Regional Processing of the N- and C-Terminal Domains of Proopiomelanocortin in Monkey Pituitary and Brain

JAMES H. MEADOR-WOODRUFF, BENEDETTO PELLERITO, HUBERT VAUDRY*, SYLVIE JÉGOU*, NABIL G. SEIDAH†, STANLEY J. WATSON and HUDA AKIL

*Mental Health Research Institute and Department of Psychiatry, University of Michigan, Ann Arbor, Michigan, 48109-0720; *Laboratoire d'Endocrinologie, Faculte des Sciences et Techniques, France; †Institut De Recherches Cliniques De Montreal, Montreal, Canada. (Reprint Requests to J.H.M.-W.)*

Abstract—The total content and extent of processing of the $_{\gamma 3}$MSH and β-endorphin-containing N- and C-terminal domains of proopiomelanocortin were determined in the anterior and intermediate lobes of the pituitaries and in 11 regions of the brains of three Rhesus monkeys. Most immunoreactive $_{\gamma 3}$MSH and β-endorphin was located in the pituitary lobes, although significant amounts were also found in several brain regions. Sephadex column chromatography revealed that $_{\gamma 3}$MSH immunoreactivity was found primarily as 4K and 9K forms; no $_{\gamma 1}$MSH was detected. β-Endorphin immunoreactivity was found as β-endorphin, β-lipotropin, and as a 5K form which may represent β-endorphin extended N-terminally by part or all of β-MSH. In the anterior lobe of the pituitary, the predominant products were 9K $_{\gamma 3}$MSH and β-lipotropin; in the intermediate lobe, more processed forms (4K $_{\gamma 3}$MSH, β-endorphin and 5K β-endorphin) appeared to be preferentially stored. The pattern of processing in various brain regions was similar to that of the intermediate lobe of the pituitary.

Introduction

Proopiomelanocortin (POMC) is a 31000 dalton propeptide containing multiple functional domains, including β-endorphin (βE), adreno-corticotropin (ACTH), and α-, β-, and γ-melano-tropins (MSHs). Although the majority of POMC is found in the pituitary, it has also been found in other sites in lower concentrations, including the

brain. The processing of POMC in various tissues has been partially elucidated in multiple species, with emphasis on the βE and ACTH-containing C-terminal domain; considerably less is known, however, about the processing of the γ-MSH-containing N-terminal region. Tissue-specific process-ing of POMC in the monkey has received little attention to date; as in other species, the process-ing of the N-terminal domain of POMC in primate neural tissue is not well understood. Patel *et al.* in our laboratories have recently cloned monkey POMC, however, which should facilitate the

investigation of the processing of POMC in this species based on the actual primary structure.

γ-Melanotropin ($_{\gamma 3}$MSH) is found within the N-terminal domain of POMC, located between the two major dibasic cleavage sites in the 16K N-terminal region (18). Within this 16K region, $_{\gamma 3}$MSH is flanked by the extreme N-terminal fragment of POMC and by joining peptide. $_{\gamma 3}$MSH can thus be theoretically found alone (molecular weight 3-4 K), in association with joining peptide or with the extreme N-terminal fragment of POMC (molecular weight 9-11 K) (22, 23), or as part of the intact 16K N-terminal domain. $_{\gamma 3}$MSH shares considerable homology between the human and the monkey. The N-terminal region of the peptide (residues 1-18) are completely conserved between the two species, an area that contains a potential dibasic cleavage site as well as a glycosylation signal. The C-terminal region of the peptide, however, shows considerable variability between the two species, and is only five residues in length in the monkey, compared with eight in the human (Patel et al., in preparation). The additional theoretical cleavage site (Arg-Arg, positions 13-14) located within $_{\gamma 3}$MSH in both the human and the monkey potentially yields a 12-residue peptide ($_{\gamma 2}$MSH), or an 11-residue, C-amidated peptide, $_{\gamma 1}$MSH (15). Neither of these products, however, have been demonstrated in the human pituitary (11, 22, 23, 25, 26).

