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Changes in Hypothalamic and Pituitary Content of Immunoreactive α-Melanocyte-Stimulating Hormone during the Gestational and Postpartum Periods in the Rat[1] (41950)

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Abstract. α-Melanocyte-stimulating hormone (α-MSH) was measured in the mediobasal hypothalamus (MH), median eminence (ME), preoptic–suprachiasmatic area (POA–SCN), anterior (AL), and posterior lobes (PL) of the pituitary gland during the gestational and postpartum periods in the rat. The content of α-MSH in the MH and POA–SCN compared to estrous levels was lower during the later days of gestation and decreased further in the MH during lactation in association with the elevated plasma prolactin (Prl). Distinct increases in the ME content of α-MSH compared to estrous levels occurred on Days 8 and 12 of the gestational period and Day 14 of the postpartum period. A significant increase in PL content of α-MSH compared to Days 5–11 and 17–20 occurred on Day 4 of gestation, and no significant changes were detected in the AP concentration of α-MSH throughout the period studied. *In vitro,* PLs and ALs from females on Day 4 of gestation secreted more α-MSH into the incubation medium than tissues from animals on Day 20. These results suggest that α-MSH of both brain and pituitary origin may play a role in mediating some of the physiological changes which occur during pregnancy and lactation.

α-Melanocyte-stimulating hormone (α-MSH) is a tridecapeptide derived from the pro-opiomelanocortin molecule. In recent years extensive efforts have been made to elucidate the role(s) for this hormone in mammals. We have recently demonstrated that intraventricular injection of α-MSH inhibits the secretion of prolactin (Prl) (1) and obtained evidence that α-MSH exerts its inhibitory effect via activation of the tuberoinfundibular dopaminergic system. As an extension of this observation, we were interested in determining if changes in the content of α-MSH in the hypothalamus and posterior lobe correlate with plasma Prl levels during physiological states in which Prl secretion changes. In the present study we report our findings in the pregnant and postpartum rat.

Materials and Methods. Adult virgin female (240–300 g) and fertile male (400–600 g) rats of the Sprague–Dawley strain (Holtzman) were maintained under controlled lighting (lights on between 0500 and 1700 hr) and temperature (24 ± 1°C) and given free access to tap water and Purina rat chow throughout the course of the study.

Females were left overnight with males (8–12 females/2 males/cage) and a vaginal lavage was obtained the following morning. The day on which spermatozoa and cornified epithelial cells were both observed in the lavage was designated as Day 0 of gestation. Pregnant females were then isolated from males and housed 1–2/cage. In our facilities, parturition usually occurred on Day 22. The day of parturition was designated as Day 0 postpartum. Parturient females were isolated in individual cages and the number of pups was reduced to 8 per dam. Animals were sacrificed between 1500 and 1600 hr by decapitation after being stunned by a quick blow to the head. Of the postpartum group, only those dams that had nursed their young for at least 30 min prior to sacrifice were used. Trunk blood was collected in glass tubes containing 300 μl of 10% EDTA and centrifuged (4°C, 1000*g*, 15 min), and the plasma was stored at −20°C until assayed for Prl. Prl was measured according to the instructions of the kit provided by NIADDK, and the results were expressed in terms of the RP-1 reference standard.

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Pituitary glands were excised and separated into anterior and posterior lobes (AL and PL, respectively). They were then weighed, frozen on dry ice, and later homogenized in 1 *M* ice-cold acetic acid. Median eminences (MEs) were microdissected from hypothalami under a stereomicroscope as described by Negro-Vilar *et al.* (2) and immediately homogenized in 300 μl of 1 *M* ice-cold acetic acid. The remaining brain tissues were frozen on dry ice to facilitate dissection of the medial hypothalamus (MH) and preoptic–suprachiasmatic area (POA–SCN). The boundaries of the former were set at the hypothalamic sulci, the anterior edge of the mammillary bodies, the dorsal surface of the third ventricle, and the rostral limit of the ME. The POA–SCN sections were outlined by the rostral border of the MH, a line 1 mm anterior to the rostral edge of the optic chiasm, lines parallel to and continuous with the hypothalamic sulci, and the anterior commissure in the dorsal plane. Tissue sections were homogenized in 1 *M* ice-cold acetic acid and stored at −70°C.

