Sexually Dimorphic Effects of Aging on Rat Somatotroph Cells. An Immunohistochemical and Ultrastructural Study

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ABSTRACT. Aging produces alterations in certain functions of the hypothalomo-pituitary axis that result in sexually dimorphic changes in the somatotrophs. Since quantitative morphological data on these age-associated alterations are scarce, we prompted to make a morphometric immunohistochemical assessment as well as undertake an ultrastructural study of the somatotrophic (GH) cell population in male and female rats of different ages. Young (3-month-old), old (20-month-old), and senescent (29-month-old) Sprague-Dawley rats of both sexes were sacrificed by rapid decapitation, their pituitaries immediately dissected out, and processed for both immunohistochemistry and electron microscopy. Analysis of different morphometric parameters revealed that surface density, volume density, and cell density significantly decreased in old and senescent rats as compared to young animals, with this reduction being clearly more marked in females. Both the GH-cell area and perimeter decreased in senescent male rats, while these parameters increased in senescent females. The ultrastructure of the GH cells from old and senescent animals of both sexes evinced changes suggestive of an immature state, with some male and female rats of different ages. Young (3-month-old), old (20-month-old), and senescent (29-month-old) Sprague-Dawley rats of both sexes were sacrificed by rapid decapitation, their pituitaries immediately dissected out, and processed for both immunohistochemistry and electron microscopy. Analysis of different morphometric parameters revealed that surface density, volume density, and cell density significantly decreased in old and senescent rats as compared to young animals, with this reduction being clearly more marked in females. Both the GH-cell area and perimeter decreased in senescent male rats, while these parameters increased in senescent females. The ultrastructure of the GH cells from old and senescent animals of both sexes evinced changes suggestive of an immature state, with some somatotrophs having the appearance of cells undergoing an involutive process. We conclude that aging has a differential impact on the GH cells of male and female rats with respect to the immunohistochemical and ultrastructural features of that cell population.

— KEY WORDS: aging, pituitary gland, quantitative immunohistochemistry, sexual dimorphism, somatotroph.


MATERIALS AND METHODS

Animals and specimen collection: Young (3-month-old), old (20-month-old), and senescent (29-month-old) male and female Sprague-Dawley rats were kindly provided by Bagó Pharmaceuticals, City Bell, Argentina. Animals were housed in a temperature-controlled room (22 ± 2°C) on a 14- to 10-hr light-dark cycle. Food and water were available ad libitum. Rats were sacrificed by rapid decapitation, their pituitary glands dissected out immediately, and processed for light and electron microscopy. Maintenance and treatment of animals were in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals.

Immunohistochemistry and morphometry: Pituitaries from 5 young, 4 old, and 4 senescent rats of each sex were fixed in Bouin’s fluid and embedded in paraffin. Serial sections (4 µm) were made at different levels of the blocks in a ventral-to-dorsal sequence and were immunostained by means of a streptavidin-biotin-peroxidase system. Stated in brief, sections were incubated for 2 hr at room temperature with 1:50 rabbit anti (rat GH) serum, kindly provided by the National Hormone and Pituitary Program, NIH, U.S.A. Thoroughly-washed sections were then treated for 1 hr with a ready-to-use multilinker, biotin-bound goat anti (rabbit IgG) serum (Biogenex, San Ramon, CA, U.S.A.), rewashed, and finally incubated for 1 hr with a ready-to-use streptavidin-peroxidase reagent from the same source. The peroxide-sensitive chromogen was diaminobenzidine. The specificity of the primary antiserum was monitored by the ability to block the immunocytochemical reaction by...
preabsorption of the rabbit antibody with an excess amount of rat GH.

In each section of the pars distalis, micrographs of all the areas containing somatotrophs were taken under a × 20 objective and enlarged up to × 750. Measurements were made by means of an image-analysis system (Mini Mop Evaluation Unit, Kontron Bildanalyse). Both the immunostained cells and the measurement-reference area were quantified by tracing the outlines of them with the stylus of a Zeiss Videoplan. For each field, an average of six micrographs from different levels (ventral, medial, and dorsal) was scanned. Between 2,500 and 3,000 GH cells were scored for each pituitary. These measurements were recorded and processed automatically by the evaluation unit attached to the Videoplan in order to calculate the following parameters [5]: reference area (RA), number of cells (N), cell area (A), cell perimeter (P), cell density (CD=N/RA), cell volume density (VD=∑A/RA), and surface density [SD=4πN(∑P/RA)].

