Endocrine (1995) 3, 711-716

Pituitary physiological and ultrastructural changes during aging

A.C. Nessi, G. De Hoz, C. Tanoira, E. Guaraglia & G. Consens

[1]*Universidad CAECE, Avda de Mayo 1400 (1085) Buenos Aires, Argentina and* [2]*Cátedra de Histología y Embriología, Facultad de Odontología, MT de Alvear 2142 (1122), Buenos Aires, Argentina*

Although it is known that both aged human beings and animals exhibit a decrease in pituitary hormone production and release, there is controversy about the true nature of these changes. Whereas some authors postulate an extra pituitary cause, i.e., a dopaminergic failure, others consider that the problem is at the level of the gland itself. CFW mice 2, 6, 12 and 18 months old, were i.p. inoculated with L-Dopa. The pituitary gland was removed and sectioned, then observed and photographed in an electron microscope. Photomicrographs were scanned into a computer and digital image analysis made to determine secretory granules and organelle kinetics. Normal GH and TSH cells of elder mice responded to stimulation with L-Dopa in a similar way as did cells of juveniles. The responsiveness rate of those cells to the amine precursor during the first hour of treatment was 38 ± 3.5% and 26 ± 0.3% of the studied cells in young and aged animals, respectively. Fully functional cells, i.e., GH and TSH cells showing 5 to 90% of their cytoplasm occupied by secretory granules (some of them immature), with a developed RER, and with absence of cell damage, were seen to be reduced from 98% in younger to 65.7% in aged animals. In successive steps, cells showed cell desquamation, darkened cytoplasm, differential swollen endoplasmic reticulum without secretory granules, increased number of secondary lysosomes (more than two per cell in a cross-section), differential swollen mitochondria, cytoplasm only containing two to five giant secondary lysosomes and finally, a complete loss of cell architecture. Therefore, GH and TSH cells at the end of their life-span were seen to derive into apoptotic images. In the whole gland, an increasing number of those pathologic images were seen as aging proceeded, thus reducing the number of normal and productive cells. From the results presented here it is proposed that an extrapituitary failure is insufficient to explain the reduced production of GH and TSH, and that the problem evolves also at the level of the gland itself.

Keywords: aging; apoptosis; dopamine; GH; pituitary gland; TSH

Introduction

Both aged human beings and animals undergo a decrease in the production and release of pituitary hormones (Georgotas, 1983; Tanaka *et al.*, 1991; Morrison *et al.*, 1993; Soule *et al.*, 1993), when comparing their values to those of juvenile counterparts. In humans it was described that, with each advancing decade, GH production decreases about 14%. Conditions are also impaired by the fact that released GH has 6% less of its half life activity (Iramnesh *et al.*, 1991), i.e., the time on which remains active and capable of producing bio-effects. Several authors (Klug & Adelman, 1979; Hylka *et al.*, 1984; Carnes *et al.*, 1993) also suggested a misregulation of the hypothalamic-pituitary axis. Moreover, it is known that plasma levels of dopamine drops as aging proceeds (Bertler, 1961; Reynolds, 1963; Robinson, 1975; Legros & Bruweir, 1982; Samorajski *et al.*, 1982; Peterson & Blackwell, 1988; Soares-Da-Silva & Fernandes, 1991; Venero *et al.*, 1991; Ollat, 1992).

In parkinsonian patients dopamine strength in both the putamen and caudate nuclei is about 50% that of normal persons. Noradrenaline in them also diminishes, albeit to a lesser extent (Vermeulen, 1987; Ganong, 1992).

In spite of much research on pituitary hormones and aging, there is little agreement regarding the true nature of their reduced levels. Some authors consider dopamine the most vulnerable substance in the process of aging (Robinson, 1975; Samorajski *et al.*, 1982; Arnetz *et al.*, 1986; Touitou, 1987; Greenspan *et al.*, 1991; Soares-Da-Silva *et al.*, 1991; Ollat, 1992), assuming therefore an extra pituitary localized process. Others propose that the problem is located at the level of the gland itself. Even more, some authors were unable to determine differences in mean ACTH plasma levels between young and aged rats, but reported a delayed response to specific stimuli in the latter (Hylka *et al.* (1984). Carnes *et al.*, 1993) have found a reduced response to corticotrophin releasing hormone in ACTH cells from aged animals, when compared to young counterparts.

The position involving an hypophyseal failure remains controversial on two possibilities. The first one concerns a possible involution in pituitary cell activity as the cause of the known lowered hormonal plasma levels. The other explanation supposes that those cells have a normal behavior, but their number is reduced.

In previous work at our laboratory we have found that L-Dopa was a stimulus strong enough to evoke the release of at least GH and TSH in young animals (Nessi & Bengtsson, 1990; Nessi *et al.*, 1992). In the latter case it was dependent on the time of day the drug was given (Nessi *et al.*, 1992). This occurred despite the fact that this catecholamine is known to have an inhibitory effect over other pituitary hormones such as prolactin. Also L-Dopa was reported to inhibit TSH release in elderly human beings (Greenspan *et al.*, 1991).