Synthetic α-MSH which is monoacetylated (Boehringer-Mannheim, Lot No. 600425) was conjugated to crystalline bovine serum albumin (Sigma, A7638) by the carbodiimide method, and antisera were raised in New Zealand white rabbits as previously described by Orth *et al.* (3). Each rabbit was injected sc with an initial dose of 200 μg of the conjugated peptide followed by three booster injections (50, 80, and 50 μg/animal, respectively) every 3–4 weeks. Rabbits were bled 2 weeks after the last injection and the sera were analyzed for their capacity to bind ^{125}I-α-MSH in a radioimmunoassay (RIA).

Five micrograms of synthetic α-MSH (Peninsula Labs) were iodinated and purified by methods previously described (2). For RIA, the buffer used was 0.01 *M* phosphate (pH 7.4) containing 0.9% NaCl, 0.1% gelatin, and 0.025 *M* EDTA.

On the first day of the assay, all tubes were brought to a volume of 200 μl with assay buffer, followed by 100 μl of antisera (1:10,000 initial dilution for KDM-1 and 1:20,000 for KDM-2) containing 2% normal rabbit serum (NRS). Nonspecific binding tubes received NRS in place of the primary antisera. After incubation at 4°C for 20–24 hr, 100 μl of tracer (10,000 cpm) was added to each tube and incubated at 4°C for an additional 20–24 hr. This was followed by addition of 100 μl of sheep anti-rabbit γ-globulin serum (1:24 dilution) and a further incubation at 4°C for 24 hr. On the fourth day bound and free ^{125}I-α-MSH was separated by centrifugation. Values were calculated after log–logit transformation of the standard curve.

For gel filtration studies acetic acid extracts of hypothalamic and pituitary tissue or synthetic α-MSH were loaded on a Sephadex G-25 column (0.9 × 100 cm) which was previously equilibrated with 0.2 *M* acetic acid. Fractions of 2 ml were collected. Aliquots were subsequently lyophilized and reconstituted in the RIA buffer for determination of α-MSH. (When the term α-MSH is used in this text, it will be understood to mean α-MSH immunoreactivity.)

The *in vitro* incubations were carried out in Krebs–Ringer bicarbonate buffer containing 0.1% glucose (KRB-G). The pituitaries (AL and PL) were randomly placed in incubation flasks containing 0.5 ml of KRB-G. After a 30-min preincubation period, the

TABLE I. SPECIFICITY OF α-MSH ANTISERA

Peptide	% Immunologic cross-reactivity	
	KDM-1	KDM-2
α-MSH	100	100
Des-acetyl α-MSH	36	0.23
γ_3-MSH	ND	ND
ACTH (1–4)	ND	ND
ACTH (4–10)	ND	ND
ACTH (1–10)	0.001	8.50
ACTH (1–24)	0.029	0.30
ACTH (ACTH (1–39))	0.004	0.20
CLIP (ACTH (18–39))	ND	ND
Ovine β-LPH	0.01	0.03
Porcine β-MSH	ND	ND
β-Endorphin	ND	ND
Ovine CRF	ND	NT
Rat Prl	ND	NT

Note. The figures given indicate the percentage cross-reactivity of various fragments of the pro-opiocortin molecule with synthetic α-MSH taken as 100%. These values were calculated on a molar basis and were based on the dose needed to displace binding of ^{125}I-α-MSH by 50%. ND: No detectable cross-reactivity at a 100 molar excess. NT: Not tested.

