Egypt. J. Anat. Vol. 26 (1) 37-55, January 2003

ISSN 1110-2144

AUGMENTATION OF AGE-RELATED CHANGES OF SOMATOTROPHS OF LIFE-LONG MELATONIN DEFICIENT RATS

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INTRODUCTION

Many studies indicate connection between pineal gland function and growth hormone (GH)-insulin-like growth factor (IGF-I) axis in mammals, but their results are not always synonymous (**Nir, 1978; Mess, 1983**). Pinealectomy abolishes a decrease of GH concentration in the pituitary gland of rats, as well as in plasma caused by constant darkness (**Relkin, 1972**). **Ronnekleiv and Mc Cann (1978)** detected the decrease of GH secretion in rats after pinealectomy during the day and a lack of the effect at night. Other investigators indicated intensification of circadian fluctuations of GH after pinealectomy in rats with small increase of daily secretion (**Niles et al., 1979**).

Progressive and irreversible physiological decline is a characteristic of all organisms late in life. Many studies indicating functional alterations in pars distalis cells accompanied with aging (**Mitchell et al., 1995; Velasco et al., 1998; Velduis, 2000**). Pinealectomy is known to result in a clear decrease in the concentration of circulating melatonin (**Gauer et al., 1992**). Growth hormone rhythm was suppressed in the pinealectomized rats (**Ostrowska et al., 2001**). The dysfunctional processes that are a result of macromolecular damage constitute what is referred to as the free radical theory of aging (**Sohal and Weindruch, 1996**).

Numerous reports documented protective actions of melatonin in various models of oxidative stress (**El-Sokkary et al., 1999; Lee et al., 2002**). This is due to its

high efficacy as a free radical scavenger and indirect antioxidant (**Tan et al., 2002**). Additionally, melatonin stimulates the activities of enzymes that metabolize reactive species (**Reiter et al., 2000**) and maintains cell membrane fluidity at an optimal level (**Garcia et al., 1998**). Melatonin was found to decrease with aging (**Lee et al., 2002**). **Reiter et al. (1999)** reported that aging in the pineal-intact animals was associated with increased levels of lipid peroxidation (in the lung, kidney and skin), together with rises in an oxidatively damaged DNA (in liver, kidney and pancreas), and in the levels of protein carbonyls (in the liver). Likewise, advanced age was associated with a significant decrease in membrane fluidity of hepatic microsomes in pineal-intact rats. For all of these parameters and in a number of organs, pinealectomy caused further increase in the indices of oxidative damage.

The aim of this work was to investigate the ultrastructural alterations in somatotroph cells that result from melatonin deficiency in aged (Pineal-intact) rats and to test whether further induced reduction of melatonin by pinealectomy augment these alterations, trying to detect the role of melaronin in the aging process.

MATERIALS AND METHODS

Animals and experimental design :

The study was performed on 33 male Sprague-Dawley rats. Initially, 25 rats were purchased when they were 2 months of age 15 of which were surgically pinealectomized {according to **Kuszak and Rodin's (1977)**} and 10 of which were sham operated. The animals were kept in a room with a temperature of 22 - 24°C and regulated light cycle : 12 hours of light : 12 hours of dark (LD 12 : 12, light from 6 am to 6 pm). Rats were given free access to standard laboratory chow and tap water. When the rats were 20 months of age, the surviving rats (aged pinealectomized and aged pineal-intact) were killed by decapitation. In addition to the old rats, 8 Sprague-Dawley rats were 2 months of age were killed for comparison.

Electron microscopy :

The pituitary glands from all animals of the three groups (adult, aged and aged-pinealectomized) were processed for ultrastructural examination. The anterior lobe were dissected and fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer. The material was cut into small pieces, post fixed in 1% osmium tetraoxide and embedded in araldite. Semithin sections (about 0.2 μm) were stained with toluidine blue, inspected by light microscopy and some fields were photographed. Selected

fields were chosen for ultrathin sections were mounted and stained with uranyl acetate and lead citrate and examined in JEM-RM 1010 Wx transmission electron microscope. Some of the examined fields were photographed.

