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# An *in vitro* study on the effects of melatonin on the ultrastructure of the hamster parathyroid gland

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**Summary.** Isolated parathyroid glands from adult female golden hamsters were incubated on a black Millipore filter in an incubation vessel containing Ham's F-12 medium, with or without melatonin at final concentration of $10^{-5}$ M for 1 hour. In the parathyroid glands used for in vitro treatments with melatonin, the Golgi complexes associated with a few prosecretory granules and cisternae of the rough endoplasmic reticulum showed a significant decrease, and lipid droplets and lysosomes appeared to be increased compared with those of the control parathyroid glands. These changes are considered to be induced by suppression of the synthesis of parathyroid hormone in parathyroid glands incubated in a vessel containing medium with melatonin.

**Key words:** Parathyroid gland, *In vitro,* Melatonin, Morphometry, Hamster

## Introduction

A few morphological studies have dealt with the problem of *in vivo* effect of pineal extract or melatonin on the parathyroid gland (Miline and Krstic, 1966; Krstic, 1968). We have previously reported that the ultrastructure of the parathyroid glands of golden hamsters is affected by melatonin treatment *in vivo* (Chen et al., 1991). The present study was performed *in vitro* to estimate whether similar ultrastructural findings could be obtained in the parathyroid glands cultured in the melatonin-containing medium, and if the direct effect of melatonin on the parathyroid glands could be expected.

## Materials and methods

Ten three-month-old female golden hamsters with an average body weight of 130 g were used in this study. In all animals two parathyroid glands were removed under sodium pentobarbital anaesthesia and immediately used for incubation.

In the control group, the glands were placed on a black Millipore filter (0.45 μm) in an incubation vessel containing 4 ml of Ham's F-12 culture medium. Incubation was carried out at 37 °C in a humidified atmosphere with 95% $O_2$ and 5% $CO_2$.

In the melatonin-treated group, the glands were incubated on a similar filter in another incubation vessel containing 4 ml of Ham's F-12 medium, to which melatonin was added at final concentration of $10^{-5}$M, at 37 °C under 95% $O_2$ and 5% $CO_2$.

At 1 hour after incubation, the glands were immersed in a mixture of 2.5% glutaraldehyde and 2%$OsO_4$ in Millonig's buffer at pH 7.4 for 1 hour, dehydrated through ascending concentrations of acetone and embedded in Epon 812. Thin sections were cut on a Porter-Blum MT-1 ultramicrotome, stained with uranyl acetate and lead salts, and examined with a Hitachi H-700H electron microscope.

In each hamster from 2 groups 20 micrographs at a final magnification of 14,000 were taken from different regions of the parathyroid glands. The area of cytoplasm, the Golgi complexes, cisternae of the rough endoplasmic reticulum, lipid droplets, large vacuolar bodies and lysosomes, and the number of secretory granules and large secretory granules were estimated with the aid of an image analyser (Digigrammer G-6, Mutoh).

Statistical analysis of data was performed by Student's t-test.

## Results

The morphology of the control parathyroid glands resembled that of normal golden hamsters as reported earlier (Emura et al., 1984; Shoumura et al., 1988a,b,c; Isono et al., 1990). The chief cells were arranged in cell clusters and cords which were surrounded by connective tissue. The chief cells were oval or polygonal in shape. The plasma membranes of adjacent cells pursued a tortuous course with occasional interdigitations (Figs. 1,

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2). The intercellular spaces were generally narrow (Figs. 1, 2), and slightly dilated intercellular spaces were sometimes observed and contained floccular or finely particulate material. The chief cells had an oval or polygonal nucleus with occasional indentations. The cytoplasm was scattered diffusely with free ribsomes and randomly with abundant mitochondria (Figs. 1, 2). Cisternae of the rough endoplasmic reticulum were randomly distributed or sometimes arranged in parallel arrays (Figs. 1, 2). Most Golgi complexes containing some prosecretory granules were relatively well developed (Figs. 1, 2). Secretory granules of 150-300 nm in diameter were sometimes scattered in the peripheral cytoplasm (Figs. 1, 2). Large secretory granules of 350-600 nm in diameter which showed a lower electron density than the secretory granules, large vacuolar bodies of 350-750 nm which contained floccular material and/or vesicles (Fig. 1), lysosomes and lipid droplets were sometimes observed in the cytoplasm. Transitional forms between the large secretory granules and large vacuolar bodies were present.