TABLE II. COMPARISON OF IMMUNOREACTIVE α-MSH LEVELS AS MEASURED BY KDM-1 AND KDM-2

Tissue	*n*	KDM-1	KDM-2
Anterior pituitary (ng/gland)	7	21.4 ± 1.5	24.2 ± 1.2
Posterior pituitary (ng/gland)	7	1400 ± 30	680 ± 30
Mediobasal hypothalamus (ng/hypothalamus)	8	3.52 ± 0.13	0.18 ± 0.02

Note. In this and subsequent figures all the levels are expressed as hormone levels ± SEM.

medium was removed and replaced with fresh medium for an additional 30 min.

In incubations employing hypothalamic fragments, the mediobasal hypothalamus (including the ME) was cut in half longitudinally. Each flask contained four pieces of tissue (2 hypothalami) in a volume of 0.5 ml KRB-G. At the end of the incubation period the media were centrifuged for 10 min at 1600*g*, acidified with 50 μl of 1 *M* acetic acid, and frozen at −30°C.

Statistics. In the *in vitro* experiments Student's *t* test was employed. When percentages were compared the arcsine transformation was performed first. For analysis of α-MSH during pregnancy analysis of variance (ANOVA) was performed followed by Dunnett's multiple comparison test in cases in which one group was compared to all others. The Student–Newman–Keul's (SNK) test was utilized when all groups were compared to each other (4).

Results. The specificity data for KDM-1 and KDM-2 are shown in Table I. KDM-1 did not cross-react with ACTH (1–39) or smaller fragments of it. This antiserum showed 36% cross-reactivity with des-acetyl α-MSH. KDM-2 cross-reacted 8.5% with ACTH (1–10) and 0.23% with des-acetyl α-MSH. This difference in cross-reactivity is reflected in the different values obtained by the two antisera when quantitating α-MSH in the same PL and mediobasal hypothalamic extract samples (Table II). KDM-1 detected 2-fold higher levels of α-MSH in the former ($P < 0.001$) and 19-fold higher levels in the latter tissue extract ($P < 0.001$). This difference was not found in AL extracts.

Further characteristics of the RIA using KDM-1 are depicted in Table III. This antiserum was used at a final dilution of 1:40,000 with a sensitivity of 4–6 pg/tube. KDM-2 was used at a final dilution of 1:80,000 and also was able to detect 4–6 pg of α-MSH/tube. Serial dilutions of lyophilized acetic acid extracts of hypothalami, AL and PL tissues, as well as nonacidified plasma gave displacement curves which were parallel to synthetic α-MSH by RIA (Fig. 1).

With both antisera, α-MSH activity in hypothalamic and pituitary extracts was detected as single peaks, eluting in the same position as the synthetic standard on a Sephadex G-25 gel filtration column (Fig. 2).

TABLE III. CHARACTERISTICS OF KDM-1 IN RIA

	KDM-1
Final dilution	1:40,000
Sensitivity ($B/B_0 \times 100$ = 90–95%)	5.9 ± 1.9 pg/tube
Linear range	6–200 pg/tube
Mid range ($B/B_0 \times 100$ = 50%)	39.4 ± 1.3 pg/tube
Intraassay C.V.	6.1%
Interassay C.V.	8.5%

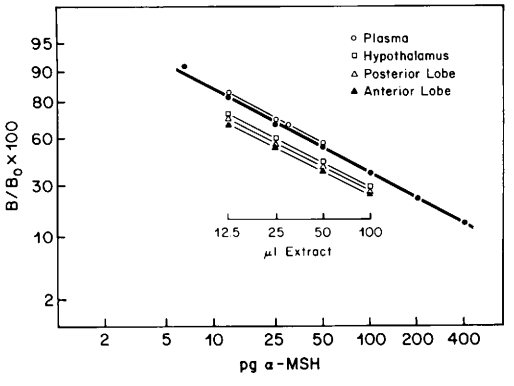

FIG. 1. Displacement of ^{125}I-α-MSH from antiserum KDM-1 by synthetic α-MSH (filled circles) extracts of anterior and posterior lobes, hypothalami, and plasma. $B/B_0 \times 100$ = % of counts bound in the presence of α-MSH standard or sample in relation to counts bound in the absence of unlabeled α-MSH.

