Long-term effect of melatonin on submandibular salivary glands in old rats

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التأثير الطويل الأمد للميلاتونين على الغدد اللعابية تحت الفكية في الجرذان المسنة

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خلاصة : تمت دراسة تأثير الميلاتونين على الغدد اللعابية تحت الفكية في الجرذان المسنة ، واستخدمت لهذا الغرض مجموعة شاهدة مكونة من عشرين جرذاً ، ومجموعة بحثية تضم عشرين جرذاً أعطيت جرعات يومية من الميلاتونين لمدة خمسة أشهر . وتم وزن الغدد أولاً ، ثم حهزت للفحص بالمجهر الضوئي والمجهر الإلكتروني . ووجد أن غدد الجرذان التي أعطيت ميلاتونين كانت أثقل بدرجة يعتد بها من غدد الجرذان الشاهدة . وبالفحص المجهري الضوئي شوهد في المجموعة الشاهدة فقدان للبنية الطبيعية للعنيبات مع تغيرات تنكسيه متعددة ، بينما في مجموعة الميلاتونين كانت بنيه العنيبات واضحة والتغيرات التنكسية قليلة . كما أظهر الفحص المجهري الإلكتروني لغدد المجموعة الشاهدة تغيرات تنكسية مع قليل من النشاط الخلوي ، بينما ظهرت في مجموعة الملاتونين علامات على حدوث نشاط خلوي زائد .

ABSTRACT The effect of melatonin on the submandibular salivary glands of old rats was studied using 20 control and 20 experimental rats which had received melatonin daily for 5 months. The glands were first weighed and then processed for light and electron microscopy. The glands of the melatonin rats were significantly heavier than the controls. With light microscopy, the control group showed a loss of normal architecture of the acini and multiple degenerative changes whereas in the melatonin group the acini had clear architecture and few degenerative changes. With electron microscopy, the control group again showed degenerative changes and little cellular activity whereas the melatonin group had features which indicated increased cellular activity.

Effet à long terme de la mélatonine sur les glandes salivaires submandibulaires chez des vieux rats

RESUME L'effet de la mélatonine sur les glandes salivaires submandibulaires chez des vieux rats a été étudié en utilisant deux groupes de 20 rats: un groupe témoin et un groupe auquel on a administré de la mélatonine quotidiennement pendant 5 mois. Les glandes ont tout d'abord été pesées puis examinées au microscope - optique et électronique. Les glandes des rats du groupe traité à la mélatonine étaient considérablement plus lourdes que celles des rats du groupe témoin. A l'examen au microscope optique, le groupe témoin montrait une perte de l'architecture normale des acini ainsi que des changements dégénératifs multiples tandis que dans le groupe traité à la mélatonine, les acini avaient une architecture claire et peu de changements dégénératifs. Au microscope électronique, le groupe témoin présentait également des changements dégénératifs et une faible activité cellulaire tandis que le groupe traité à la mélatonine avait des caractéristiques indiquant une activité cellulaire plus intense.

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Introduction

The pineal gland was formerly considered to be a vestigial organ with little or no function, but it is now known to be an active endocrine gland [*1*]. The principal hormone of the pineal gland is indolamine melatonin, which is released without prior storage [*2*]. The mechanism of action of melatonin remains controversial but it is known to inhibit aging in the rat [*3*].

Just as life always ends in death, aging is inescapable and begins at birth. Aging constitutes a decreasing ability to survive and, even in the absence of disease, changes occur throughout life at the level of the organelles, cells, organs and systems. But what actually stops the clock of life remains an enigma.

Submandibular salivary glands, like many other organs, undergo changes with age. The gland parenchyma is atrophic in aged individuals [*4*] and shows morphological alterations [*5*], changes in the acinar cell organelles [*6*] and a reduction in the proportional volume of acini [*7*]. Total saliva shows a decrease [*8*], and the level of secretory granules in submandibular salivary glands also decreases with age [*9*]. Age-related decreases in flow rates of saliva have been observed by Percival et al. [*10*] and an age-related decline in melatonin production both in rats and men has been found by Laudon et al. [*11*]. In addition, decreased saliva production is common among elderly people [*12*].

However one cannot exclude the possibility that melatonin can affect the salivary tissue changes that are age-related. This study was undertaken to study the effect of melatonin on the submandibular salivary gland using light and electron microscopy.

Materials and methods

For this study 40 old albino rats (27–28 months) were used and divided into two groups.

- group A control group (20 rats) which did not receive any drug
- group B experimental group (20 rats) which received melatonin orally in a dose of 1 mg per kg body weight per day [*13*].

All rats were kept on the same type of food (milk and bread) and in the same room (4 rats per cage) in order to keep the temperature. light and humidity for both experimental and control groups the same.