Bearing in mind those results, we have repeated the experimental design applied to young animals, to groups of mice with several degrees of aging. Our interest was to determine whether or not the same doses of L-Dopa given to a juvenile provoke a parallel response in the old animal.

Results

Ten minutes after L-Dopa administration a number of GH cells of any group responded with RER enlargement (Figures 1 and 2) and newly formed secretory granules (Figure 2, arrows). Immature granules were seen in the neighborhood of a growing Golgi apparatus (Figures 2 and 8). As it is known, immature granules have a variable space between their content and the surrounding membrane, which can also be irregular (Figure 2, arrows). Nevertheless, the amount of responding cells varied from group to group.

Thirty minutes after L-Dopa was administered to age 2 and 6 groups, 38 ± 3.5% of the studied GH cells showed an almost depleted cytoplasm. RER appeared to grow, forcing the remaining secretory granules to spread towards the cell membrane (Figures 3 (inset) and 5). On the other hand, control GH cells showed this shape in only 3.3 ± 0.1% of the

Correspondence: A.C. Nessi
Received 16 January 1995; accepted 28 June 1995

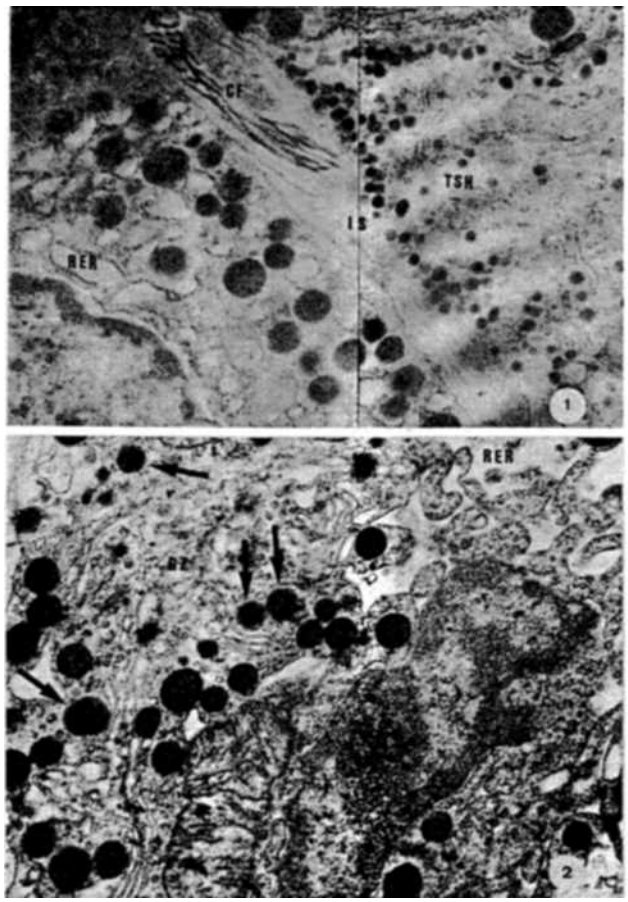

Figure 1 Age 12 GH (left) and TSH (right) cells after 10 min of L-Dopa administration. Compare the rough endoplasmic reticulum (RER) development: whereas in GH cells it is enlarged, in TSH cells it remains unchanged. CF: collagen fibers at the interstitial space (IS). × 22,000. **Figure 2** Age 2 cells after 10 min of treatment. The Golgi zone (GZ) is enlarged, with several immature granules in its neighbourhood (arrows). As in Figure 1, RER is also enlarged. × 22,000