RESULTS

1. Group I (Adult rats) :

In the semithin sections, the cells of pars distalis appeared as loose cords surrounded by rich network of large capillaries. Somatotrophs were demonstrated scattered among the other cells. They were generally rounded or oval. Their nuclei were rounded euchromaic with prominent nucleoli. The secretory granules were numerous, spherical and scattered through the cell (Fig. 1 - A).

In the ultrathin sections, three *varieties of somatotrophs* were observed. The first type of cells (type I) was the commonest one. The cells were rounded or polygonal with large rounded euchromatic nucleus nearly occupying the center of the cell. The most prominent feature was the presence of numerous parallel cisternae of RER surrounding the nucleus. Immature vesicles were frequently seen adjacent to these cisternae. The Golgi complex was moderate in size and present on one side of the nucleus. The secretory granules were electron dense rounded and their sizes were nearly equal (Fig. 1 - B).

The second frequent type of cells (type II) was ovoid or rounded with a more electron dense cytoplasm compared to the previous one. The nucleus was rounded euchromatic and eccentric in position. A well-developed Golgi complex occupied a zone near the nucleus. The RER consisted of long flattened sacs, scattered throughout the cytoplasm. Round or oval mitochondria, occasional lysosomes and numerous free ribosomes could also be observed. The secretory granules were electron dense, rounded and variable in size (Fig. 1 - C).

The third type of somatotrophs (type III) was the least frequently seen. They were oval cells with more electron dense cytoplasm as compared to the previous two types. The nucleus was heterochromatic with irregular outlines and is eccentric in position. The Golgi complex was very large with moderately dilated sacules occupying a large area of the cytoplasm on one side of the nucleus. The RER cisternae were moderately dilated and scattered throughout the cytoplasm up to the periphery of the cell. The secretory granules were electron dense, rounded and variable in size but the majority of them were small (Fig. 1 - D).

2. Group II (Aged rats) :

In the semithin sections, some somatotrophs appeared large in size while others were shrunken. The nuclei of most cells were dense and irregular. The cytoplasm appeared vacuolated (Fig. 2 - A).

In the ultrathin sections, type I cells appeared shrunken and most of the mitochondria were seen with destructed cristae. Some secretory granules were seen large, pleomorphic and less electron dense than the normal secretory granules (Fig. 2 - B).

The second type of cells appeared shrunken with heterochromatic nuclei having slightly irregular outlines. The nuclear envelope and RER cisternae appeared dilated. The Golgi sacules were mildly dilated and some mitochondria with destructed cristae could be observed. The secretory granules were relatively scanty (Fig. 2 - C).

The third type of somatotrophs appeared hypertrophied and it is the most type, which displayed prominent atrophic changes. The nuclei were very dense with greatly irregular outlines. The RER were severely dilated. The mitochondria appeared swollen with destructed cristae. The secretory granules were ouevcrowded with altered electron density (Fig. 2 - D).

3. Grup III (Aged pinealectmized rats) :

In the semithin sections, the capillaries were dilated and engorged with blood. The intercellular spaces were more pronounced and dilated. Most of the somatotrophs were seen with dense irregular nuclei and the cytoplasm showed many vacuoles (Fig. 3 - A).

In the ultrathin sections, the ultrastructural atrophic changes detected in the somatotrophs were increased in both frequency and intensity after pinealectomy. The most conspicuous characteristics were the shrunken of the cells and the dense electron cytoplasm (Fig. 3 - B). No different varieties of cells could be detected. All the cells displayed the same atrophic changes. The nuclei were seen very dense with very irregular outlines. The RER cisternae, the nuclear envelope and the Golgi sacules were markedly dilated (Fig. 3 - C).

