Changes in Dopaminergic Control of Circulating Melanocyte-Stimulating Hormone-Related Peptides at Puberty

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ABSTRACT

Desacetyl α-melanocyte-stimulating hormone (MSH) (ACTH 1–13) is the main form of immunoreactive α-MSH circulating in human plasma. This study evaluates the possibility that a dopaminergic inhibitory mechanism could be operative during human development. Thus, α-MSH and ACTH 1–13 plasma levels were measured after dopaminergic blockade (domperidone (0.3 mg/kg body weight, maximum 10 mg, p.o.) in 13 prepubertal (aged 4.5–12.3 y) and 12 pubertal (aged 10.2–16.9 y) children. Both peptides were measured by RIA after plasma extraction on Sep-pak C-18 cartridges and reverse phase HPLC. The chromatographic profile of α-MSH immunoreactivity falls into two main peaks, corresponding to the retention time of α-MSH and ACTH 1–13. Moreover, in prepubertal children domperidone induced a significant increase of α-MSH from 1.7 (median) to 5.0 pmol/L, whereas no changes in α-MSH plasma levels were found in pubertal subjects (from 5.0 to 4.1 pmol/L). Similarly,

ACTH 1–13 plasma levels significantly increased from 3.0 to 19.8 pmol/L in prepubertal children remaining stable in pubertal ones (from 7.8 to 4.6 pmol/L). Moreover, a significant negative correlation was found between basal DHEA-S levels and the plasma α-MSH increase after domperidone. These data demonstrate that: *1*) ACTH 1–13 is the main form of immunoreactive α-MSH in prepubertal life and *2*) the dopaminergic inhibition of both ACTH 1–13 and α-MSH plasma levels is apparent only in prepubertal subjects. (*Pediatr Res* **38: 91–94, 1995**)

Abbreviations

α-**MSH**, α-melanocyte-stimulating hormone
ACTH 1–13, desacetyl α-melanocyte-stimulating hormone
POMC, proopiomelanocortin
DHEA-S, dehydroepiandrosterone sulfate

α-MSH is a peptide deriving from a two steps cleavage of POMC for the formation of which the activity of α-*N*-acetyltransferase is required (1). In lower mammals, these enzymatic events are absent from the anterior pituitary, whereas they are typical of the neurointermediate lobe, which is well developed in these species (2). In humans, the neurointermediate lobe is virtually absent in adults (3), whereas it has been detected in the fetal pituitary (4) and in a subgroup of patients with Cushing's disease (5).

In contrast to the studies of late 1970s, detectable levels of α-MSH plasma have been found in healthy adult volunteers (6, 7), and they have been found higher than normal in patients affected by Cushing disease (8). The results of the above-mentioned studies were obtained coupling the classical α-MSH RIA to purification procedures, such as extraction and HPLC, and revealed that the des-acetylated form of α-MSH (*i.e.* ACTH 1–13 amide) is the main form of plasma immunoreac-

tive α-MSH. This finding also suggests a possible functional activity of neurointermediate lobe in some physiopathologic conditions.

We previously demonstrated that, in contrast to ACTH levels, plasma β-endorphin concentrations increased progressively throughout prepuberty, reaching adult levels at the beginning of pubertal development (9). These data were in keeping with the suggested role of POMC-related peptides in adrenarche and raised the hypothesis that sources other than the anterior pituitary might contribute to circulating peptides (10).

Inasmuch as α-MSH secretion is under a unique dopaminergic inhibitory control in rats (11), this study evaluates the possibility that a similar mechanism could be operative during human development. Thus, α-MSH plasma levels were measured after dopaminergic blockade in prepubertal and pubertal children.

METHODS

Patients. The study protocol was approved by the Local Ethics Committee. After informed consent was obtained from

parents and/or patients, 25 subjects (13 boys, 12 girls; age ranging from 5 y and 6 mo to 16 y and 3 mo) entered the study. Patients were referred to the Department of Pediatrics for the assessment of the hormonal/metabolic state, and clinical and laboratory findings excluded the presence of any endocrine disorder. Discharge diagnosis was mainly that of short-normal child.

According to pubertal development, patients were subdivided into prepubertal (13 subjects, six boys, seven girls) and pubertal subjects (12 subjects, eight boys, four girls) (Table 1).

