Differential Effect of Aging on Serum Levels of Prolactin and α-Melanotropin in Rats (43183)

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Abstract. **Prolactin (PRL) and α-melanocyte-stimulating hormone (α-MSH) are the only two pituitary hormones whose basal secretion is under tonic dopaminergic inhibition exerted by the hypothalamus. In the female rat, continuous exposure to estrogens is believed to depress hypothalamic dopaminergic activity and lead to the appearance of PRL-secreting pituitary adenomas during aging. Since there is no information about the impact of aging on circulating α-MSH levels, it was of interest to assess and compare the serum levels of PRL and α-MSH in male and female rats of different ages. Young (3–4 months) and old (24–25 months) male and female Sprague-Dawley rats as well as senescent (33–35 months) females were killed by decapitation between 10 AM and 1 PM, and pituitaries were immediately removed and dissected. Hormones were measured in unextracted trunk serum by radioimmunoassay. Serum PRL levels were ($\bar{X}$ ± SE), 18.4 ± 2.0, 26.8 ± 3.8, 19.8 ± 2.5, 43.0 ± 7.5, and 193.5 ± 47.6 ng/ml for young and old males, and young, old, and senescent females, respectively. Serum α-MSH levels were 243.2 ± 15.2, 252.9 ± 24.8, 320.0 ± 31.3, 234.7 ± 19.1, and 374.0 ± 29.7 pg/ml for young and old males, and young, old and senescent females, respectively. Anterior pituitary and neurointermediate lobe weights increased significantly with age in both sexes, although the change was particularly conspicuous in the females. We conclude that aging does not have a major impact on circulating α-MSH levels in rats and that melanotrophs probably have a greater ability than prolactotrophs to withstand age-associated alterations in central regulatory mechanisms.** [P.S.E.B.M. 1991, Vol 196]

The more striking neuroendocrine alteration associated with aging in female, and to a lesser extent in male, rats is the development of prolactin (PRL)-secreting pituitary adenomas (1). It now seems clear that chronic exposure to endogenous estrogens is the key etiologic factor of this pathology. Thus, ovariectomy in young rats prevents both the occurrence of prolactinomas and the age-associated hyperprolactinemia which typically develops during the constant estrus stage in intact animals (2–4). Estrogens affect PRL secretion by at least two mechanisms: (i) by a direct stimulatory action on the prolactotrophs (5) and (ii) by depressing the activity of the tuberoinfundibular dopaminergic neurons of the arcuate nucleus which exert a tonic inhibitory control on PRL secretion (6). Interestingly, there is another pituitary hormone, namely, α-melanocyte-stimulating hormone (α-MSH), which is also under tonic dopaminergic inhibition exerted by neurons of the arcuate nucleus (7). Furthermore, like PRL, pituitary α-MSH secretion is also stimulated by estrogens and depressed by ovariectomy (8).

In view of the above similarities in the regulation of these two hormones, and taking into account that there is, to our knowledge, no information about the impact of aging on the circulating levels of α-MSH, it was of interest to assess and compare the age changes in serum levels of PRL and α-MSH in rats of both sexes.

Materials and Methods

Animals. Young (3–4 months), old (24–26 months), and senescent (33–35 months) female, and young (3–4 months) and old (24–26 months) male Sprague-Dawley rats were obtained from Harlan Industries (Indianapolis, IN). The old and senescent animals

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were purchased as retired breeders at 8–10 months of age and maintained in our animal facilities until they reached the above ages. All animals were housed in a temperature-controlled room (22 ± 2°C) on a 14:10-hr light:dark cycle and subjected to regular handling. Food and water were available *ad libitum*. In our laboratory the mean life span of Sprague-Dawley rats is 26.4 and 35.1 months for males and females, respectively, the more typical age-related pathologies being mammary and pituitary tumors in females and chronic progressive nephropathy in males. Mammary tumors were systematically removed to preserve the well being of the animals. All animals were necropsied at the end of the experiments described below. Rats showing overt signs of disease, detectable by gross examination, were not included in this study.

Experimental Procedures. Young and old animals were killed by decapitation in a staggered sequence between 10 AM and 1 PM, trunk blood was collected, and the serum obtained was stored at −25°C until ready for hormone determinations. Pituitaries were immediately dissected and weighed.

Hormones were measured by radioimmunoassay in unextracted serum. Prolactin was assayed in duplicate with the reagents provided by the NIDDKD. Iodination grade PRL was radiolabeled by a lactoperoxidase-glucose oxidase method. The iodinated hormone was purified on a 1.5- × 40-cm Sephacryl S-200 column equilibrated with 0.01 *M* phosphate-buffered saline at pH 7.6. IgGsorb (protein A; Enzyme Center, Malden, MA) was used to separate bound from free hormone. Rat PRL-RP-3 was used as standard, the average intraassay coefficient of variation being 9.3%.

α-MSH was assayed according to a modified version of a previously described protocol (9). ^{125}I-α-MSH was puchased from Melloy Laboratories (Springfield, VA), while anti-α-MSH serum was kindly provided by Dr. Mark Goldman (Merck, Sharp & Dohme Co., West Point, PA). Synthetic α-MSH to be used as standard was purchased from Peninsula Laboratories (Palo Alto, CA). On the first day of the assay, α-MSH antiserum (final dilution 1/18,000), tracer, and standards or samples in assay buffer were added to plastic tubes and incubated at 4°C for 48 hr. The incubation was terminated by addition of 200 μl of dextran-coated charcoal suspended in 30% horse serum, 0.9% NaCl. After 15-min incubation at 0°C, tubes were centrifuged for 15 min at 4°C and the radioactivity in the pellets counted. When stored frozen in distilled water, the α-MSH tracer remains stable for 3 months. Samples were assayed in duplicate and, when necessary, at several dilutions to ensure that sample values were obtained from the linear portion of the standard curve (10–300 pg of α-MSH). The α-MSH antiserum used is specific for the C terminus of α-MSH and does not cross-react with the following ACTH fragments: ACTH(1–24), ACTH(1–16), ACTH(4–10), ACTH(25–38), and ACTH(11–24). It slightly cross-reacts (1%) with ACTH(1–10). The intraassay coefficient of variation for α-MSH was 5.0%.