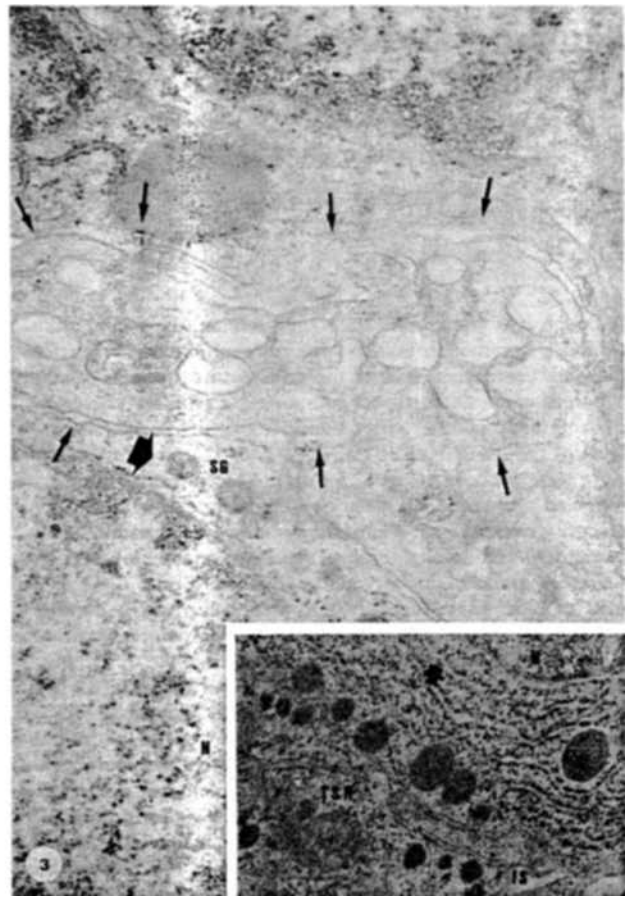

Figure 3 Age 6, control. Early stage of a desquamating body, on which an amount of swollen vesicles are protruded towards the interstitial space (small arrows). An amount of immature secretory granules (SG) are placed in the direction of the big arrow, between the nucleus and cell membrane. × 16,000. Inset: Thirty min after treatment. RER forces the SG to distribute towards the cell membrane. TSH: partially degranulated thyrotrope cell. × 14,000

studied cells. In 9% of control GH cells, the amount of cytoplasm occupied by RER was 34 ± 2.8%, being 46 ± 3% (in 16%), whereas in 13% of that cells an 84 ± 6% of their cytoplasm was occupied by this organelle.

In age 12 and 18 groups, GH cells showed a similar response to L-Dopa as did cells of juveniles (Figures 1, 9 (inset) and 10 (lateral cells). The number of responding cells was also different (Tables 1 and 2).

In TSH cells these findings were less noticeable, since the secretory granules distribution is always peripheral (Figures 1 and 3 (inset) (left cell)). The dinamics of granules depletion *vs* RER enlargement, however, was similar to that one of GH cells.

Age 6 group animals showed a similar response as those of age 2. The difference was that in age 6 group a number of multivesicular bodies and desquamating cells appeared (Figures 3 and 4, Table 1). A discrete number (1.89%) of darkened cytoplasm cells were also observed (Table I).

As shown in Table I, there is an increase in anomalous features as aging proceeds. Successively they include:

1 Cell desquamation (Stage 0) (Figures 3 and 4);
2 Darkened cytosplasm with cell shape preservation (Stage 1) (Figure 9);
3 Differential swollen endoplasmic reticulum without secretory granules (Stage 2) (Figure 7);
4 Increase in secondary lysosomes (more than 2 per cell in a cross-section) (Stage 3) (Figure 6);
5 Differential swollen mitochondria (Stage 4);

Table 1 Degrees of GH and TSH cells damage in the studied groups

	D0	*D1*	*D2*	*D3*	*D4*	*D5*	*D6*
Age 2	0.1 ± 0.009	1.9 ± 0.04					
Age 6	8.4 ± 1.05	4.9 ± 0.22	0.3 ± 0.001	1.4 ± 0.03			
Age 12	9.0 ± 0.81	8.3 ± 0.75	2.1 ± 0.02	1.8 ± 0.09	2.3 ± 0.01	0.4 ± 0.003	
Age 18	8.6 ± 0.03	14.3 ± 1.26	3.1 ± 0.42	3.5 ± 0.02	2.5 ± 0.009	2.1 ± 0.04	0.2 ± 0.08

References: D0: Cell desquamation. D1: Slightly darkened cytoplasm. D2: Differential swollen endoplasmic reticulum without secretory granules. D3: Increment of secondary lysosomes (more than 2 per cell). D4: differential swollen mitochondria. D5: Cytoplasm only containing 2 to 5 giant secondary lysosomes. D6: Cell death and complete architecture loss. Figures are expressed as the mean from the study of about 400 cells per group, in experiments repeated 5 times. ± 95% confidence

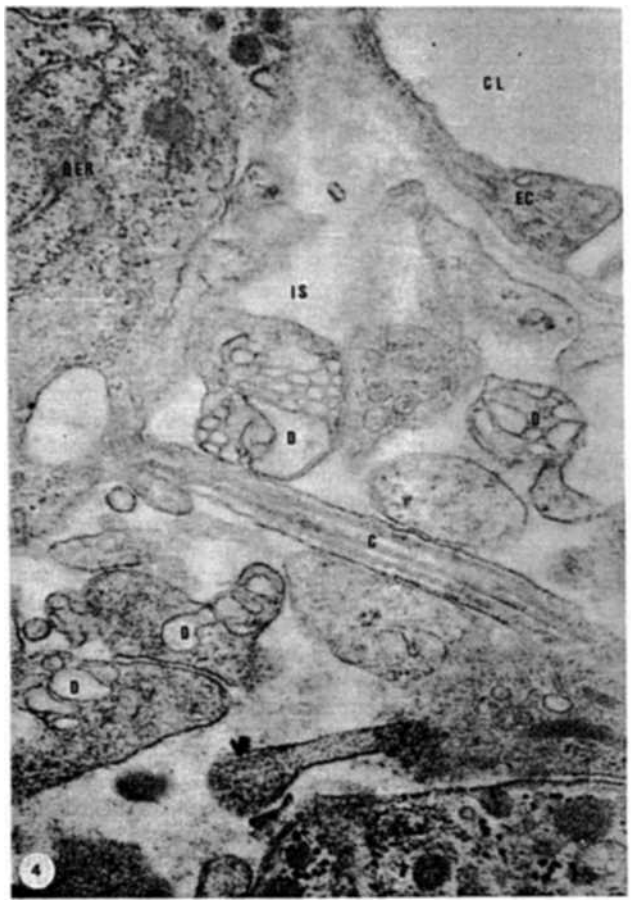

Figure 4 Age 12, controls. Several desquamating bodies (D) are seen at the interstitial space (IS). C: cilia, CL: capillary lumen, ec: endothelial cell, RER in a GH cell. × 16,000