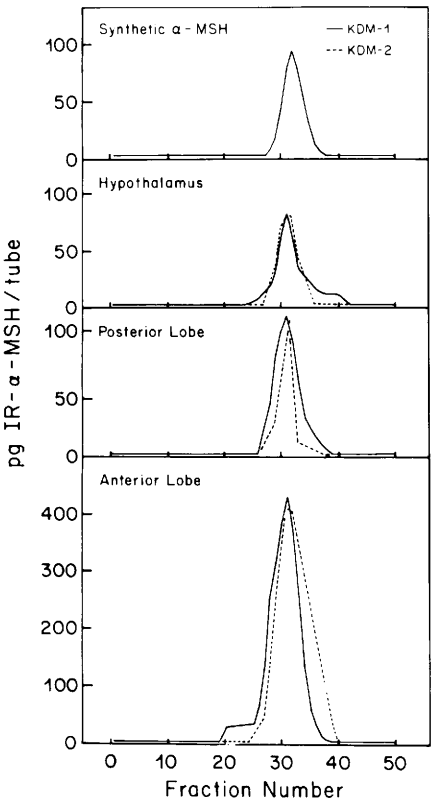

FIG. 2. Comparative (KDM-1, KDM-2) gel chromatography elution profiles of hypothalamic (upper panel), posterior lobe (middle panel), and anterior lobe (lower panel) α-MSH from a Sephadex G-25 fine column (0.9 × 100 cm). V_0 = 32 ml.

The afternoon (1500 hr) plasma Prl profile during pregnancy and the postpartum period is shown in Fig. 3. The levels of Prl were relatively high on Days 0, 5, and 7 but not on Days 2 and 4 of pregnancy. Prolactin levels had declined to low values by Day 8 of pregnancy. This low level of Prl secretion was maintained until Day 20, at which time a marked increase occurred. Prl levels were markedly elevated in the postpartum period but were significantly lower on Day 7 than on Day 3 ($P < 0.05$) and not different from Day 14. The levels on Days 7 and 14 were not different from each other. Prl levels at the time of delivery were not measured.

The changes in the AL and PL contents of α-MSH during pregnancy and the postpartum period are shown in Fig. 4. ANOVA analysis of AL α-MSH produced an F probability of 0.024. However, due to the large variation in the results, no significant difference was obtained by Dunnett's test comparing estrous levels of α-MSH with different days of pregnancy or the postpartum period. The SNK test also failed to detect any significant difference when the mean α-MSH concentrations from different days were compared to each other.

ANOVA analysis of the PL content of α-MSH during pregnancy indicated that a significant difference between the group means exists (F probability = 0.001). The concen-

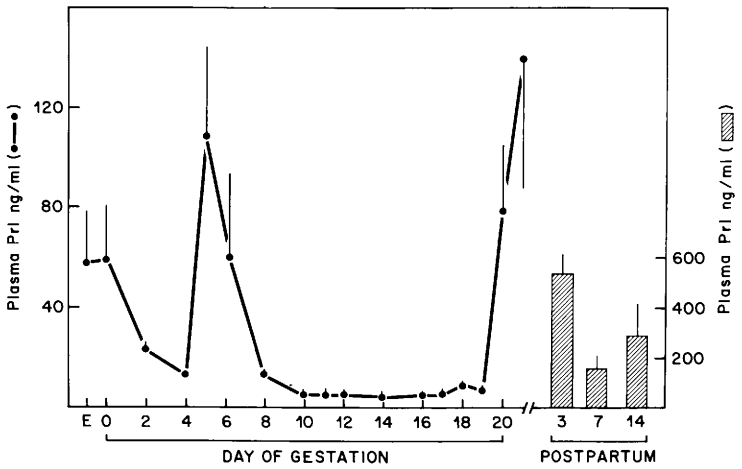

FIG. 3. Profile of plasma Prl (ng/ml) during gestation and the postpartum period. The error bars depict the standard error of the mean. There were seven or eight animals per each day of pregnancy in this and subsequent figures.

