CHAPTER 1

The Distribution of MSH and ACTH in the Rat and Human Brain and Its Relation to Pituitary Stores

Dick F. Swaab, Peter W. Achterberg, Gerard J. Boer,
Jan Dogterom, and Frederik W. van Leeuwen

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Substances with melanocyte-stimulating activity were found 50 years ago in the pars intermedia of the pituitary and in the periventricular regions of the human brain. The pituitary was, however, long considered to be the sole source of "intermedin," which was thought to be transported to the brain via the portal vessels or the cerebrospinal fluid. Although this view is still held by some, evidence is rapidly accumulating that melanocyte-stimulating hormone (MSH), adrenocorticotrophic hormone (ACTH), and a family of related peptides are not only produced by the pituitary, but also by the nervous system. This family of peptides has been shown, at least in the pituitary, to be derived from a 30 kdalton precursor.

The adult rat pituitary contains about three times more α -MSH than ACTH; 97% of the total α -MSH content is found in the neurointermediate lobe and 95% of the total ACTH content is found in the pars distalis. Comparable assay data do not exist for the human pituitary. The rat brain has high levels of α -MSH and ACTH in the area of the arcuate nucleus of the hypothalamus, but these peptides are found, in addition, in cell bodies and fibers in a number of extrahypothalamic areas.

Immunocytochemical observations are consistent with the notion that opiomelanocortin peptides are present in neurons of the arcuate nucleus and in pathways running from this nucleus to the limbic system, midbrain, and hindbrain. However, extrahypothalamic cell bodies positive for anti- α -MSH or anti-ACTH have been described as well. Cross-reaction of the antibodies to as yet unidentified, related compounds is the most probable explanation for this discrepancy and illustrates that specificity is a crucial problem in immunocytochemistry.

α -MSH, ACTH, and other peptides of the opiomelanocortin family are present early in the development of fetal brain and pituitary of man and rat and might influence development and labor.

Evidence indicating that peptides related to opiomelanocortin are synthesized in the vertebrate nervous system has been obtained recently by means of bio- and radioimmunoassays, biochemistry, immunocytochemistry, hypophysectomy, mechanical and chemical brain lesions, and tissue culture. Other data indicate the presence of these peptides in neuronal tissue in lower animals. The importance of peptides of pituitary origin for brain peptide content and function remains to be determined.

I. HISTORICAL INTRODUCTION

A pituitary hormone was discovered 50 years ago that was capable of inducing changes in the skin color of some species, such as bridal staining in the fish and the darkening of frog skin. This hormone is also found in the mammalian pituitary gland. In the human pituitary, it is present in similar amounts in Blacks and Caucasians. Because the highest concentration of this hormone is in the pars intermedia of the pituitary, it was called "intermedin" (Zondek, 1935; Zondek & Krohn, 1932). The locus of intermedin in the pars intermedia was established in 1930 when Bayer found one light-colored frog among a shipment of 70 esculenta. The color of this animal remained light even after many days in the dark, and histological investigation revealed that its pars intermedia had been selectively destroyed by a parasite (see Zondek, 1935). The high amount of chromatophoric activity found by Zondek can, however, certainly not be attributed solely to α -melanocyte-stimulating hormone (α -MSH, see Section II), as this hormone represents only 2% of the bioassayable melanotropic activity in the human pituitary (Abe, Island, Liddle, Fleischer, & Nicholson, 1967).

In addition to the localization of intermedin in the pituitary, Zondek reported the presence of this hormone in the human fetal, neonatal, and adult brain. Although he found intermedin in the choroid plexus of the brain of eclamptic women, and in areas around the third ventricle, Zondek could not find any hormone in the cerebrospinal fluid itself. The earlier identification of intermedin in this fluid by Trendelenburg and Ehrhardt (see Zondek, 1935) were therefore attributed by Zondek to aspecific effects due to their extraction procedure. Zondek concluded that intermedin was transported from the pituitary via the pituitary stalk to the vegetative centers in the periventricular brain area,

where it exerted its actions. He concluded that transport of intermedin to the brain is very rapid because little intermedin was found in the pituitary stalk itself. Popa (1938) proposed that the transport of "xanthomélanophorine" from the pituitary to the brain takes place via both the portal vessels and the cerebrospinal fluid. Little further work was done until 1958 when Mialhe-Voloss reported the disappearance of melanotropic activity from the rat brain after hypophysectomy, and restoration of the activity after intermedin substitution via the carotid artery. She thus came to the conclusion that the pituitary was the sole source of intermedin. Later, however, Rudman and coworkers provided evidence by means of similar experiments that the melanotropic peptides in the brain were not derived from the pituitary but from the central nervous system (CNS) itself (Rudman, Del Rio, Hollins, Houser, Keeling, Sutin, Scott, Sears, & Rosenberg, 1973; Rudman, Scott, Del Rio, Houser, & Sheen, 1974). Meanwhile, Guillemin, Schally, Lipscomb, Andersen, and Long (1962) had found, in addition to melanotropic activity, adrenocorticotrophic activity in the hypothalamus and proposed a diencephalic origin for such peptides. Despite the discovery in the 1930s of intermedin in the brain, little attention was paid to its possible central functions until the mid-1950s, when a number of central actions of MSH and ACTH were discovered, such as the effects of ACTH and α -MSH on shuttlebox avoidance behavior (Appelzweig & Moeller, 1959; de Wied, 1964, 1965; Murphy & Miller, 1955), facilitation of monosynaptic spinal reflexes by β -MSH (Guillemin & Krivoy, 1960), effects of MSH and ACTH on grooming, scratching, and stretching (Ferrari, Gessa, & Vargiu, 1963), and alterations of the electroencephalogram by α - and β -MSH (Sandman, Denman, Miller, Knott, Schally, & Kastin, 1971). We became interested in the possibility that the brain produces peptides related to MSH when we found that antibodies raised against α -MSH and used for immunofluorescence microscopy also persistently stained homogenized brain tissue that was put around fetal pituitaries as support for the cryostat sections. The immunoreactivity of MSH was indeed found in nerve cells and fibers, suggesting a central origin of such compound(s) (Swaab, 1976; Swaab & Visser, 1977). Although the "classical" view that the pituitary is the only source for MSH, ACTH, and related peptides in the brain has again recently been advocated (Moldow & Yalow, 1978a), evidence is accumulating that a family of peptides related to MSH and ACTH is not only produced by the pituitary but also by the nervous system.

II. PRO-OPIOMELANOCORTIN, MSH, AND ACTH

During the last decades, it has become apparent that MSH and ACTH belong to a family of related peptides that are all derived from one large precursor.

