Thirty-Five Years of Progress in the Study of MSH

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In this paper, initial work on MSH at Dr. Lerner's laboratory in Portland, Oregon, from 1952 to 1954 is presented. The development of an in vitro bioassay method enabled us to show increased urinary excretion of MSH in Addison's disease. The ability of MSH to increase skin pigmentation in man was also demonstrated. Subsequent work on MSH during the past thirty years is reviewed, such as characterization of α- and β-MSH and their precursors in the pituitary gland and localization of MSH-like peptides in various regions of the brain.

Finally there are presented the characterization of γ-MSH, the hypothermic effect of intracisternal administration of γ-MSH, the effect of corticortropin releasing factor on increased secretion of α-MSH from rat pituitary, and the effect of arginine vasopressin on secretion of α-MSH from pituitary adenoma.

It is a great honor and pleasure for me to participate in the Aaron B. Lerner symposium, honoring his thirty years of dedication and accomplishment at Yale University.

From 1952 to 1954 I worked with him on MSH in Portland, Oregon, before he moved to this university in 1955. During the three decades after I left Dr. Lerner's laboratory, remarkable progress has been made on MSH. In this paper I will first introduce our research on MSH at Lerner's laboratory in Portland, Oregon, from 1952 to 1954 and will then review some of the work on MSH during the past 30 years.

While I was working as a fellow in the department of medicine at the University of Kansas Medical Center in 1951, several patients were observed whose skin pigmentation increased noticeably after ACTH therapy. This was explained in an article by Lerner and Fitzpatrick, entitled "Biochemistry of Melanin Formation," [1], which reported that a patient receiving ACTH developed marked pigmentation of the skin. The ACTH preparation contained appreciable MSH, a finding which may be relevant to the development of pigmentation. The content of melanophore hormone in human pituitary was demonstrated elsewhere [2]. I wrote Drs. Lerner and Fitzpatrick at Ann Arbor, Michigan, discussing this problem. This event began my association with Dr. Lerner, and, in September 1952, we started our work on MSH in Portland, Oregon.

First an in vitro quantitative method was developed [3] for measuring MSH, using the change of light reflection from frog skin before and after immersion in solutions containing MSH. The change in light reflection was measured by a photoelectric reflection meter.

We showed that this method could be used to determine MSH in body fluids and tissue extracts [4]. We measured MSH in blood and urine and showed that it is increased in patients with Addison's disease [4]. We also administered hog MSH preparation to five men and two women and observed increased pigmentation in all of them [5].
Lerner and Lee isolated α-MSH from porcine pituitary in 1955 [6]. Its primary structure was subsequently determined by Harris and Lerner in 1957 [7]. This peptide was later found and chemically identified in pituitaries from other species, including the cow, sheep, horse, macaque, camel, and dogfish [8]. α-MSH is a thirteen amino acid residue peptide, as shown in Fig. 1. Because its structure is identical with the N-terminal tridecapeptide sequence of ACTH, it was considered that α-MSH might be derived from ACTH.

Another MSH, β-MSH, was also isolated from porcine pituitary glands in 1956 by Geschwind et al. [9] and its amino acid sequence was determined the next year [10]. Later it was determined that the amino acid sequence of β-MSH corresponds to β-lipotropin (β-LPH) (41–58) [11]. The amino acid sequence of β-endorphin, which was isolated in 1975, also corresponds to the C-terminal portion (61–91) of β-LPH, (Fig. 2), and so it was speculated that β-MSH and β-endorphin are derived from β-LPH [12].

In 1977, Mains, Eipper, and Ling [13] disclosed that ACTH and β-endorphin are derived from a 31 K common precursor. In their series of studies on the biosynthesis of the ACTH/β-endorphin precursor in the anterior and intermediate lobes of rat pituitary, they demonstrated that post-translational processing of the 31 K common precursor is different between anterior and intermediate lobes. As shown in Fig. 3, in the anterior lobe the 31 K common precursor consists of a 16 K fragment, and ACTH and β-LPH sequences. It was also found that ACTH and β-LPH are the main products, along with small amounts of γ-LPH and β-endorphin in the anterior lobe, whereas in the intermediate lobe α-MSH, corticotropin-like intermediate lobe peptide (CLIP), β-MSH, and β-endorphin are the predominant final peptides.