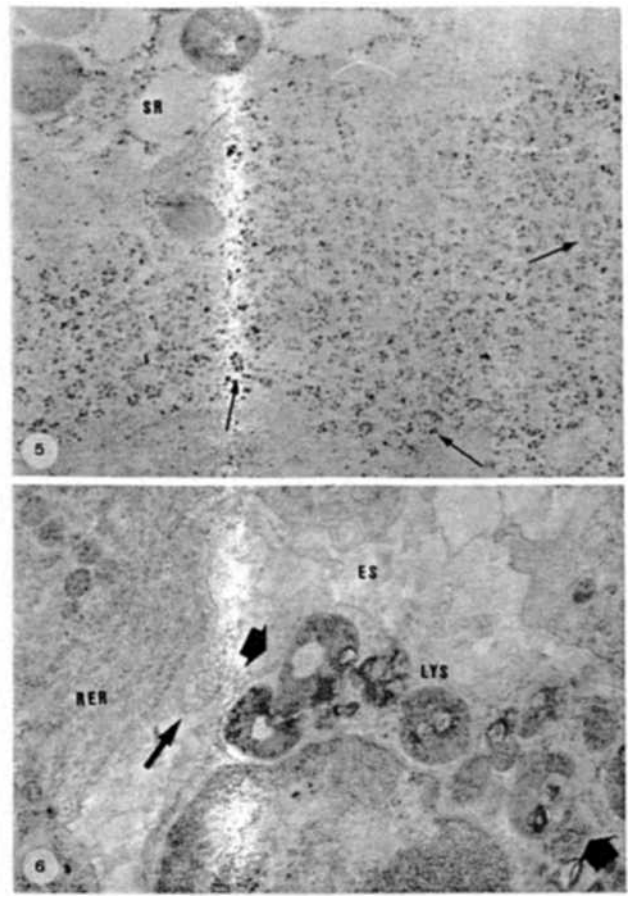

Figure 5 A degranulated GH cell of the Age 6 group after 30 min of L-Dopa treatment. A remaining secretory granule is seen towards the cell membrane, embedded in a considerable amount of polysomes, which occupy 84% of the cross-section cytoplasmic surface. Some of them show the characteristic spiral aspect (arrows). A swollen RER is seen in the cell at the upper left-hand corner.

Figure 6 Age 18. Control at 2:00 PM. A conspicuous amount of lysosomes (Lys) are seen between the two large arrows. There is an apparent fusion of both close to the left pointer. Small arrow: Desquamating body at the extracellular space (ES). × 12,000

6 Cell compression and density increment. Cells predominantly round or at least convex started to show a polygonal-like aspect, and some of their sides were concave (Stage 5) (Figure 9 (inset, arrow)).

7 Complete loss of cell architecture. In the last step cells were compressed, their fine structure lost, and dendrite-like dark extensions were observed (Stage 6) (Figure 10, central cell).

Among animals from the same group there were no differences in the amount of damaged cells between treated and controls. On the contrary, as animals grew older, there was an increase in pathological shapes (Table 1). Moreover, the presence of tissue macrophages was more evident from age 12 group onwards (Figure 6).

During the first hour of L-Dopa administration, a parallel fact was observed with GH and TSH circulating levels. The difference was in magnitude, since the first one increased about three fold (Table 3), whereas TSH only responded with a 20 to 25% of control values (Table 3). This finding agrees with those reported for electron microscopy and image analysis, on which TSH cells showed a less evident response than GH cells. The other samples did not show significant differences. Nevertheless, a decrease in hormone levels was observed in 12 ($P < 0.001$) and 18-month-old mice (Table 3).

Although normal cells response observations were independent of age, a significant ($P < 0.001$) decay of reacting cells in the old animal is shown in Tables 1 and 2.

