Original Paper

Acta Anat 1995;152:28–32

G.M. Cónsole[a]
C.L.A. Gómez Dumm[a, b]
R.G. Goya[a, c]

[a] Cátedra de Histología y Embriología 'B',
[b] CENEXA, and
[c] INIBIOLP, Facultad de Ciencias Médicas, Universidad Nacional de La Plata, La Plata, Argentina

Immunohistochemical and Radioimmunological Assessment of Thyrotrophs in the Pituitary of Aging Rats

Key Words
Aging
Adenohypophysis
Pituitary gland
Thyrotroph
Quantitative immunohistochemistry

Abstract
Thyrotrophs were studied by quantitative immunohistochemistry in the pituitary gland of young (4 months), old (20 months) and very old (29 months) male rats. An attempt was also made to correlate morphometric parameters with serum levels of thyrotropin (TSH), thyroxine (T_4) and triiodothyronine (T_3). Cells were immunostained by the peroxidase-antiperoxidase method and hormones were measured in serum by specific radioimmunoassays. There was a marked age-related reduction in TSH cell number, volume density and surface density but a significant increase in TSH cell area and perimeter. Basal serum levels of TSH increased, T_4 decreased and T_3 remained unchanged with age. There was a highly significant ($p < 0.001$) negative correlation between serum TSH and T_4, but no significant correlation was found between TSH and morphometric parameters. The present results suggest that aged rats possess a reduced but functionally preserved thyrotrophic cell population. The coexistence of high circulating levels of TSH with reduced serum T_4 suggests that aging brings about a progressive desensitization of the thyroid to TSH.

Introduction

Pituitary enlargement is a common finding in old rats, particularly in females [Huang et al., 1976; Takahashi and Kawashima, 1983]. This change is usually associated with lactotroph hyperplasia or the presence of prolactin (PRL)-secreting adenomas or microadenomas [Berkvens et al., 1980; Takahashi and Kawashima, 1983; Nagatani et al., 1987]. It has been reported that approximately one-fourth of the pituitary adenomas of aging male Wistar rats contain growth hormone (GH)- and/or thyrotropin (TSH)-immunoreactive cells coexisting with the lactotrophs [Fong et al., 1982]. Pituitary adenomas containing all known adenohypophysial hormones, separately or in combination, have been reported in old Sprague-Dawley rats [McComb et al., 1984]. Although most rats do not develop pituitary adenomas during aging, normal pituitary morphology in aged rats has aroused much less interest than tumors. Thus, except for PRL [Takahashi and Kawashima, 1983] and GH [Cónsole et al., 1993], there is virtually no information regarding the impact of aging on specific cell populations in the nontumorous rat pituitary.

Received:
March 9, 1994
Accepted:
December 9, 1994

Rodolfo G. Goya
INIBIOLP
Facultad de Medicina, UNLP
CC 455
1900 La Plata (Argentina)

0001–5180/95/1521–0028
$ 8.00/0

In previous studies we showed that the progressive hypothyroidism that develops with age in rats is paralleled by an overall increase in TSH secretion and a loss of circadian rhythmicity in the plasma levels of this hormone [Goya et al., 1990]. Since these functional changes could reflect morphologic alterations at the pituitary level, it was of interest to perform a quantitative immunohistochemical assessment of the thyrotrophs in young, old and very old rats and to correlate morphometric parameters with serum levels of TSH and thyroid hormones. The present report describes our findings.

Materials and Methods

Animals and Specimen Collection

Young (4 months), old (20 months) and very old (29 months) male Sprague-Dawley rats were kindly provided by Bagó Pharmaceuticals, City Bell, Argentina. The number of animals varied according to the parameter assessed (immunohistochemistry or hormone radioimmunoassay, see tables). Animals were housed in a temperature-controlled room (22 ± 2 °C) on a 14:10-hour light/dark cycle. Food and water were available ad libitum. Rats were killed by decapitation, trunk blood was collected and the corresponding serum stored at −20 °C until hormone assays. Pituitaries were removed immediately and processed as described below.