In the parathyroid glands treated with melatonin *in vitro*, many chief cells contained rich free ribosomes, abundant mitochondria, poorly-developed Golgi complexes associated with a few prosecretory granules

**Table 1.** Volume density of the Golgi complex (G), rough endoplasmic reticulum (ER), lipid droplet (LD), large vacuolar body (VB) and lysosome (Ly): the volume density is presented as percentage of cytoplasmic volume. Number of secretory granules (SG) and large secretory granules (LG) per 100 $\mu m^2$ in the cytoplasm.

| | G | ER | LD | VB | Ly | SG | LG |
|---|---|---|---|---|---|---|---|
| Control group | 6.05±0.32 | 8.46±0.42 | 0.20±0.06 | 0.35±0.04 | 0.39±0.05 | 6.39±0.47 | 0.18±0.04 |
| Melatonin-treated group | 4.94±0.39* | 7.12±0.51* | 0.33±0.07 | 0.36±0.05 | 0.51±0.06 | 5.66±0.42 | 0.13±0.03 |

Values are mean±SEM. *: $p<0.05$.

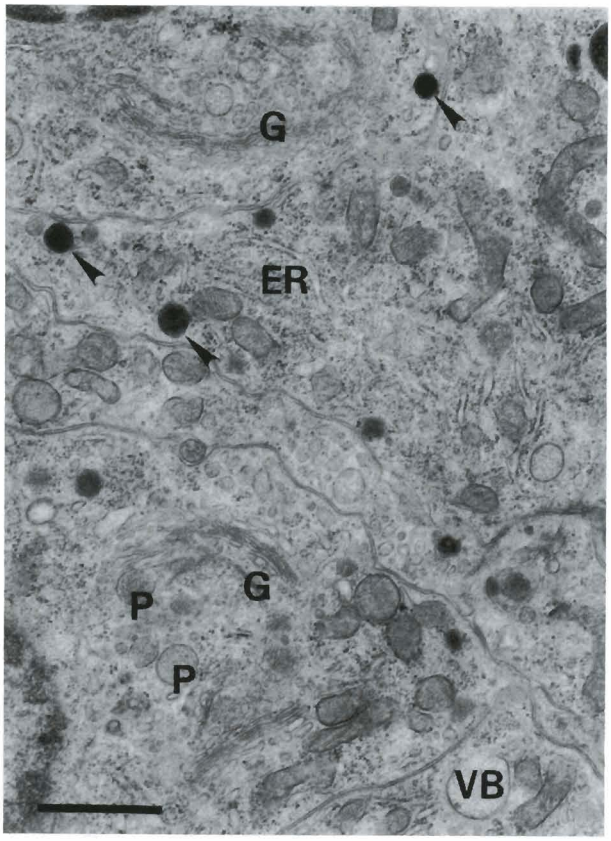


**Fig. 1.** Chief cells of the control parathyroid gland. Relatively well-developed Golgi complexes (G) containing some prosecretory granules (P) and cisternae of the rough endoplasmic reticulum (ER) are observed. Secretory granules (arrow heads) are scattered in the peripheral cytoplasm. VB: large vacuolar body. x 16,000. Bar=1 μm