The possible chemical relationship between MSH and ACTH was suggested as early as the 1950s by the observation that in patients with Addison's disease or Cushing's syndrome, the levels of both MSH and corticotropic activity were elevated. Two forms of MSH, α - and β -MSH, were quickly isolated. Structural elucidation revealed that their amino acid sequences of 13 and 18 amino acids, respectively, had a central core of 7 amino acids (ACTH/ α -MSH₄₋₁₀) in common with each other and with ACTH. In addition, the entire sequence of α -MSH was found to be present within the ACTH molecule (cf. Harris, 1960). The sequence of β -MSH was later retrieved in β -lipotropic hormone (β -LPH) (Li, Barnafi, Chrétien, & Chung, 1965). The factor necessary for both melanocyte-expanding and corticosteroidogenic activity was found to be ACTH/ α -MSH₆₋₉ (cf. Schwyzer & Eberle, 1977). These findings suggested the existence of a family of related peptides containing two classes: (1) ACTH and its cleavage products of α -MSH and corticotropin-like intermediate lobe peptide (CLIP); and (2) β -LPH and its fragments γ -LPH and β -MSH (Scott, Ratcliffe, Rees, Landon, Bennett, Lowry, & McMartin, 1973). It is not surprising, therefore, that ACTH and β -LPH were immediately regarded as precursor molecules. A remarkable difference from prohormones that were already known, such as insulin (Steiner, Rubenstein, Peterson, Kemmler, & Tager, 1973) and vasopressin (Sachs & Takabatake, 1964), was that the "prohormone," ACTH itself, also had a known biological function.

Interest in the peptides of the ACTH/LPH family was further heightened when Hughes, Smith, Kosterlitz, Fothergill, Morgan, and Morris (1975) reported the structure of a morphine-like pentapeptide in the brain, which they named *enkephalin*, and of which the Met-derivative turned out to be present in its entirety in the β -LPH molecule. The part of β -LPH remaining after splitting off γ -LPH appeared to have even more potent opiate-like properties and was named β -endorphin (Bradbury, Smyth, & Snell, 1976; Guillemin, Ling, & Burgus, 1976). β -Endorphin was thought to fragment successively into γ - and α -endorphin, which in turn were both thought to be precursors of enkephalin (Austen, Smyth, & Snell, 1977). Thus, β -LPH is a prohormone for melanocyte-expanding and opiate activities and can be named pro-opiomelanin.

Studies of high-molecular-weight forms of ACTH, first described by Yalow and Berson (1971) as "big ACTH," indicated the existence of a larger precursor protein, in which both immunoreactive ACTH and β -endorphin were present. Other observations pointing to a 30 kdalton precursor came from studies of a pituitary tumor cell line (Mains, Eipper, & Ling, 1977; Roberts & Herbert, 1977), of bovine pituitaries (Nakanishi, Inoue, Taii, & Numa, 1977), and of human pulmonary carcinoma cells (Bertagna, Nicholson, Sorenson, Pettengill, Mount, & Orth, 1978). Recently, the entire nucleotide sequence of a cloned DNA insert encoding the bovine corticotropin- β -lipotropin precursor has been determined (Nakanishi, Inoue, Kita, Nakamura, Chang, Cohen, & Numa,

1979), which was named *pro-opiomelanocortin* (Chrétien, Benjannet, Gossard, Gianoulakis, Crine, Lis, & Seidah, 1979). This sequencing study defined not only the precise locations of ACTH and β -LPH (Fig. 1) but also the amino acid composition of the remaining NH_2 -terminal portion, which was known to exist but had never been determined (Mains et al., 1977; Roberts & Herbert, 1977) and in which the sequence -His-Phe-Arg-Trp- (ACTH/ α -MSH₆₋₉ and β -MSH₉₋₁₂) appeared to be present for a third time. On the basis of the occurrence of two adjacent pairs of basic amino acids, which are apparent sites of chain splitting, Nakanishi et al. (1979) postulated this sequence to be part of a not yet identified decapeptide that they called γ -MSH (Fig. 1).

The rat intermediate lobe pituitary cells split off the 30 kdalton precursor first between the sequences of ACTH and β -LPH, whereby a 20 kdalton fragment (promelanocortin) and β -LPH are produced. From the 20 kdalton fragment, α -MSH, CLIP plus a 16 kdalton fragment are derived, and from β -LPH, γ -LPH plus β -endorphin are derived (Mains & Eipper, 1979). The pro-opiomelanocortin molecule of the mouse pituitary tumor cell line has been shown to fragment, after the same first cleavage into ACTH, β -LPH, and β -endorphin molecules (Mains et al., 1977). This seems to suggest the possibility that different cells (e.g., pars intermedia versus pars distalis of the pituitary or versus brain) or cells under different circumstances (e.g., in the course of development) might process the precursor in different ways. The large number of possible sites of fragmentation (i.e., between each of the pairs of basic amino acids of pro-opiomelanocortin; Fig. 1) may account for the high number of different ACTH- and MSH-like compounds that appear to exist (see Section III-B). This, and the great number of members of the pro-opiomelanocortin family, as revealed so far from pituitary sources (Fig. 1), indicates that problems of specificity will be encountered in radioimmunoassay and immunocytochemical studies.

III. ASSAY OF α -MSH AND ACTH IN THE PITUITARY AND THE BRAIN

As has already been suggested by Zondek (1935), peptides of the opiomelanocortin family are present both in the pituitary and in the brain.

A. Pituitary Content of α -MSH and ACTH

Reports by various authors on the rat pituitary content of α -MSH and ACTH are in reasonable agreement (Table 1), although assay data vary according to a number of factors, such as the strain of rat, the ages and sexes of the animals,

Fig. 1. The peptides of the pro-opiomelanocortin family. Bovine pro-opiomelanocortin is a protein of presumably 265 amino acids numbered according to Nakanishi et al. (1979). A pituitary extract splits the molecule at site 40/41, resulting in promelanocortin and pro-opiomelanin (β -LPH) parts. Thus far, no physiological function is known for either of these peptides, other than being prohormones, that may serve as a storage mechanism until further cleavage is needed. Promelanocortin can give rise to either ACTH and a 16 kdalton fragment (e.g., in the pars distalis of the pituitary) or, in addition, to α -MSH and CLIP as further cleavage products of ACTH (e.g., in the pars intermedia). The pro-opiomelanin molecule is physiologically cleaved at site 102/103, liberating γ -LPH and β -endorphin.

Successive cleavage of pro-opiomelanocortin probably occurs at sites of paired basic amino acids, whereby direct amidation of the terminal carboxyl group is concomitant (cf. Bradbury et al., 1976). It is assumed, therefore, that the pituitary β -MSH is liberated from the γ -LPH fragment and, consequently, both ACTH and γ -LPH might be called promelanins.

