Exogenous melatonin: Morphology and kallikrein activity of male Syrian hamster submandibular gland

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Summary. Seven weeks of daily melatonin administration resulted in substantial reduction of convoluted duct cell granule population and kallikrein activity. Some reduction of intercalated duct cell granules was also observed. Testes weight and size was also dramatically reduced. All these changes were not observed after three weeks of melatonin injection.
Key words. Melatonin; kallikrein; morphology; Syrian hamster; submandibular.

Melatonin or 5-methoxy-N-acetyl tryptamine is a biologically active amine and was first identified in pineal some 60 years ago[2]. It is synthesized from tryptophan within the pineal parenchymal cells and is secreted in blood stream[3,4]. Recent evidence suggests that melatonin is also present in the hypothalamus[5].

Although exact physiological role of this neuroendocrine hormone is not clear, melatonin has been implicated in many biological phenomena, e.g. photoperiodism[7]; circadian rhythm[6]; behavior[8]; depression[9]; sexual function and development[10,11] and regulation of neuroendocrine mechanism.

The last-mentioned phenomenon has been the subject of intense investigation and evidence suggest melatonin's involvement in the regulation of gonadotrophons[12,13], prolactin[17,18], luteinizing hormone[19], thyroid hormone[14,15,16], testes[18,37] and somatomedin[20]. However very little is known about melatonin's effect on exocrine glands.

In recent years there has been renewed interest in the elucidation of exocrine tissue function. In this context investigations have been carried out in an attempt to evaluate functional importance of different secretory components of salivary glands. Evidence suggests that ducts of salivary glands are not mere conduits for the passage of saliva and cells which make up these ducts are involved not only in electrolyte and water balance[27], but also synthesize and store many biologically active proteins[21]. These polypeptides, upon appropriate hormonal and neuronal stimuli, are actively secreted. One such polypeptide is the enzyme kallikrein. This 38 000 kDa protein is present in the duct cells[32–34]; most likely in the secretory granules because it is possible to isolate kallikrein-rich secretory granules which resemble duct cell granules[28]. Although the enzyme itself is not biologically active, it cleaves a small polypeptide (bradykinin) from α-2 globulin fraction of the plasma and it is the cleaved peptide which exhibit biological activity.

In the present study effects of daily exogenous melatonin administration were evaluated on the morphology and kallikrein activity of adult male Syrian hamster submandibular gland.

Material and method

Twenty adult male Syrian hamsters were used in this experiment. They were kept in a light, temperature and humidity controlled room and were provided rat feed and water ad libitum. Throughout the experiment they were kept on 14 h light and 10 h dark cycle. Lights were turned off at 19.00 h and were turned on at 05.00 h daily. Between 17.00 and 17.30 h half of the group received 25 µg of melatonin (sigma) in 250 µl of saline by subcutaneous injection. The other half of the group received 250 µl of saline only.

After three and seven weeks of daily treatment, the melatonin- and saline-only-injected animals were sacrificed by decapitation between 13.00 and 14.00 h. Their submandibular glands were immediately exposed and small pieces of tissue were excised and processed immediately for light and electron microscopy. The rest of the submandibular was then quickly frozen in liquid nitrogen for enzyme and protein assays.

For microscopy, small pieces of submandibular glands were fixed in a mixture of 4% paraformaldehyde and 2% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, for 1 h at room temperature. Following a wash in distilled water pieces were postfixed in 1.5% osmium tetroxide made up in 0.1 M cacodylate buffer, pH 7.2, for 1 h. These pieces were then thoroughly washed in distilled water, dehydrated in ascending series of ethyl alcohol; passed through propylene oxide (3 changes 10 min each) and embedded in epon. For light microscopy 1-µm thick sections were cut and stained with 1% methylene blue. For electron microscopy silver to gold colour sections were cut, stained with uninyl acetate (5% uranyl acetate dissolved in 70% ethyl alcohol) for 5 min and lead citrate for 1 min and examined in a Philips 410.

For kallikrein assay frozen glands were homogenized in distilled water using Dounce homogenizer. Homogenate was then frozen and thawed twice to break all cells. Finally homogenate was microfuged and the supernatant was used to measure kallikrein activity by using the spectrophotometric method of Trautschold[35].

Protein concentrations were determined by the method of Lowry et al.[36].

Results

Effects on morphology. Following seven weeks of daily melatonin injection, no effects were observed on acinar component of the submandibular gland. The cells were full of secretory granules that showed variation in their content; some showed an electron dense core and a concentric less dense region. The dense core region also exhibited variation and in some granules it was much lighter in appearance than others. The nuclei were pushed toward the basal side of these cells and appeared rather flat and indented. The endoplasmic reticulum was arranged in the form of parallel lamellae and was mostly confined to the basal regions of these cells. All in all these cells looked very much like cells in animals injected with saline only.