Table 2 Percentage of GH and TSH functional cells at different ages in mice

	FF	*F*	*NR*
Age 2	98.1 ± 3.3	1.5 ± 0.02	0.4 ± 0.001
Age 6	85 ± 2.4	31.3 ± 1.16	1.4 ± 0.003
Age 12	76.1 ± 1.7	17.3 ± 0.81	4.5 ± 0.003
Age 18	66.7 ± 1.6	22.9 ± 0.14	11.4 ± 0.02

References: FF: Fully functional cells were considered those showing any of the following features: A degree of granulation from 5 to 90%, developed rough endoplasmic reticulum, immature secretory granules, and absence of cell damage. However, follicular, undifferentiated or non hormone producing cells are also included. F = Functional cells were those fulfilling the above characteristics plus one of the two (D0 and D1) stages of cell damage. NR = Non responsive were those showing any of D3 to D6 characteristics from Table 3 ±: 95% confidence

Discussion

Most extra-pituitary factors during aging relate to blood supply failure, to the existence of neurotransmission alterations, these last ones due to synthesis and release deficiency, post-synaptic effectiveness and degradation. That general failure compromises substances like neuropeptides, noradrenaline, dopamine, serotonin, GABA and acetylcholine. This is true also for their enzymes of synethesis, such

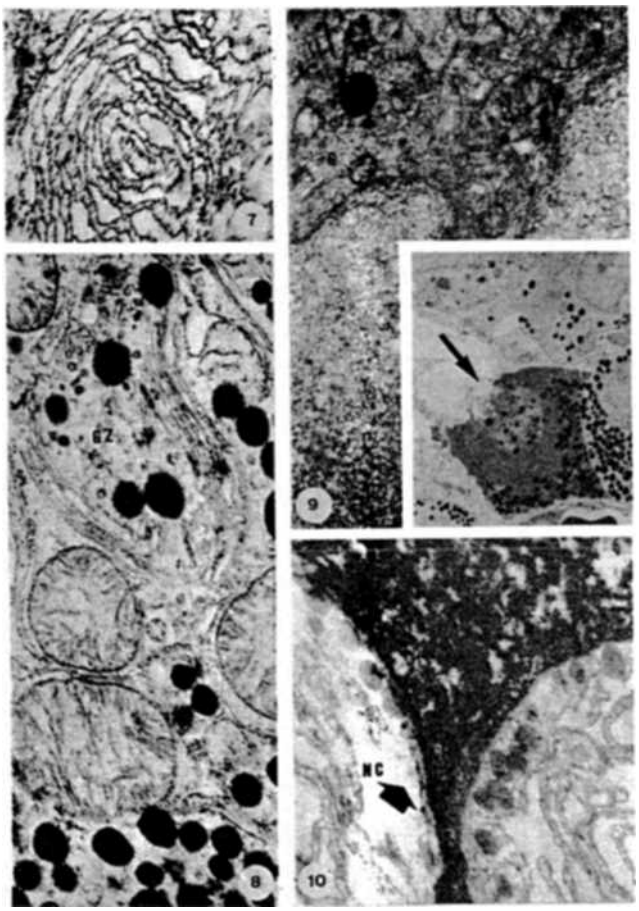

Figure 7 Nebenkern-like RER on a 18-month-old mouse, after 2 h of L-Dopa treatment. The cell, having a loose architecture, showed a delayed response to treatment, when compared to young animals. A few secretory granules of the GH type were seen in similar shaped cells. × 20,000. **Figure 8** Age 18, GH cell from the control group. Mitochondrial structure is preserved, and the Golgi zone (GZ) has a half-developed aspect, with immature granules in its neighbourhood. × 24,000. **Figure 9** Age 18. Dark GH cell at 1 h after L-Dopa treatment, on which an almost depleted cytoplasm and a spared nucleus are seen. × 24,000. Inset: GH cell in the process of programmed death (arrow). Compare its negative polygonal-like and compressed aspect with the positive one (rounded) of its neighbouring active counterparts. Note the different granulation rate of contiguous GH cells in a control animal. × 5,000. **Figure 10** A necrotic cell (NC) in a 18-month-old mouse, after 30 min of treatment. The normal architecture is lost. Its nearby resident cells responded to the stimulus in a similar way as in the younger groups, with a developing RER, which forces the granules to distribute towards the cell membrane. × 20,000

as cholin-acetyltransferase, dopadecarboxylase, and neurotransmitter metabolites, such as 3-methyl-hydroxyfenilglycol, vanilmandelic acid, 5-hydroxy-indolacetic acid, etc.

Dopamine is capable of promoting at least GH and TSH release in young animals (Nessi & Bergtsson, 1990; Nessi *et al.*, 1992). Neverthless, pituitary cells' response to this catecholamine was reported to follow a circadian pattern, related to the drug administration time (Nessi *et al.*, 1992). In this work, GH cells' response to L-Dopa is strong, whereas in TSH cells only a weak reaction is evoked by L-Dopa (Table 3).

Control pituitary cells of the same kind and group are not in phase (Nessi & Bozzini, 1982) (Figure 9 (inset)). This means that they do not show a homogeneous granulation rate, a fact that results in ultradian pulses pattern in the release of their hormones. In both young and aged animals not all cells responded to the stimulus. In this sense, secretory granule's depletion, which was observed 30 to 60 min after L-Dopa administration, was detected in a mean of only 38% of the studied cells.