In 1979, Nakanishi and his co-workers characterized the complete sequence of the mRNA isolated from the intermediate lobe of bovine pituitary, which codes for the 31 K precursor of ACTH/β-lipotropin by using techniques of DNA cloning and nucleotide sequence analysis (Fig. 4) [14]. They found in the precursor an amino acid sequence which is strikingly similar to the sequence of α-MSH and β-MSH, and named the sequence γ-MSH. According to the mRNA sequence, the nucleotide sequence was

\[
\begin{align*}
\text{ACTH} & : \quad H-\text{Ser-Tyr-Ser-Met-Glu-His-Arg-Trp-Gly-Lys-Pro-Val-Gly-Lys-Arg-Arg-Pro-Val-Lys-Val} \\
\alpha-\text{MSH} & : \quad CH_2CO-\text{Ser-Tyr-Ser-Met-Glu-His-Arg-Trp-Gly-Lys-Pro-Val-NH}_2 \\
\end{align*}
\]

FIG. 1. The structures of human ACTH and α-MSH.

\[
\begin{align*}
\beta-\text{LPH} & : \quad H-\text{Glu-Leu-Thr-Gly-Gln-Arg-Leu-Arg-Gln-Gly-Aaa-Gly-Pro-Aaa-Ala-Gln-Gly-Pro-Aaa-Ala} \\
\beta-\text{MSH} & : \quad \text{HO-Glu-Gly-Lys-Tyr-Ala} \\
\beta-\text{endorphin} & : \quad \text{HO-Glu-Gly-Lys-Tyr-Ala} \\
\end{align*}
\]

FIG. 2. The structures of β-MSH, β-endorphin, and β-LPH.
sequences encoding the common amino acids are conserved except for a very few changes.

In 1978, Shibasaki et al. isolated γ-MSH from bovine pituitary extracts. They developed radioimmunoassays for two possible γ-MSHs which were synthesized according to the sequence of the precursor molecule, γ₁-MSH and γ₃-MSH, as shown in Fig. 5 [15,16].

According to the results (Fig. 6), there are two γ₃-MSH-like peptides in bovine pituitary: one slightly smaller than β-LPH and the other with almost the same molecular size as that of ACTH. “Small” γ₃-MSH was found to be a glycosylated Lys-γ₃-MSH [17,18]. “Big” γ₃-MSH is dominant in the anterior pituitary but both γ₃-MSHs have almost the same distribution in the intermediate lobe. In addition to γ₃-MSH, Lys-γ₁-MSH and related “big” γ₁-MSH exist in the intermediate lobe extract of bovine pituitary [16,19]. Therefore, in the anterior pituitary, “big” γ₃-MSH, ACTH, and β-LPH are predominant. On the other hand, in the intermediate lobe, each peptide is further processed into smaller ones such as “small” γ₃-MSH plus Lys-γ₁-MSH, α-MSH plus CLIP, and β-endorphin plus β-MSH, respectively.

FIG. 4. The structure of ACTH-β-LPH precursor.
\( \gamma_1 \)-MSH  
\[ \text{Tyr-Val-Met-Gly-His-Phe-Arg-Trp-Asp-Arg-Phe-NH}_2 \]

\( \gamma_3 \)-MSH  
\[ \text{Tyr-Val-Met-Gly-His-Phe-Arg-Trp-Asp-Arg-Phe-Gly-Arg-Arg-Asn-Gly-Ser-Ser-Ser-Gly-Val-Gly-Gly-Ala-Ala-Gln-OH} \]

**FIG. 5.** Possible \( \gamma \)-MSHs.

A. Anterior lobe extract  
B. Intermediate lobe extract

**FIG. 6.** Sephadex G-75 gel permeation chromatography of the anterior lobe extract A and intermediate lobe extract B of bovine pituitary.

**FIG. 7.** The effect of intracisternally administered bovine \( \gamma_3 \)-MSH on rat core temperature at room temperature.
γ3-MSH-positive neurons are found in hypothalamus, and their positive fibers are widely distributed in the brain [20].

A rat which has been intraventricularly or intracisternally injected with synthetic γ3-MSH shows transient behavioral hyperactivity, cortical desynchronization, and hypothermia [21]. As shown in Fig. 7, Shibasaki demonstrated that intracisternal administration of 10 μg synthetic γ3-MSH significantly lowered core temperature by 1.25°C, 30 minutes after injection into rats at room temperature. This action of γ3-MSH was more manifest when the experiment was performed at 4°C. The administration of 100 ng γ3-MSH significantly lowered core temperature, and the effect lasted two hours (Fig. 8).