Intercalated or neck region cells, although without alteration of their morphology following three weeks of daily melatonin injection, did exhibit small reduction in the size of their secretory granules after seven weeks of melatonin injection. However, no appreciable decrease in the population was observed.

Convoluted duct cells showed distinct alteration of their morphology following seven weeks of daily melatonin injection. Most of the effect was on secretory granules. Figure 2 shows that the number of secretory granules was significantly lower when compared with saline-only-in-

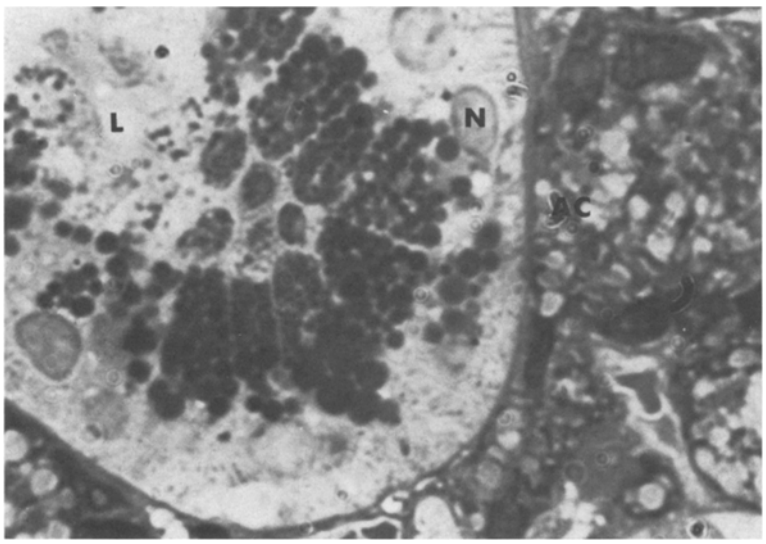

Figure 1. Light micrograph of 1-μm thick epon embedded section showing normal submandibular gland convoluted duct cells. Note the presence of dark stained secretory granules in these cells. On the right side a few acinar cells can also be seen. N, nucleus; L, lumen; AC, acinar cell. 1900×.

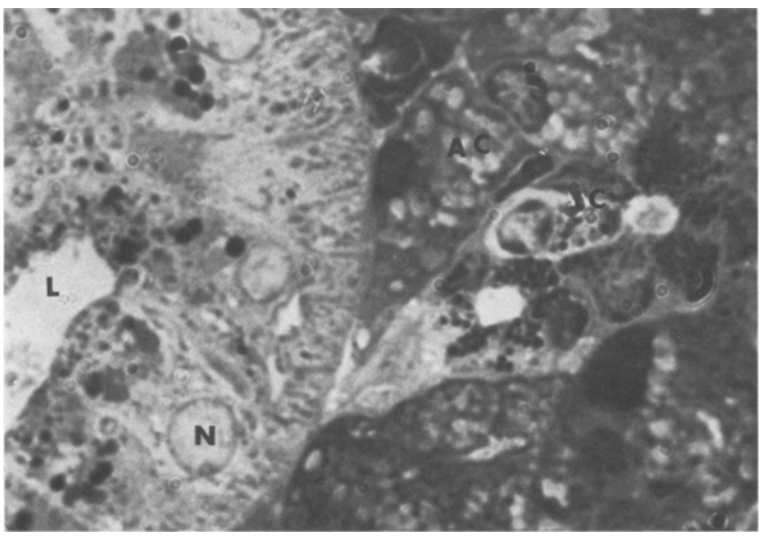

Figure 2. Light microscope photograph of 1-μm thick epon embedded section showing submandibular gland convoluted duct cells following seven weeks of daily melatonin injection. Note reduction of secretory granules on the left side, in the middle, a few neck cells with secretory granules can also be seen. Above and below these neck cells acinar cells are also visible. N, nucleus; L, lumen; AC, acinar cells; IC, intercalated duct cells. 1900×.