Past efforts to determine the distribution and extent of processing of immunoreactive-γ-melanotropin in primate neural tissue have focused on the pituitary. γ-MSH has been found primarily as larger molecular forms (i.e., 9K or larger) in the human pituitary (4, 22, 23, 25). The distribution and extent of processing γ-melanotropin and βE in the primate brain have been rarely reported. Osamura and colleagues (19) studied γ-MSH in the human hypothalamus by immunocytochemistry and found that it tended to be colocalized with βE, and located primarily in the arcuate nucleus; these investigators also performed electron microscopic studies in this tissue, finding γ-MSH localized in nerve terminals and in synapse-like structures, suggesting a possible neuromodulatory role for this peptide. Emson et al. (10) quantitated some POMC products, including βE but not γ-MSH, in human brain, finding relatively increased amounts in the hypothalamus, amygdala, periventricular grey, substantia nigra, and the superior colliculus. Column chromatography revealed that the primary peptide forms in the hypothalamus and

periventricular grey are fully-processed (i.e., β-endorphin).

Because of the similarities between human and monkey POMC, the processing of monkey POMC is potentially relevant to human physiology. We previously reported on the content and partial processing of $_{\gamma 3}$MSH and βE in the monkey pituitary (16). In this paper, we extend these earlier observations, reporting content and complete processing of both γ-MSH and βE in monkey pituitary and in selected brain regions.

Methods

Tissue preparation. Three post-menopausal Rhesus monkeys (*Macaca mulatta*) were anesthetized with pentobarbital and sacrificed by exsanguination. The brain and pituitary were rapidly removed and kept on wet ice for dissection. The pituitary was dissected into anterior and intermediate lobes. Due to the adhesion of the intermediate lobe to the other lobes, this tissue was somewhat contaminated by both anterior and posterior lobe tissue, hence, in this study, "intermediate" lobe actually refers to a mixture of all three pituitary lobes, although predominately intermediate lobe. The brain was dissected on wet ice into the following regions: hypothalamus, hippocampus, septum, colliculi, amygdala, striatum, thalamus, midbrain, pons, medulla, and portions of frontal and occipital cortex. Immediately following dissection, individual tissue sections were frozen on dry ice and stored at −80°C.

Prior to extraction, individual tissue sections were weighed. Extraction was accomplished by subjecting tissue in 2 ml acetone/0.1 N HCl (3:1, v/v) to a Polytron for 60 s, followed by a 1 ml rinse in acetone/HCl. The homogenate was centrifuged at 12000 Xg for 10 min and the supernatant collected and lyophilized. The dried extract was resuspended in 1 ml 1.0% formic acid.

Radioimmunoassays. βE was assayed by a previously validated radioimmunoassay (1, 6). The antiserum is directed against a midportion of the βE molecule (residues 17-27), and is 100% cross-reactive with all forms of βE, β-lipotropin, and POMC. $_{\gamma 3}$MSH was assayed using a radioimmunoassay that we have previously standardized (16, 17, 27). This antiserum is directed against a mid-portion region of bovine $_{\gamma 3}$MSH (residues 5-14),

which shares complete homology with both monkey and human $_{\gamma 3}$MSH. This antisera does not cross-react with $_{\gamma 1}$MSH or $_{\gamma 2}$MSH.

$_{\gamma 1}$MSH was quantitated by a recently developed radioimmunoassay. The antiserum was raised in rabbits against synthetic $_{\gamma 1}$MSH (monkey, human, and bovine sequences are identical) conjugated to thyroglobulin. The assay was performed in 150 mM phosphate buffer, pH 8.2, containing 0.1% bovine serum albumin and 1.0% NaCl. Standards (synthetic Lys-$_{\gamma 1}$MSH) and samples were added in 50 uL of 0.1% bovine serum albumin, pH 3.0. The antiserum was added in 100 uL of buffer at a final titer of 1:3000. Due to difficulties of [^{125}I] $_{\gamma 1}$MSH instability in this assay, the radioligand was rat [^{125}I] Lys-$_{\gamma 3}$MSH which was iodinated by the chloramine-T method (13); 20 000 counts in 100 uL of buffer containing 5% rabbit serum were added per tube. The assay was run in triplicate in a final volume of 250 uL under disequilibrium conditions for 48-72 h at 4°C. Separation was by immunoprecipation following the addition of 15 uL of sheep-anti-rabbit IgG. This assay typically has an IC_{50} of 60 fmoles $_{\gamma 1}$MSH/tube, and a sensitivity of 5 fmoles/tube. The antiserum has 57% cross-reactivity with synthetic human $_{\gamma 3}$MSH, 2% with ACTH$_{1-24}$, and <0.1% with α-MSH, β-MSH and βE. The intra- and inter-assay coefficients of variation are 5.3% and 8.4%, respectively.

