothalamus), until a systematic survey of the entire central nervous system is performed it will not be possible to fully ascertain the anatomical distribution of these hormones or their concentrations relative to each other. At least some fraction of the total immunoreactive ACTH, TSH, and growth hormone-like material detected in brain appears to be active in bioassay systems, indicating some degree of structural similarity to that of the respective pituitary hormone. The concentrations of all of these hormones in the central nervous system are unaltered or actually increased after hypophysectomy.

Evidence for Pituitary Site of Origin

Although the presence of pituitary hormones in the brain of hypophysectomized animals has suggested a central nervous system origin of such peptides, this interpretation has been questioned (*35, 48, 50–53*) and a pituitary origin has been suggested.

One possible mode of transport from pituitary to brain is by way of the vascular system. There is no evidence that significant amounts of pituitary hormones are taken up by the brain when these hormones are injected intravenously. Whole brain uptake was less than 1 percent (*54*) and 0.07 percent (*53*) after the intravenous injection of tritiated ACTH or ACTH analogs, and no radioactivity was found in brain after systemic injection of ^{125}I-labeled growth hormone (*5*).

Over the past four decades, since the observations of Wislocki and King (*55*) and Green and Harris (*56*), it has been generally agreed that the direction of blood flow in the pituitary portal system is from the hypothalamus to the pituitary. This has subsequently been confirmed by direct observation of portal blood flow in the mouse (*57*), and further strengthened by the findings of hypophysiotropic substances in pituitary portal blood (*58*). There was, however, one earlier report noting blood flow from the pituitary to the hypothalamus in cats and dogs (*50*). This observation, together with the finding of pituitary hormones in rat pituitary portal blood (*51*) and the anatomic demonstration of a paucity of direct venous connections from the adenohypophysis to the cavernous sinus (*52*), has been cited in support of the suggestion that venous blood may leave the adenohypophysis by routes other than venous outflow to the cavernous sinus, that is, by retrograde flow to brain by way of the pituitary portal vessels (*59*). That the actual volume of the central nervous system area containing either ACTH (*35*) or TSH (*48*) is similar in a variety of species (so that the larger the brain size, the smaller the anatomic area containing such peptides) has been cited as evidence that diffusion from the pituitary (via blood or cerebrospinal fluid) is the source of these hormones in brain.

In interpreting these findings, several factors have to be considered. The anatomical demonstration (*52*) of possible retrograde vascular connections from pituitary to brain requires substantiation that the retrograde flow actually does occur under various physiological conditions. It will be important to ascertain the pressure relationships within the various portions of the hypothalamic-pituitary vasculature, to see if conditions are indeed present where such flow reversal may take place. It will also be important to conduct such studies in unanesthetized animals, since many forms of anesthesia (*60*) affect hypophyseal portal blood flow. It also appears that passive diffusion from the vascular compartment cannot alone account for the concentrations of pituitary hormones in brain, since neither intrapituitary nor intracellular injection of [^{3}H]ACTH analog (*53*) revealed any relation between the distribution of radioactivity and the distance of various anatomical areas from the pituitary; more important, the distribution was not similar to the endogenous anatomic sites of localization of ACTH.

Another possible pathway from pituitary to brain is by way of the cerebrospinal fluid. Biogenic amines (*61*), pituitary hormones (*62*), and hypophysiotropic hormones (*63*) can be transported from the third ventricle to pituitary portal blood. Such transport is believed to occur through specialized ependymal cells (tanycytes) that have both long apical processes that extend into the third ventricle lumen and elongated basal processes that extend throughout the entire thickness of the median eminence from the ventricle to the primary portal capillary plexus (*64*). More recently, the question has been raised as to whether the direction of tanycyte transport may also be toward the ventricle, creating a pathway whereby pituitary hormones, if they reach the median eminence via retrograde portal flow, can then be transported throughout the central nervous system. An alternative transport route to cerebrospinal fluid might be from the pituitary to its neighboring subarachnoid space (*65*). Such a route was suggested by the pattern of distribution of radioactivity in the intrasellar injection studies of Mezey *et. al.* (*53*), which showed that such distribution (except for that to hypothalamus) was unchanged after stalk transection. There is disagreement as to whether blood to cerebrospinal fluid transport can occur. This does not seem to be present in the case of ACTH (*66*); it may occur in the case of prolactin (*67*).