The recognition of a third ACTH₆₋₉ sequence (-51/-48) in the pro-opiomelanocortin as well as pairs of basic amino acids around this sequence have been regarded as possible evidence for the existence of more "family members" than have been recognized so far. The 16 kdalton fragment might split off γ -MSH (-55/-44; Nakanishi et al., 1979), but a fragment -55/-29 or -55/-3 is also possible.

The peptides γ - and α -endorphin and Met-enkephalin can be liberated *in vitro* from β -endorphin. However, because of the absence of paired basic amino acids, processing of β -endorphin is enzymatically different (the site 131/134 has not yet been mentioned as a site of cleavage). Moreover, in this scheme, bovine β -LPH contains 93 amino acids instead of the usually mentioned 91 (Li et al., 1965). Two additional amino acid residues (Ala and Glu- β -LPH₃₅₋₃₆) have been added on the basis of DNA-sequencing (Nakanishi et al., 1979).

TABLE 1

Summary of Data on the α -MSH and ACTH Content of the Rat Pituitary

	α -MSH(ng/mg)	Assay ^a	Reference	ACTH(ng/mg)	Assay ^a	Reference
Total pituitary	210	(BIO)	Shapiro et al., 1972	85	(RIA)	Usategui et al., 1976
	200 ^b	(BIO)	Tilders, 1975	38	(RIA)	Moldow and Yalow, 1978a
	220	(RIA)	Usategui et al., 1976	100	(RIA)	Vaudry et al., 1978
	140	(RIA)	Oliver and Porter, 1978			
	610	(RIA)	Vaudry et al., 1978			
	80	(RIA)	Eskay et al., 1979a			
	260	(RIA)	Visser and Swaab (see Fig. 4)			
(Mean)	(250)			(74)		
Pars distalis	17	(BIO)	Shapiro et al., 1972	50	(BIO)	Scott et al., 1974
	4	(RIA)	Shapiro et al., 1972	95	(RIA)	Scott et al., 1974
	0.7 ^b	(RIA)	Thody et al., 1975	110	(RIA)	Scott et al., 1974
	6 ^b	(BIO)	Tilders, 1975	65	(BIO)	Moriarty and Moriarty, 1975
	18	(RIA)	Usategui et al., 1976	92	(RIA)	Usategui et al., 1976
	5	(RIA)	Oliver and Porter, 1978	105	(RIA)	Vaudry et al., 1978
	13	(RIA)	Vaudry et al., 1978	87	(RIA)	van Dijk, 1979
(Mean)	(9)			(86)		
Neurointermediate lobe	3200	(BIO)	Thody, 1974	18	(BIO)	Scott et al., 1974
	3560	(BIO)	Thody et al., 1975	63	(RIA)	Scott et al., 1974
	2770 ^b	(RIA)	Thody et al., 1975	15	(BIO)	Moriarty and Moriarty, 1975
	3350 ^b	(BIO)	Tilders, 1975	33	(RIA)	Usategui et al., 1976
	3490 ^b	(BIO)	Kraicer et al., 1977	60	(RIA)	Vaudry et al., 1978
	1920	(BIO)	Vaudry et al., 1978			
	6220	(RIA)	Vaudry et al., 1978			
	1100	(RIA)	Oliver and Porter, 1978			
(Mean)	(3200)			(38)		

^a Bioassay (BIO); radioimmunoassay (RIA).^b Wherever total content is given, ng/mg was calculated based on the assumption that the weight of the pars distalis was 9 mg and that of the neurointermediate lobe was 1 mg.

the time of sacrifice, the stage of the estrous cycle, the tissue extraction procedure, the type and specificity of the assay, and the standards used (e.g., Celis, 1975; Tilders, 1975; Tilders & Smelik, 1975).

The rat pituitary contains about three times as much α -MSH as ACTH on a weight basis; 97% of the total α -MSH content is found in the neurointermediate lobe and 95% of the total pituitary ACTH content is in the pars distalis. Some extremely low α -MSH concentrations (500 ng/mg by bioassay and 59 ng/mg by radioimmunoassay, 0.04 ng/mg for the pars distalis, and 72 ng/mg for the intermediate lobe) were reported by Shapiro, Nicholson, Orth, Mitchell, Island, and Liddle (1972) and Dubé, Lissitzky, Leclerc, and Pelletier (1978), respectively. The anomalous results may be explained by the neutral extraction medium used by these investigators (P. Janssens, unpublished observations). Some exceptionally high ACTH levels have been reported in the neurointermediate lobe (890 ng/mg: Moriarty & Moriarty, 1975; 2190 ng/mg: Scott, Lowry, Ratcliffe, Rees, & Landon, 1974; and 480 ng/mg: van Dijk, 1979). The apparent high levels may be due to the use by these authors of antibodies directed to the C-terminal part of ACTH, which probably cross-react with CLIP (Moriarty & Moriarty, 1975; van Dijk, 1979). This could explain why these levels are in the same range as the α -MSH content of this structure (cf. Table 1). No assay data exist for *human* pituitaries that allow an estimation of the distribution of ACTH and α -MSH. On the basis of the bioassay data of Morris, Russell, Landgrebe, and Mitchell (1956) and the individual weights of the three parts of the human pituitary (Romeis, 1940; Table 1), it appears that the distribution of melanotropic and corticotropic activity in the pars distalis, pars intermedia, and posterior lobe is very similar (96%; 0.2%; 3.8%, and 89.9%; 0.1%; 10%, respectively). However, because the α -MSH content of the human pituitary was estimated to represent only 2% of the bioassayable activity (Abe et al., 1967), Morris's melanotropic activity data do not reflect the distribution of α -MSH in the human pituitary. The only information on this subject is based upon immunocytochemistry (see Section V-B).

B. Brain Content of α -MSH and ACTH

Consistently high levels of α -MSH have been found in the arcuate nucleus in the rat (Table 2). The same nucleus was also found to contain relatively large amounts of ACTH (van Dijk, 1979). Assay data for ACTH are highly variable, as can be seen by the more than hundredfold difference in hypothalamic ACTH content found in different studies. Table 2 also reveals that there is not a complete overlap in distribution of α -MSH and ACTH. In this respect, it is remarkable that the ventral hypothalamus seems to contain more radioim-