Domperidone (0.3 mg/kg body weight, maximum 10 mg) was administered orally at 0900 h, 1 h after catheter placement in an antecubital vein. All subjects remained supine throughout the test, and blood samples (6–8 mL) were collected before (−30, −15, and 0 min) and after (60, 75, 90, and 120 min) drug administration. Plasma of the three basal samples as well as that of the four poststimulation samples (always the same amount) was pooled and stored at −20°C until assays.

Because ethical reasons do not allow a time course study on a single subject, the sampling times poststimulation were chosen on the basis of the pattern of the plasma prolactin response which normally peak between 75th and 90th min. No side effects were observed upon domperidone administration.

Peptide assays. Peptides were measured by RIA after plasma extraction and chromatography (12). Plasma (6–14 mL) was acidified with an equal volume of O.1 N hydrochloric acid and centrifuged, and the supernatant was extracted through Sep-pak C-18 cartidges (Waters Instruments, Rochester, MN). Cartridges were first eluted with a 10% methanol in 0.5 M acetic acid mixture to eliminate interfering substances.

The peptides were then recovered eluting three times with 3 mL of 90% methanol in 0.5 M acetic acid. The mixture was evaporated to dryness and reconstituted in 0.3 mL of acetonitrile/0.01 M hydrocloric acid (18:82), to be injected in the chromatograph. HPLC (Waters) was performed using a reverse-phase C-18 column (μBondapack, 39 × 300 mm, 10 μm size) eluted with a linear gradient from 18 to 40% acetonitrile in 0.01 M hydrochloric acid, for 22 min, at a flow rate of 1.5 mL/min. For each sample, 44 fractions were collected (every 30 s), evaporated to dryness, and redissolved in 1 mL of 0.12 M phosphate buffer, pH 7.4, and 0.1% BSA. To evaluate the amount of material retained in the column, 1 ng of each of the three peptides was injected into the system and processed as described above. This experiment was replicated five times. Recoveries were 92.4 ± 7.2% for ACTH 1–13 and 90.3 ± 5.5% for α-MSH. In view of the retention times of α-MSH (11.6 min) and ACTH 1–13 (8.5 min), fractions were tested for their α-MSH immunoreactivity. Standard α-MSH and ACTH 1–13, as well as anti α-MSH serum (M2) were provided by Dr. V. Wiegant (University of Utrecht, The Netherlands). M2 serum cross-reacts at 100% with α-MSH, 24.8% with ACTH 1–13, 27.1% with di-acetyl α-MSH, and less than 0.1% with ACTH and its fragments. RIA was performed using 100 μL of M2 serum at 1/15000 final dilution, 25 μL of iodinated tracer (3,000 cpm), and 100 μL of sample. The incubation lasted 48 h at 4°C and free from antibody-bound tracer was separated using polyethylene glycol 18%. Sensitivity was 1.0 fmol/tube. Intraassay coefficient of variation was 6.6 ± 1.0% at 4 fmol, 5.4 ± 1.2 at 32 fmol, and 5.2 ± 0.8 at 128 fmol. Interassay coefficient of variation at medium dose was 11.9 ± 1.7%.

Table 1. *Clinical and auxological data*

No.	Sex	Age	BMI*	Breast	Pubic hair	Testis (mL)
Prepubertal						
1	F	5.06	15	1	1	
2	F	5.09	13	1	1	
3	M	6.00	15	1	1	2
4	F	6.06	15	1	1	
5	M	7.10	14	1	1	1
6	F	8.02	19	1	1	
7	F	8.08	15	1	1	
8	M	8.11	21	1	1	1
9	F	8.11	14	1	1	
10	M	9.00	14	1	1	1
11	M	11.09	14	1	1	2
12	M	12.03	28	1	1	2
13	F	10.03	22	1	1	
Pubertal						
1	M	10.02	15	1	3	5
2	F	10.06	15	3	3	
3	M	11.06	19	1	3	15
4	M	12.01	20	1	4	17
5	M	12.10	17	1	3	6
6	F	13.00	16	3	3	
7	M	13.08	14	1	3	5
8	M	14.00	14	1	3	8
9	M	15.01	17	1	3	6
10	M	16.03	21	1	3	12
11	F	14.04	18	3	4	
12	F	12.02	27	3	3	

* BMI, body mass index.

Statistical analysis. Data are reported as median (with 25th and 75th centiles, in brackets) for peptides concentrations. DHEA-S levels are reported as mean ± SD. Comparison between groups (prepubertal *versus* pubertal children) was made using Kruskal-Wallis test (Z score). Wilcoxon test was applied for paired observations. Correlations were evaluated by using the Spearman rank correlation test.