In humans, most measurements of the pituitary hormone content of cerebrospinal fluid have been made on fluid obtained from the spinal canal. In these studies, IRGH, prolactin, and gonadotropin concentrations were either undetectable or below the limits of assay sensitivity (*68–70*); concentrations of ACTH were slightly greater or less than those in

plasma (*66*). In contrast, although there is disagreement about the actual amounts present, the concentrations of β-endorphin-like material in cerebrospinal fluid are greater than those in plasma (*71, 72*). To date, therefore, when a normal pituitary gland is present there does not appear to be evidence in favor of significant transport of hormones from pituitary to cerebrospinal fluid, so that further transport from cerebrospinal fluid to other areas of the central nervous system would be an unlikely source of such hormones in brain.

Last, retrograde axonal transport of molecules, such as horseradish peroxidase and certain amino acids, has been demonstrated. In view of the close contact of nerve terminals and vascular elements within the median eminence, if one accepts the presence of pituitary hormones within the hypophyseal portal vein, it is possible (but has not been demonstrated) that central nervous system transport of such hormones may occur by such retrograde transport. The presence of specific cellular central nervous system localization could then be explained by the presence of specific presynaptic or dendritic neuronal uptake mechanisms.

Evidence for Central Nervous System Site of Origin

That the concentrations of ACTH, α-MSH, β-endorphin, prolactin, TSH, and growth hormone in the central nervous system are essentially unchanged after hypophysectomy is consistent with synthesis of these hormones in the central nervous system. It has been implied, however (*35*), that the animals used in these experiments were not completely hypophysectomized and that, therefore, pituitary hormones found in the brains of "hypophysectomized" animals resulted solely from their secretion to the brain by residual microscopic pituitary remnants. Data supporting this view were not provided.

In view of the known difficulties of performing a totally complete hypophysectomy (even in instances where pituitary hormones can no longer be detected in peripheral blood, and where there is evidence of target organ atrophy), several investigators have used more direct approaches to establish the presence of central nervous system synthesis of "pituitary hormones." Recent studies of Mezey *et al.* (*73*) have demonstrated enhanced concentrations of immunoreactive ACTH-like material in hypothalamus after removal of both median eminence and pituitary (in which there is no possibility of diffusion from pituitary to brain, or any type of retrograde flow). Pacold and co-workers (*5*) demonstrated that when cells from the amygdaloid nucleus were grown in tissue culture there was progressively increasing release of IRGH into the medium, even when the medium was completely replenished every 3 to 5 days; a comparable phenomenon was seen in amygdala cells derived from hypophysectomized animals. We have also noted (*74*) that rat arcuate nucleus cells maintained in culture for 17 days release approximately 24 times the immunoreactive ACTH and β-endorphin present in the tissue on day 1. We have recently (*75*) reported that in cultures of enzymatically dispersed bovine hypothalamic cells, ^{3}H-labeled amino acids are incorporated into material with the immunological and size characteristics of the pituitary precursor molecule. Similarly treated cell preparations derived from cortex failed to show such specific incorporation. This direct evidence of brain synthesis of ACTH-like peptides is further strengthened by our observation (*38*) that destruction of the arcuate nucleus in newborn rats by injection of monosodium glutamate is associated with a marked decrease in brain content of immunoreactive ACTH and endorphin-like material; no such change occurs in the concentration of pituitary ACTH. Watson *et al.* (*8*) have also reported that unilateral hypothalamic lesions, which destroy β-LPH positive cells, reduce the staining of ACTH and β-LPH fibers on the side of the lesion, as well as decrease the immunoassayable β-LPH content (*43*). (As noted before, it is only within the arcuate nuclear area that ACTH, β-LPH, and endorphin-like material occur within cell bodies in contrast to their occurrence in fibers elsewhere in the central nervous system.) Several investigators (*8, 42*) have also noted that in animals treated with colchicine before being killed there is a significant increase in the immunocytochemical staining of arcuate nucleus cell bodies for ACTH-related material (ACTH, β-endorphin, α-MSH), a result similar to that for peptides of known central nervous system origin.