Changes related at the level of the gland itself were the following: There was no difference in cell damage ratio between treated and control mice from the same group. The affected cells were surrounded by normal cells (Figures 9 and 10), a fact which matches to the concept of apoptosis or programmed cell death.

In this sense, we have paid particular attention to recognize a differential swelling in an image when it can be distinguished from a fixation artifact. In this regard, we admitted the pathological changes only if other cells of the same section maintained their normal structure.

In a classical work Smith & Farqhuar (1966) described an alternative lysosomal activity as an intracellular disposal mechanism for the turnover of secretory protein, as a regulatory process when pituitary hormones are overproduced. According to the scheme suggested by these authors, an important amount of secretory material follows the lysosomal pathway when the release stops, while an enhancement of the phenomenon appears to be common during aging. Lysosomes were the most outstanding organelles and occupied almost the whole cytoplasm (Figure 6). Actually, in apoptotic cells not only the secretory material is destroyed by lysosomes, but also the entire cell itself, thus reducing the number of hormone productive units.

Nève *et al.* (1981) described a similar finding in the thyroid cells of aged hamsters. Nevertheless, they failed to observe a similar pathway in other glands, such as the parathyroid, and speculated whether that process was specifically related to the thyroid gland.

The results reported here suggest that the mechanism of dopamine-GH and TSH control is maintained in the aged animal. As a matter of fact, both GH and TSH cells responded to the catecholamine stimulation in a similar way as in the young animal. Indeed, secretory granule depletion was observed 30–60 min following L-Dopa administration, with a further RER enlargement (Figure 3, inset). The presence of immature granules in the neighbourhood of a growing Golgi complex during the recovery phase of hormone production was also a common finding in age 2 animals (67% of studied cells). Nevertheless, these immature granules were only seen in 62% of studied cells in age 6 animals and 18% in age 18 groups.

However, the enhanced amount of pathological images, which were found in pituitary cells of the aged animal, suggest the following:

1. There is a decrease in the number of normal cells which are still fully functional (Tables 1 and 2).
2. On the other hand, the presence of normal cells with a densitometric activity variation similar to that of young

Table 3 Increment percent of L-Dopa treated GH and TSH cells against the controls

Age	C_1	*L-Dopa*	IP_1	C_2	*I-Dopa*$_2$	IP_2
2	4.7 ± 0.3	12.9 ± 1.7	274%	14.9 ± 1.1	18.1 ± 1.2	21.4%
6	4.6 ± 0.3	14.7 ± 0.4	319%	15.9 ± 1.3	19.9 ± 2.1	25.1%
12	3.6 ± 0.08	11.5 ± 0.6	319%	13.1 ± 0.8	15.8 ± 1.3	20.6%
18	3.1 ± 0.02	10.2 ± 0.1	326%	10.6 ± 1.4	12.9 ± 0.8	21.8%

References: The subscript (1) refers to GH, and (2), to TSH levels. All figures, except IP are expressed as ng/ml. IP: increment percent after 1 h L-Dopa administration. ± 95% confidence

Table 4 Experimental design

H	*12:00*	*12:10*	*12:30*	*1:00*	*2:00*
Age 2	⇧	◆	◆	◆	◆
C	◆	◆	◆	◆	◆
Age 6	⇧	◆	◆	◆	◆
C	◆	◆	◆	◆	◆
Age 12	⇧	◆	◆	◆	◆
C	◆	◆	◆	◆	◆
Age 18	⇧	◆	◆	◆	◆
C	◆	◆	◆	◆	◆

References: H: hours of the day (PM). ◆: Times of sampling. C: Controls. ⇧: Time point on which L-Dopa was administered. Experiments were repeated five times

Table 5 Ultrastructural pathways to differentiate the pars distalis cells

			Secretory granules		
	Cell shape	*Size*	*Density*	*Shape*	*Distribution*
ACTH	Negative	300–400	ED	circular	P-LD
GH	Positive	300–400	ED	circular	WC
LTH	Positive	200–600	ED	IE	WC
TSH	Negative	100–180	ED	circular	peripheral
LH	Positive	<200	DE	circular	WC
FSH	Positive	200–300	Var	circular	WC

References: Granules size is expressed as nm in diameter, ED: electron dense, IE: irregular edges, with depression and prominences, P-LD: predominantly peripheral, and surrounding lipid droplets, Var: variables from lucent to several degrees of darkness, WC: whole cytoplasm

animals, rules out the possibility of a full pituitary regression.

3. Our findings also question a dopaminergic insufficiency as the single source of lowered pituitary gland hormone plasma levels during aging.

Materials and methods

CFW mice 2, 6, 12 and 18 months old were maintained in standard conditions (Halberg *et al.*, 1955) for circadian studies, 21 days before treatment and sampling. This strain, obtained in Lyon, France, for prolificit and size 10, is documented in a strain reference published by the Medical Research Council, England. It arrived in Argentine 12 years ago, and sustained with its characteristics in the CONEA (Comisión Nacional de Energía Atómica). It was maintained in our laboratory by inbreeding since 1984.