Peptide measurement paradigm. Aliquots of extracted tissue were lyophilized, resuspended in the appropriate solvent, and assayed for $_{\gamma 3}$MSH and βE immunoreactivity. From those regions with sufficiently high concentrations of peptides (both pituitary lobes, hypothalamus, pons, septum, and colliculi), aliquots (300-400 uL) were chromatographed on a Sephadex G-50 column (0.9 × 90 cm). The column was developed in 1.0% formic acid, and 2.2 ml fractions collected. Individual fractions were lyophilized, and resuspended in the appropriate solvent for βE, $_{\gamma 3}$MSH and $_{\gamma 1}$MSH quantitation by radioimmunoassay. Aliquots of fractions contituting the void volumn from this column were pooled, lyophilized, resuspended in 300 uL of 1.0% formic acid, and rechromatographed on a Sephadex G-100 column (0.9 × 90 cm) in 1.0% formic acid to better resolve larger molecular weight forms. The total amount of βE, $_{\gamma 3}$MSH and $_{\gamma 1}$MSH found in each observed peak from these two columns was calculated, and expressed as a percentage of the total immuno-

reactivity for that peptide for each region examined. Recovery of all peptides from the Sephadex columns exceeded 90%.

Concavalin A affinity chromatography. Fractions from Sephadex G-50 columns corresponding to a 4000 dalton form of $_{\gamma 3}$MSH from the pituitary were pooled and applied to a Concanavalin A-Sepharose 4B affinity column. This method has been used previously to demonstrate that bovine (24) and human (17) $_{\gamma 3}$MSH is glycosylated. Briefly, samples were resuspended in 500 uL of buffer and applied to a 500 uL affinity column. Following extensive washing, glycosylated material was eluted by increasing concentrations of α-methyl-D-mannopyranoside and by acetic acid. 1 ml fractions were collected from this column and lyophilized. $_{\gamma 3}$MSH content was determined in each fraction by radioimmunoassay.

Results

Peptide content. The total amount of $_{\gamma 3}$MSH and βE immunoreactivity found in the individual brain and pituitary regions is summarized in Table 1. Both $_{\gamma 3}$MSH and βE were found concentrated in the two pituitary lobes, although significant levels were also detected in the hypothalamus, colliculi, septum and pons. Lower levels were detected in the remaining brain regions. There was no significant difference between total immunoreactive (IR)-βE and IR-$_{\gamma 3}$MSH in any given tissue. Inspection of Table 1 reveals fairly large standard errors, partially due to the small number of animals studied.

Processing of γ-MSH in brain and pituitary regions. The percentage of $_{\gamma 3}$MSH immunoreactivity found as fragments of various molecular weights was calculated from Sephadex column chromatography, and is summarized in Table 2. As shown in the representative elution profiles of γ-MSH in the "intermediate" and anterior lobes of the pituitary in the top panels of Figures 1 and 2, IR-$_{\gamma 3}$MSH was found in peaks of 4 K, 9 K, 16 K (POMC N-terminal to ACTH), and in the void volume (presumably intact POMC). In all studied tissues, most IR-$_{\gamma 3}$MSH was found as 4 K and 9 K forms. In the anterior lobe of the pituitary (Fig 2), the predominant form was 9 K. In "intermediate" lobe (Fig 1) and the various brain regions, however, the 4 K form either predominated or was

nearly equal to the amount of 9 K $_{\gamma3}$MSH. In spite of the obvious extent of processing of $_{\gamma3}$MSH to 4 K and 9 K forms in all tissues, a significant amount (up to 25%) of $_{\gamma3}$MSH immunoreactivity was found as the 16 K form. $_{\gamma1}$MSH was not found in any of the tissues listed in Table 2.

Processing of βE. As a comparison control, the extent of processing of βE immunoreactivity was determined in parallel with $_{\gamma3}$MSH. These results are summarized in Table 3. As shown in represent-

ative elution profiles (lower panels, Figs 1 and 2), βE immunoreactivity was found as βE, β-lipo-tropin, and a larger form (presumably POMC). Additionally, some was found as a 5 K form, which is presumably βE extended to N-terminally by part or all of β-MSH. In anterior pituitary (Fig 2), 62% of IR-βE was found to exist as β-lipotropin; in contrast, in "intermediate" lobe (Fig 1) and the various brain regions, most was found as the smaller, more processed forms (βE and the 5 K form).