RESULTS

A chromatographic profile of α-MSH immunoreactivity in a pool of subjects' plasma revealed the existence of two main peaks corresponding to the elution position of authentic α-MSH and ACTH 1–13. Few, if any, immunoreactivity corresponds to the elution of diacetyl α-MSH.
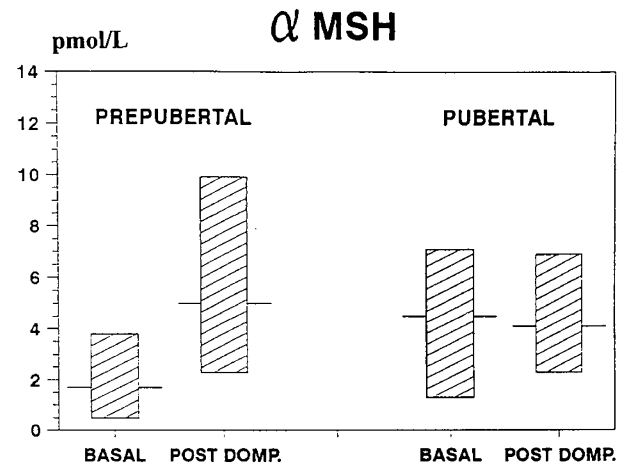
Individual data are reported in Table 2. In prepubertal children α-MSH increased from 1.7 (0.5–3.8) (median with quartiles in brackets) to 5.0 (2.3–9.9) pmol/L (Z = 3.05, p = 0.02), whereas no changes in peptide levels were found in pubertal subjects [(from 4.5 (1.3–7.1) to 4.1 (2.3–6.9) pmol/L, Z = 0.18)] (Fig. 1). Similarly, ACTH 1–13 levels in the same subjects increased from 3.0 (1.7–8.2) to 19.8 (4.5–33.2) pmol/L (Z = 2.87, p = 0.004) in prepubertal children, remaining stable in pubertal ones [(from 7.8 (3.3–12.7) to 4.6 (2.2–12.4) pmol/L, Z = 0.09)] (Fig. 1). According to the Kruskal-Wallis results, baseline MSH and ACTH 1–13 levels do not differ between prepubetal and pubertal subjects. In basal conditions, the ACTH 1–13/α-MSH ratio was similar in prepuber-
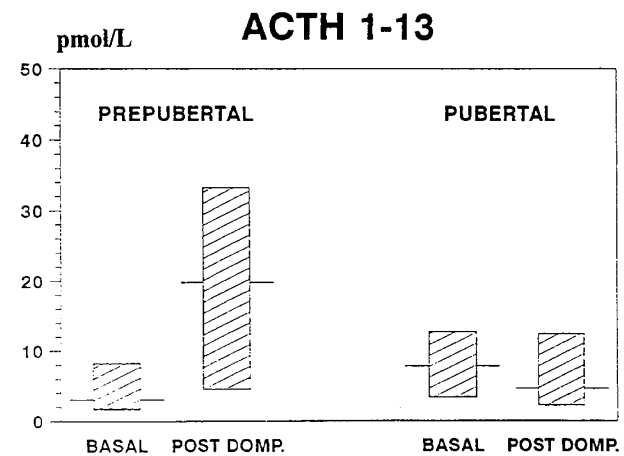

Figure 1. Median (*line*) and both 25th and 75th centiles (*bars*) of α-MSH and ACTH 1–13 plasma levels changes in prepubertal and pubertal subjects, before and after domperidone stimulation (*post domp.*). For statistical evaluation, see "Results."

tal (2.78 ± 1.90) and pubertal (2.17 ± 1.62) subjects. This finding was replicated in poststimulation samples (prepuberty, 2.96 ± 1.35; puberty, 1.89 ± 1.45).

Baseline concentrations of prolactin and cortisol were similar among groups, whereas DHEA-S plasma levels were lower in prepubertal (43.1 ± 38.5 μg/L) than in pubertal subjects (443.8 ± 356.1 μg/L, p = 0.006). No correlations between plasma DHEA-S levels and both α-MSH and ACTH 1–13 levels in basal condition and after domperidone stimulation were found. A significant negative correlation was found between the net increase of α-MSH (poststimulation minus basal level) and the basal DHEA-S levels (r = −0.501, p < 0.05).