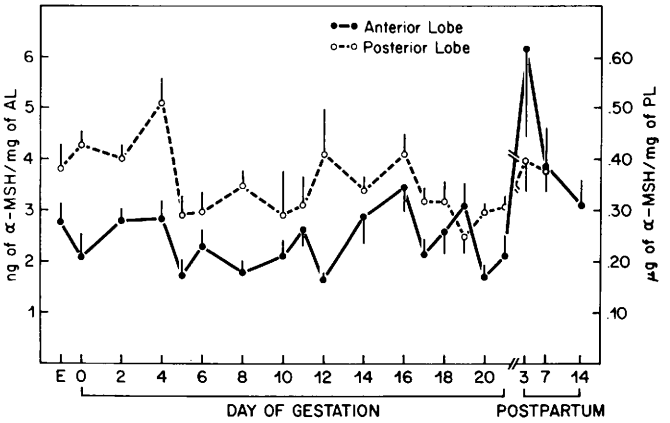

FIG. 4. Content of α-MSH in the anterior lobe (AL) and posterior lobe (PL) of the pituitary gland during gestation and the postpartum period. In this and subsequent figure, KDM-1 was used for measurement of α-MSH concentrations.

trations of α-MSH on estrus and on Days 0, 2, 4, and 16 of pregnancy were not significantly different from each other. A marked increase in the PL concentration of α-MSH occurred on Day 4 of pregnancy, at which time the α-MSH concentration was significantly higher than the levels on Days 5, 6, 10, 11, 17–20 ($P < 0.01$) and Days 8 and 14 ($P < 0.05$). The PL content of α-MSH during the postpartum period was not different from estrus or Day 0 levels of α-MSH.

α-MSH in the MH (Fig. 5) underwent marked changes during pregnancy (F probability < 0.001). The concentrations of α-MSH on days 0, 5, 6, 8, and 10 were high compared to those on other days of pregnancy and not significantly different from each other. Dunnett's test indicated that the estrous levels of α-MSH were significantly higher than those on Days 16–21 ($P < 0.01$) and on Days 2, 4, and 11 ($P < 0.05$), but not different from the levels on Days 0, 5, 6, 8,

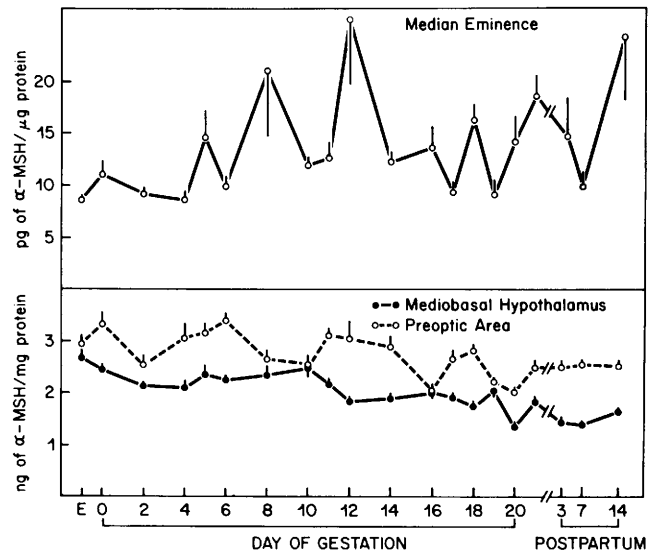

FIG. 5. Content of α-MSH in the median eminence (ME), medial hypothalamus (MH), and preoptic-suprachiasmatic region (POA–SCN) during gestation and the postpartum period.