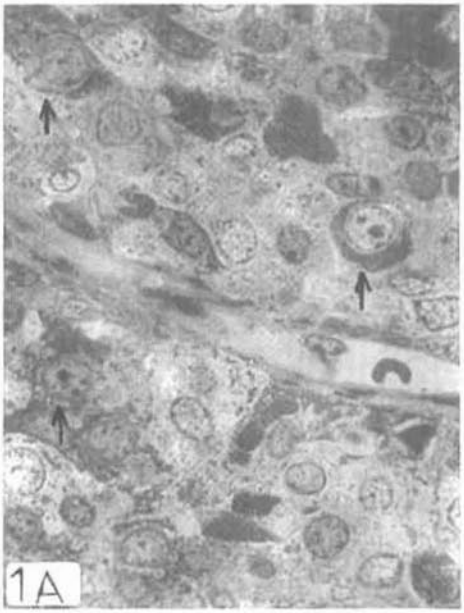
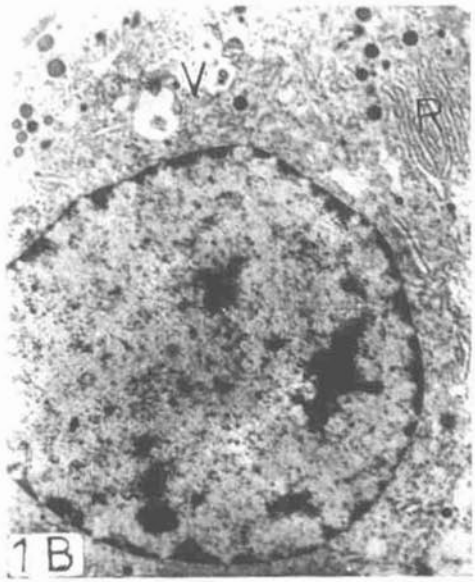
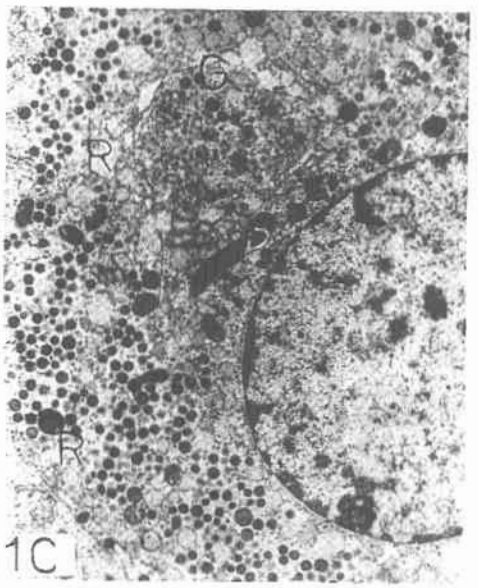


Fig. (1) : A semithin section and electron micrographs of pars distalis of adult rat.
1 - A : A semithin section showing cords of different cells with the intervening capillaries. Note the variations in the appearance of somatotrophs (arrows).
(Toluidine blue; x 1000)

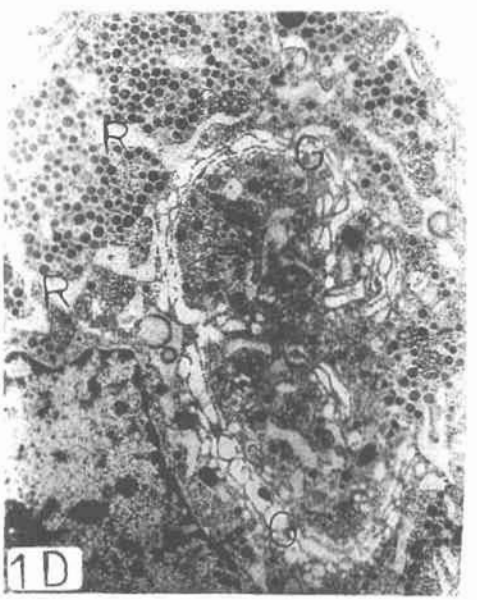
1 - B : An electron micrograph of type I somatotroph showing a large central rounded euchromatic nucleus, parallel cisternae of RER (R). Note the frequent immature vesicles (V).
(x 6000)





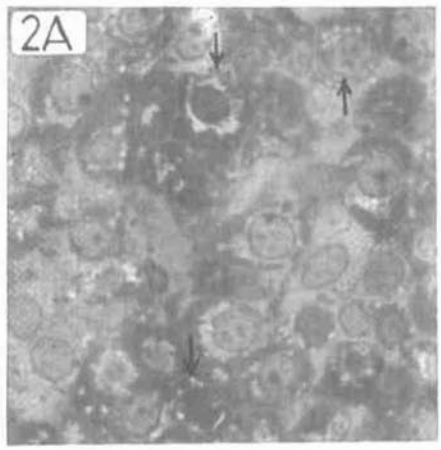
1 - C : An electron micrograph of type II somatotroph. The cytoplasm shows large number of variable sized secretory granules. Note the moderate Golgi (G) and the scattered RER (R) with slightly dilated cisternae.