DISCUSSION

The processing of children plasma through HPLC and RIA allows the identification of α-MSH and related peptides. Thus, besides fetal (12) and adult life (5–8) these data demonstrate the existence of circulating α-MSH-like peptides in the prepu-

Table 2. *Chromatographic profile*

No.	Sex	α-MSH		ACTH 1–13	
		Basal	Post domperidone	Basal	Post domperidone
Prepubertal					
1	F	9.2	28.3	7.6	35.3
2	F	5.0	4.8	9.8	10.6
3	M	3.5	7.7	23.8	21.4
4	F	0.5*	2.1	1.4	4.8
5	M	1.7	5.0	1.6	21.4
6	F	1.6	3.9	8.3	10.7
7	F	0.5	2.5	1.2	8.4
8	M	0.5	0.5	1.8	3.2
9	F	0.5	2.4	2.6	4.2
10	M	1.5	1.6	3.0	3.6
11	M	3.1	9.3	6.8	19.8
12	M	2.1	10.5	2.1	41.5
13	F	4.1	9.3	8.1	31.0
Pubertal					
1	M	1.2	1.7	1.0	4.4
2	F	5.0	4.8	9.8	10.6
3	M	1.7	12.8	8.0	9.1
4	M	10.0	15.1	7.5	2.6
5	M	0.5	0.5	2.8	1.4
6	F	13.9	5.7	17.9	27.6
7	M	7.5	3.4	15.2	2.0
8	M	4.4	2.1	7.4	3.7
9	M	3.1	7.3	10.5	13.0
10	M	5.9	4.3	13.4	16.4
11	F	1.0	2.7	0.5	0.5
12	F	4.6	3.8	4.6	4.7

Values are expressed as pmol/L.
* Samples below the limits of detection were assigned the value of 0.5.

bertal period. From the chromatographic profile and the quantitative data it appears that the des-acetylated form of α-MSH, called ACTH 1–13, predominates over the authentic molecule. This observation agrees with a series of studies in which the amount of these peptides in amniotic fluid (13), fetal pituitary (14, 15), fetal (12), and adult plasma was determined (5–8). Therefore, it can be concluded that in physiologic conditions, the posttranslational processing allowing the acetylation of POMC-related peptides is of minor importance, in humans.

The source of circulating MSH-like peptides in humans is not clear. Coates *et al.* (16) showed that ACTH 1–13 can be synthesized by corticotropes from both anterior and intermediate lobe. However, using immunohistochemistry they were unable to find authentic α-MSH in fetal and adult pituitary extracts. These findings are in contrast with the results of a study carried out with HPLC coupled to RIA in which the fetal pituitary extracts were shown to contain significant amount of both α-MSH and ACTH 1–13, this latter being the main form. Moreover, the pituitary content of both peptides seems responsive to the mode of delivery suggesting that a melanotropic stress-related circuitry is already established from mid-gestation (15). Domperidone, an antagonist of dopamine receptors, was able to stimulate both α-MSH and ACTH 1–13 plasma levels, in the present study, in agreement with the well-known inhibitory role exerted by dopamine on α-MSH release in different species (11, 17, 18). However, domperidone-induced melanotropic hormone release is evident in prepubertal children although absent in pubertal boys and girls, suggesting an enhanced dopaminergic tone and/or an increased sensitivity to dopamine antagonist, in prepubertal life. Considering that in lower mammals dopamine acts on the intermediate pituitary lobe for the inhibition of α-MSH, our data could led to the hypothesis that remnants of the fetal pituitary intermediate cell layer are still functioning during prepubertal life.

Whatever the reason, the prompt and significant release of α-MSH related peptides observed in prepubertal children could be related to the endocrine effects displayed by melanotropic hormones. In fact, α-MSH exerts a gonadotropin releasing activity in patients with functional hypothalamic hypogonadotropic amenorrhea, a pathologic condition sharing the hypogonadic state with prepubertal subjects (19). Moreover, domperidone treatment of sexually immature dogs stimulated the growth of adrenal zona reticularis and was accompanied by the enhancement of delta-5 androgen response to ACTH (20). Confirming the *in vitro* steroidogenic effects of melanotropic hormones, such a treatment was accompanied by an increase in the ACTH 1–13 pituitary content (21). A similar trophic effect of α-MSH on adrenal gland was also reported in the fetus (22). Considering the negative correlation existing between DHEA-S and α-MSH response to domperidone, the actual study suggests that a role for α-MSH could possibly be hypothesized also for human adrenarche (22).

In conclusion, apart from speculations on the possible participation of such peptides on the neuroendocrine development, these data demonstrate that the dopaminergic control of MSH-related peptides released from the anterior and intermediate pituitary lobes is modulated by pubertal development.

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