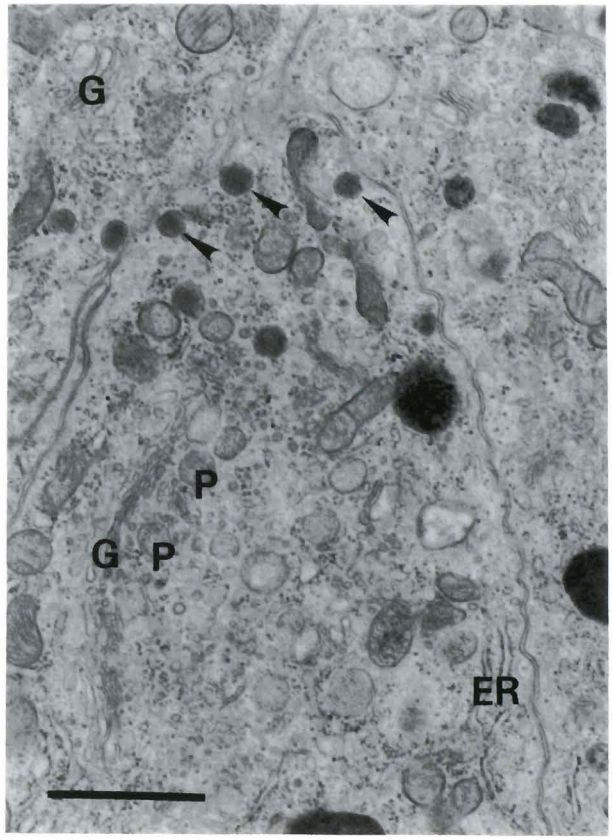


**Fig. 2.** Chief cells of the control parathyroid gland. Relatively well-developed Golgi complexes (G) associated with some prosecretory granules (P) and secretory granules (arrow heads) located in the peripheral cytoplasm are observed. ER: cisternae of the rough endoplasmic reticulum. x 21,000. Bar=1 μm

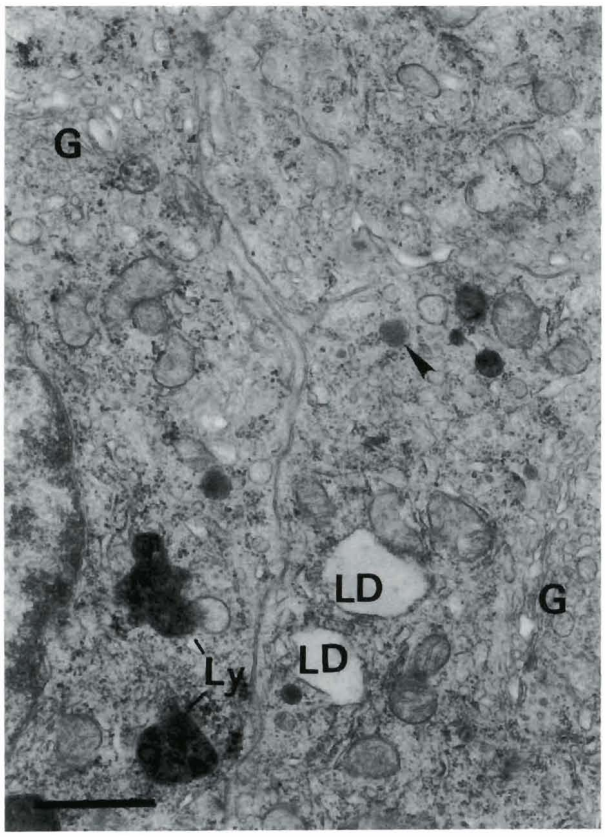


**Fig. 3.** Chief cells of the melatonin-treated parathyroid gland. Poorly-developed Golgi complexes (G) and numerous lipid droplets (LD) and lysosomes (Ly) are observed. Arrow head: Secretory granule. x 16,000. Bar=1μm