(x 6000)



1 - D : An electron micrograph of type III somatotroph. The nucleus is heterochromatic, eccentric with irregular outlines. The Golgi sacules (G) and the RER cisternae (R) are moderately dilated.

(x 6000)



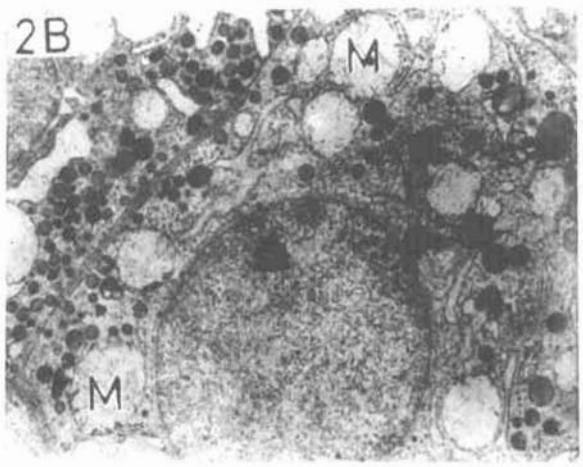
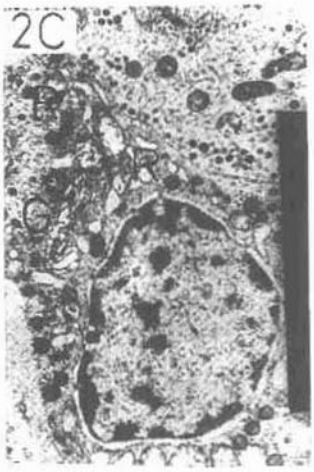


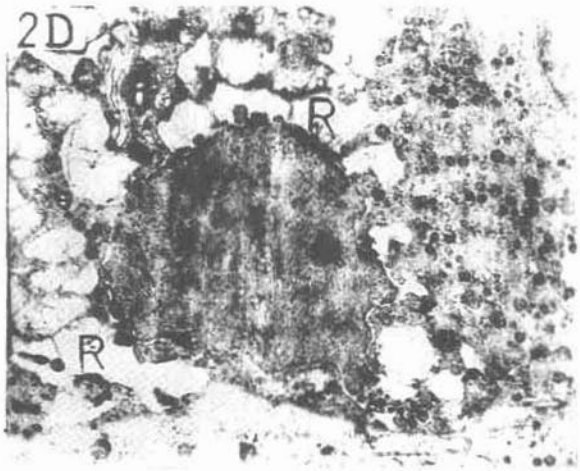
Fig. (2) : A semithin section and electron micrographs of pars distalis of aged-pineal-intact rat.
2 - A : A semithin section showing the presence of numerous vacuoles in the somatotrophs (arrows).

(Toluidine blue; x 1000)

2 - B : An electron micrograph of type I somatotroph showing shrunken cell, and the mitochondria with destructed cristae (M). Note the presence of large pleomorphic secretory granules (S).

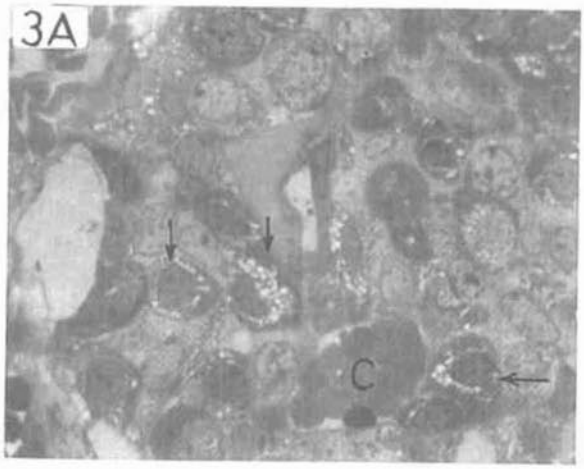
(x 6000)





2 - C : An electron micrograph of type II somatotroph showing shrunken cell. The Golgi sacules (G), the RER (R) and the nuclear envelope are moderately dilated.
(x 6000)

2 - D : An electron micrograph of type III somatotroph showing hypertrophied cell. The RER cisternae (R) are highly dilated. The nucleus is irregular and electron dense. Note the numerous secretory granules with variable electron density.
(x 6000)