munoassayable ACTH than does the dorsal hypothalamus, whereas the reverse is found for α -MSH. The huge differences sometimes reported in the MSH/ACTH contents of the various brain areas might be explained by the assay variables mentioned before (see also O'Donohue, Miller, Pendleton, & Jacobowitz, 1979c), particularly in view of the low amounts that are present in the brain, as compared to the pituitary, and the variability in the way the brain areas are dissected. An additional source of variability might be the different sensitivities of the various assays for the known or unknown members of the opiomelanocortin family. For instance, there are good reasons to assume that α -MSH itself is not present in the pineal gland but that, instead, an unknown related immunoreactive compound is synthesized in this structure (Pévet, Ebels, Swaab, Mud, & Arimura, 1980). The differences between the pineal α -MSH content (< 2 up to 380 pg/mg—see Table 2) might therefore be related to the differences in sensitivity for the unknown compound(s) of the various assays. Other compounds have been demonstrated by Loh and Gainer (1977) and Loh, Zucker, Verspaget, and van Wimersma Greidanus (1979), who found most of the bioassayable melanotropic activity in the rat brain and pituitary to be due to compounds that showed distinct cross-reactivity in a radioimmunoassay for α -MSH. These compounds appeared to be identical neither to α -MSH, ACTH₄₋₁₀, 1-10, 1-24, nor to N-Ac-ACTH₁₋₁₀, a conclusion fully consistent with our immunocytochemical data (see Section IV). High-performance liquid chromatography (HPLC) also indicates the existence of radioimmunoassayable peptides that are not identical to α -MSH (O'Donohue, Miller, & Jacobowitz, 1979a). Rudman, Chawla, and Hollins (1979) showed that an α -MSH-like peptide of bovine, rabbit, guinea pig, and rat pituitaries is N,O-diacetyl serine- α -MSH, and suggested that α -MSH may need to be acetylated before it is released from the pituitary. There may, in fact, be a host of converted peptides of the opiomelanocortin family in the brain and pituitary. One of the two peaks found by O'Donohue et al. (1979a) using HPLC might represent the compound recently described by Rudman et al. (1979). The presence of the melanotropic peptide "II F," as claimed earlier by Rudman et al. (1974), has been shown by means of HPLC to be due to a minor (1%) contamination of α -MSH in this fraction (Rudman et al., 1979). However, γ -MSH (Nakanishi et al., 1979) might be another candidate for an α -MSH-like peptide (see Fig. 1).

In the human brain, β -endorphin concentrations of about 100 pg/100 g protein were recently reported in the arcuate nucleus, median eminence, suprachiasmatic area, preoptic nucleus, supraoptic and paraventricular nuclei, and ventro- and dorsomedial nuclei. The highest concentrations were observed in the ventral hypothalamus (Wilkes, Watkins, Stewart, & Yen, 1980). Other quantitative data on the opiomelanocortin family in the human brain are not presently available.

TABLE 2

Summary of Data on the α -MSH and ACTH Content of the Rat Brain

	α -MSH(pg/mg)	Assay ^a	Reference	ACTH(pg/mg)	Assay ^a	Reference
Hypothalamus (total)	645	(RIA)	Vaudry et al., 1978	1500-4500 ^b	(RIA)	Krieger et al., 1977a
	140	(RIA)	Oliver and Porter, 1978	800	(RIA)	Krieger et al., 1977b
	35	(RIA)	Dubé et al., 1978	1000 ^b	(BIO)	Krieger et al., 1977a
	250	(RIA)	Eskay et al., 1979b	450	(BIO)	Krieger et al., 1977b
	80	(RIA)	Orwoll et al., 1979	14	(RIA)	Moldow and Yalow, 1978a
	180	(RIA)	Warberg et al., 1979	<8	(BIO)	Vaudry et al., 1978
Ventromedial nucleus	320 ^b	(RIA)	O'Donohue et al., 1979a	29	(RIA)	Orwoll et al., 1979
	290 ^b	(RIA)	Eskay et al., 1979b	54	(RIA)	van Dijk, 1979
	895 ^b	(RIA)	O'Donohue et al., 1979a	40	(RIA)	Moldow and Yalow, 1978a
	550 ^b	(RIA)	Eskay et al., 1979b	61 ^b	(RIA)	van Dijk, 1979
	220	(RIA)	Eskay et al., 1979a	10	(RIA)	Moldow and Yalow, 1978a
	690 ^b	(RIA)	Eskay et al., 1979b	36 ^b	(RIA)	van Dijk, 1979
Arcuate nucleus	870 ^b	(RIA)	O'Donohue et al., 1979a	105 ^b	(RIA)	van Dijk, 1979
	25	(RIA)	Oliver and Porter, 1978			
	30	(RIA)	Vaudry et al., 1978			
	6-12 ^b	(RIA)	Eskay et al., 1979a,b			
	42-719 ^b	(RIA)	O'Donohue et al., 1979a			
	0.2-1.0	(RIA)	Eskay et al., 1979b, a			
Thalamus	25 ^b	(RIA)	O'Donohue et al., 1979a			
				24 ^b	(RIA)	Krieger et al., 1977a
				4	(RIA)	Moldow and Yalow, 1978a
				<8	(RIA)	Vaudry et al., 1978
				31 ^b	(RIA)	van Dijk, 1979
				105 ^b	(BIO)	Krieger et al., 1977b
Hippocampus				120 ^b	(RIA)	Krieger et al., 1977b
				120 ^b	(RIA)	Krieger et al., 1977a
				3	(RIA)	Moldow and Yalow, 1978a
				<8	(RIA)	Vaudry et al., 1978
				15 ^b	(RIA)	van Dijk, 1979
				150 ^b	(BIO)	Krieger et al., 1977b
Septum	32	(RIA)	Eskay et al., 1979a	190 ^b	(RIA)	Krieger et al., 1977b
	47	(RIA)	Eskay et al., 1979b			

Amygdala	12-87 ^b	(RIA)	Eskay et al., 1979b	12 ^b	(RIA)	van Dijk, 1979
	15-130 ^b	(RIA)	O'Donohue et al., 1979a			
	90-355 ^b	(RIA)	O'Donohue et al., 1979a			
Cerebellum	6	(RIA)	Eskay et al., 1979a	180 ^b	(BIO)	Krieger et al., 1977b
	17-19	(RIA)	Eskay et al., 1979b	260 ^b	(RIA)	Krieger et al., 1977b
	2	(RIA)	Oliver and Porter, 1978	3	(RIA)	Moldow and Yalow, 1978a
	4.1	(RIA)	Vaudry et al., 1978	20 ^b	(BIO)	Krieger et al., 1977b
	0.2	(RIA)	Eskay et al., 1979b	40 ^b	(RIA)	Krieger et al., 1977b
Cortex (parietal or total)	8.5	(RIA)	Achterberg (unpubl.)	3.4 ^b	(RIA)	Krieger et al., 1977a
	1.5-3.2	(RIA)	Vaudry et al., 1978			
	3.6	(RIA)	Dubé et al., 1978	40 ^b	(BIO)	Krieger et al., 1977b
	5.7	(RIA)	Oliver and Porter, 1978	50 ^b	(RIA)	Krieger et al., 1977b
	0.07-1.4 ^b	(RIA)	Eskay et al., 1979a,b	46 ^b	(RIA)	Krieger et al., 1977a
Brain stem/medulla	1.8	(RIA)	Achterberg (unpubl.)	<1	(RIA)	Moldow and Yalow, 1978a
	11	(RIA)	Oliver and Porter 1978	<4 ^b	(RIA)	van Dijk, 1979
	3.6	(RIA)	Eskay et al., 1979a,b	<10 ^b	(BIO)	Krieger et al., 1977b
	6.9	(RIA)	Achterberg (unpubl.)	<20 ^b	(RIA)	Krieger et al., 1977b
	0.6	(RIA)	Eskay et al., 1979b	3	(RIA)	Moldow and Yalow, 1978a
Spinal cord	2.4	(RIA)	Achterberg (unpubl.)			
	27	(RIA)	Achterberg (unpubl.)			
Spinal ganglia Pons	2.4	(RIA)	Eskay et al., 1979a,b			
Pineal	2.2	(RIA)	Dubé et al., 1978	23 ^b	(BIO)	Krieger et al., 1977b
	160	(RIA)	Oliver and Porter, 1978	23 ^b	(RIA)	Krieger et al., 1977b
	380	(RIA)	Vaudry et al., 1978	4	(RIA)	Moldow and Yalow, 1978a
	27	(RIA)	Eskay et al., 1979b	<8	(BIO)	Vaudry et al., 1978
	240	(RIA)	O'Donohue et al., 1979a			
	<2	(RIA)	Achterberg (unpubl.)			