Table 1 β-Endorphin and $_{\gamma3}$MSH Content in Various Monkey Brain and Pituitary Regions

Region	Peptide content (fmoles/mg)	
	IR-$_{\gamma3}$MSH	IR-βE
Pituitary, anterior lobe	120 400 ± 33 000	91 200 ± 25 000
Pituitary, intermediate lobe*	68 500 ± 19 800	97 500 ± 45 600
Hypothalamus	25.2 ± 21.2	49.9 ± 44.1
Colliculi	10.6 ± 4.5	8.1 ± 4.0
Septum	7.4 ± 3.2	3.3 ± 3.1
Pons	5.2 ± 5.0	7.0 ± 4.1
Hippocampus	2.5 ± 2.2	4.7 ± 4.7
Medulla	1.9 ± 0.6	11.3 ± 8.9
Midbrain	1.1 ± 0.9	2.0 ± 0.8
Striatum	1.0 ± 0.4	2.6 ± 1.7
Amygdala	0.9 ± 0.6	3.1 ± 1.5
Thalamus	0.4 ± 0.2	1.8 ± 1.5
Cortex	0.2 ± 0.2	0.4 ± 0.4

* "intermediate" lobes contain small amounts of both anterior and posterior lobes well, as described in the text.
Results are means ± S.E.M. of values from three animals.

**Table 2** Distribution of Immunoreactive $_{\gamma3}$MSH by Molecular Weight as Determined by Sephadex Column Chromatography

Region	% IR-$_{\gamma3}$MSH			
	4 K	9 K	16 K	Void
Pituitary, anterior lobe	22%	60%	18%	1%
Pituitary, "intermediate" lobe	48	35	16	1
Hypothalamus	40	34	25	0
Pons	38	48	13	1
Colliculi	44	49	7	0
Septum	50	36	10	4

4 K presumably represents glycosylated $_{\gamma3}$MSH; 9 K, $_{\gamma3}$MSH extended with the extreme N-terminal fragment of POMC; 16 K, the N-terminal region of POMC less ACTH and β-lipotropin; void, intact POMC. "Intermediate" lobes are contaminated by small amounts of anterior and posterior lobes, as discussed in the text.

Table 3 Distribution of Immunoreactive β-Endorphin by Molecular Weight

Region	% IR-β-endorphin			
	βE	5 K	β-lipotropin	Void
Pituitary, anterior lobe	21%	16%	62%	1%
Pituitary, "intermediate" lobe	37	42	20	1
Hypothalamus	28	44	22	7
Pons	6	56	36	3
Colliculi	26	41	30	3
Septum	46	15	35	4
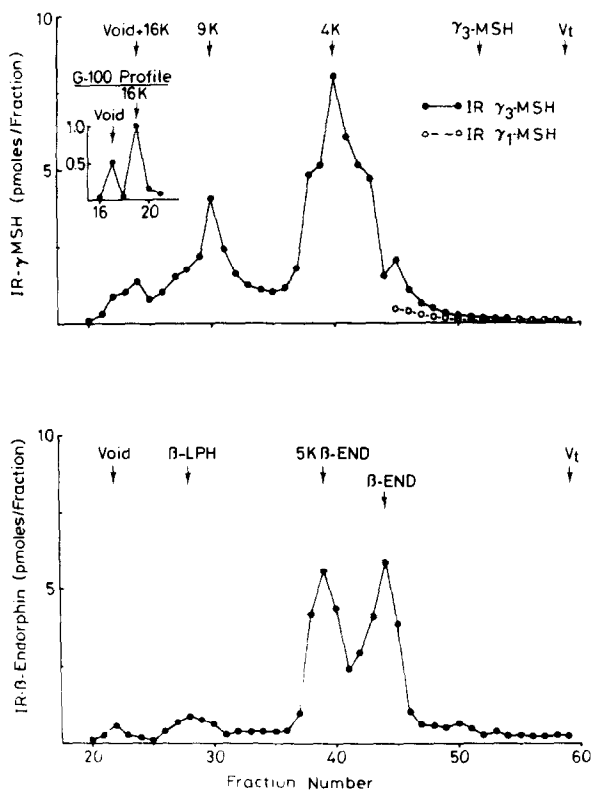
Fig 1 Representative elution profiles of γ-MSH and βE immunoreactivity from a Sephadex G-50 column. The two profiles represent parallel determinations of γ-MSH (top panel) and βE (lower panel) in the "intermediate" lobe of the pituitary. The void volume peak from the γ-MSH determination was pooled and re-chromatographed on Sephadex G-100 (insert, top panel), as described in the text.