As dopamine cannot cross the blood-brain barrier, its precursor L-Dopa was given instead. L-Dopa was dissolved in balanced salt solution (BSS) and administered at noon with an i.p. 0.0143 mg/kg body weight single injection, which is equivalent to the therapeutic dose prescribed to parkinsonian patients.

Experimental design

Animals were divided into four main groups, of 20 mice each, aged 2, 6, 12 and 18 months old. Throughout the experiment the groups are named: Age 2, 6, 12 and 18, respectively. For the main groups, Age 2 mice served as controls. For each group it was applied the drug schedule and sampling model outlined in Table 4.

Animals were killed by decapitation 10, 30, 60 and 120 min after being injected with L-Dopa. They were allowed to bleed into heparinized tubes in order to obtain plasma for radioimmunoassay (RIA). Mice not receiving the amine precursor, but the same amount of BSS, served as controls. Each batch consisted of two treated and two untreated animals. Experiments were repeated five times.

After opening the skull, the encephalic mass was removed and five drops of 2% glutaraldehyde in phosphate buffer (pH 7.2) were added to the exposed pituitary gland, to allow simultaneous fixing during dissection. Once the gland was removed, the lateral wings were sectioned into small 1 mm^3 cubes and fixed in the same reagent for 2 h, at room temperature. After 4 h of rinsing in BSS, samples were post-fixed in 1% osmium tetroxide in Millonig's buffer. After dehydration they were embedded in araldite (Polysciences). Nine to 10 nm thick sections were obtained in a Porter Blum ultramicrotome. Sections were stained with uranyl acetate and lead citrate (Reynolds, 1963).

Samples were observed and photographed in a Siemens Elmiskop electron microscope. In order to evaluate the secretory granule dynamics, 74 to 92 photographs of each group were scanned and digitized with the help of a video camera (Crest), connected to an analog-digital converter (Digi-View) and an Amiga 4000 computer. The same sections used for cell counts were used for scanning analysis. Digitized images were then processed by commercial software (McCormic, 1987; Nickerson, 1987; Peterson & Blackwell, 1988). The scanned cross sections were measured in pixels amounts. The cytoplasmic percentage occupied by secretory granules in GH and TSH cells was used to determine hormone production.

As it is known, pituitary cells of the same kind are not in phase (Nessi & Bozzini, 1982) (Figure 9 (inset)). This means that they do not show a homogeneous granulation rate either in the controls, or at different time points of the day. Therefore, in order to estimate the cell response to L-Dopa, in this study we used the quadratic index (Nessi & Bozzini, 1982) (sum of percentage granular surface multiplied by the observed frequency). This is a special technique previously developed and updated for mixed populations (Nessi, 1995).

Blood samples were centrifuged at 3000 r.p.m. at 4°C, and plasma separate and frozen at −20°C until assay. GH and TSH titers in plasma were determined by RIA, which was carried out with protocols and reagents kindly provided by the NIADDK and NHPP, University of Maryland, School of Medicine, Baltimore, MA, USA.

TSH ^{125}I-labelling was done using the stoichiometric chloramine T method, the limit of sensitivity being 0.7 μU/ml. Separation of free and antibody bound ^{125}I-labeled TSH was performed by the double antibody technique (Nessi *et al.*, 1992). The limit of detection was 10 nMol/L. To calculate hormone concentrations the Pall-M-4-logistic analysis method (Compact 120; Picker CO) was used, with a maximal standard error of 5%. GH last detectable concentration was 0.5 ng/ml plasma. The intra and interassay coefficients of variation were less than 5%. All samples for each hormone were measured in the same assay.

As many dyes have been used in an attempt to discriminate the pituitary gland cells (albeit with little success) the only reliable criteria are those based on immunocytochemistry or electron microscopy. In the present work we have used the latter method. GH cells were recognized as rounded cells with also round or elliptical electron dense secretory granules. Their size ranged from 300 to 400 nm in diameter. Those cells are regarded as positive by the fact that they maintain the rounded shape even when degranulated, in which case the granular volume is replaced by a similar amount of rough endoplasmic reticulum (RER). On the other hand, stellate shaped cells, which quite always are placed at the sites allowed by their nearby residents, with 100 to 180 nm electron dense secretory granules, were recognized as TSH cells. As they fail to adopt a rounded contour, even when laden with granules, TSH-producing units belong to the negative group of pituitary cells. The ultrastructural pathways to differentiate the pars distalis cells are given in Table 5.

Experimental data were processed separately. Statistics were done by means of variance analysis.

Acknowledgements

This work was financially supported by the Consejo Nacional de Investigaciones Cientificas Y Técnicas (CONICET), University of Buenos Aires and University CAECE. We thank Mrs Maria Marta Fagilde, Mr Claudio Saggese and Mr Daniel Franzetti for technical assistance.

References

Arnetz, B.B., Lahnborg, G. & Eneroth, P. (1986). *Life Sci.*, **39,** 135–139.

Bertler, A. (1961). *Acta Physiol. Scand.*, **51,** 97.