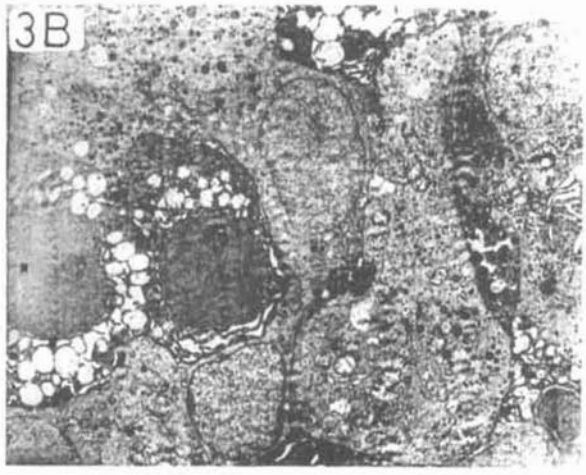


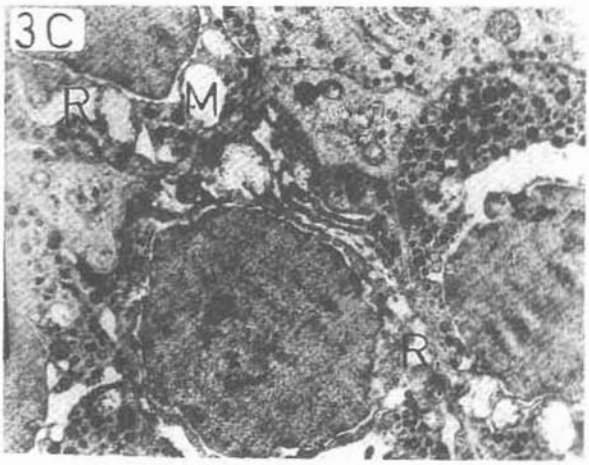
Fig. (3) : A semithin section and electron micrographs of pars distalis of aged-pinealectomized rat.

3 - A : A semithin section showing the presence of large number of vacuolated somatotrophs (arrows). Note the large intercellular spaces (S) and the dilated engorged capillaries (C).

(Toluidine blue; x 1000)

3 - B : An electron micrograph showing somatotrophs with very dense irregular nuclei and electron dense highly vacuolated cytoplasm.

(x 2500)



3 - C : An electron micrograph showing the shrunken cells. The cytoplasm with highly dilated RER cisternae (R) and swollen mitochondria (M) with destructed cristae.

(x 6000)

DISCUSSION

Many of experimental works have not been devoted to the problem of assessment of influence of pinealectomy on CH-IGF-I axis function. They mainly regarded GH secretion, more rarely IGF-I in rodents at morning and evening hours **(Relkin, 1972; Smythe and Lazarus, 1973; Nir, 1978; Ronnekleiv and Mc Cann, 1978; Mess, 1983; Griffiths et al., 1987; Galstain et al., 1994)**. The stimulating or suppressive effect of pinealectomy on GH and / or IGF-I concentrations, dependent on the time of the day was shown **(Niles et al., 1979; Vaughan et al., 1994)**. The work on the influence of pinealectomy on the morphological changes of GH-secretory cells in pituitary gland is too scarce.

In the present study, the adult rat somatotrophs showed morphologically different three populations. The cells of the first type (Type I) were demonstrated as polygonal large cells with central, rounded and euchromatic nuclei, numerous RER cisternae, moderate Golgi complex and equal-sized secretory granules. The cells of the second type (Type II) were ovoid or rounded with rounded eccentric and euchromatic nuclei, well developed Golgi, scattered RER cisternae and numerous secretory granules which were variable in size. These morphological features indicate that type I and II cells are active secretory somatotrophs. The cells of the third type

(Type III) have similar morphology to type II cells but with heterochomatic irregular nuclei, moderately dilated Golgi sacules and RER cisternae and numerous small secretory granules. This appearance suggests that the cells of this type are the same cells of type II at initial degenerative changes that start to occur in early adult life. Also, this explains the early decline in GH release that starts to occur in early adult life **(Liberman and Hoffman, 1997)**.