Last, α-MSH-like material has been demonstrated in human hypothalamus (*76*), although none has been conclusively detected in normal human pituitary. The demonstration of pituitary hormones, as well as gastrointestinal peptides (*77*), in brain has raised the question of how neurons and endocrine cells can contain similar polypeptide hormones. Such findings are in keeping with the concept of an APUD (amine precursor uptake and decarboxylation) system of cells. This postulates in its present version that all peptide hormone-producing cells are derivatives of specialized ectoderm derived from cell lines arising from the epiblast; such cells therefore have ontogenic commonality with neurons (*77*), which have a similar embryological derivation and, thus, such endocrine cells should be considered as specialized neurons. This theory is also consistent with the presence of ACTH-like material within the gastrointestinal tract and pancreas (*9, 18*), an observation which further weakens the hypothesis that the presence of pituitary hormones in extrapituitary locations is solely due to diffusion from a pituitary site.

Although pituitary hormones appear to occur only in cell bodies in limited central nervous system areas, their specific pattern of distribution (*78*) in other areas may be explained by recent electrophysiological studies. These have demonstrated that axon collaterals of hypothalamic tuberoinfundibular neurons extend to brain sites quite distant from the hypothalamus, such as thalamic nuclei, supporting the neuroanatomical evidence of hypothalamic projections to limbic areas, cerebral cortex, and brainstem (*79*). Material synthesized in hypothalamic cell bodies could therefore be processed and transported to other central nervous system sites by such axon processes or collaterals. The decreases in hypothalamic ACTH content in animals with lesions of the arcuate nucleus would also be in keeping with these observations.

In summary, (i) anatomical studies of pituitary vasculature, (ii) the demonstration of pituitary hormones in hypophyseal portal blood, and (iii) studies of the central nervous system distribution of pituitary hormones (either under basal conditions or after intrapituitary or intracellular injection of such hormones) have suggested to some investigators that the pituitary is the source of these hormones when they are found in brain. There has thus far been no physiological validation of this suggestion. In contrast, (i) the persistence of these hormones in brain after hypophysectomy, (ii) the evidence for secretion of these hormones into culture medium by brain slices or dispersed central nervous system cell preparations, (iii) the intraneuronal localization of these hormones, (iv) the demonstration in vitro of biosynthesis of precursor ACTH- or β-endorphin-like material by hypothalamic cells, and (v) the decrease

in central nervous system concentrations of ACTH-related peptides in animals with lesions of the central nervous system cell bodies containing such peptides, have all suggested to other investigators that the pituitary hormones found in brain are of central nervous system origin.

Regulation of Central Nervous System Concentrations of "Pituitary Hormones"

As already noted, there is no decrease in central nervous system concentrations of pituitary hormones after hypophysectomy. The few studies evaluating other factors that may regulate such hormone concentrations have been concerned with ACTH and related peptides. Studies from our laboratory have shown (*80*) that concentrations of hypothalamic immunoreactive ACTH-like material are not significantly altered 24 hours or 2 weeks after adrenalectomy, or after dexamethasone or corticosterone administration, or by the stresses of ether administration or chronic immobilization. All of these procedures are associated with alterations in the concentrations of ACTH in the pituitary and in the plasma. Rossier *et al.* (*16*) have noted no changes in the concentrations of immunoreactive β-endorphin-like material in whole brains of adrenalectomized animals. In these studies, animals subjected to the stress of foot shock showed a decrease of β-endorphin-like immunoreactivity in hypothalamus, but not in other parts of brain (*81*). No change has been noted in concentrations of β-endorphin-like material in hypothalami of animals treated with morphine or naloxone (*37*). Akil *et al.* (*82*) noted an increase in β-endorphin-like immunoreactivity in the ventricular cerebrospinal fluid of humans undergoing local stimulation of medial thalamic sites for relief of chronic intractable pain. In all of these instances the β-endorphin-like material was not characterized by gel filtration or other methods. This is an important consideration in view of the cross-reactivity of the β-endorphin antibodies used with both β-lipotropin and the precursor molecule. The limited studies to date, therefore, indicate that the concentrations of ACTH and endorphin in the brain are not affected by positive or negative hormonal feedback signals, which affect the concentration of these peptides in the pituitary. The concentrations of such peptides in the central nervous system may be affected by certain stresses, but additional studies with other types of stresses are needed, and whether such changes reflect increased turnover or decreased synthesis has yet to be determined.