^a Radioimmunoassay (RIA); bioassay (BIO).
^b Originally expressed as pg/mg protein and transferred to pg/mg wet tissue by multiplication by 0.1. The data of Krieger et al. (1977a,b) were obtained by the paradoxical binding phenomenon.

Although the exact nature of the compounds measured radioimmunochemically in the brain as α -MSH and ACTH is not known (see, e.g., the second α -MSH-like material containing peak in O'Donohue et al., 1979a), the literature is consistent in that these compounds do not disappear from the brain after hypophysectomy and are, thus, most probably synthesized (see Section VI) by the brain itself (Krieger, Liotta, & Brownstein, 1977a,b; Liotta, Hauser, Brownstein, & Krieger, 1978; O'Donohue, Holmquist, & Jacobowitz, 1979b; Oliver & Porter, 1978; Vaudry, Tonon, Delarue, Vaillant, & Kraicer, 1978; van Dijk, 1979).

IV. IMMUNOCYTOCHEMICAL LOCALIZATION OF α -MSH AND ACTH IN THE BRAIN

Immunocytochemical techniques, in principle, enable the precise localization of the opiomelanocortin peptides in the brain. However, due to the current limitations of these techniques, a number of questions still remain unanswered. In agreement with the assay data (Table 2), cell bodies immunoreactive with antisera raised against ACTH₁₋₂₄, 17-39 and ACTH₁₋₃₉ and α -MSH have all been found in the arcuate nucleus, the periarculate area, and the ventromedial nuclei of the *rat* hypothalamus. The same cells can also be stained with anti- β -MSH, β -LPH, and α - and β -endorphin (Bloch, Bugnon, Fellmann, Lenys, & Gouget, 1979; Sofroniew, 1979; Watson & Akil, 1980; Watson & Barchas, 1979). These neurons are distinct from the monoamine-containing cells in the same region (Bugnon, Bloch, Lenys, Gouget, & Fellmann, 1979a). Peptide-containing hypothalamic cell bodies send projections positive for α -MSH, ACTH, and β -LPH (Fig. 2) to the medial and lateral hypothalamus, zona incerta, lateral septum, nucleus accumbens, periventricular thalamus, periaqueductal grey, locus coeruleus, nucleus tractus solitarius, reticular formation, stria terminalis, and medial amygdala (Bloch et al., 1979; Dubé et al., 1978; Jacobowitz & O'Donohue, 1978; O'Donohue et al., 1979a; Sofroniew, 1979; Watson & Barchas, 1979). In addition, ACTH immunoreactive fibers were observed in the spinal cord (Pelletier & Leclerc, 1979) and occasionally in the hippocampus (Larsson, 1978).

Jacobowitz and O'Donohue (1978) have shown that α -MSH and β -LPH do not always occur together. Positive β -LPH fibers were found in the locus coeruleus and substantia nigra zona compacta, but essentially no α -MSH-containing fibers were seen. However, it is not known whether the obvious overlap in the localization of the ACTH-MSH-LPH immunoreactivity is due to the presence of all of these compounds and their family members in the same cells and fibers, or to cross-reaction of the different antibodies with one or more related or unrelated compounds, because it is impossible at present

Fig. 2. Parasagittal schematic illustration of the arcuate cells and fibers that contain peptides of the opiomelanocortin family in the rat brain (after Watson & Barchas, 1979, with permission). (a) Cells are found in and near the arcuate nucleus with fibers running via the n. accumbens and periventricular thalamus to the periaqueductal central grey. (b) A caudo-dorsally directed pathway; and (c) a centro-caudo-laterally running pathway, arising in the arcuate region and running dorsally via the nucleus hypothalamicus posterior to the nucleus cuneiformis where the fibers join the dorsal ones. (Data from O'Donohue et al., 1979a.)

to sufficiently elaborate specificity in immunocytochemical studies (see the following).

A striking coincidence exists between the distribution of opiomelanocortin-containing fibers and noradrenergic projections (Jacobowitz & O'Donohue, 1978), on the one hand, and the localization of exohypothalamic vasopressin and oxytocin pathways, on the other (Buijs, 1978; Buijs, Swaab, Dogterom, & van Leeuwen, 1978). There is indeed pharmacological evidence for a functional relationship between the brain levels of these peptides and catecholamine metabolism (Kovács, Bohus, & Versteeg, 1979; Versteeg, de Kloet, & de Wied, 1979). Exactly how these systems are related in the various brain areas is not clear.

Apart from the opiomelanocortin system arising from the arcuate nucleus, α -MSH and ACTH cell bodies and fibers have been demonstrated in a number of other brain sites. Anti- α -MSH-positive cell bodies that did not react with antibodies to ACTH₁₋₃₉, 1-24 and ACTH₄₋₁₀ were observed by Swaab and Fisser (1978) in the spinal cord motoneurons, the pineal gland, dorsal root ganglion cells, and neurons of the nucleus reticularis gigantocellularis (Fig. 3). Cerebellar basket cell fibers also stained positively. It is of interest that the cells in the

Fig. 3. Staining of cells in (a) the nucleus reticularis gigantocellularis; and (b) the pineal gland, using antibodies raised against α -MSH; (a) 4394 9/4 and (b) 4372 26/3. (For details see Swaab & Fisser, 1978, and Pévet et al., 1980.)

nucleus reticularis have also been reported to be immunoreactive to enkephalins (Watson & Barchas, 1979). Enkephalins have, on the basis of their immunocytochemical distribution, been considered to be distinct from the ACTH- α -MSH- β -LPH-stained cells and fibers (Watson & Barchas, 1979), which is puzzling, taking the common precursor model into consideration. Immunocytochemical observations point either to an additional, separate pathway for enkephalin synthesis (cf. Rossier, 1981) or to a rapid degradation, release, or masking of the remaining fragments of the opiomelanocortin family. Larsson (1978) found ACTH-positive cells in the supraoptic nucleus, an observation that was extended to the paraventricular and suprachiasmatic nucleus by Sternberger (1979).^{*} Watson and Akil (1979, 1980) described a group of α -MSH-positive cells arching over the top of the third ventricle, continuing laterally into the zona incerta, down toward the fornix, the lateral hypothalamic sulcus, and the insertion of the optic tract, whereas α -MSH-positive fibers, which did not stain for β -endorphin or β -LPH, could be demonstrated in the hippocampus.