The previous ultrastructural studies of somatotrophs had ignored the heterogenecity of their populations. Using immunocytochemical study, **Takahashi (1991)** classified somatotrophs on the basis of the size of the secretory granules. He reported that type I cells contain large secretory granules, type II cells contain variable-sized granules and the cells of type III contain only small granules. Also, he suggested that the cells of type III are immature and may be converted to the more mature type II then to type I cells. **Dabado - Berrios et al. (1996)** recognized 2 types of somatotrophs in aged male rats, by centrifugation of dispersed cells in density gradient; low and high-density cells.

The biological meaning of the two different active secretory cells, type I and type II, demonstrated here is not known. They may be developed independently from the stem or progenitor cells. Alternatively, they may represent the same type in different functional activity but this would not be consistent with their morphology.

Distinct ultrastructural changes were demonstrated in somatotrophs of aged rats. Most of type I cells were small in size with degenerative changes including dilated Golgi sacules and destructed mitochondrial cristae. Most of these cells showed some large and pleomorphic secretory granules with less electron density, which indicate that a number of type I cells, were converted to somatomammotrophs. **Borrelli et al. (1989)** reported that GH and prolactin (PRL) cells are derived from a common GH-expressing stem somatotrophs. Our results are in consistent with the previous report of **Shinkai et al. (1995)** regarding the increase in the number of somatomammotrophs in aged rats. Shinkai and co-workers suggested that this is might be an indication of reductin in the stability of gene expression in cell differentiation during aging. The decrease in hypothalamic growth hormone releasing hormone (GHRH) and its gene expression that occurs during aging may be responsible for that increase **(Morimoto et al., 1988)**. **Shinkai et al. (1991)** reported that GHRH suppressed the increase in the number of mammotrophs even at very low concentration in vitro.

In the current study, type II cells showed atrophic changes in aged animals. These cells became small in size with heterochromatic nuclei with irregular outlines.

The RER and Glogi were dilated and the mitochondria showed destructed cristae. Type III cells were demonstrated with marked degenerative changes. The nuclei were greatly electron dense and irregular. The Golgi sacules and RER cisternae appeared severely dilated. These cells were observed to be large in size, a finding indicate cell hypertrophy, probably as a compensation for the pronounced histological destructions in these cells.

Although quantitative immunohistochemical studies have been reported for the somatotrophs of old rats (**Rossi et al., 1991; Takahashi, 1992**), few attempts have been made to estimate the progressive ultrastructural changes that occur in these cells along with the aging in general and after pinealectomy in particular. The demonstrated atrophic changes will be reflected on the decreased secretory capacity of the somatotrophs in aged rats. In mammals, it was found that aging is associated with a decrease in GH and this thought to be responsible for most of the age-deterioration of several tissue and organ functions (**Ceda et al., 1986**). The age-related GH-deficiency state was termed as somatopause (**Martin et al., 1997**).

The previous reports tried to hypothesize the age-related decline of GH secretion. **Morimoto et al. (1988) and Colonna et al. (1989)** reported that the decline of GH secretion was due to the decrease in GHRH and its gene expression in the hypothalamus. **Ceda and co-workers (1986)** claimed that this occurs due to the decrease of responsiveness of somatotrophs to the GHRH. Also, it was found that somatostatin increase gradually with age in rats (**Ge et al., 1989; Veldhuis et al., 1997**). In addition to these explanations about the age-related disturbance in the hypothalamic-pituitary axis, the reduction in GH secretion that was observed previously may be attributed to the intrinsic atrophic lesions in the somatotrophs that were demonstrated in the current study.

The demonstrated degenerative atrophic changes in aged animals were more pronounced after pinealectomy both in frequency and intensity. The populations of the somatotrophs could not be determined. All the cells showed severe atrophic changes. In male rats, **Ostrowska and co-workers (2001)** reported marked reduction in GH level after pinealectomy, which indicate that the pineal gland has an influence on GH-secretory cells.

The somatotroph atrophic changes that start in adult, increase in senescent rats, coinciding with the gradual decrease in melatonin secretion during aging (**Lee et al., 2002**). Accordingly, the morphological and ultrastructural changes of somatotrophs in the current investigation may give a hypothesis for the reduction of GH level in both aged and aged pinealectomized animals. The augmentation of these changes

after pinealectomy suggests that melatonin is responsible for at least great degree for these atrophic changes. Whether, melatonin only or other pineal hormones participates in generating these changes needs further investigations.