TABLE IV. PATTERN OF α-MSH RELEASE FROM AL AND PL FROM DAYS 4 AND 20 OF PREGNANCY

Tissue	Day	*n*	Gland content (ng/mg)	Media content (ng/mg)	% Release
AL	4	6	0.70 ± 1.14	0.21 ± 0.05**	2.5 ± 0.28*
	20	7	0.46 ± 0.10	0.065 ± 0.02	1.3 ± 0.56
PL	4	7	430 ± 30 ***	21.0 ± 2.2 ***	4.6 ± 0.37
	20	8	210 ± 20	9.0 ± 1.6	4.2 ± 0.66

Note. Medium content is the amount of hormone released in 30 min. Gland content is the amount of hormone remaining in the tissue, expressed in terms of the tissue weight, at the end of the incubation period. % Release = medium content/(medium content + gland content) × 100. In this and the subsequent table, KDM-1 was used for measurement of α-MSH concentration.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus Day 20 values.

and 10. A marked decrease in the MH concentration of α-MSH occurred 3, 7, and 14 days postpartum ($P < 0.001$) when compared to estrous levels, during the time when plasma Prl was elevated in the lactating mothers.

The α-MSH content of the POA–SCN also showed some changes during pregnancy (*F* probability = 0.001). A significant decrease in the POA concentration of α-MSH occurred on Days 16 and 20 ($P < 0.05$) when compared to estrous levels. The SNK test indicated that the levels of α-MSH on Days 16, 19, and 20 were significantly lower than those examined on all the other days with the exception of Days 2, 8, 10, 17, 18, and 21. No significant changes occurred in this region during the postpartum period.

α-MSH in the ME appeared to show cyclical variation during pregnancy. ANOVA analysis produced an *F* probability value of 0.001. A significant increase in the ME content of α-MSH occurred on Day 8 ($P < 0.05$) and Day 12 ($P < 0.01$) compared to estrous levels. The marked elevation on Day 12 was significantly different from the levels measured on all the days examined except Days 8, 16, 18, and 21. The concentration of α-MSH during the postpartum period was comparable to estrous levels except at 14 days postpartum, at which time a significant increase ($P < 0.01$) in the ME concentration of α-MSH occurred.

The patterns of α-MSH release *in vitro* from the AL, PL, and mediobasal hypothalamus from 2 different days of pregnancy are depicted in Tables IV and V. ALs from rats on Day 4 of pregnancy released more α-MSH into the incubation medium than glands from animals on Day 20 ($P < 0.01$). The content of α-MSH in these glands was slightly higher than that of glands from rats on Day 20 of pregnancy, although this change was not statistically significant. When the *in vitro* secretion data were expressed in terms of percentage release, i.e., (media α-MSH/gland α-MSH × 100), anterior lobes from Day 4 of pregnancy secreted a slightly higher percentage of their content than glands from rats on Day 20 of pregnancy ($P < 0.05$).

The content of α-MSH in the PL of rats on Day 4 was found to be significantly higher ($P < 0.001$) than that of glands from pregnant animals at Day 20, and they also secreted more α-MSH *in vitro* ($P < 0.001$). However, in contrast to the AL, there was no significant difference in the percentage release of α-MSH from the PL between Day 4 and Day 20.

The results of the α-MSH release study from mediobasal hypothalamic fragments are

TABLE V. PATTERN OF α-MSH RELEASE FROM MEDIAL BASAL HYPOTHALAMIC FRAGMENTS FROM DAYS 4 AND 20 OF PREGNANCY

Day	*n*	I_1 (pg/flask)	I_2 (pg/flask)	MBH content (ng/mg protein)
4	5	22.9 ± 2.7	50.4 ± 4.7**	1.1 ± 0.04*
20	4	27.9 ± 3.1	50.8 ± 3.2***	0.92 ± 0.05

Note. Following a 30-min preincubation, the medium was replaced and the tissue was further incubated for 30 min (I_1). The medium was then replaced with KRB-G containing 56 m*M* K^+ (I_2). Each flask contained four tissue fragments (two hypothalami).