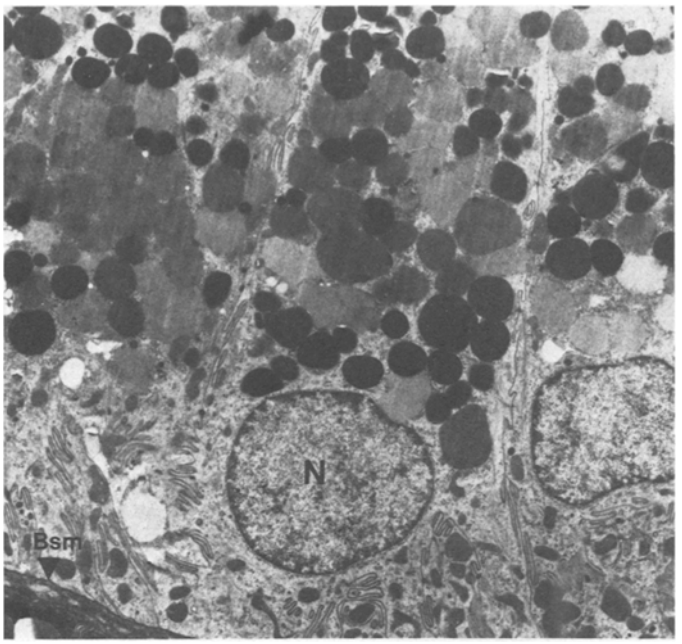

Figure 3. Electronmicrograph of normal submandibular gland convoluted duct cells. Note tight packaging of secretory granules. Some granules are more electron-dense than others. Despite tight packaging no fusion of granules was observed. In the basal part mitochondria and basal plasma membrane infoldings can be seen. N, nucleus; BSM, basement membrane. 7000 ×.

jected animals (see fig. 1). It is also obvious from these figures that the greatest decrease occurred in large-size granules which in control glands are more centrally located.

Figures 4 and 5 B are electron micrographs showing convoluted duct cells of melatonin-injected hamster. When compared with saline-only-injected animal as shown in figures 3 and 5 A it is clear that the number of secretory granules is much lower in the experimental gland. Another feature is that in melatonin-treated animals these secretory granules are much smaller and are located more towards the apical pole of the cell. Some of these granules, particularly those located in the apical region, are quite different from those seen in saline-only-injected animals (arrows in fig. 5 B). Here they are not very electron dense and seem to contain amorphous looking material in them.

These granules are not completely surrounded by their membrane and gaps with protruding amorphous material can be seen. These granules also appear irregular in outline and near the apical border they completely lose their shape and form.

The Golgi apparatus and rough endoplasmic reticulum do not show change in their morphology. The Golgi

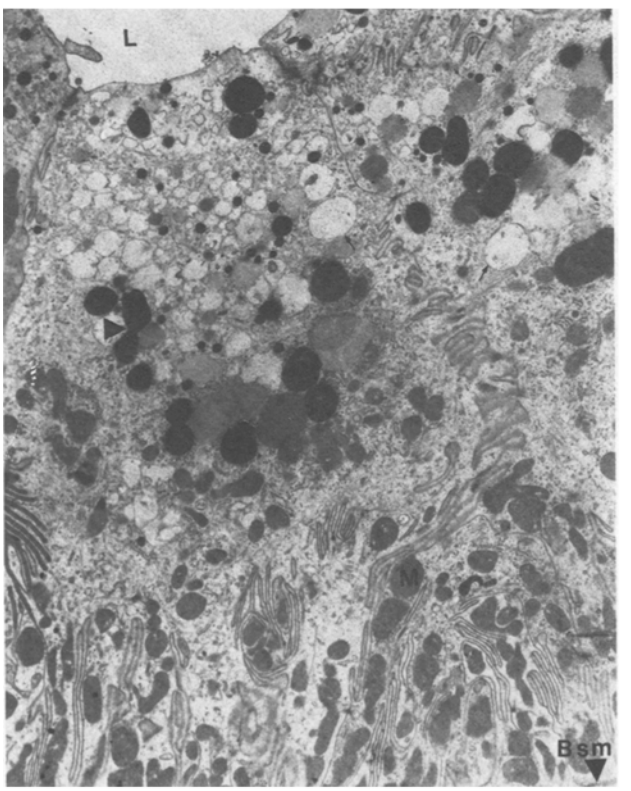

Figure 4. Electron micrograph of convoluted duct cells following seven weeks of daily melatonin injection. Note the decrease in number and size of secretory granules. Some granules show amorphous looking material in them (arrows). Fusion of electron dense granules (arrow heads) can also be seen. Due to the decrease of secretory granules, basal plasma infoldings and mitochondria become more apparent. L, lumen; M, mitochondria; BSM, basement membrane. 7000 ×.