Immunohistochemistry and Morphometry

Pituitaries were fixed in Bouin's fluid and embedded in paraffin. Serial sections (4 μm) were obtained at three different levels of the blocks following a ventral to dorsal sequence, and were immunostained by means of the peroxidase-antiperoxidase (PAP) technique for immunohistochemical identification of TSH [Nakane, 1970]. Briefly, sections were incubated for 2 h, at room temperature, with 1/100 rabbit anti-rat TSH serum (kindly provided by Dr. Salvatore Raiti from the National Hormone and Pituitary Program, NIDDK, NICHHD, USDA, USA). After washing, sections were treated for 1 h with 1/20 goat anti-rabbit IgG (Sigma Chemical Co., St. Louis, Mo., USA), washed again and incubated for 1 h with 1/100 PAP (Sigma). Diaminobenzidine was used as the chromogen. The specificity of the primary antiserum was assessed by blocking the immunocytochemical reaction by preabsorption with an excess of ovine TSH. A negative control was carried out by replacing the primary antiserum with normal rabbit serum. In each section of the pars distalis, micrographs of all the areas showing TSH cells were taken with a ×20 objective and enlarged to ×750. Sections from three different levels (dorsal, medium and ventral) were studied. In addition, a low magnification view from each pituitary section was photographed in order to record the complete area where TSH cells appeared. Measurements were made by means of an image analysis system (Mini-Mop Evaluation Unit, Kontron Bildanalyse). Immunostained cells stood out in sharp definition and were measured by tracing them with the stylus of a Zeiss Videoplan. Specifically stained TSH cells were scored: 876, young; 1,096, old; 723, very old. The whole area of the pars distalis was also delimited as a reference area, using the above low magnification micrographs appropriately enlarged. These measurements were recorded and processed automatically by the evaluation unit attached to the Videoplan, which then calculated the following parameters [MiniMop Manual, 1985]: reference area (RA), number of cells (N), cell area (A), cell perimeter (P), cell density (CD) = N/RA, volume density (VD) = ΣA/RA and surface density SD = $(4/\pi) \times (\Sigma P/RA)$.

Hormone Radioimmunoassays

Both TSH and PRL were assayed by specific RIAs using the materials provided by Dr. Salvatore Raiti (NHPP). Values for TSH and PRL were expressed in terms of NHPP rTSH-RP-2 and rPRL-RP-3, respectively. Both hormones were iodinated by the Iodogen® method [Franker and Speck, 1978] and purified on a 1.6×55 cm Sephacryl S-200 column equilibrated with 0.01 *M* phosphosaline, pH 7.6. A 2% suspension of protein A (Sigma Chemical Co.) in 0.9% NaCl was used to separate bound from free hormone. Serum T_4 and T_3 were radioimmunoassayed by means of commercial kits (DPC, Allegro, Calif., USA).

Statistical Analysis

Data were expressed as mean ± SEM, unless otherwise indicated. Statistical comparisons among age groups were performed by ANOVA followed by Duncan's multiple range test, when appropriate. The correlation between morphological parameters and hormone levels was evaluated by means of the Spearman's correlation test.

Results

Pituitary weight showed a significant increase from 3 to 20 months of age but remained stable afterwards up to 29 months (data not shown). One old and 2 very old rats that showed gross pituitary enlargement (> 20 mg) were excluded from the study. In order to avoid including in this study animals with active microprolactinomas, rats with serum PRL values above 2SD of the average level for the corresponding age group were discarded (table 1).

Immunostained TSH cells stood out in sharp definition showing the characteristic shape for this cell type (fig. 1). Analysis of morphometric parameters revealed a clear age-related reduction in VD, SD and CD. On the other hand, A and P values showed a significant age-related increase.

Serum levels of T_4, but not of T_3, were low in the older animals. This hypothyroid status was paralleled by a progressive increase in serum TSH in the old and very old rats (table 2). There was a highly significant ($p < 0.001$) inverse correlation between serum TSH and serum T_4 in the animals studied. On the other hand, there was no significant correlation between morphometric parameters and serum TSH levels.