* $P < 0.05$ vs day 20 hypothalamic content of α-MSH.

** $P < 0.01$, *** $P < 0.001$ vs I_1 values.

shown in Table V. No difference could be found in the total α-MSH released into the medium on Days 4 and 20 of pregnancy. A depolarizing concentration of K^+ significantly stimulated α-MSH release from tissues from Day 4 ($P < 0.01$) and Day 20 ($P < 0.001$) animals. As shown in an earlier experiment (Fig. 5), hypothalami from rats on Day 4 contained more α-MSH than those from animals on Day 20 ($P < 0.05$).

Discussion. The cross-reactivity data and the gel filtration study indicate that both KDM-1 and KDM-2 specifically recognize monoacetylated and to different extents des-acetyl α-MSH. The antisera are directed toward different portions of the molecule. KDM-1 is more C-terminally directed than KDM-2 and consequently cross-reacts with des-acetyl α-MSH, whereas KDM-2 requires the presence of the *N*-acetyl group for recognition. The values of α-MSH measured by KDM-1 (Table II) are comparable to those reported by others using C-terminally directed antisera (5). The levels of α-MSH measured by KDM-2 are lower than those reported by most investigators probably because it does not cross-react with des-acetyl α-MSH. Our results suggest that the majority of α-MSH reported to be found in the hypothalamus is des-acetyl α-MSH, and that both forms exist in the pituitary gland. It is possible that the extraction procedure may cause conversion of one form of α-MSH into another as has recently been reported (6–9). However, our observations utilizing KDM-1 or KDM-2 in immunohistochemical staining of the hypothalamus (unpublished observations) corroborate our results from RIA, i.e., KDM-2 stained fewer neurons than KDM-1, thus ruling out the extraction procedure as a cause of conversion of one form of α-MSH into another. Furthermore, these data are in agreement with the findings in humans in which des-acetyl α-MSH is thought to be the predominant form of α-MSH in the brain (7). The physiological importance of acetylation has not yet been elucidated although the des-acetyl form has lower activity than *N*-acetyl α-MSH in certain behavioral tests (8).

The plasma Prl levels reported in this study agree with previously reported data (10, 11), although the high levels measured on Day 5 of pregnancy were not observed by others. We are not certain why this peak was observed at this time but it could be due to an acute stress response during the handling procedure prior to decapitation in some of the animals.

All measurements of α-MSH in this study were of tissue content. Since this is a reflection of rates of biosynthesis, release and intracellular degradation of the peptide, interpretation of the meaning of changes in tissue content is speculative. In some instances release of the peptide *in vitro* was evaluated. In these cases the *in vitro* release was presumed to be correlated to the release *in vivo*.

No marked changes in AL α-MSH occurred during pregnancy although the levels were slightly higher on Day 4 (Table IV) than on Day 20. This pattern was statistically evident in the PL α-MSH measurements. Since the ALs on Day 4 released more α-MSH *in vitro* than those from Day 20 animals, in terms of both the media content and total percentage released, an increase in both the synthesis and release of the peptide on Day 4 as compared to Day 20 is indicated.

In the case of the PL (intermediate lobe portion of the PL) the levels of α-MSH tended to be higher on the first 3 days of pregnancy and comparable to estrous levels. The most remarkable change in the PL content of α-MSH occurred on Day 4. Not only was the content higher, but a greater release of α-MSH *in vitro* from PLs on Day 4 of pregnancy also was evident. However, the percentage of the total content that was released was comparable between the 2 days, indicating that probably a greater synthesis of α-MSH occurs on Day 4 than on Day 20.