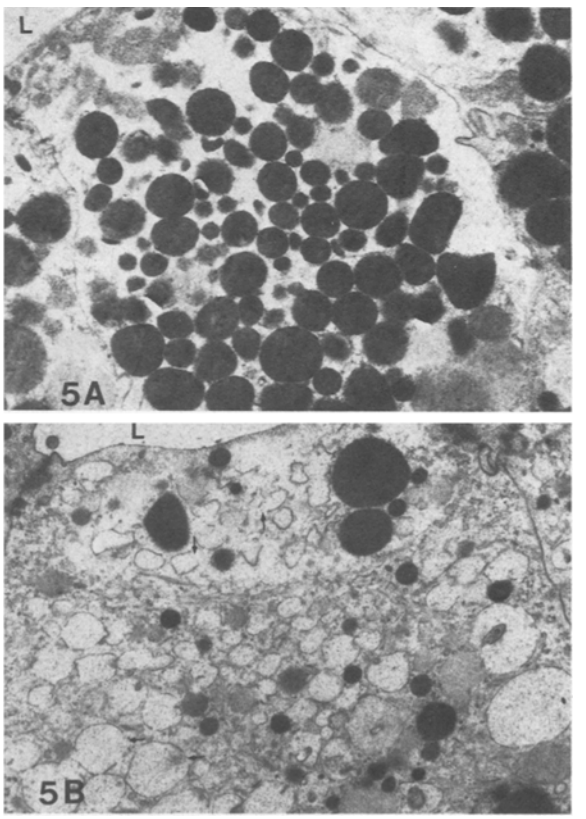

Figure 5. Top part (*A*) shows the apical region of normal striated duct cell. Note the abundance of electron-dense secretory granules. At the bottom (*B*) is the apical region of striated duct cell from seven weeks of daily melatonin injected gland. Note there are very few normal looking secretory granules. Most of them show signs of disintegration. The granular material is amorphous, electron opaque and the surrounding membrane is incomplete (arrows). L, lumen. 15000 ×.

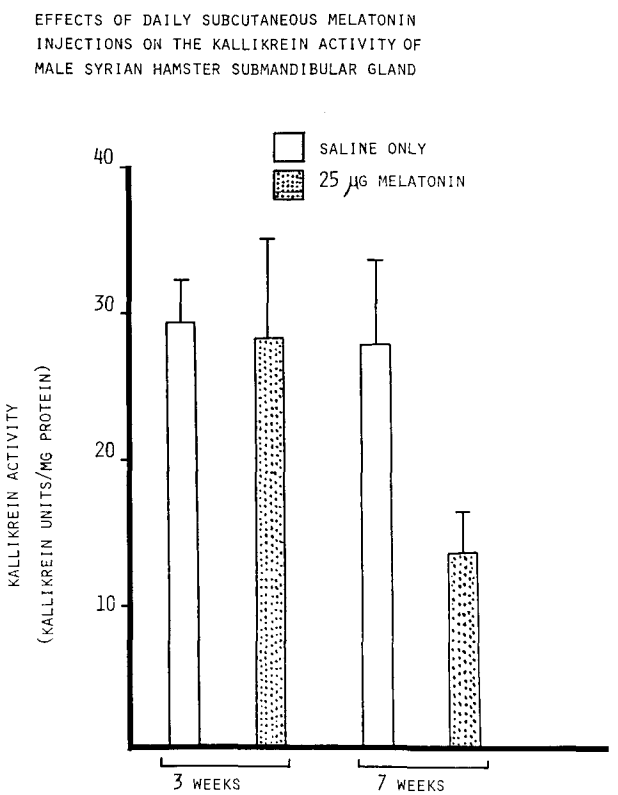

Figure 6. Kallikrein activity following three and seven weeks of daily melatonin injection. Note significant decrease of kallikrein after seven weeks of melatonin treatment. Standard deviation is on top of the bar. Each bar represents five animals.

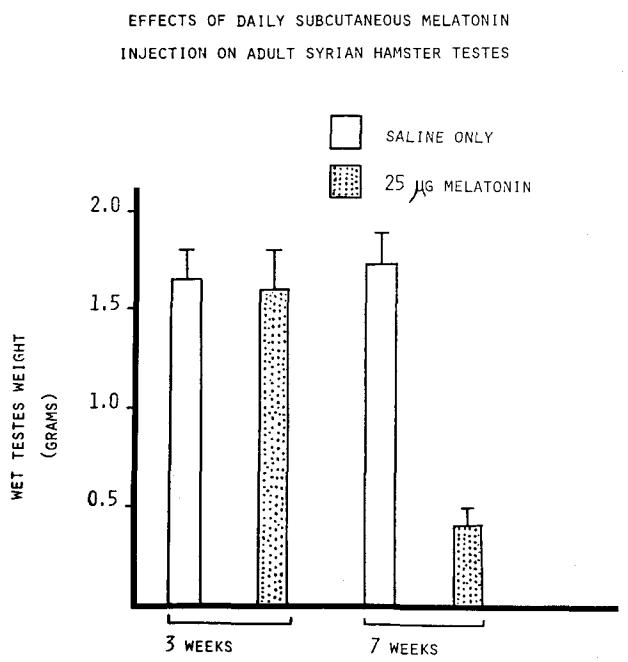

Figure 7. Wet testes weight following three and seven weeks of daily melatonin injections. Note dramatic reduction of testes weight following seven weeks of melatonin treatment. Standard deviation is shown on top of the bar. Each bar represents five animals.