RA represents the adenohypophyseal (pars distalis) area scanned, in which GH cells were scored. N means the absolute number of GH cells counted. Then, cell density (CD) represents the relative number of GH cells referred to as RA, in order to establish actual differences between groups. Moreover, with the sum (Σ) of the individual areas (A), referred to as RA, we obtained VD, which indicates cell mass according to a usually accepted concept. Finally, the sum of the individual perimeter (P), also referred to as RA, was used to obtain SD, as a complement of VD.

Electron microscopy: Pituitary glands from 4 animals from each group mentioned above were processed for ultrastructural examination. The anterior lobe was dissected away and fixed in 2% (v/v) glutaraldehyde in 0.05 M cacodylate buffer. The material was cut into small pieces (1 × 1 mm), postfixed in 1% (w/v) osmium tetroxide, and embedded in Araldite. Thin sections (about 1 µm) were stained with toluidine blue and inspected by light microscopy in order to select fields. Ultrathin sections were mounted on 200-mesh copper grid, stained with uranyl acetate and lead citrate, and examined in a JEM-1200 EX transmission electron microscope at 80 kV.

Immunoelectron microscopy: Ultrathin sections from the Araldite-embedded blocks were mounted on 200-mesh nickel grids. Tissues were etched with 10% (v/v) hydrogen peroxide for 10 min. For GH detection, the grids were incubated in anti (rat GH) serum (NIADDK, Bethesda, Maryland, U.S.A.) at a dilution of 1:200 in phosphate buffer containing 1% (v/v) bovine-serum albumin. After several washings with this supplemented buffer, immunoreactive sites were labelled by means of the protein A-colloidal gold technique (20-nm particles; EY Laboratories, San Mateo, CA, U.S.A.) for 1 hr at room temperature. The grids were then stained with uranyl acetate and lead citrate.

For both light and electron immunocytochemistry, the specificity of the staining was monitored as follows: (1) incubation with normal rabbit serum instead of anti (rat GH) serum, (2) preabsorption of the antiserum with rat GH (NIADDK rGH Y-5, 50 µg/ml of diluted serum) at 24 hr before immunostaining. No immunostaining was observed in these controls.

Statistical analysis: Data were expressed as the mean ± SEM. Statistical comparisons among age groups were performed by ANOVA followed by Duncan’s multiple-range test, when appropriate.

RESULTS

Age-associated quantitative immunohistochemical changes: Immunostained GH cells stood out in sharp relief, exhibiting a definite granular cytoplasmic pattern. In all the sections of the pars distalis, these cells showed a homogeneous distribution in all three age groups.

Both age- and sex-associated variations were observed in the GH-cell population (Fig. 1). In both sexes, a decreased number of somatotrophs were found in old (b, e) and senescent (c, f) animals relative to the young (a, d) ones, but this decrease being more marked in females than in males. All of these initial observations were confirmed statistically when CD values were obtained.

Analysis of the morphometric parameters revealed age-related changes in the GH cells from animals of both sexes (Figs. 2–6). The SD, VD and CD decreased significantly in the old and senescent animals of both sexes compared to the young ones. By contrast, although the values for cell A and P decreased significantly in senescent males, these same parameters were found to be increased in senescent females.

Moreover, when the effect of aging was compared between sexes, clear dimorphic changes within the GH-cell population also became evident. The parameters SD, VD, and CD within each age group were lower in females than in males. Conversely, the cell A and P values for the senescent animals were found to be higher in the females than in the males. The p value was <0.01 for all the observed differences.

Age-associated ultrastructural changes: The immunocytochemical analyses revealed two types of GH cells: type I contained large secretory granules with a diameter of 250–400 nm (Fig. 7), while type II possessed these large granules as well as small (100–150 nm) ones (Fig. 8). Type-I GH cells were more prevalent than type II in young male rats, while type-II cells were predominant in senescent animals of both sexes, though more so in females.

In the young animals of both sexes (Fig. 9), most of the somatotrophs were ovoid or round in shape. The nucleus was ovoid and usually occupied an eccentric position. The secretory granules were numerous, round, and very dense; they ranged in size from 100 to 400 nm, and were scattered throughout the cytoplasm. Although a number of granules could be seen in contact with the plasma membrane, secretory material extruding from the cell was not a common finding. A well-developed Golgi complex occupied a zone near the nucleus. The rough endoplasmic reticulum (RER) was composed of long parallel flattened sacs, although sometimes these structures were moderately dilated. Round
or oval mitochondria, occasional lysosomes, and abundant free ribosomes could also be observed.

In old rats, distinct changes could be found in a number of the GH cells (Fig. 10). The Golgi complex appeared widely extended, with dilated cisternae and immature granules of variable density, as well as lipid droplets. The RER displayed highly-dilated cisternae containing visible material in the interior. The secretory granules were less numerous and mainly located near the plasma membrane. In addition, some GH cells undergoing an involutive process were also detected: they consisted of dark elements containing an irregular and electron-dense nucleus. In some of these cells, both the Golgi complex and the RER exhibited remarkably dilated cisternae. Scarce dense secretory granules and crinophagic profiles could also be encountered.