Our *in vitro* data are further supported by plasma α-MSH measurements in which Day 4 plasma levels (440 ± 83 pg/ml; $n = 9$) were significantly higher ($P < 0.05$) than those of Day-20 females (179 ± 28 pg/ml; $n = 5$). Volosin and Celis (12) also found higher bioassayable α-MSH in plasma on the afternoon of Day 4 of pregnancy as compared to Day 20. The pituitary content of α-MSH was not measured on these 2 days in their study. Plasma Prl levels were low on Day 4 of pregnancy raising the possibility that the greater release of α-MSH on Day 4 may mediate lower Prl release by activation (Day

4) of the dopaminergic system which is inhibitory to Prl release. Conversely, on Day 20, lesser release of MSH on Day 20 with lesser activation of the dopaminergic system may account for higher Prl values on that day. This hypothesis is supported by our recent finding that α-MSH can lower Prl release via a dopaminergic mechanism (1) and by the results of Ben-Jonathan *et al.*, who found high levels of dopamine in the portal plasma on Day 4 of pregnancy and lower levels on Day 20 (13).

The increased synthesis and release of α-MSH on Day 4 of pregnancy may be a consequence of the increased secretion of estrogen and progesterone which occurs prior to ovum implantation (14) since estrogen and progesterone are thought to increase the secretion of bioassayable (15) and immunoassayable α-MSH (16).

No marked changes occurred in the PL content of α-MSH during lactation. These results are in contrast to those of Taleisnik *et al.* (17) who showed a decrease in pituitary bioassayable α-MSH following 1 hr of suckling. This discrepancy may be explained by the difference in the protocol in the two studies. In our study the pups were not separated from the mother and subsequently returned. According to Taleisnik *et al.*, the drop in content that they measured was restored within 4 hr of continuous suckling. Therefore, the α-MSH values in our study represent those of continuous suckling. Furthermore, α-MSH was measured by two different methods, i.e., bioassay in their study vs RIA in our study.

The MH did not show the cyclical variation in α-MSH as seen in the ME, although a trend of a gradual decline throughout gestation and the postpartum period was evident. The comparison of the release pattern of α-MSH between Days 4 and 20 *in vitro* indicated that equal amounts of α-MSH were secreted in these 2 days. Therefore, the difference in the content of α-MSH between these 2 days after the incubation period probably reflects decreased synthesis of α-MSH on Day 20 as compared to Day 4. The response of the tissue to high K^+ (56 m*M*) was identical between the 2 days. The decline in α-MSH content during the postpartum period may be indicative of a lower release of the peptide, which would diminish the influence of the peptide on the dopaminergic neurons and hence lead to greater secretion of prolactin.

Much evidence suggests that α-MSH in the brain is involved in certain types of behavior (18, 19). Whether certain types of behavior associated with α-MSH are exhibited to a greater extent during one stage of pregnancy as compared to another or whether the changes in MH α-MSH in the postpartum period affects maternal behavior remains to be elucidated. In agreement with the results of this study demonstrating a decline in hypothalamic α-MSH concentrations during the postpartum period, Wardlaw *et al.* (21) found a significant drop in another POMC-derived peptide, β-endorphin, in the hypothalamus 1–2 days postpartum.

α-MSH in the POA–SCN showed similar variations as in the ME, with the exception of Days 8 and 20. The relevance of the POA–SCN and ME α-MSH fluctuations is not known since no physiological function has yet been attributed to the peptide in these regions.

In summary, we have measured immunoreactive α-MSH in the ME, MH, POA–SCN, and AL and PL by a specific antiserum to α-MSH which recognizes the *N*-acetyl form and cross-reacts 36% with the des-acetyl form. Although no clear relationship exists between the content of α-MSH in the various areas examined and plasma Prl during pregnancy, distinct changes in the content of the peptide occurred in different tissues on specific days of pregnancy. There was a decline in MH α-MSH content which was associated with elevated plasma Prl values in lactating rats.

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