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Effects of melatonin on the ultrastructure of the golden hamster parathyroid gland

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Summary. Ultrastructural changes of the parathyroid glands of melatonin-treated golden hamsters were studied. Many chief cells in the parathyroid glands after 1 hour of administration of melatonin contained poorly-developed Golgi complexes associated with a few prosecretory granules and numerous lipid droplets as compared with those of the control animals. The morphology of the parathyroid glands after 5 hours of administration resembled that of the control animals. Many chief cells in the parathyroid glands after 24 hours of administration had well-developed Golgi complexes and cisternae of the granular endoplasmic reticulum, numerous prosecretory granules, a few lipid droplets and many secretory granules in the peripheral cytoplasm as compared with those of the control animals. The ultrastructure of the parathyroid glands after 48 hours of administration was almost similar to that of the control animals.

It is considered that melatonin affects the secretory activity of the parathyroid gland.

Key words: Parathyroid gland, Melatonin, Golden hamster, Ultrastructure, Morphometry

Introduction

Some studies have dealt with the relationship between the pineal gland and the parathyroid gland (Miline and Krstic, 1966; Krstic, 1967, 1968; Kiss et al., 1969; Semm et al., 1981). Morphological alterations suggest that the pineal gland may inhibit (Miline and Krstic, 1966; Krstic, 1967, 1968) or stimulate parathyroid function (Kiss et al., 1969). There are a few light microscopic studies on the effects of pineal extract on the parathyroid gland (Miline and Krstic, 1966; Krstic, 1968). However, there is no study on the effects of melatonin on the ultrastructure of the parathyroid gland.

We investigated ultrastructural changes in the parathyroid glands of golden hamsters after administration of melatonin.

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Materials and methods

Three- to 4-month-old female golden hamsters with an average body weight of 130 g were divided into 5 groups of 5 animals each. One group was given 0.2 ml of distilled water intraperitoneally as controls. The parathyroid glands of the control group were removed under sodium pentobarbital anesthesia at 24 hours after injection. The remaining groups were given intraperitoneally 0.2 ml of melatonin solution at a dose of 0.8 mg/100 g. body weight. The parathyroid glands of the melatonin-treated groups were removed under sodium pentobarbital anesthesia at 1, 5, 24 and 48 hours after injection, respectively. The glands were immersed in a mixture of 2.5% glutaraldehyde and 2% OsO4 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-300 or Hitachi H-700 H electron microscope.

Stereological investigations were performed using the parathyroid glands of 5 golden hamsters of each group. Twenty micrographs at final magnifications of 14,000 were taken from different regions of the parathyroid glands of animals from each group. The area of cytoplasm, nuclei, cisternae of the granular endoplasmic reticulum, the Golgi complexes, lysosomes, lipid droplets and large vacuolar bodies, and the number of large secretory granules and secretory granules were estimated with the aid of an image analyser (Digigrammer G-6, Mutoh).

The serum calcium levels of all animals were measured using a Cornning calcium analyser 940.

All data were presented as the mean ± SEM.

One-way analysis of variance (ANOVA) was used to detect significant differences among the 5 groups and then Duncan's multiple comparison test was used to determine differences between pairs of means. Significance was accepted at $p < 0.05$.

Results

Serum calcium level

The mean serum calcium concentrations (mg/100ml) of the control and experimental groups are shown in Table 1. The calcium concentration in serum of golden hamsters after 1 hour of administration of melatonin was significantly low ($p < 0.05$) as compared with that of the control golden hamsters and 5- and 48-hour-melatonin-treated animals.

Fine structure of the parathyroid gland

Control group

The morphology of the parathyroid glands of the control group resembled that of normal golden hamsters as reported earlier (Emura et al., 1984; Shoumura et al., 1988a,b,c). The chief cells were oval or polygonal in shape. The plasma membranes of adjacent cells pursued a tortuous course with occasional interdigitations (Fig. 1). The intercellular spaces were generally narrow, and slightly enlarged intercellular spaces which contained floccular or finely particulate material were sometimes observed. The chief cells had an oval or polygonal nucleus with occasional indentations. Many chief cells were rich in free ribosomes and mitochondria. Cisternae of the granular endoplasmic reticulum were randomly distributed or sometimes arranged in parallel arrays (Fig. 1). Most Golgi complexes were relatively well developed and associated with some prosecretory granules (Fig. 1). Secretory granules of 150-300 nm in diameter which were filled with a finely particulate material were sometimes scattered in the Golgi areas as well as in the peripheral cytoplasm (Fig. 1). Large secretory granules of 350-600 nm in diameter which showed lower electron density than the secretory granules, large vacuolar bodies of 350-750 nm in diameter which contained floccular material and/or vesicles, lysosomes and lipid droplets were sometimes seen in the cytoplasm. Occasional transitional forms between large secretory granules and large vacuolar