The experiment continued for 5 months. Then the rats were killed by cervical dislocation. The submandibular salivary glands of each rat were dissected; each was separated from the sublingual salivary gland, rinsed in physiological saline, bottled and weighed. Each submandibular salivary gland was then divided in half. One half was fixed in 10% neutral formalin, processed and prepared for light microscopic examination using H&E stain. The other half of each gland was fixed in 2% glutaraldehyde solution and post-fixed in 1% osmic acid, processed and prepared for electron transmission microscopic examination using uranyl acetate and lead citrate double stain.

Results

The mean weight of the submandibular salivary glands in the experimental group (63.730 ± 0.228 mg) was found to be significantly higher compared with the controls (62.948 ± 0.197 mg) ($t = 16.42$, $P < 0.001$).

Light microscopic findings

Light microscopic examination of the submandibular gland (H&E stained) of the

control group revealed loss of the normal architecture in most of the acini and multiple degenerative changes in the acinar cells, including the cytoplasm and the nuclei, which were of variable size and different degrees of staining (Figure 1). These included both serous and mucous acini.

In the experimental group the submandibular salivary gland (H&E stained) showed small areas of degeneration and clear acinar architecture; some nuclei were deeply stained and others were lightly stained. The degenerative changes included some nuclei and the nuclei were of different sizes (Figure 2).

Electron microscopic findings

Examination of the acini of the submandibular salivary glands of the control group at the electron microscopic level showed pyknotic nuclei, no clearly visible nucleolus, and degenerated cytoplasm, heterochromatic nuclei with a small amount of euchromatin, restricted rough endoplasmic reticulum (RER) and smooth endoplasmic reticulum (ER) that were located near the nucleus (Figures 3 and 4). Lysosomal activity and electron-dense crystalloids were also found (Figure 5).

In the experimental group, the acini of the submandibular salivary gland showed enlarged nucleoli, numerous, prominent

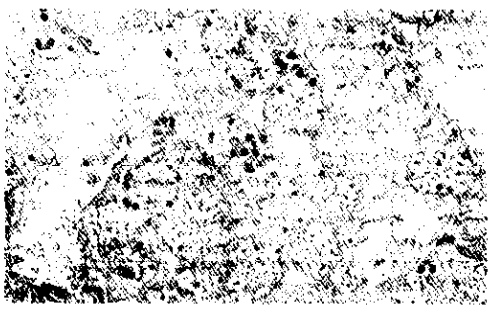

Figure 1 **Light micrograph of submandibular salivary gland of old rat (control). Most of the acinar cells show degenerative changes and the normal architecture of the cells and acini are mostly lost (H&E × 400)**

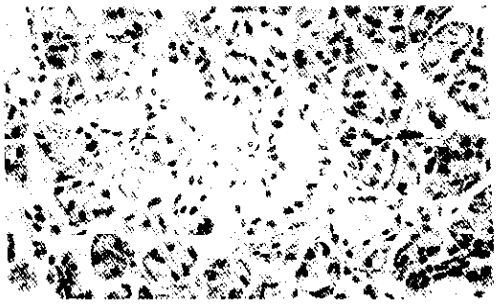

Figure 2 **Light micrograph of submandibular salivary gland of old rat which had received melatonin (experimental group). The acinar architecture is clear with some areas of degenerative change. The nuclei are of variable size and different degrees of staining. Lumina of the acini are mostly unclear (H&E × 400)**

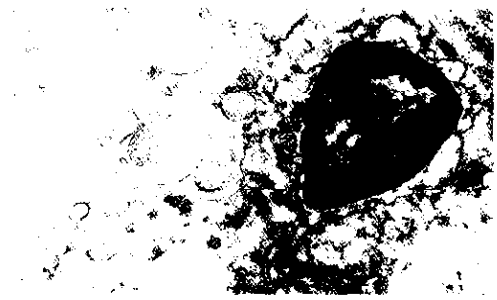

Figure 3 **Electron micrograph of submandibular salivary gland of old rat (control group) showing serous acinar cells. Note the degenerated cell organelles, pyknotic nucleus, unclear nucleolus and the heterochromatic appearance of the nucleus with a restricted amount of euchromatin. RER and smooth ER are not clear except for a small amount near the nucleus (EM × 8000)**

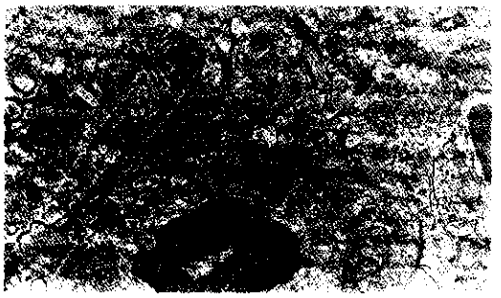

Figure 4 **Electron micrograph of submandibular salivary gland of old rat (control group) showing serous acinar cells. The heterochromatin in the pyknotic nucleus is more prominent than euchromatin which can be seen as a very small area in the centre of the nucleus. There is little smooth ER and very little RER. Crystalloids and numerous mitochondria are also present. The nucleolus cannot be seen (EM × 9000)**