In the senescent rats (Fig. 11), the age-associated ultrastructural changes described above for GH cells were increased in both frequency and intensity. In the somatotrophs of this group, the most conspicuous characteristics were the following: (1) Golgi complex with dilation of cisternae and increase in immature granules, (2) expansion and remarkable dilation of the RER, (3)
mitochondrial swelling, (4) increased lysosomal activity and crinophagy, and (5) presence of dark involutive cells.

DISCUSSION

A hormonal disbalance, characterized by decrease in GH-RH and increase in GIIH, together with a higher sensitivity of the pituitary to the latter, appears to occur in aged rats of both sexes [8, 11, 12, 14]. In addition, the reduced response to GH-RH with respect to the output of GH observed in old rats probably results from both their limited hypophyseal GH content and the diminished secretion of that hormone [2, 13].

The hypothalamic GH-RH content and gh-rh gene expression have also been reported to be reduced in aged rats [3, 6]. Thus, this neuropeptide, a major mitogen for GH cells [1], appears to be a potential regulator of somatotroph-population size during aging. In this regard, changes in pituitary response distal to receptor binding also contribute to the diminution of hypophyseal sensitivity of GH-RH with age [14].

Senescence represents the terminal segment of a mammalian life span, a period where pathologic changes have as yet been little studied. Our morphometrical results have revealed a clear-cut decline in the SD, VD, and CD of the GH cells from old and senescent rats as compared to the data obtained in young animals. Moreover, among these parameters, the values measured in females were lower than those seen in males of the same advanced age, a finding constituting a well-defined expression of sexual dimorphism. Finally, both the A and the P of the somatotrophs were observed to be significantly decreased in the senescent male animals, whereas these values were higher in the comparably aged females—a finding indicative of cell hypertrophy, probably as a compensation for the more pronounced diminution in the number of GH cells in the latter sex.

The different parameters described here have not previously been investigated in senescent animals, only in young and old rats [9, 16, 17]. Accordingly, in that species [9], aging had been shown to produce a moderate rise in somatotroph GH content in males, with a decline in this parameter being seen in females. Moreover, the number of immunoreactive GH cells appeared to be reduced in animals
of both sexes. Likewise, in 12- and 18-month-old male or female rats, the VD of GH cells was seen to be decreased, consistent with the observed diminution in the percentage of immunoreactive GH cells [16–18]. This decrease, however, appeared more marked in the males than in the females, in disagreement with our present findings. In adult mice [10], distinct sex differences have been reported with respect to the proportion of somatotrophs within the

Fig. 7. Immunoelectron microscopy of a type I GH cell from a young male rat showing large secretory granules (×20,000). Insert: colloidal gold particles are selectively located in the secretory granules (×40,000).

Fig. 8. Immunoelectron microscopy of a type II GH cell from a young rat. Both large and small secretory granules are observed in the cytoplasm (×24,000). Insert: colloidal gold particles are labeling the secretory granules (×48,000).

Fig. 9. Ultrastructure of a GH cell from a young female rat showing the habitual characteristics (×18,000).
Fig. 10. Electron micrograph of a GH cell from an old male rat. The mitochondria are swollen and the Golgi complex (G) is dilated. The cytoplasm contains a diminished number of secretory granules. On the upper part, a very dense involutive cell can also be seen (× 15,000).

Fig. 11. Ultrastructure of a GH cell from a senescent female rat exhibiting swollen mitochondria (m) and a highly dilated endoplasmic reticulum (asterisks). A decreased number of secretory granules is also observed (× 19,000).
adenohypophysis, which proves to be more numerous in males than in females. Finally, the present results are consistent with the data documented in humans [15] that indicated a decrease in the number and size of GH cells with age.

Kurosumi et al. [4] described three types of GH cells in the rat: I, II, and III. Types II and III—considered to be intermediate and immature cell states, respectively—seem to increase gradually with age [16, 17]. Our results revealed only type-I and -II GH cells, with the latter being predominant in senescent rats, a stage in life which has not been studied previously. It is thus possible that the reduced stimulation by GH-RH with age causes a decrease in the number of type-I somatotrophs and that this effect, in turn, is more pronounced in females than in males.

We thus conclude that in rats the aging process produces a well-defined and sex-dependent impact on the immunohistochemistry and ultrastructure of the somatotroph population of the adenohypophysis, with these cells exhibiting conspicuous morphometric changes that become the most fully manifest in senescent animals.

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