apparatus, like the normal gland cell, is dilated with very few cisternes and is usually located in the perinuclear area. The rough endoplasmic reticulum is located in this area as well. Most mitochondria are located in the basal region of the cell in between the folds of basal plasma membrane, however, few of them can also be seen in the apical region (fig. 5). In some cells of the experimental glands, fusion of electron-dense secretory granules was also observed (fig. 4, arrow heads). Overall convoluted duct cells of melatonin-treated glands gave the appearance of inactivity. These changes were not observed in glands after three weeks of melatonin injections.

Effects on kallikrein activity. Submandibular gland kallikrein specific activity of animals injected with melatonin for 7 weeks was significantly reduced. Figure 6 shows that the activity of kallikrein is decreased by more than 55% when compared with saline-only-injected animals. However 3 weeks of daily melatonin injection did not decrease kallikrein activity significantly.

Effects on testes. Following three weeks of daily melatonin injection there was no significant change in either the size or the weight of testes. However seven weeks of daily melatonin injection caused dramatic reduction of testes size and weight when compared to saline-only-injected animals (fig. 7).

Discussion

From the results obtained in the present investigation it appears that convoluted granular cells of hamster submandibular gland showed altered morphology following seven weeks of daily melatonin injection. The concentration of secretory polypeptide kallikrein was also reduced significantly. Decrease kallikrein activity of the gland and reduction of convoluted secretory granules, in the present investigation, is consistent with other findings in the cat, dog and guinea pig submandibular glands and tend to suggest kallikrein's localization in duct cell secretory granules[29, 30].

Melatonin is rapidly cleared from the blood and metabolized in the liver largely through 6-hydroxylation[22, 23]. It has also been shown that some of it is metabolized in the brain where the calculated half life is approximately 40 min[24]. Because of its rapid clearance, it is unlikely that in the present investigation there was an accumulation of the biogenic amine in injected animals. Since three weeks of daily melatonin injections neither altered the convoluted cell secretory granule population nor decreased the enzyme activity of the gland, the question arises whether changes observed following seven weeks of daily injection were due to direct effect of this indolamine or were a consequence of melatonin's well-studied and established effect on hypothalamus-pituitary-target organ axis. In the present investigation positive correlation between decreased size and testes weight and number of duct cell secretory granules with concomitant reduction of kallikrein activity tend to suggest that melatonin may exert its action via hypothalamus-pitu-

itary-gonad axis. It should be pointed out that similar changes were observed in mouse submaxillary gland following castration and that these changes were reversible after androgen administration[25,26].

Since no apparent morphological changes were observed in the rough endoplasmic reticulum and the Golgi apparatus, it is rather unlikely that melatonin exerted its effects directly or indirectly in the increased synthesis of granule-bound secretory proteins. On the other hand, lack of electron-dense secretory material (secretory proteins) in these granules raises the possibility of a decrease in protein synthesis undetectable by other morphological criteria such as the Golgi apparatus activity and association and disassociation of ribonucleoprotein particles with the endoplasmic reticulum. This decrease coupled with prolonged normal release of secretory protein containing granules would explain the reduction of secretory granule population.

It is well known that as secretory granules move towards the apex of the cell, contents of these granules undergo condensation and compaction and the membrane surrounding these granules is believed to play an important part in these processes. Also, immature granules which are located close to the Golgi apparatus contain more amorphous looking material and appear less electron dense than mature granules which are located away from the Golgi area usually near the apex and appear more electron dense. Disintegration of granule membrane coupled with the lack of electron-dense material (secretory proteins) in granules located near the apex of the cell tend to suggest impairment of condensation mechanism of secretory granules.

Thus, prolonged melatonin injection results in altered morphology of Syrian hamster submandibular gland convoluted duct cells. Secretory granules are affected most and activity of kallikrein is reduced substantially. Cells, in general, show inactivity. The possibility is raised that melatonin exerts its action affecting the hypothalamus-pituitary-gonad axis.

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