Pituitary growth hormone and hypothalamic somatostatin in young female rats versus old constant estrous female rats[1]

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Summary. Pituitary content and concentration of growth hormone was significantly reduced, and hypothalamic somatostatin content significantly increased, in old constant estrous as compared to young female rats. Increased levels of somatostatin may contribute to the decrease in pituitary growth hormone levels in these animals.
Key words. Aging; growth hormone; somatostatin.

In young male[3] and female[4,5] rats, the release of growth hormone (GH) from the pituitary into the plasma occurs in secretory bursts. Recent evidence suggests that the release of growth hormone from the pituitary is regulated, in part, by the hypothalamic GH release inhibiting hormone, somatostatin[6,7]. In old male rats, the amplitude of GH pulses is diminished as is the content and concentration of GH in the pituitary. Also characteristic of aged male rats is an increase in the content of somatostatin in the hypothalamus[8]. This increase in hypothalamic somatostatin may contribute to the decrease in pituitary and plasma GH levels in these old animals.

In male and female rats, gonadal steroids appear to participate in the regulation of GH release from the pituitary[9]. As female rats age, regular estrous cycles become lengthened and irregular, and are followed by constant vaginal estrus or prolonged recurrent pseudopregnancies[10]. Old female rats in constant estrus (CE) are characterized by a persistant elevation in plasma levels of estrogen[11], unlike the aged male rat, in which gonadal steroid levels are reduced[12]. These observations suggest that the age-related changes in the regulation of GH release may differ according to the sex of the animal. In the present study, the content and concentration of pituitary GH were compared in young female rats and old female rats in constant estrus. Also compared in the two age groups was the content of somatostatin in the hypothalamus. Similarities and differences between the present observations and those made in aging male rats, are discussed.

Materials and methods. Young (3–4 months of age) and old (16–20 months of age) female Long-Evans rats were used in the present study. The old female rats were obtained as retired breeders (8 months of age) and were maintained in our animal quarters until they reached 16–20 months of age. All animals were housed in a temperature-controlled room (22±2 °C) and were kept on a 14:10 light/dark cycle (lights on at 05.00 h). Food and water were available ad libitum. Daily vaginal smears were obtained from young and old female rats for 1 month prior to the initiation of each experiment. Young female rats demonstrating regular 4–5 day vaginal estrous cycles were selected to comprise the young control group. They were compared with old female rats which demonstrated periods of prolonged vaginal estrus. All animals were killed between 10.00 and 12.00 h. Young female rats were killed randomly during the estrous cycle since pituitary[13] and plasma[4,5] GH levels do not appear to vary during the cycle.

Pituitaries were removed and homogenized in 2 ml of 0.05 M sodium bicarbonate buffer (pH 8.2) and frozen at −20 °C until assayed for GH. Protein was determined by the method of Lowry et al.[14]. Immediately before assay, the homogenates were centrifuged at 2000 × g for 15 min, and the supernatant was diluted in 0.02 M phosphate buffered saline containing 1% bovine serum albumin (pH 7.6). At the time of decapitation, the whole brain was quickly frozen on dry ice and stored at −20 °C. The brains were subsequently thawed and the hypothalami were dissected into rostral and caudal hypothalamic areas by cutting coronally 3 mm anterior to the optic chiasm, rostral to the emerging median eminence, and at the mammilary bodies. Sections were bordered laterally by the lateral hypothalamic sulci and dorsally by a cut at the top of the 3rd ventricle. The rostral area included the organuum vasculosum of the laminae terminalis, the preoptic area, the supraoptic nucleus, the suprachiasmatic nucleus and the anterior hypothalamic area. The caudal area included the median eminence, the infundibular stalk, the arcuate nucleus, the ventromedial nucleus and the dorsomedial nucleus. Tissues were homogenized in cold 0.2 N acetic acid and immersed in boiling water for 5 min. The extracts were stored at −20 °C until ready for RIA. Immediately before assay, samples were centrifuged at 2000 × g for 15 min, and the supernatant was diluted in 0.05 M phosphate buffer, 0.08 M NaCl, 0.025 M EDTA, 0.02% NaN_3, pH 7.5.

Plasma and pituitary GH were measured with materials provided by Dr A. Parlow (NIADDK, Bethesda, MD) and the data were expressed in terms of GH-RP-1. A detailed description of this assay has been reported elsewhere[8]. Hypothalamic somatostatin was assayed after the method of Arimura et al.[15], who also supplied the antibody to somatostatin. A complete description of the assay appears in an earlier report[8]. Differences in pituitary GH content and concentration, and hypothalamic somatostatin content in young and old rats were tested using Student's t-test.

Results and discussion. The content and concentration of GH were greater ($p < 0.05$) in the pituitaries of young than of old CE female rats (table 1), in agreement with the earlier report of Dickerman et al.[13]. These data suggest that the synthesis of GH by the pituitary of the old CE female rat is reduced. The content of somatostatin was significantly greater ($p < 0.05$) in the rostral portion of the hypothalamus of old CE as compared to young female rats. There was no significant difference in somatostatin content in the caudal region of the hypothalamus in young and old animals (table 2). It is possible that increased levels of hypothalamic somatostatin may contribute to the decrease in pituitary GH in the old CE female rats.