Although radioimmunoassayable α -MSH was found in the pineal gland (Table 2), no positive immunocytochemical reaction with anti- α -MSH was reported until recently (Swaab & Fisser, 1978). Pévet et al. (1980) extended this observation and found a positively-staining population of cells in the rat pineal using one antibody against α -MSH, whereas a second antibody stained less well, and a third antibody did not stain these cells at all. By means of pineal cultures, it was demonstrated that this staining remained present during a culture period of 6 days, suggesting that the immunoreactive substance is synthesized in the pineal gland. Because the three antibodies against α -MSH stained α -MSH-containing agarose beads and sections of the intermediate lobe of the rat pituitary equally well, and showed different staining potencies for the pineal and the rest of the brain (see Fig. 3), Pévet et al. (1980) suggested that the stained compound is different from α -MSH. The finding of Dubé et al. (1978) that the pineal did not stain with an antibody against α -MSH, whereas other brain sites were found to be positive, is consistent with this idea. Cross-reaction of antibodies raised against α -MSH with as yet unidentified compounds in the brain that resemble α -MSH immunologically, was also inferred on the same grounds by Swaab and Fisser (1978) and by Dubé, Côte, and Pelletier (1979), and is fully consistent with the presence of α -MSH-like peptides as demonstrated by means of the separation and assay techniques of Loh and Gainer (1977), Loh et al. (1979), O'Donohue et al. (1979a), and Rudman et al. (1979; see Section III-B).

In the *human* brain, neurons in the infundibular nucleus stain with antisera raised against α - and β -endorphin, ACTH₁₇₋₃₉ and ACTH₁₋₂₄, α - and β -MSH,

^{*}Recently, this staining appeared to be due to neurophysin II reactivity in the antiserum. However, the localization of β -LPH-like reactivity in this area was in all probability specific (Joseph & Sternberger, 1979).

and β -LPH. Their axonal fibers either appear to terminate around the blood vessels in the neurovascular zone and in the pituitary stalk, or to establish contacts with nonimmunoreactive perikarya of the infundibular nucleus (Bugnon, Bloch, Lenys, & Fellmann, 1979b). Pelletier and Désy (1979) localized ACTH-positive cell bodies in the arcuate nucleus of the human hypothalamus. These authors found ACTH-containing fibers throughout the hypothalamus, with the greatest density in the paraventricular nucleus. They could not, however, confirm Bugnon et al.'s (1979b) observation of fibers positive for ACTH in the neurovascular zone of the pituitary stalk. Although less numerous and less extensively distributed throughout the hypothalamus, a similar distribution of cells and fibers was found in the human hypothalamus using antibodies against α -MSH (Désy & Pelletier, 1978) and β -LPH (Pelletier, Désy, Lissitzky, Labrie, & Li, 1978).

The crucial question of immunocytochemistry, namely, exactly which compound in the tissue is stained, can only be solved by combining immunocytochemical procedures with separation techniques. Although most authors claim to use "specific antibodies" for their immunocytochemical work, this claim is generally based on false assumptions (cf. Swaab, Pool, & van Leeuwen, 1977; van Leeuwen, 1980). Until now, no immunocytochemical study on the localization of α -MSH-ACTH in the brain has fulfilled the criteria for a specificity test as formulated by C. W. Pool (see Swaab & Boer, 1980; van Leeuwen, 1981). Procedures that will fulfill these criteria are currently under development in our institute (Boer, 1979). Thus, at present, extreme care is necessary in interpreting data on α -MSH localization in the brain, including such unexpected findings as the presence of Met-enkephalin-like material in pituitary somatotrophs (Weber, Voigt, & Martin, 1978), of an ACTH-like substance in hypothalamic LHRH neurons (Tramu, Leonardelli, & Dubois, 1977), and of the simultaneous presence of ACTH-like and prolactin-like material in arcuate and ventromedial neurons (Toubeau, Desclin, Parmentier, & Pasteels, 1979).

V. THE OPIOMELANOCORTIN FAMILY IN DEVELOPMENT

In various species, peptides of the opiomelanocortin family in fetal brain seem to determine, to an important degree, normal intrauterine growth and the initiation of labor. Because the various peptides of this family are thought to influence these processes in different ways, increasing attention is now being paid to developmental aspects of this group (for recent reviews, see Boer, Swaab, Uylings, Boer, Buijs, & Velis, 1980; Swaab, 1980; Swaab, Visser, & Dogterom, 1979).

A. The Rat Pituitary and Brain

Endogenous fetal α -MSH was found to stimulate intrauterine body and brain growth at the time of the intrauterine growth spurt that takes place in the Day 19 fetal rat (Honnebier & Swaab, 1974; Swaab, Boer, & Visser, 1978; Swaab, et al., 1976). By means of immunofluorescence microscopy, α -MSH-containing cells were observed from Day 18 onward in the intermediate lobe of the fetal pituitary and in a few scattered cells in the pars distalis (Chatelain, Dubois, & Dupouy, 1976; Swaab et al., 1976). Radioimmunoassayable α -MSH was even present 2 days earlier (Fig. 4). A remarkably rapid rise in fetal pituitary α -MSH was found around Day 19 of pregnancy—the day of the intrauterine growth spurt—both by bioassay (Swaab et al., 1976) and by radioimmunoassay (Fig. 4). The fact that the absolute values of the radioimmunoassay were some fivefold lower than those obtained by means of the bioassay suggests that melanocyte-stimulating substances, other than α -MSH are also increasing rapidly at this time.