Extent of Glycosylation of ₓMSH. A representative profile of pituitary-derived 4 K ᵧ3MSH from a Concanavalin A affinity column is shown in Figure 3. Similar to findings in human plasma (17) and bovine pituitary (24), most (76 ± 10%) of this material is glycosylated. As a control, less than 10% of synthetic, nonglycosylated, human ᵧ3MSH is retained by these affinity columns.

Discussion

The largest concentrations of ᵧ3MSH and βE were found in the anterior and "intermediate" lobes of the pituitary, although significant amounts were also found in the hypothalamus, pons, septal region, and colliculi. This is in agreement with previous reports of substantial amounts of γ-MSH in the human pituitary (4, 22, 23, 25). Although

the distribution of γ-MSH has not been determined in the primate brain, it has been studied in the rat brain immunohistochemically (5, 14). These studies have demonstrated that γ-MSH parallels the distribution of βE; γ-MSH cell bodies are localized in the intermediate lobe of the pituitary, corticotrophs in the anterior lobe, and the arcuate nucleus. Kawai et al. (14) also demonstrated a collection of γ-MSH-containing neurons in the nucleus commissuralis. γ-MSH-immunoreactive nerve fibers have been demonstrated within the diencephalon, pons, hypothalamus, amygdala, and the central grey of the midbrain.

Several other investigators have studied the distribution of other POMC products in brain, although γ-MSH itself was not explored. Dupont and co-workers (8) quantitated βE in bovine and rat brain, finding the largest concentration in the hypothalamus of both species, followed by the periaqueductal grey, thalamus and amygdala in
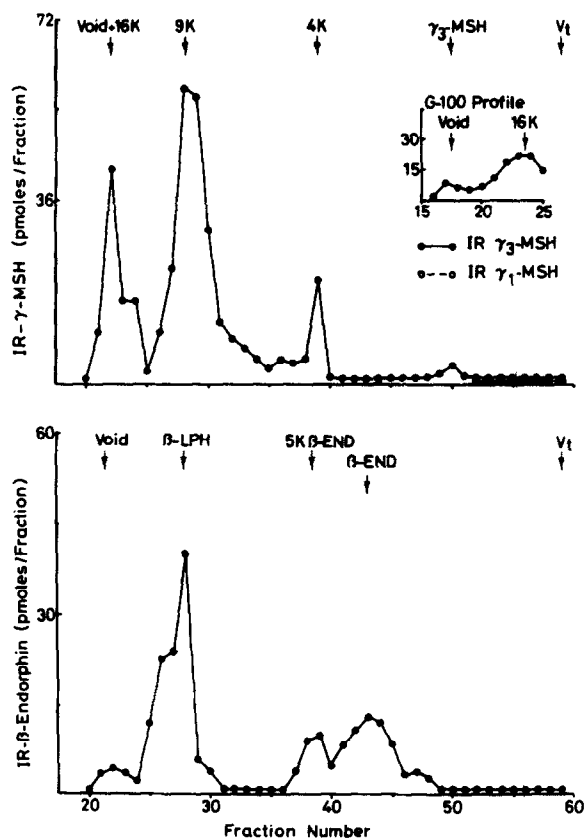
Fig 2 Parallel determinations of γ-MSH (top panel) and βE (lower panel) immunoreactivities in the anterior lobe of the pituitary following Sephadex G-50 column chromatography.
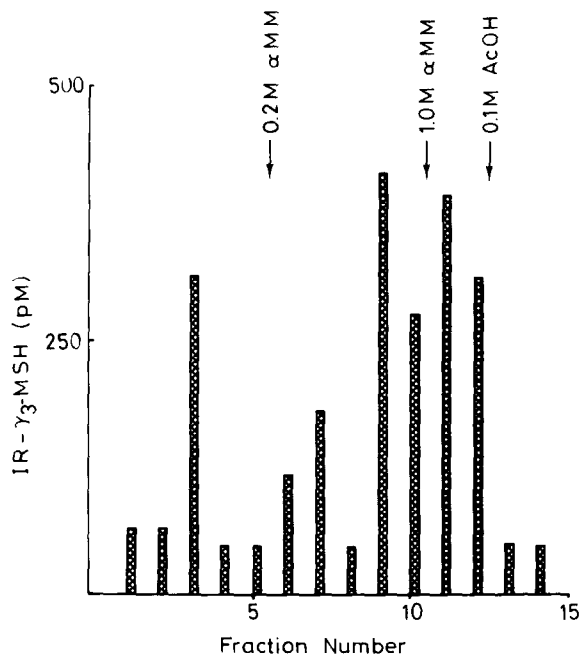
Fig 3 Representative elution profile of $_{\gamma2}$MSH immunoreactivity in pituitary from a Concanavalin A-Sepharose 4B affinity column. Aliquots of fractions comprising the 4 K $_{\gamma3}$MSH peak were pooled and chromatographed on the affinity column. Glycosylated material was eluted with increasing concentration of α-methyl-D-mannopyranoside (αMM) and 0.1 N acetic acid.