SUMMARY

The relationship between growth hormone producing cells in the pars distalis of the pituitary gland (somatotrophs) and the pineal gland function in rats is nearly not elucidated, particularly in the aspect of melatonin participation. The current study tested whether the life-long reduction of endogenous melatonin levels due to pinealectomy would influence the morphological and the ultra structural changes of somatotrophs as the animals aged. Aging in the pineal-intact animals was associated with large-sized and shrunken somatotrophs (Type I, II and III) with dense and irregular nuclei. The cytoplasm appeared vacuolated. Electron microscopy revealed somatotrophs with destroyed mitochondrial cristea. The secretory granules were large, pleomorphic and less electron dense (Type I), scanty (Type II) and overcrowded with altered electron density (Type III). The rough endoplasmic reticulum (RER) cisternae and Golgi sacules were dilated in all cell types. Aging in the pinealectomized animals was associated with dilated intercellular spaces and capillaries engorged with blood. Electron microscopy indicated increased atrophic changes in both frequency and intensity. No different varieties of cells could be detected. The nuclei were very dense with irregular outlines. The RER cisternae, the nuclear envelope and the Golgi sacules were markedly dilated. The findings are consistent with the idea that the severe cytological changes after pinealectomy were due to reduction in melatonin since it functions as free radical scavenger and antioxidant. On the other hand, other pineal secretory products that were reduced as a consequence of pineal removal may have also been responsible for some of the observed changes, but this will need further studies.

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زيادة التغيرات المرتبطة بتقدم العمر للخلايا المفرزة لهرمون النمو فى الفئران البيض ذوات النقص فى الميلاتونين لفترة طويلة

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ان العلاقة بين الخلايا المختلفة لهرمون النمو بالفص القصى للغدة النخامية ووظيفة الغدة الصنوبرية فى الفئران البيض لا تزال غير واضحة وخصوصا ما يخص الميلاتونين ، وقد أجريت هذه الدراسة لتوضيح ما إذا كان نقص مستويات الميلاتونين الداخلية نتيجة استئصال الغدة الصنوبرية يمكن أن يكون له تأثير على الشكل والتركيب المجهرى للخلايا المفرزة لهرمون النمو عندما تتقدم أعمار هذه الحيوانات .

وقد وجد أن تقدم العمر فى الحيوانات السليمة كان مصحوبا بوجود خلايا كبيرة وأخرى منكمشة والتى لها أنوية غير منتظمة وداكنة الاصطباغ ، وظهر السيتوبلازم به فراغات ، وأوضح المجهر الاليكترونى أن الشبكة الاندوبلازمية الخشنة فى هذه الخلايا تبدو لها صهاريج واسعة والميتوكوندريا لها أرفف مكسرة وأكياس جولجى متسعة ، كما ظهرت الحبيبات المفرزة فى النوع الاول كبيرة ومختلفة الاحجام وذات كثافة اليكترونية منخفضة وبدت قليلة فى النوع الثانى بينما كانت مزدحمة جدا فى النوع الثالث . وفى الحيوانات المتقدمة فى العمر والتى أجرى لها عملية استئصال الغدة النخامية فقد وجد أن هناك اتساع فى المسافات الموجودة بين الخلايا وظهرت الشعيرات الدموية محتقنة بالدم ، وبالمجهر الاليكترونى ثبت أن التغيرات المصاحبة لتقدم العمر قد ازدادت من حيث درجتها وتكرارها ، وأصبحت معظم الخلايا ضامرة ولا يمكن التعرف على نوعها ، وهذه النتائج تبدو متفقة مع فكرة أن هذا الضمور الذى ينشأ فى الخلايا نتيجة استئصال الغدة الصنوبرية هو على الأقل جزئيا نتيجة لنقص

الميلاتونين حيث أنه يعمل كماسح للشوارد الحرة ومضاد للاكسدة ، وعلى الجانب الآخر فان الافرازات الاخرى لهذه الغدة التى نقصت أيضا نتيجة لنزع الغدة الصنوبرية يمكن أن تكون أيضا مسؤولة عن هذه التغيرات ولكن ذلك يحتاج إلى دراسات أخرى .