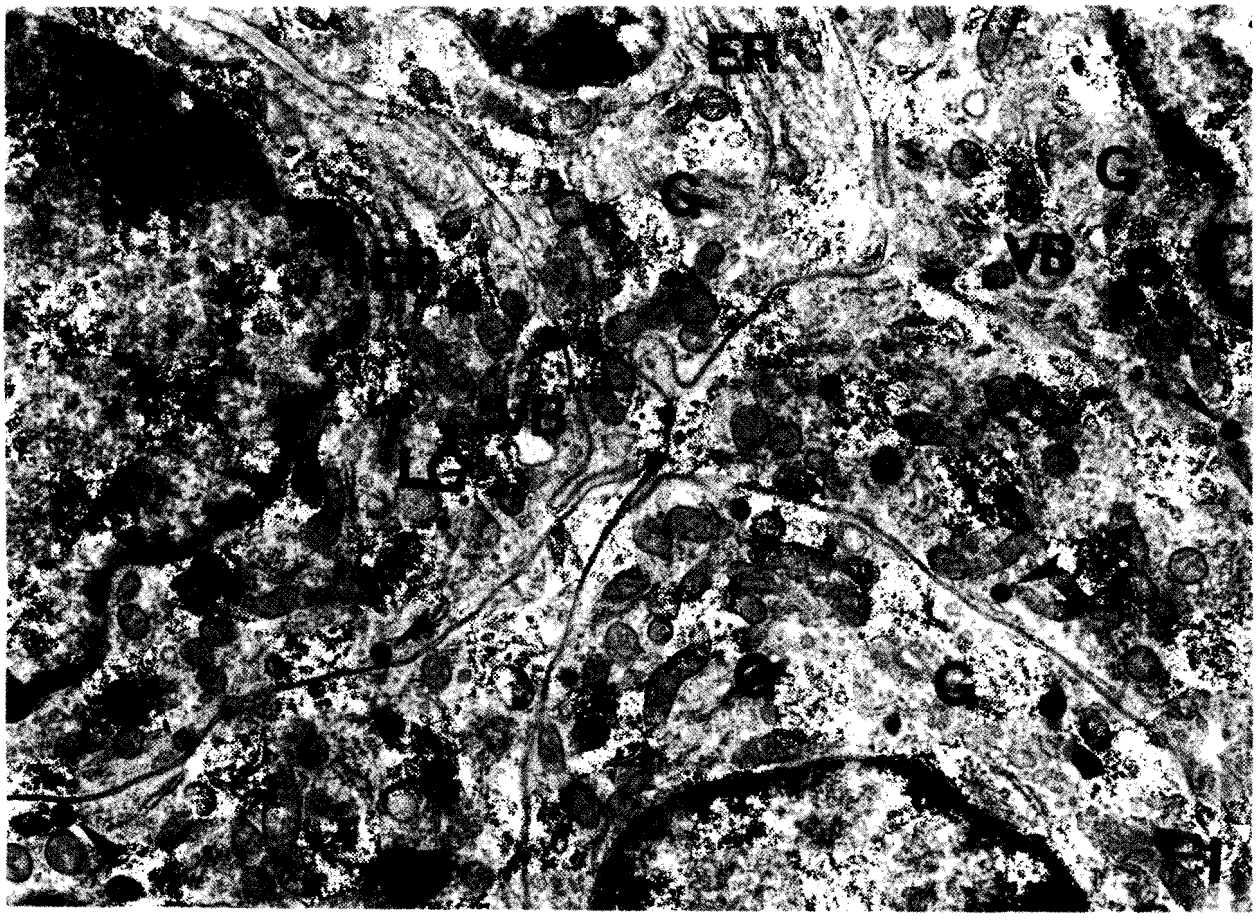

Fig. 1. Parathyroid chief cells of a control golden hamster. Golgi complexes (G) are relatively well developed and cisternae of the granular endoplasmic reticulum (ER) are arranged in parallel arrays. Secretory granules (arrowheads), large secretory granule (LG) and large vacuolar bodies (VB) are seen. × 15,000

Table 1. Serum calcium level (mg/100 ml): values are mean ± SEM

Control group	11.00 ± 0.17
Experimental group	
1-hour-treated-group	10.38 ± 0.13[a]
5-hour-treated group	11.22 ± 0.09[b]
24-hour-treated group	10.88 ± 0.20
48-hour-treated group	11.13 ± 0.19[b]

[a]$p < 0.05$ vs. control group; [b]$p < 0.05$ vs.1-h-treated group (ANOVA and Duncan's multiple comparison test).

bodies were present. Several vesicles were observed near some of the large secretory granules, large vacuolar bodies and transitional forms.