Statistical Analysis. All statistical analyses were performed with an HP-41 CX computer (Hewlett-Packard, Corvallis, OR) using specific programs for one-way analysis of variance (10) and Duncan's multiple range test (11).

Results

The effects of age on the serum levels of PRL and α-MSH in male and female rats are shown in Figure 1. The young and old animals were killed in a staggered sequence (i.e., a young male, then a young female, then an old male, then an old female, then a senescent female, then a young male, and so on). Therefore, the indicated values represent average hormone concentrations between 10 AM and 1 PM. Old males did not show significant differences in the serum levels of either PRL or α-MSH as compared with their young counterparts. As expected, serum PRL increased markedly with age in females (young versus old versus senescent, $P < 0.001$). On their part, serum α-MSH levels showed a bimodal age change in females, with a moderate but significant decline around the second year of life (young versus old, $P < 0.05$) followed by a full recovery by the third year of age (old versus senescent, $P < 0.01$).

The weights of the anterior pituitary and the neurointermediate lobe increased significantly with age in both males and females (Table I). The anterior pituitary size became particularly large in 33- to 35-month-old females.

Discussion

Our PRL data confirm a well-established sex difference which was also evident in the effect of aging on serum α-MSH levels. Thus, while serum α-MSH

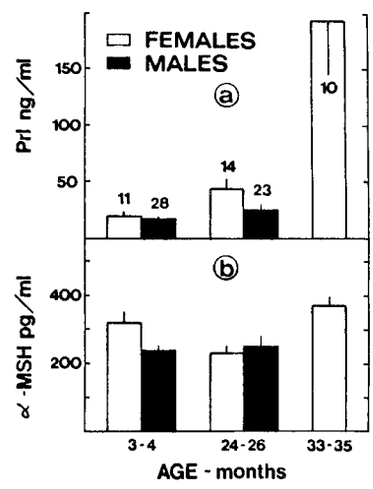

Figure 1. Serum levels of PRL (a) and α-MSH (b) in male and female rats of different ages. Numbers above columns represent number of rats per group. Bars above columns represent SEM.

Table I. Pituitary Weights in Male and Female Rats of Different Ages

Age (months)	Males		Females	
	Anterior pituitary	Neurointermediate lobe	Anterior pituitary	Neurointermediate lobe
3–4	8.7 ± 0.2 (28)[a]	1.7 ± 0.4 (20)	9.8 ± 0.5 (11)	1.8 ± 0.5 (11)
24–26	11.5 ± 0.4 (23)	2.5 ± 0.6 (18)	22.5 ± 3.2 (14)	2.9 ± 0.8 (14)
	$P < 0.001$[b]	$P < 0.001$	$P < 0.01$	$0.05 < P < 0.10$
33–35	—	—	41.3 ± 10.7 (10)	4.3 ± 1.4 (10)
			$P < 0.01$	$P < 0.01$

[a] Pituitary weights (mg) are expressed as $\bar{X}$ ± SE (sample size).
[b] Probability values represent the level of significance of the difference from the corresponding young counterpart.

showed almost no age-related change in males up to 2 years, females showed a moderate reduction in α-MSH levels around the second year of life that was followed by a full recovery by the third year of age. A comparison between the age changes in PRL and α-MSH serum levels in our females suggests that aging has a different impact on the regulatory mechanisms controlling the circulating levels of these two hormones. Unlike prolactotrophs, the rat melanotrophs are able to reestablish basal secretory levels very quickly after disconnection from hypothalamic control (12) and, similarly, dopamine antagonists evoke only a short-lasting elevation of plasma α-MSH (13). It seems therefore reasonable to hypothesize that the absence of major changes in the basal levels of α-MSH during aging in females may be due, at least in part, to the ability of the pars intermedia for compensating alterations in central regulatory mechanisms (especially in tuberoinfundibular dopaminergic activity).

It is of interest to mention that although our data show little change in serum α-MSH during aging, it has been reported that brain α-MSH content diminishes markedly in old rats (14, 15). The content of another pro-opiomelanocortin-derived peptide, β-endorphin, has also been reported to decline in the hypothalamus but increase in the pituitary and plasma of old rats (16, 17). A similar situation has been reported in female mice where the content of pro-opiomelanocortin mRNA was reported to decrease in the hypothalamus, but not in the pituitary of old animals (18). Thus, it seems that, at least in rodents, the synthesis and/or processing of pro-opiomelanocortin-derived peptides is differently affected by aging in brain and pituitary.

The age-related increase in both anterior pituitary and neurointermediate lobe weights reported here is in agreement with data previously reported in Wistar/Tw and Sprague-Dawley/Tw rats ranging from 3 to 17 to 18 months of age (19). In this study the relative volume of the pars intermedia was reported not to change significantly with age in either strain. We have recently reported a significant correlation between anterior pituitary weight and serum PRL levels in our old and senescent animals (20). In the same study we found a significant decline of serum testosterone in old males but not of estradiol or progesterone in old and senescent females, which usually are in constant estrus.

We conclude that aging does not have a major impact on circulating α-MSH levels in rats. Whether the same is true for other MSH peptides (e.g., β-MSH and γ-MSH) remains to be assessed.

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