the rat, and by the pons and septum in bovine tissue. Multiple POMC products, including βE, ACTH, and α-MSH were studied in the human brain (10), with significant amounts found in the hypothalamus, amygdala, periventricular grey, substantia nigra, and superior colliculus. The distribution of $_{\gamma3}$MSH and βE in the monkey agrees well with these studies in other species; the largest amounts of immunoreactivity are found in tissues rich with POMC-containing neurons (pituitary and hypothalamus), and relatively less peptide is found in tissues associated with fiber tracts.

The processing of γ-MSH in the monkey pituitary appears to be similar to the processing in other species. For example, γ-MSH is processed to a smaller form (glycosylated $_{\gamma3}$MSH), and is also found as a larger form (10-11 K) in rat (20) and bovine pituitaries (24). In both species, the smaller form predominates in the intermediate lobe, whereas the somewhat larger form is preferentially stored in the anterior lobe, in agreement with our results in the monkey. γ-MSH has also been studied in the human pituitary. Only a larger form

of γ-MSH-immunoreactivity is found, however (22, 23, 25), which in the human corresponds to $_{\gamma3}$MSH extended with the extreme N-terminal fragment of POMC (22, 23). Based on findings in other species, 9 K $_{\gamma3}$MSH is likely $_{\gamma3}$MSH extended with the extreme N-terminal fragment of POMC, although it is possible that 9 K form in monkey is $_{\gamma3}$MSH extended by joining peptide. It is of note that in the anterior lobe of the monkey, some of this intermediate-sized product is further processed to 4 K $_{\gamma3}$MSH, a processing step that does not appear to occur in human pituitary tissue. 4 K $_{\gamma3}$MSH is likely glycosylated $_{\gamma3}$MSH, as found in other species. Because of slight differences in the primary structure of monkey POMC compared to that of rat and human, the identification of the 4 K and 9 K fragments is tentative. While there are two minor amino acid differences between the human and monkey sequences of the extreme N-terminal fragment of POMC, differences in joining peptide are more substantial. In monkey joining peptide, positions 11-12 are Arg-Gly, compared to Cys-Gly in the human (and absent in the rat), resulting in an additional possible single basic cleavage site. 4 K $_{\gamma3}$MSH in the monkey, then, could conceivably also be deglycosylated $_{\gamma3}$MSH extended C-terminally by the first eleven residues of joining peptide.

βE is processed similarly to γ-MSH in the monkey pituitary; the "intermediate" lobe tends to store more processed forms of βE (βE and a 5 K form), and the anterior lobe β-lipotropin. Of interest is that the anterior lobe is able to further process β-lipotropin to smaller forms in significant amounts, in agreement with a previous report (7). An unexpected finding is the 5 K-sized form of βE. This fragment contains βE, and is presumably extended N-terminally by some or all of β-MSH. This may represent an alternate processing pathway for βE in some tissues in the monkey.