Radioimmunoassayable α -MSH is also present early in the fetal rat brain. The content and concentration of α -MSH in this organ is described by a U-shaped curve, between Day 16 of pregnancy and birth. The high levels at Day 16 of pregnancy are within the same range as the adult brain concentrations, whereas the absolute amount in the fetal brain on Day 16 is of the same magnitude as that of the fetal pituitary (Fig. 4). In view of the possible function of peptides of the opiomelanocortin family in brain development (for review, see Swaab, 1980), knowledge of the developmental course of the other members of this family would be of great interest. This is particularly true because opiate receptor binding has been found to be present in the fetal rat brain as early as Day 14 (Clendeninn, Petraitis, & Simon, 1976). The majority of the developmental observations are, however, based only on qualitative, immunocytochemical studies. Immunofluorescence microscopy has revealed the presence of ACTH and β -MSH in the anterior lobe of the fetal pituitary at Days 16–17 and in the pars intermedia at Days 17–18 (Chatelain et al., 1976). The α -MSH-like material stained in the rat pineal gland by immunocytochemistry is present in higher amounts in young (21 days postnatally) than in adult animals (Pévet et al., 1980). The only other data on the developmental pattern of the opiomelanocortin family comes from Bayon, Shoemaker, Bloom, Mauss, and Guillemin's (1979) study on the developmental pattern of radioimmunoassayable endorphin and enkephalins in the fetal brain. Endorphin levels revealed that the adult regional distribution is present at fetal Day 16. In contrast, enkephalin systems were not as developed, supporting the hypothesis that enkephalin occurs in interneurons that usually differentiate later than the endorphin-containing neurons that form long pathways. A decline was found in endorphin levels in the telencephalon, hippocampus, and striatum after Day

Fig. 4. α -MSH content and concentration in (a) rat pituitary and (b) brain during the course of intrauterine development (Days 16–21) and on the first postnatal day (0) as measured by radioimmunoassay. (P means parturition.) The day on which spermatozoa were found in the vaginal smear was taken as Day 0 of pregnancy. Note the rapid rise in pituitary α -MSH content on Day 19 of pregnancy and the relatively high levels and concentrations of α -MSH in the fetal brain early in development. Male adult pituitary levels in (a) are $2.6 (\pm 0.08) \mu\text{g/pituitary}$ and $13.0 (\pm 4.1) \text{ ng/g body}$, and male adult brain levels in (b) are $12.3 (\pm 2.1) \text{ ng/brain}$ and $5.7 (\pm 0.9) \text{ ng/g brain}$. (From Visser & Swaab, unpublished observations.)

16 of pregnancy. A relationship to α -MSH, which also decreases in the brain after Day 16 of pregnancy, is plausible, and underscores the desirability of obtaining a more complete picture of the different components of the opiomelanocortin family during development.

B. The Human Pituitary and Brain

Because the human fetus has a distinct pars intermedia in the pituitary (Romeis, 1940), it might be expected that α -MSH is present during human development. However, the evidence is conflicting. It has been stated that α -MSH

1. is not present in the fetal pituitary (Dubois, Vargues-Regairaz, & Dubois, 1973);
2. is present only in the fetal pituitary (Silman, Chard, Lowry, Smith, & Young, 1976);
3. is not present in the adult pituitary (Scott et al., 1973);
4. is present in 30% of the adult pituitaries (Nieuwenhuyzen Kruseman & Schröder-van der Elst, 1976);
5. is present in only a very few cells in adult pituitaries (Dubois, 1973); and
6. is present in all adult pituitary cells that contain ACTH (Phifer, Orth, & Spicer, 1974).

Because these discrepancies seemed at least partially explainable by the technical pitfalls of immunocytochemistry, we have studied the pituitary distribution of α -MSH and ACTH using antisera that were purified and characterized for their staining capabilities on a number of peptides of the opiomelanocortin family (Visser & Swaab, 1979).

In all fetal pituitaries, the pars intermedia was found to consist mainly of α -MSH-containing cells and only a few ACTH-containing cells. In the pars distalis, the opposite was observed. In the youngest fetus (15 weeks) the pars intermedia contained many α -MSH cells, but no ACTH cells, whereas ACTH cells were present in the pars distalis (Visser & Swaab, 1979). These observations agree with the fact that melanocyte-stimulating activity has been detected by bioassay in the human fetal pituitary from 11 weeks of pregnancy onward (Kastin, Gennser, Arimura, Miller, & Schally, 1968; Levina, 1968). During development the pars intermedia was found to develop gradually into a "zona intermedia," in which there was a large amount of cyst formation. This remnant of the pars intermedia contains α -MSH and ACTH immunoreactive cells, which also invade the posterior lobe. In our study, α -MSH immunoreactive cells were observed in the zona intermedia and pars distalis of all pituitaries throughout life, irrespective of age, sex, pregnancy, therapy, or cause of death.

However, from birth to 19 years of age, progressively fewer α -MSH cells and more ACTH cells were detected in the pars distalis and zona intermedia (Visser & Swaab, 1979). The gradually decreasing ratio of α -MSH–ACTH-containing cells in the human pituitary during development agrees fully with the biochemical work of Silman et al. (1976) and is considered to be of importance, both for intrauterine development and for timing the moment of labor onset (Swaab et al., 1979). The few α -MSH-positive cells found in adulthood is consistent with the low levels of α -MSH in human pituitaries found by radioimmunoassay (Abe et al., 1967). Earlier data (Phifer et al., 1974), indicating that each ACTH cell in the human adult also contained α -MSH can be explained by cross-reactivity, as the antibodies used in these studies were not purified prior to use in immunocytochemistry (Swaab et al., 1976).

The total absence of the pars intermedia and of any α -MSH-containing cells in the pars distalis of the seven anencephalics in our study supports the hypothesis of a stimulatory effect of the hypothalamus on the development of the pars intermedia (Gaillard, 1937). Our study is consistent with the suggestion of Celis (1977) that oxytocin, which we did not find in the anencephalic pituitaries (Swaab & Visser, 1977), might be a prohormone for an α -MSH-releasing factor. This is of particular interest because the influence of the adult mammalian brain on MSH production is generally thought to be inhibitory (Tilders & Smelik, 1978).

Previously, hypothalamic stimulation was not considered to be essential for the development of fetal ACTH cells in the pars distalis (Bégeot, Dubois, & Dubois, 1977). However, we found very few fluorescent cells in the pars distalis of one anencephalic and no ACTH cells in the other six pituitaries when using purified anti-ACTH₁₋₂₄ plasma (Visser & Swaab, 1979). Thus, the human fetal pituitary seems to be dependent on the hypothalamus for the development of ACTH. The staining of "ACTH cells" as reported by Bégeot et al. (1977), might be explained by the staining of a precursor molecule, because when we used anti-ACTH₁₋₃₉ plasma, which cross-reacted with β - and γ -lipotropic hormone, fluorescent cells were found in anterior lobes of *all* the anencephalics (Visser & Swaab, 1979).