Recent immunohistochemical studies by several investigators have revealed that α-MSH-like immunoreactivity is widely distributed not only in the intermediate lobe of pituitary but also in various regions of the brain [22]. In the brain, α-MSH-positive neurons exist in the hypothalamus, thalamus, midbrain, and cortex. Nerve fibers with α-MSH-like immunoreactivity are found in the septum, hypothalamus, amygdala, and cortex.

α-MSH-positive neurons in the arcuate nucleus are also stained by β-endorphin antiserum. However, neurons stained by α-MSH antiserum in the subthalamus and dorsomedial hypothalamic and lateral hypothalamic nuclei are not stained by β-endorphin antiserum. The latter α-MSH neuron group is therefore called the secondary melanotropinergic system [23]. This may imply that each α-MSH neuronal group has a different role in neurotransmission or neuromodulation in the brain. The different staining pattern may be due to variation in post-translational processing.
within different branches of the same neuron: α-MSH and β-endorphin are the final products in some fiber branches, while α-MSH is the only final product in the branches. It is also possible that different neurons have specific processing systems which yield the same single or multiple final products at every secretory site in the neuron. Diacetyl α-MSH, in addition to authentic acetylated α-MSH, exists in the intermediate lobe of the pituitary [24]. On the other hand, the acetylation of α-MSH in
brain is a controversial issue [25,26]. Some groups have provided evidence for the presence of authentic acetylated α-MSH in extracts of rat brain tissue; however, the presence of des-acetyl α-MSH instead of authentic α-MSH has also been reported.

In peripheral tissues, α-MSH-like immunoreactivity is present in both mucosal and muscular layers in all areas of the gastrointestinal tract [27]. The highest concentra-
tions were found in the duodenum. This fact may suggest that α-MSH is involved in digestion and absorption of food in the gastrointestinal tract.

Shibasaki has shown that the secretion of α-MSH is stimulated by CRF in rat and inhibited by dopamine. Figure 9 shows that synthetic ovine CRF stimulates the secretion of α-MSH from monolayer-cultured intermediate lobe cells in a dose-dependent manner.

Figure 10 shows that dopamine decreases the secretion of α-MSH in a dose-dependent manner and also attenuates the CRF-induced secretion at a concentration of $1 \times 10^{-6}$ M. Shibasaki and Hotta have recently observed that, in a perfusion system, the addition of ovine CRF stimulates secretion of α-MSH from rat fetus pituitary obtained at 16.5 days of gestation (Fig. 11). Daikoku et al. have reported that beaded CRF-positive fibers are observed in the median eminence on day 17.5 of gestation in the rat [28]. It was therefore concluded that the CRF receptor system in the intermediate lobe cells of the pituitary is already present in advance of the appearance of the hypothalamic regulatory mechanism of CRF.

We found an α-MSH-like substance secreted by cultured pituitary adenoma cells obtained from patients with Cushing's disease or Nelson's syndrome. Figure 12 shows the elution profile of ACTH, β-LPH, and γ3-MSH in gel filtration chromatography of Sephadex G-75 of the culture medium in the upper panel; the elution profile of an α-MSH-like substance is shown in the lower panel. A peak of α-MSH-like immuno-reactivity, expressed as closed circles, was found at the position where $^{125}$I-authentic α-MSH eluted.

FIG. 13. The effect of arginine, vaso-pressin, and VIP on the secretion of POMC-derived peptides by a pituitary adenoma causing Nelson's syndrome.
Figure 13 shows that the amount of \( \alpha \)-MSH secreted into medium from cultured pituitary adenoma cells causing Nelson's syndrome was increased dose-dependently by arginine, vasopressin, and VIP. Patients with these diseases show hyperpigmentation in skin and mucosa. Therefore, \( \alpha \)-MSH-like substance secreted from pituitary adenoma cells may contribute to the hyperpigmentation in these patients.

In addition to papers on skin darkening, many papers on the action of \( \alpha \)-MSH have been published, including those on stimulation of sebum secretion and dermal lipogenesis [29], increase of intrauterine growth in the rat [30], induction of growth hormone and luteinizing hormone secretion in man [31,32], stimulation of aldosterone secretion in the rat [33], and elevation of plasma glucagon, insulin, and free fatty acid levels in the rabbit [34]. Furthermore, the entire amino acid sequence of the \( \alpha \)-MSH precursor and its metabolism in the pituitary has been characterized. However, the physiological roles of \( \alpha \)-MSH, especially in the brain and GI tract, are still unknown. Therefore I look forward to further studies on MSH, based upon those which have been initiated by Dr. Lerner.

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