The processing of γ-MSH and βE in various brain regions was similar to that in the intermediate lobe of the pituitary, with the primary products identified as a mixture of the most processed form and the immediate precursor to this product for both peptides ($_{\gamma3}$MSH and the 9 K form, and βE and the 5 K form). There have been few reports detailing the processing of $_{\gamma3}$MSH in brain regions. Emeson and Eipper (9) demonstrated that the majority of γ-MSH in rat hypothalamus is glycosylated $_{\gamma3}$MSH. Although γ-MSH was not studied, Emson et al (10) demonstrated that the majority of βE immunoreactivity in the human hypothalamus and periventricular grey is βE-sized. The finding in monkey brain of substantial processing of POMC

to $_\gamma3$MSH and βE agrees well with these two reports in other species.

These results in the monkey agree fairly well with the limited γ-MSH processing data published for other species. Several notable differences, however, are apparent from our data. In the human pituitary, γ-MSH processing appears to stop at the 9K form, and no smaller forms are found. In the monkey, however, significant amounts of glycosylated $_\gamma3$MSH are found, indicating an additional processing step absent in the human. A second unexpected finding was the processing of IR-βE to a 5K form; as previously mentioned, this may represent an alternate processing route for the C-terminal domain of POMC in the monkey. Although γ-MSH has a peripheral role potentiating ACTH-induced adrenocortical steroidgenesis (2, 3, 12, 21), its role in the central nervous system is unknown. It has been demonstrated by electron microscopic studies (19) to exist in nerve terminals and synapse-like structures. It exists in processed forms in various brain regions, as we and others have demonstrated. These data suggest that γ-MSH may have a neuromodulatory role in the central nervous system.

Acknowledgements

References

1. Akil, H., Ueda, Y. and Lin, H.-L. (1981). A sensitive coupled HPLC/RIA technique for separation of endorphins: Multiple forms of β-endorphin in rat pituitary vs. anterior lobe. Neuropeptides 1: 429-446.
2. Al-Duijaili, E. A. S., Hope, J., Estivariz, F. E., Lowry, P. J. and Edwards, C. R. W. (1981). Circulating human pituitary pro-γ-melanotropin enhances the adrenal response to ACTH. Nature 291: 156-158.
3. Al-Duijaili, E. A. S. Williams, B. C., Edwards, C. R. W., Salacinski, P. and Lowry, P. J. (1982). Human γ-melanotropin precursor potentiated corticotropin-induced adrenal steroidogenesis by stimulating mRNA synthesis. Biochem. J. 204: 301-305.
4. Bertagna, X., Seurin, D., Pique, L., Luton, J. P., Bricaire, H. and Girard, F. (1983). Peptides related to the NH₂-terminal end of proopiocortin in man. J. Clin. Endocrinol. Metab. 56: 489-495.
5. Bloom, F. E., Battenberg, E. L. F., Shibasaki, T., Benoit, R., Ling, N. and Guillemin, R. (1980). Localization of γ-melanocyte stimulating hormone (γ-MSH) immunoreactivity in rat brain and pituitary. Reg. Peptides 1: 205-222.