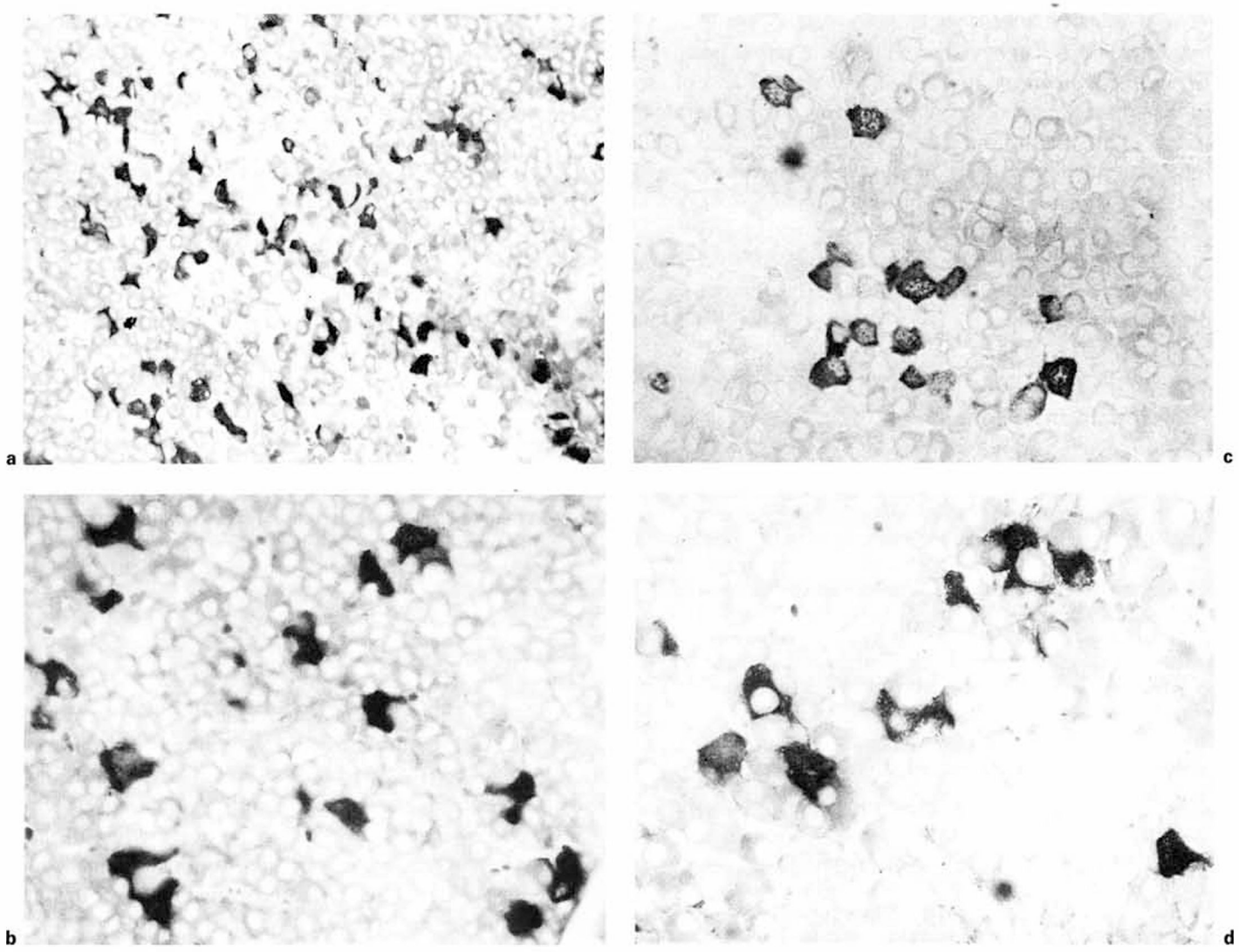

Fig. 1. Specifically immunostained TSH cells from young (**a**, **b**), old (**c**) and very old (**d**) rats. Immunoperoxidase method. Objectives ×10 (**a**) and ×40 (**b**–**d**). Final magnification: ×118 (**a**) and ×470 (**b**–**d**).