Table 1. Pituitary content and concentrations of GH in young cycling and old constant estrous female rats

Group	N	μg GH/pituitary[c]	μg GH/mg protein[c]
Young[a]	9	1335 ± 79	484 ± 34
Old[b]	9	777 ± 66[d]	228 ± 15[d]

[a] Young female rats demonstrating 4–5 day vaginal estrous cycles. [b] Old female rats in constant estrus. [c] Each value represents the mean ± 1 SE. [d] Significantly diminished when compared to young female rats ($p < 0.05$).

Table 2. Hypothalamic content of somatostatin in young cycling and old constant estrous female rats

Group	N	Rostral[c] (ng/section)	Caudal[c] (ng/section)
Young[a]	9	13.7 ± 0.8	44.3 ± 2.3
Old[b]	9	17.1 ± 0.7[d]	36.1 ± 1.9

[a] Young female rats demonstrating 4–5 day vaginal estrous cycles. [b] Old female rats in constant estrus. [c] Each value represents the mean ± 1 SE. [d] Significantly greater ($p < 0.05$) as compared to young female rats.

However, before a causal relationship between the increase in hypothalamic somatostatin and the decrease in pituitary GH can be suggested with certainty, the release of somatostatin from the hypothalmus will have to be measured.

Plasma levels of GH in old female rats have been reported to be decreased[13] or unchanged[16] when compared to young female rats. The reason for this difference may reside in the fact that neither study accounted for the pulsatile release of GH into the plasma, by measuring GH levels in the same animal over time, as was done in aged male rats[8]. A decrease in plasma levels of GH in old CE female rats is suggested by the growth stasis observed in these old animals[17] which preliminary findings suggest can be reversed by the administration of exogenous GH (Forman, Sonntag and Meites, unpublished observation).

Sonntag et al.[18] have reported that various centrally acting drugs increase GH to a greater extent in young as compared to old male rats. In addition, these investigators reported that systemic administration of growth hormone releasing factor (hp-GRF) increased plasma levels of GH more in young than in old animals[19]. By contrast, passive immunization with antiserum to somatostatin increased GH levels to the same or greater extent in old than in young animals[18]. Moreover, when pituitaries from young and old rats were exposed to hp-GRF in vitro, the release of GH from these pituitaries was essentially the same[19]. These data appear to indicate that the decreased release of GH characterizing aged male rats may be attributed to the observed increase in hypothalamic somatostatin[8].

Similar to the aged male rat, centrally acting drugs were observed to increase plasma levels of GH to a greater extent in young than in aged CE female rats (Forman, Sonntag and Meites unpublished observation). The decreased ability of old CE female rats as compared to young female rats to increase plasma levels of GH may partially be attributed to the decrease in pituitary GH levels, and the increase in hypothalamic somatostatin levels observed in these old animals in the present study.

Thus in the present study, hypothalamic somatostatin was increased and pituitary GH decreased, in old CE as compared to young female rats. Furthermore, these age-related alterations in the hypothalamic-pituitary unit in the old CE female rat, appear to be similar to those changes observed in aged male rats.

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An *Anolis* skin melanophore assay suitable for photoaffinity labeling studies with α-MSH[1]

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Summary. Visual determination of MSH-induced pigment migration in melanophores of small pieces of *Anolis carolinensis* skin is standardized by first measuring photoelectrometrically the change in reflection/transmission of the whole dorsal skin in response to different hormone concentrations. This method allows the rapid and precise recording of time-response curves after photoaffinity labeling of MSH receptors or of dose-response curves of large series of synthetic compounds.
Key words. α-MSH; bioassay; *Anolis carolinensis;* melanophores; photoaffinity labeling.

In vitro bioassays for the measurement of melanocyte-stimulating hormones (MSHs) are based on the ability of these peptides to induce pigment dispersion in the melanophores of amphibian *(Rana, Xenopus)* or reptilian *(Anolis)* skin. The biological response is usually quantitated photoelectrometrically *(→Rana pipiens)* by measuring the reflectance change of the isolated skin[2-4] or microscopically *(→Xenopus laevis)* by visual comparison of the degree of pigment dispersion with that of the Hogben-Slome index[5,6]. This latter method is very rapid and although subjective, is precise as demonstrated recently with a new assay using tail-fin pieces of *Xenopus* tadpoles[7].

Pigment dispersion in melanophores of *Anolis carolinensis* skin cannot easily be observed under the microscope. The skin has, however, the advantage that it changes color from bright green to dark brown in response to increasing hormone concentrations. This color change forms the basis for the quantal *Anolis* skin assay[8,9] in which the degree of pigment dispersion in small skin pieces is assessed visually by comparison with a standard series. The method is particularly useful for titrating the lowest hormone concentration that produces a response, but it is not suitable for constructing dose-response curves. So far the latter could only be obtained with the reflectometric assay[10,11] or with the rate assay[12] which measures the time for the generation of the response. Since neither method is suitable for recording time-response curves of large series of skin pieces, we have developed an assay in which the entire dorsal skin of each