Figure 6 **Electron micrograph of submandibular salivary gland of old rat (experimental group) showing serous acinar cells. Note the enlarged nucleolus, the numerous, prominent dilated Golgi apparatus, pleomorphic mitochondria, electron-dense crystalloids with immature secretory granules which are less electron-dense than normal, numerous lipodroplets and dilated perinuclear cisternae. Euchromatin is more prominent than heterochromatin, which is peripherally located (EM × 6700)**

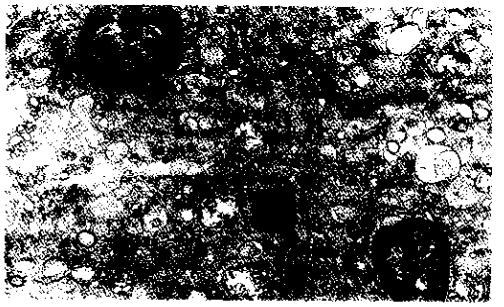

Figure 5 **Electron micrograph of submandibular salivary gland of old rat (control group) showing serous acinar cells. Note the pyknotic nucleus with prominent heterochromatin, degenerated mitochondria, lipodroplets, unclear nucleoli and lysosomal activity (EM × 4000)**

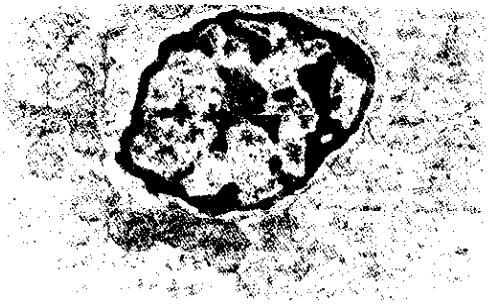

Figure 7 **Electron micrograph of submandibular salivary gland of old rat (experimental group) showing serous acinar cells. The cytoplasm contains stalks of pleomorphic mitochondria together with smooth ER and some RER, especially near the nuclear envelope. Euchromatin is more prominent than the peripherally located heterochromatin. There is a clear nucleolus which is nearly centrally located in the nucleus and the perinuclear cisterna is dilated (EM × 8000)**

and dilated Golgi apparatus, pleomorphic mitochondria, dilated perinuclear cisternae and prominent euchromatin in comparison with heterochromatin (Figures 6 and 7).

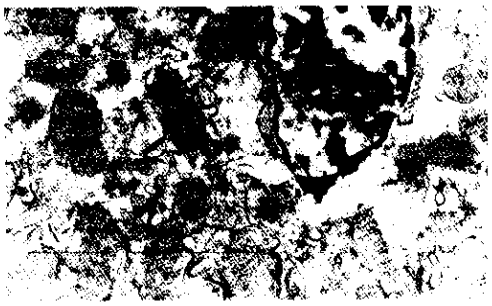

Figure 8 **Electron micrograph of submandibular salivary gland of old rat (experimental group) showing serous acinar cells. The euchromatin occupies most of the nucleus with a small amount of heterochromatin. The nucleolus appears very large, pleomorphic mitochondria are seen. there is abundant smooth ER with some RER, especially near the perinuclear membrane. Crystalloids are still a prominent feature (EM × 8000)**

Figure 9 **Electron micrograph of serous acinar cells in submandibular salivary gland of old rat (experimental group). The euchromtin is more prominent than heterochromatin in the nucleus. There are two nucleoli and open nuclear pores; the perinuclear cisterna is dilated. Note there are numerous mitochondria of different sizes, smooth ER, some RER and the nuclear envelope is studded with ribosomes in some areas (EM × 8000)**

with mature and immature secretory granules. In addition, the RER and smooth ER were prominent, the nucleolus occupied a great part of the nucleus and the heterochromatin was very restricted (Figure 8). In some cases two nucleoli were found with open nuclear pores, abundant RER and smooth ER, and a nuclear membrane studded with ribosomes (Figure 9). The perinuclear membrane in some acinar cells was significantly dilated and there was prominent RER with free ribosomes (Figure 10).

Figure 10 **Electron micrograph of serous acinar cells in the submandibular gland of old rat (experimental group). Note the dilated perinuclear cisterna, dilated RER, free ribosomes and ribosomes attached to the perinuclear membrane and the large nucleolus (EM × 16 000)**

Discussion

The activity of the pineal gland and melatonin production is influenced by the daily cycle of light and dark, and there is an important link between the environment and the physiology of the organism responding to annual changes in day-length [*14*], which is why we fixed the environment (light and darkness) and kept all the rats in cages in the same room with the same type of food.