Fluorescent cells have been found in the anterior and intermediate lobes of the pituitary of the human fetus as early as the eighth week of fetal life by the use of antibodies to ACTH₁₇₋₁₉, β -MSH, β -LPH, and α - and β -endorphins (for review see Bégeot, Dubois, & Dubois, 1978). The specificity of these immunocytochemical observations is not sufficiently elaborated (see preceding), but the various studies show that fragments of the opiomelanocortin family are present very early in human fetal development. The same is true for fetal brain; in the infundibular nucleus of the hypothalamus, neurons stained with antibodies against β -endorphin or ACTH₁₇₋₃₉ from the eleventh week of fetal life onward (Bugnon et al., 1979b).

Various compounds of the opiomelanocortin family have been demonstrated during pregnancy in the human amniotic fluid and maternal blood. Generally, it is unknown, however, what the relative contributions of the fetus, placenta, and mother are to the levels of these peptides in these fluids. In the amniotic fluid, ACTH levels reach a peak between the twenty-sixth and thirty-fourth week of pregnancy and then level off (Schollberg, Seiler, & Schmidt, 1978; Tuimala, Kauppila, & Haapalahti, 1976). However, β -MSH levels in maternal serum rise progressively throughout pregnancy. Because fetal umbilical cord serum and amniotic levels were found to be higher than maternal levels, synthesis by the fetus was suggested (Ances & Pomerantz, 1974). However, data indicate that postpartum maternal serum β -MSH levels are not different from the levels in midpregnancy (Thody, Plummer, Burton, & Hytten, 1974), and, thus, the fetal contribution to the β -MSH levels seems unimportant. β -endorphin in the amniotic fluid probably does not originate in the fetal brain or pituitary either, because anencephalic amniotic fluid contains a high level of this material (Gautray, Vielh, Levailant, & Rotten, 1979). Corresponding data on the measurement of circulating fetal levels of α -MSH are lacking. Reported levels of more than 0.1 ng of α -MSH/ml in maternal and cord plasma (Clark, Thody, Shuster, & Bowers, 1978) are, in our opinion, at least tenfold too high. This might be explained by the fact that these authors used unextracted plasma in their radioimmunoassay which, with our antisera, yields high nonspecific values.

VI. THE SYNTHESIS OF MSH AND ACTH BY THE BRAIN

Although it is now half a century since Zondek and Krohn (1932) proposed that brain "intermedin" is synthesized in the pituitary, and 20 years since Guillemin et al. (1962) proposed that MSH- and ACTH-like peptides are also synthesized in the brain, the relative contributions of both potential peptide sources is still a matter of intense controversy.

The fact that concentrations of brain peptides, which are identical to pituitary hormones, are unchanged after hypophysectomy (Krieger et al., 1977a,b; Liotta et al., 1978; O'Donohue et al., 1979a,b; Oliver & Porter, 1978; Swaab, 1980; Vaudry et al., 1978; van Dijk, 1979) has not been accepted as proof of their central origin by Moldow and Yalow (1978a,b). These authors proposed that pituitary remnants found in the sella after commercial hypophysectomy could still be the origin of brain peptides. In addition, as concentrations of ACTH and MSH in various brain sites have been found to be inversely related to the distance of these areas from the hypophysis, they suggested that the pituitary may be the sole site of synthesis of these hormones. This idea is, of course,

consistent with observations that behavioral deficits after hypophysectomy can be restored by peripheral injections of ACTH or MSH (de Wied, 1964, 1965). Although others have found much less ACTH in the sella after hypophysectomy, and blood levels are apparently affected by "pituitary remnants" (van Dijk, 1979), it is still possible that the pars tuberalis remains functional after hypophysectomy (Ordronneau, 1979). Also, pituitary tissue, which is present in the pharyngeal canal and even the pharynx itself (McGrath, 1976, 1978) might be an additional source of hormones after hypophysectomy. Retrograde transport of peptides from the pituitary to the brain or CSF, as has been proposed by Popa (1938), is also a realistic possibility (Mezey, Kivovics, & Palkovits, 1979).

Nevertheless, evidence for production of "pituitary" hormones by the nervous system is accumulating:

1. Pituitary hormones remain present in the brain after hypophysectomy as shown by means of bioassay, radioimmunoassay, and immunocytochemistry (for references see Section III-B and preceding).

2. α -MSH disappears from the brain sites that are innervated (cf. Section IV) by arcuate nucleus neurons after electrolytic, knife, or sodium glutamate lesions of this area, whereas the pituitary store of this hormone does not diminish (Eskay, Brownstein, & Long, 1979a; Eskay, Giraud, Oliver, & Brownstein, 1979b; O'Donohue et al., 1979a). Sodium glutamate lesions of the arcuate nucleus cause a four- or fivefold decrease of ACTH and β -endorphin in the hypothalamus and a twofold decrease of these peptides in the amygdala, whereas pituitary ACTH content remains the same (Krieger, Liotta, Nicholson, & Kizer, 1979). These observations argue strongly for the arcuate nucleus as a site of peptide synthesis.

3. Enkephalins have been found in cultured spinal cord neurons (Neale, Barker, Uhl, & Snyder, 1978) by immunofluorescence microscopy, whereas in explants of dorsal root ganglia (van Leeuwen, unpublished observations) and organ cultures of the pineal gland (Pévet et al., 1980) α -MSH-like material can be stained immunocytochemically. For the latter experiment, a synthetic culture medium was used, which excludes uptake of exogenous peptides.

4. The localization of "pituitary" peptides in the nervous system is not restricted to site that are immediately connected to the hypophyseal portal vessels as has been claimed by Moldow and Yalow (1978a,b). For example, α -MSH immunoreactivity has been found in neurons of the spinal cord, dorsal root ganglia (Swaab & Fisser, 1978), and the pineal gland (Table 2).

5. Animals that do not have a pituitary have ACTH- and α -MSH immunoreactivity in their nervous systems (Boer, Schot, Roubos, ter Maat, Lodder, Reichelt, & Swaab, 1979).

6. The intracellular localization of "pituitary" hormones observed by light microscopy also supports a central site for their origin (see preceding). Immunoelectron microscopy has shown that immunoreactivity for α -MSH and

ACTH is present in vesicles of arcuate nucleus neurons (Pelletier & Dubé, 1977; Pelletier & Leclerc, 1979).

7. α -MSH is releasable by potassium from hypothalamic synaptosomes (Oliver, Barnea, Warberg, Eskay, & Porter, 1977; Warberg, Oliver, Barnea, Parker, & Porter, 1979).

8. Hippocampal ACTH levels change independently of hypophyseal release (van Dijk, 1979).

From these observations and from the fact that a significant transfer of ACTH from the blood to the brain is very unlikely in view of the existence of an effective blood-brain barrier for such peptides (for references see van Dijk, 1979), it is most probable that opiomelanocortic peptides are produced by the nervous system. Any additional contribution of peptides of pituitary origin to the physiology of the various brain sites remains to be determined.

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