Function of "Pituitary Hormones" in Brain

Investigators attempting to assess the functional significance of pituitary hormones in brain should entertain the possibility that the role of the hormones within the central nervous system differs from their currently assigned physiological role within anterior pituitary. For example, it is known that TRH-like activity is present in the neural tissue of certain invertebrates even though evidence of a role for it in the regulation of pituitary secretion has only been found in animals higher up in the evolutionary scale (*83*). Within recent years the concept of "peptidergic neurons," referring not only to the well-defined neurosecretory cells of the hypothalamus (that is, those producing vasopressin and hypophysiotropic hormones), but also to those neurons containing additional peptides (such as substance P, neurotensin, angiotensinogen, gastrointestinal peptides, and pituitary hormones), has attracted considerable interest (*83*). It has also been postulated that in some neurosecretory neurons biosynthesis of the peptide precursor occurs within the perikarya, with posttranslational cleavage events, occurring during axonal transport (*84*).

Within the neuron these peptides usually appear as membrane-bounded, electron-dense cytoplasmic granules, which are transported by axoplasmic flow and are considered to be released by exocytosis. These peptides, when secreted at peptidergic terminals, may act on a variety of effector cells, including neurons, functioning as either neurotransmitters or neuromodulators, rather than by acting within the narrow definition of a "hormone" (that is, a product secreted into the bloodstream and acting at a distance). For example, immunoreactive somatostatin has been demonstrated in dendritic contacts with other dendrites and in dendritic-soma contact in recipient neurons (*85*), and we have also demonstrated in hypothalamic dendrites substances that react with antiserums to ACTH and β-lipotropin (*13*).

Barker (*86*) has noted numerous differences in the actions of the classical neurotransmitter substances (that is, amino acids, catecholamines, acetylcholine) and the peptide substances found in the central nervous system. Although they are similar with regard to calcium-dependent evoked release and axonal transport, the peptidergic hormones, compared to neurotransmitters, have a relatively slower action at onset (this being measured in seconds to minutes, rather than the millisecond-to-second action of onset of classical neurotransmitters) and a longer duration of action (minutes to hours, rather than milliseconds to seconds). It has been hypothesized that neurotransmitters are involved in the momentary mediation of single cell-to-cell interactions, whereas neurohormones are involved in the sustained modulation of specific sets of target neurons (*86*). The concept of neuromodulation implies an action of the secreted peptide in altering the effect of the classical neurotransmitter on its receptors. There are other possible interactions of peptides and neurotransmitters. Hökfelt *et al.* (*78*) indicated that some neurons may contain both peptides and classical neurotransmitters, seemingly in violation of Dale's concept of one neuron, one transmitter. It has been reported (*87*) that ACTH and vasopressin interact with opiate receptors; affect acetylcholine, norepinephrine, and serotonin content or turnover of various brain regions; selectively modify unit activity in midbrain limbic structure; and enhance the dephosphorylation of membrane proteins, leading to a change in membrane permeability.

Most of the speculation about the functions of "pituitary hormones" in brain within recent years has centered on the endorphins, which are the only peptides for which central nervous system neuronal receptors have been identified. A voluminous literature has appeared with regard to their possible effects on pain perception, addictive states, psychiatric disorders (*88*). Here we review only some of the major findings.

Intracerebral administration of β-endorphin in rats is associated with marked analgesic effects, β-endorphin being 18 to 33 times more potent than morphine on a molar basis. Its actions are blocked by the specific opiate antagonist, naloxone (*89*). Relief of intractable pain was reported in six human patients receiving stimulation through electrodes permanently implanted in the periventricular and periaqueductal gray matter (*90*); naloxone administration reversed this stimulation-induced pain relief. Such stimulation of periaqueductal gray matter is associated with the appearance of β-endorphin-like immunoreactivity in human ventricular cerebrospinal fluid (*82*). Relief of intractable pain has also been produced in human pa-

tients by intraventricular administration of human β-endorphin (*91*). All of these findings imply a central effect of β-endorphin (or fragments thereof) with regard to analgesia. The mechanism of action of acupuncture in producing analgesia has been a topic of much interest. It has been reported that intravenous administration of naloxone blocks acupuncture analgesia; also, that hypophysectomy abolishes the effect of acupuncture analgesia (*92*). These studies have suggested that acupuncture analgesia may involve the release of pituitary endorphin; to date, the amount of endorphin in either the blood or cerebrospinal fluid of humans undergoing acupuncture has not been measured.