The results have demonstrated that the weight of the submandibular salivary gland significantly increased with melatonin administration, which may be a result of increased cellular activity compared with the controls. The increased cellular activity and inhibited degeneration seen in the experimental group was evident from the retained acinar architecture, cellular behaviour and retarded degenerative processes, and reflects the effect of melatonin on the gland.

It has been shown by Scott that the salivary gland parenchyma is atrophic and often replaced by connective tissues in aged individuals [*4*]. Also Gresik and Azmitia noted morphological age-related alterations in salivary glands [*5*]. Scott et al. also reported that the proportional volume of acini was reduced with age [*7*]. In general, cellular function declines progressively with age and aging constitutes a decreasing ability to survive. Even in the absence of disease the "human machine" eventually wears out.

Ultrastructural investigation of the submandibular salivary glands of the experimental group revealed changes in different intracellular organelles in comparison with those of the control group. Euchromatin was more prominent than heterochromatin in the nucleus of the acinar cells in comparison with those of controls. In the acinar cells of the control group, heterochromatin occupied most of the nucleus with little or no euchromatin. In the experimental group, enlarged nucleoli were seen and in some cases two nucleoli were found. The amount of euchromatin usually associated with a large nucleolus (or nucleoli) can be used as an indicator of the metabolic activity of a specific cell or cell type because euchromatin is usually active in RNA synthesis; conversely a high proportion of heterochromatin indicates a cell with low metabolic activity. Koller et al. found a significant age-related decline in DNA synthesis in submandibular glands, and total saliva showed a decrease with age [*8*].

In our study, mitochondria were degenerated in the acinar cells of controls, while in those of the experimental group mitochondria were pleomorphic and numerous. Mitochondria tend to hypertrophy and increase in number with accelerated cell activity, but in aged cells vacuolated mitochondria, decreased RER, vesicular smooth ER and distorted Golgi apparatus have been found. Aged mitochondria have a decreased ability to survive hypoxic insult [*15*]. Also, in the experimental group, the acinar cells showed other different features which reflect increased cellular activity, such as numerous prominent and dilated Golgi apparatus, abundant RER, smooth ER, free ribosomes, open nuclear pores, dilated perinuclear cisternae and mature and immature secretory granules, in comparison with the controls in which the RER and smooth ER were restricted, there were increased lysosomal activity and electron-dense crystalloids, and pyknotic nuclei were seen, all of which indicate that the cellular activity in the controls was significantly reduced. Pyknotic nuclei and an increase in size and number of lipofuscin granules are characteristic in parenchymal cells of the submandibular cells of the submandibular salivary gland of aged rats [*6*]. Ribosomes and granular endoplasmic reticulum have a role in protein synthesis. Crystalloids have been found predominantly in submandibular salivary glands of aged rats and may thus be a manifestation of their altered cell function [*6*].

It has been proposed that the aging process may simply be the sum of deleterious free radical reactions continuously going on throughout cells and tissues. Free radicals cause random damage to DNA, RNA, proteins and enzymes and induce polymerization of membranes; they are capable of eventually

causing cell death [*16*]. Antioxidants retard cellular senescence [*17*] and dietary antioxidants increase life expectancy in rats [*18*]. There is a progressive decline in the rate of protein synthesis with age in submandibular glands [*19*] and a decreased level of secretory granules [*9*].

Comparison of the experimental and control groups in the present study may indicate that melatonin administration corrected the cellular structure and activity, and the gland in the controls was affected by aging. This is not in agreement with Bodner and Baum [*20*], who concluded that rat submandibular salivary secretions remained relatively constant with age. Our results in the control group support those found by many authors [*7,10*] and explain the noted xerostomia which is a common complaint among elderly people, and they also support the morphological evidence for a loss of acinar tissue among elderly humans which is well established [*4,21*]. Decreased saliva production is common among elderly people and may compromise oral health with implications for systemic health nutrition and quality of life [*12*]. There is also an age-related decline in melatonin production both in rats and humans [*11*].

The effect of melatonin in our study could be attributed to its antioxidant properties as a scavenger of free radicals, or its effect on the blood supply and immune system [*22*]. In a study conducted by Bellavia et al. melatonin was found to affect amylase secretion in the parotid glands in rats [*23*]. Weekly demonstrated that both the pulmonary artery and vein relax in response to melatonin which could explain the effect of melatonin on the whole body [*24*]. The immune function has also been reported to be affected by melatonin [*13*]. Gilad et al. suggested that melatonin may affect all growth and viability [*25*].

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