6. Cahill, C. A., Matthews, J. D. and Akil, H. (1983). Human plasma β-endorphin-like peptides: A rapid, high recovery extraction technique and validation of radioimmunoassay. J. Clin. Endocrinol. Metab. 56: 992-997.
7. Cahill, C. A., Watson, S. J., Knobloch, M. and Akil, H. (1983). POMC in rhesus anterior pituitary and plasma: Evidence of N-acetylated β-endorphin and α-MSH. Life Sci. 33: 53-55.
8. Dupont, A., Lépine, J., Langelier, P., Mérand, Y., Rouleau, D., Vaudry, H., Gris, C. and Barden, N. (1980). Differential distribution of β-endorphin and enkephalins in rat and bovine brain. Reg. Peptides 1: 43-52.
9. Emeson, R. B. and Eipper, B. A. (1986). Characterization of Pro-ACTH/endorphin-derived peptides in rat hypothalamus. J. Neurosci. 6: 837-849.
10. Emson, P. C., Corder, R., Ratter, S. J., Tomlin, S., Lowry, P. J., Rees, L. H., Arrequi, A. and Rosser, M.N. (1984). Regional distribution of pro-opiomelanocortin-derived peptides in the human brain. Neuroendocrinology 38: 45-50.
11. Estivariz, F. E., Hope, J., McLean, C. and Lowry, P. J. (1980). Purification and characterization of a γ-melanotropin precursor from frozen human pituitary glands. Biochem. J. 191: 125-132.
12. Farese, R. V., Ling, N. C., Sabir, M. A., Carsin, R. E. and Trudeau, W. L. III (1983). Comparison of effects of adrenocorticotropin and lys-γ3-melanocyte-stimulating hormone on steroidogenesis, adenosine 3', 5'-monophosphate production, and phospholipid metabolism in rat adrenal fasciculata-reticularis cells in vitro. Endocrinology 112: 129-132.
13. Hunter, W. M. and Greenwood, F. C. (1962). Preparation of ¹³¹I-labelled human growth hormone of high specific activity. Nature 194: 495-496.
14. Kawai, Y., Inagaki, S., Shiosaka, S., Shibasaki, T., Ling, N., Tohyama, M. and Shiotani, Y. (1984). The distribution and projection of γ-melanocyte stimulating hormone in the rat brain: An immunohistochemical analysis. Brain Res. 297: 21-32.
15. Ling, N., Ying, S., Minick, S. and Guillemin, R. (1979). Synthesis and biological activity of γ-melanotropin peptides derived from the cryptic region of the adrenocorticotropin/β-lipotropin precursor. Life Sci. 25: 1773-1780.
16. Meador-Woodruff, J. H., Knobloch, M., Vaudry, H., Seidah, N. G., Watson, S. J. and Akil, H. (1986). Gamma-3-MSH and β-endorphin in monkey pituitary. In: Holaday, J. W., Law, P.-W. and Herz, A. (eds.) Progress in Opioid Research. National Institute on Drug Abuse. Rockville, Maryland. p. 307-310.
17. Meador-Woodruff, J. H., Watson, S. J., Murphy-Weinberg, V., Jégou, S., Vaudry, H., Seidah, N.G., Rivier, J., Vale, W. and Akil, H. (1987). Gamma-Melanotropin response to ovine corticotropin releasing factor in normal humans. Neuropeptides 9: 269-282.
18. Nakanishi, S., Inoue, A., Kita, T., Nakamura, M., Chang, A. C. Y., Cohen, S. N. and Numa, S. (1979). Nucleotide sequence of cloned cDNA for bovine corticotropin-β-lipotropin precursor. Nature 278: 423-427.
19. Osamura, R. Y., Komatsu, N., Watanabe, K., Nakai, Y., Tanaka, I. and Imura, H. (1982). Immunohistochemical and immunocytochemical localization of γ-melanocyte stimulating hormone (γ-MSH)-like immunoreactivity in human and rat hypothalamus. Peptides 3: 781-787.
20. Pedersen, R. C., Ling, N. and Brownie, A. C. (1982). Immunoreactive γ-melanotropin in rat pituitary and

plasma: A partial characterization. Endocrinology 110: 825-834.

21. Pedersen, R. C., Brownie, A. C. and Ling, N. (1980). Pro-adrenocorticotropin/endorphin-derived peptides: Coordinate action on adrenal steroidogenesis. Science 208: 1044-1046.

22. Seidah, N. G. and Chrétien, M. (1981). Complete amino acid sequence of a human pituitary glycopeptide: An important maturation product of proopiomelanocortin. Proc. Natl. Acad. Sci. USA 78: 4236-4240.

23. Seidah, N. G., Rochemant, J., Hamelin, J., Lis, M. and Chrétien, M. (1981). Primary structure of the major human pituitary proopiomelanocortin NH_2-terminal glycopeptide. J. Biol. Chem. 256: 7977-7984.

24. Shibasaki, T., Ling, N. and Guillemin, R. (1980). Pituitary immunoreactive γ-melanotropins are glycosylated oligopeptides. Nature 285: 416-417.

25. Tanaka, I., Nakai, Y., Jingami, H., Fukata, J., Nakao, K., Oki, S., Nakanishi, S., Numa, S. and Imura, H. (1980). Existence of γ-melanotropin (γ-MSH)-like immunoreactivity in bovine and human pituitary glands. Biophys. Res. Commun. 94: 211-217.

26. Tanaka, I., Nakai, Y., Nakao, K., Yoshimasa, T., Oki, S. and Imura, H. (1984). Big form of γ-melanotropin-like immunoreactivity in normal human plasma. Peptides 5: 913-916.

27. Vaudry, H., Jenks, B. G. and van Overbeeke, A. P. (1984). Biosynthesis, processing and release of pro-opiomelanocortin related peptides in the intermediate lobe of the pituitary gland of the frog (*rana ridibunda*). Peptides 5: 905-912.