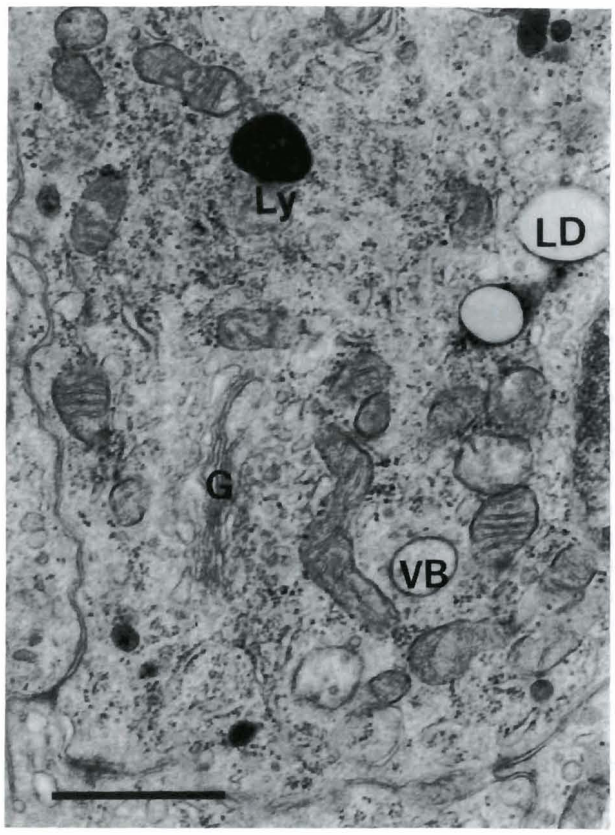


**Fig. 4.** Chief cells of the melatonin-treated parathyroid gland. Poorly-developed Golgi complex (G), lipid droplet (LD), large vacuolar body (VB) and lysosome (Ly) are observed. x 23,000. Bar=1 μm

and numerous lipid droplets and lysosomes (Figs. 3, 4). Cisternae of the rough endoplasmic reticulum were randomly distributed or occasionally arranged in parallel arrays (Figs. 3, 4). Secretory granules (Fig. 3), large secretory granules and large vacuolar bodies (Fig. 4) were sometimes seen in the cytoplasm.

The results of the stereological investigation are given in Table 1. In the parathyroid glands used for *in vitro* treatments with melatonin, the volume density occupied by the Golgi complexes and cisternae of the rough endoplasmic reticulum was significantly decreased ($p<0.05$), and the volume density occupied by lipid droplets and lysosomes appeared to be increased as compared to that of the control pararathyroid glands.

## Discussion

Application of pineal extract or melatonin causes a decrease in parathyroid activity (Miline and Krstic, 1966; Krstic, 1968), while the symptoms of hyperfunction are observed in the parathyroid gland after pinealectomy (Krstic, 1967; Chen et al., 1990). On the other hand, secretory activity of the parathyroid gland is suppressed in response to pinealectomy (Kiss et al., 1969).

Our previous study demonstrated that the parathyroid glands of golden hamsters 1 hour after intraperitoneal injection of melatonin contained poorly-developed Golgi complexes associated with a few prosecretory granules and numerous lipid droplets as compared to those of the control animals (Chen et al., 1991).

In the present study, the principal changes in the *in vitro* effects of melatonin on the parathyroid glands of golden hamsters, compared with the control parathyroid glands, were a significant decrease of the Golgi complexes and cisternae of the rough endoplasmic reticulum. In addition, many chief cells contained a few prosecretory granules in the Golgi areas and numerous lipid droplets. The fine structure of the *in vitro* effects of melatonin on the parathyroid gland was almost stimilar to that of the *in vivo* study (Chen et al., 1991). These results are consistent with the observations of a decrease in functional activity of the parathyroid gland (Roth and Schiller, 1976; Isono et al., 1977, 1980, 1981, 1982, 1983, 1985, 1990; Wild and Becker, 1980; Hayashi et al., 1981; Wild et al., 1982; Emura et al., 1984; Iwasaki et al., 1987; Shoumura et al., 1988a, 1989, 1990; Ishizaki et al., 1989). Therefore, these changes are considered to be induced by suppression of the synthesis of parathyroid hormone in many chief cells of the

parathyroid glands used for *in vitro* treatments with melatonin.

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