Table 1. Serum levels of TSH, PRL, T_4 and T_3 in male rats of different ages

Hormone	Age group		
	young	old	very old
TSH, ng/ml	4.8±0.5 (13)	6.2±1.2 (11)	10.1±1.6** (13)
PRL, ng/ml	21.9±2.5 (13)	36.3±7.1* (11)	39.4±7.9* (13)
T_4, µg/dl	4.1±0.3 (13)	3.1±0.2** (11)	2.7±0.5** (11)
T_3, ng/dl	77.4±7.1 (6)	74.6±6.7 (6)	63.5±6.5 (6)

Values are expressed as mean ± SEM; number of rats between parentheses. Significant difference from young counterparts is denoted by * for $p < 0.05$ or ** for $p < 0.01$.

Table 2. Morphometric profiles of thyrotrophs in the pituitary of young, old and very old rats

Age group	Volume density $\times 10^{-3}$	Surface density $\mu m^{-1} \times 10^{-3}$	Cell density cells/$\mu m^3 \times 10^{-5}$
Young (4)	4.78±0.88	5.42± 1.00	13.70±2.34
Old (5)	1.92±0.38*	2.10±0.41**	4.84±0.96**
Very old (4)	2.13±0.6*	2.45±0.69*	5.75± 1.67**

Values are expressed as mean ±SEM; number of rats between parentheses. Significant difference from young counterparts is denoted by * for $p < 0.05$ or ** for $p < 0.01$.

Table 3. Average dimensions of thyrotrophs in young, old and very old rats

Age group	Cell area, μm^2	Cell perimeter, μm
Young (4)	33.45± 1.82	31.25±0.58
Old (5)	39.50± 1.73*	34.38±0.63**
Very old (4)	38.40±0.51	33.70±0.06*

Values are expressed as mean ±SEM; number of rats between parentheses. Significant difference from young counterparts is denoted by * for $p < 0.05$ or ** for $p < 0.01$.

Discussion

We are unaware of any previous immunohistochemical studies of the impact of aging on the thyrotrophic cell population in rats. In humans, it has been reported that thyrotroph hypertrophy was present in pituitaries from aged individuals [Zegarelli-Schmidt, 1985] as well as in those from patients with asymptomatic atrophic thyroiditis [Bonnyns et al., 1972].

The increased A and P values in the aged rats indicate that thyrotrophic cell enlargement occurs with age. Our morphometric and hormonal data suggest that, while aged rats possess less TSH immunoreactive cells than their young counterparts, the remaining thyrotrophs become hyperactive, perhaps in response to the low levels of circulating T_4 present in the aging animals.

The serum TSH and thyroid hormone levels recorded here agree with previous studies in chronically cannulated rats, which showed that plasma T_4 deficiency in aging animals was associated with an overall increase in TSH secretion and a loss of circadian rhythmicity in the plasma levels of this hormone [Goya et al., 1987, 1988, 1990]. Low levels of serum T_4 are a consistent finding in old rats [Valueva and Verzhikovskaya, 1977; Chen and Walfish, 1979; Pekary et al., 1983; Goya et al., 1990] and may account for the above age-associated changes in the secretory patterns of TSH in this species. This hypothesis is supported by the fact that experimental hypothyroidism not only results in increased levels of circulating TSH but also abolishes the circadian variations of the hormone in plasma [Fukuda et al., 1975].

As expected, there was a moderate weight increase in the pituitaries from aged males. The hyperprolactinemia recorded in the old males (table 3) suggests that this age-related pituitary enlargement is probably accounted for by hyperplasia of the lactrotrophs, which is known to occur with age in both male and female rats [Takahashi and Kawashima, 1983].

We conclude that aged rats possess a reduced but functionally preserved thyrotroph cell population. The present data also reveal a coexistence of high circulating levels of TSH with reduced serum T_4, thus suggesting that in rats, aging brings about a progressive desensitization of the thyroid gland to TSH.

Acknowledgements

The authors are grateful to Ms. Yolanda E. Sosa and Ms. Georgina C. Luna for technical help and to Dr. Néstor Carri, IMBICE, La Plata, for making available the Mini-Mop system in the present study. This work was supported in part by grants to RGG from the Argentine Research Council (CONICET) and the INSERM, France, through its program 'Réseau Nord-Sud'. CLAGD is a member of the Researcher's Career, CICPBA, Argentina.

References

Berkvens, J.M., J.H.J. van Nesselrooy, R. Kroes (1980) Spontaneous tumours in the pituitary gland of old Wistar rats. A morphological and immunocytochemical study. J Pathol *130:* 179–191.