Although several models have been proposed, there is still no agreement about the nature of the addictive state or the abstinence syndrome at the molecular level. Tolerance to, and physical dependence on, β-endorphin have been demonstrated. There has been a preliminary report of improvement in some signs and symptoms of the abstinence syndrome in man after intravenous β-endorphin administration, although rigorous double-blind studies have yet to be performed (*93*). To account for this effect of endorphin in amelioration of the abstinence syndrome it has been suggested that when exogenous opioids are ingested, the body reduces its own production of such substances; therefore, with withdrawal, a deficiency of endogenous endorphin exists, which might be corrected by further administration of exogenous material.

One of the early striking effects of intracerebral administration of endorphin has been the profound sedation and immobilization (considered to be similar to catatonia) that occurs (*94, 95*). The similarity of behavioral effects with those following exogenous administration of neuroleptic drugs has suggested to some investigators that endorphins might be involved in certain psychiatric states. There have been reports that suggest (*96*) and deny (*97*) therapeutic effects of intravenous naloxone in some populations of schizophrenic subjects. The need for accurate clinical characterization of such patients in controlled double-blind studies is evident.

The initial reports on behavioral effects of anterior pituitary hormones centered on ACTH and MSH (*87*). Administration of ACTH and ACTH analogs affects a variety of behaviors in the rat, such as active and passive avoidance behavior, approach behavior, memory, reverse learning behavior, and sexually motivated behavior. Some of these effects are seen only in hypophysectomized animals in whom behavioral deficits are corrected by administration of these fragments, while others are seen after administration to intact animals, usually by intracerebral administration. The increased rate of acquisition of avoidance behavior and inhibition of extinction of avoidance behavior after administration of ACTH and ACTH fragments has been interpreted as being equivalent to increased memory retention and increased persistence of the learned response. It has been suggested that these compounds affect learning and memory by increasing the alertness of the animal. The questions of the relation of these behavioral effects (most of which have been measured under stressful conditions) to normal learning processes, the physiological relevance of doses administered, and the circadian appropriateness of the time of day when these were performed still remain to be fully investigated. To date there have been no reports of these peptides and their fragments affecting human behavior. Of interest are the observations that fragments of the ACTH molecule [such as ACTH(4-10), ACTH(4-7)] which in themselves have no steroidogenic potency, are as effective as the intact hormone. Whether such fragments can arise normally through cleavage by specifically localized proteases in brain still remains an important unanswered question.

A direct effect of prolactin on the central nervous system is suggested by reports that impotence present in males with hyperprolactinemia and normal testosterone concentrations may be corrected by lowering the prolactin concentrations by bromocryptine administration (*98*). Iontophoretic studies have demonstrated the presence of prolactin-sensitive neurons in the hypothalamus, but not elsewhere within the central nervous system (*99*).

In addition to the postulated behavioral effects of pituitary hormones in brain, such hormones may also have effects on endocrine regulatory processes. In constructing a scheme of brain-pituitary-target organ interrelations, the concept of "short-loop" feedback has been invoked as one regulatory component (*100, 101*). This implies that the pituitary hormone, while stimulating its target organ, will also inhibit its own release—presumably acting either directly on secretion of its central nervous system derived releasing factor, or on central nervous system mechanisms involved in the regulation of releasing factor. This has been extensively studied in the case of ACTH; most experiments demonstrating such an inhibitory effect have required large doses and long periods of treatment (*102*). Similar effects have been postulated for the effect of growth hormone and prolactin on their putative brain releasing factors.

Studies with opiate-like compounds (including morphine and enkephalin, as well as endorphin) have demonstrated that intracerebral administration is associated with an increase in serum growth hormone and prolactin concentrations, such effects being blocked by prior naloxone treatment. Intravenous β-endorphin administration is associated with significant increases in plasma arginine vasopressin concentrations without any effect on release from isolated rat neural lobes in vitro. It remains to be determined whether such observations reflect a direct effect of these compounds on releasing factors or on the neurotransmitters that regulate such releasing factors (*103*).

In conclusion, it may well be that pituitary hormones found in brain are derived from two different sources. Those arising in brain may be involved in central coordination of responses independent of, or even concomitant with, those affected by peripheral secretion of such hormones by the pituitary. Those present by virtue of possible retrograde portal blood flow may participate in short-loop feedback effects of such hormones in the regulation of anterior pituitary function. It is apparent that attempts to answer the question of the distribution, origin, regulation, and function of such hormones will lead to new concepts of both normal and abnormal brain function.

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105. This work was supported in part by NIH grant NB 02893-16 and the Lita Annenberg Hazen Charitable Trust Fund.