Carnes, M., Goodman, B.M., Lent, S.J. & Vo, H. (1993). *Endocrinology*, **133,** 608–616.

Cizza, G., Skudinski, M., Blackman, M.R., Laglia, G. & Gold, P.W. (1993). Abstract of the 75th Annual Meeting of the Endocrine Society, Las Vegas, Nevada, p. 82.

Corpas, E., Harman, S.M. & Blackman, M.R. (1993). *Endocrine Rev.*, **14,** 20–39.

Ganong, W. (1992). Fisiología Médica. Ed Manual Moderno, 12th ed. Mexico.

Georgotas, A. (1983). *Age & Aging*, **12,** 1–10.

Greenspan, S.L., Sparrow, D. & Rowe, J.W. (1991). *Horm. Res.*, **36,** 41–46.

Halberg, F., Barnum, C.P., Silber, R.H. & Bittner, J.J. (1955). *Proc. Soc. Exp. Biol.*, **97,** 897–900.

Hylka, V., Sontag, W. & Meites, J. (1984). *Proc. Soc. Exp. Biol. Med.*, **175,** 1–14.

Iramnesh, A., Lizaralde, G. & Velhis, J.D. (1991). *J. Clin. Endrocinol. Metab.*, **73,** 1081–1088.

Klug, T. & Adelman, R.A. (1979). *Endocrinology*, **104,** 1136–1142.

Legros, J.J. & Bruwier, M. (1982). *Gerontologie: Biologie et Clinique.* F Bourlire (Ed), Flammarion, Paris, pp. 63–80.

McCormic, J.V. (1987). Pixmate. Total Image Processing System. Progressive Peripherals & Software. Owner's Manual.

Morrison, T., Storm, D., Zhou, H. & Rosen, C. (1993). Abstract of the 75th Annual Meeting of the Endocrine Society, Las Vegas, Nevada, p. 222.

Nessi, A.C. & Bozzini, C.E. (1982). *Acta Physiol. Latinoam*, **32,** 175–183.

Nessi, A.C. (1983). *Com. Biol. (Buenos Aires)*, **3,** 289–296.

Nessi, A.C. & Altschuler, L.R., Clement, J.L., Korzin, E.A. & Slaen, A.P. (1989). *Com. Biol.*, **7,** 219–224.

Nessi, A.C., Bengtsson, M.C. (1990). *Acta Physiol. et Pharmacol. Latinoam*, **40,** 309–318.

Nessi, A.C., Altschuler, L.R., Clement, J.L., Vázquez, S. & Slaen, A.P. (1992). *Com. Biol.*, **10,** 149–159.

Nessi, A.C. (1995). *Analysis Digital De Imagenes: Technicas Clasicas Y Nuevas Tendencias en Morfometria*, A.C. Nessi (Ed), ed. INARBITE, Buenos Aires, Argentina.

Nève, P., Authelet, M. & Golstein, J. (1981). *Cell Tissue Res.*, **220,** 499–509.

Nickerson, J. (1987). Butcher 2.0. Eagle Tree Software. Image processor users' manual.

Ollat, H. (1992). *J. Neurol. (Germany)*, **293,** 13–16.

Peterson, S. & Blackwell, R. (1988). Digi-View 3.0. New Teck, Inc, User's Manual.

Reynolds, E. (1963). *J. Cell Biol.*, **17,** 208–213.

Robinson, D.S. (1975). *Fed. Proc.*, **34,** 103.

Saegar, W. (1992). *Microsc. Res. Tech.*, **20,** 162–176.

Samorajski, T., Ho, B., Kralik, P.M. & Hartford, J.T. (1982). *The Aging Brain.* Raven Press, NY, **20,** 259–269.

Schwartzman, R.A., Cidlowski, J.A. (1993). *Endocrine Rev.*, **14,** 133–151.

Smith, R.E. & Farqhuar, M.G. (1966). *J. Cell Biol.*, **31,** 319–347.

Soares-Da-Silva, P. & Fernandes, M.H. (1991). *Acta Physiol. Scand.*, **143,** 287–293.

Soule, S.G., Macfarlane, P.W., Levitt, N.S. & Millar, R.P. (1993). Abstract of the 75th Annual Meeting of the Endocrine Society, Las Vegas, Nevada, p. 222.

Tanaka, K., Inoue, S., Shiraki, J., Shishido, T., Saito, M., Numata, K. & Takamura, Y. (1991). *Metabolism (USA)*, **40,** 1257–1262.

Tokuyama, T., Yoshinari, M., Okamura, K., Ikenoue, H., Sato, K., Kuroda, T. & Fujishima, T. (1991). *Nipp Naib Gakk Zass*, **67,** 65–74.

Touitou, Y. (1987). *Path. Biol.*, **35,** 1005–1012.

Venero, J.L., Machado, A. & Cano, J. (1991). *Brain Res. (Netherlands)*, **557,** 109–114.

Vermeulen, A. (1987). *J. Clin. Endocr. & Metab.*, **64,** 884–889.