Bonnyns, M., J.L. Pasteels, M. Herlant, L. Vanhaelst, P.A. Bastenie (1972) Comparison between thyrotropin concentration and cell morphology of anterior pituitary in asymptomatic atrophic thyroiditis. J Clin Endocrinol Metab *35:* 722–728.

Chen, H.J., P.G. Walfish (1979) Effects of age and testicular function on the pituitary-thyroid system in male rats. J Endocrinol *82:* 53–59.

Cónsole, G.M., C.L.A. Gómez Dumm, R.G. Goya (1993) Impact of aging on the morphology and function of the somatotroph cell population in rats. Mech Ageing Dev *70:* 45–51.

Fong, A.C.O., J.M. Hardman, E.A. Porta (1992) Immunocytochemical hormonal features of pituitary adenomas of aging Wistar male rats. Mech Ageing Dev *20:* 141–154.

Fraker, P.J., J.C. Speck (1978) Protein and cell membrane iodinations with a sparingly soluble chloramide, 1,3,4,6-tetrachloro-3a,6a-diphenylglycoluril. Biochem Biophys Res Commun *80:* 849–857.

Fukuda, H., M.A. Greer, L. Roberts, C.F. Allen, V. Critchlow, M. Wilson (1975) Nyctohemeral and sex-related variations in plasma thyrotropin, thyroxine and triiodothyronine. Endocrinology *97:* 1424–1431.

Goya, R.G., S. Takahashi, K.L. Quigley, Y.E. Sosa, A.L. Goldstein, J. Meites (1987) Immune-neuroendocrine interactions during aging: Age-dependent thyrotropin-inhibiting activity of thymosin peptides. Mech Ageing Dev *41:* 219–227.

Goya, R.G., Y.E. Sosa, K.L. Quigley, P.E. Gottschall, A.L. Goldstein, J. Meites (1988) Differential activity of thymosin peptides (TF5) on plasma thyrotropin in female rats of different ages. Neuroendocrinology *47:* 379–383.

Goya, R.G., K.L. Quigley, S. Takahashi, Y.E. Sosa, J. Meites (1990) Changes in somatotropin and thyrotropin secretory patterns in aging rats. Neurobiol Aging *11:* 625–630.

Huang, H.H., S. Marshall, J. Meites (1976) Capacity of old versus young female rats to secrete LH, FSH and prolactin. Biol Reprod *14:* 538–543.

McComb, D.J., K. Kovacs, J. Beri, F. Zak (1984) Pituitary adenomas in old Sprague-Dawley rats: A histological, ultrastructural and immunocytochemical study. J Natl Cancer Inst *73:* 1143–1166.

Mini-Mop Manual. Rel. 1.0. Kontron Bildanalyse. Munich, Germany, 1985.

Nagatani, M., K. Miura, M. Tsuchitani, I. Narama (1987) Relationship between cellular morphology and immunocytological findings of spontaneous pituitary tumours in the aged rat. J Comp Pathol *97:* 11–20.

Nakane, P.K. (1970) Classifications of anterior pituitary cell types with immunoenzyme histochemistry. J Histochem Cytochem *18:* 9–16.

Pekary, A.E., J.M. Hershman, M. Sugawara, K.I. Gieschen, P.B. Sogol, A.W. Reed et al. (1983) Preferential release of triiodothyronine: An intrathyroidal adaptation to reduced serum thyroxine in aging rats. J Gerontol *38:* 653–659.

Takahashi, S., S. Kawashima (1983) Age-related changes in prolactin cells in male and female rats of the Wistar/Tw strain. J Sci Hiroshima Univ Ser B Div 1 *31:* 185–191.

Valueva, G.V., N.V.Verzhikovskaya (1977) Thyrotropic activity of hypophysis during aging. Exp Gerontol *12:* 97–105.

Zegarelli-Schmidt, E., X.R. Yu, C.M. Fenoglio-Preiser, K. O'Toole, N. Pushparaj, G. Kledzik, D. West King (1985) Endocrine changes associated with the human aging process: II. Effect of age on the number and size of thyrotropin immunoreactive cells in the human pituitary. Hum Pathol *16:* 277–286.