Experimental groups

In the parathyroid gland of the golden hamsters after 1 hour of administration of melatonin, many chief cells had rich ribosomes, poorly-developed Golgi complexes associated with a few prosecretory granules (Fig. 2) and many lipid droplets (Fig. 3). Occasional secretory granules were observed in the peripheral cytoplasm (Fig. 3). Cisternae of the granular endoplasmic reticulum were randomly distributed or sometimes arranged in parallel arrays (Fig. 2). Large secretory granules, large vacuolar bodies, transitional forms and lysosomes were sometimes observed in the cytoplasm.

Table 2. Volume density of the Golgi complex(G), granular 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 100μm^2 in the cytoplasm

	G	ER	LD	VB	LY	SG	LG
Control group	5.57 ± 0.25	7.75 ± 0.26	0.17 ± 0.03	0.29 ± 0.02	0.29 ± 0.02	5.99 ± 0.32	0.19 ± 0.04
Experimental groups							
1-hour-treated group	4.33 ± 0.21[a]	7.88 ± 0.11	0.37 ± 0.03[a]	0.31 ± 0.04	0.31 ± 0.03	5.98 ± 0.33	0.27 ± 0.04
5-hour-treated group	5.10 ± 0.19	7.84 ± 0.24	0.21 ± 0.02[b]	0.27 ± 0.02	0.35 ± 0.02	5.53 ± 0.19	0.22 ± 0.02
24-hour-treated group	7.02 ± 0.04[a,b,c]	8.09 ± 0.38	0.09 ± 0.03[a,b,c]	0.53 ± 0.04[a,b,c]	0.34 ± 0.02	6.32 ± 0.45	0.20 ± 0.03
48-hour-treated group	6.03 ± 0.26[b]	8.51 ± 0.16[a,c]	0.14 ± 0.02[b]	0.34 ± 0.03[d]	0.37 ± 0.03[a]	5.97 ± 0.25	0.24 ± 0.04

Values are mean ± SEM.
[a]$p < 0.05$ vs. control; [b]$p < 0.05$ vs.1-h-treated group; [c]$p < 0.05$ vs.5-h-treated group; [d]$p < 0.05$ vs.24-h-treated group (ANOVA and Duncan's multiple comparison test).

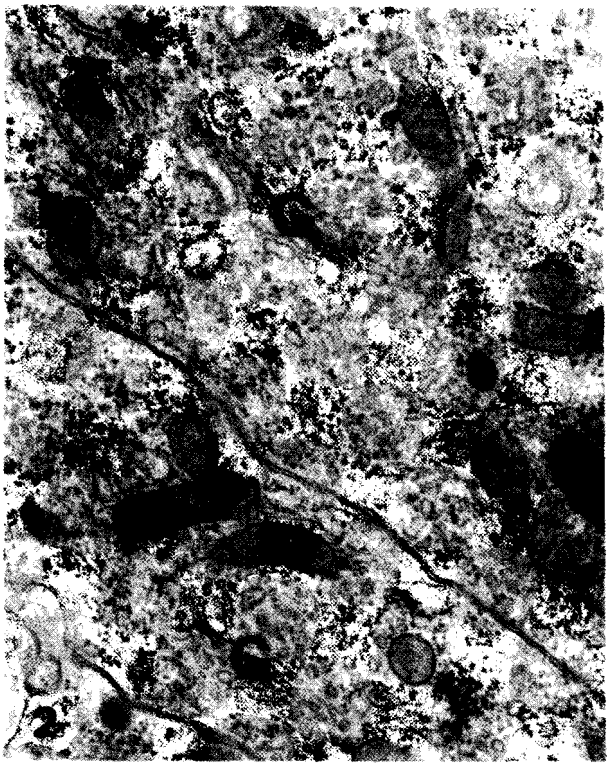

Fig. 2. Poorly-developed Golgi complexes (G) in the chief cells of the 1-hour-melatonin-treated golden hamster. × 21,000

The morphology of the parathyroid glands of the golden hamsters after 5 hours of administration of melatonin resembled that of the control animals.

In the parathyroid glands of the golden hamsters after 24 hours of administration of melatonin, many chief cells had rich free ribosomes, abundant mitochondria, well-developed Golgi complexes containing numerous prosecretory granules (Fig. 4), a few lipid droplets and numerous large vacuolar bodies (Fig. 5). Cisternae of the granular endoplasmic reticulum were sometimes arranged in parallel arrays. Many secretory granules were located in the peripheral cytoplasm adjacent to the plasma membranes (Fig. 4). Enlarged intercellular spaces, containing a finely particulate material, were sometimes observed (Fig. 5). Large secretory granules, transitional forms and lysosomes were sometimes seen in the cytoplasm (Fig. 5).

In the parathyroid glands of the golden hamsters after 48 hours of administration of melatonin, cisternae of the granular endoplasmic reticulum were frequently arranged in parallel arrays. Many chief cells had rich free ribosomes, abundant mitochondria and relatively well-developed Golgi complexes. Secretory granules, large secretory granules, large vacuolar bodies, transitional forms, lysosomes and lipid droplets were sometimes present.

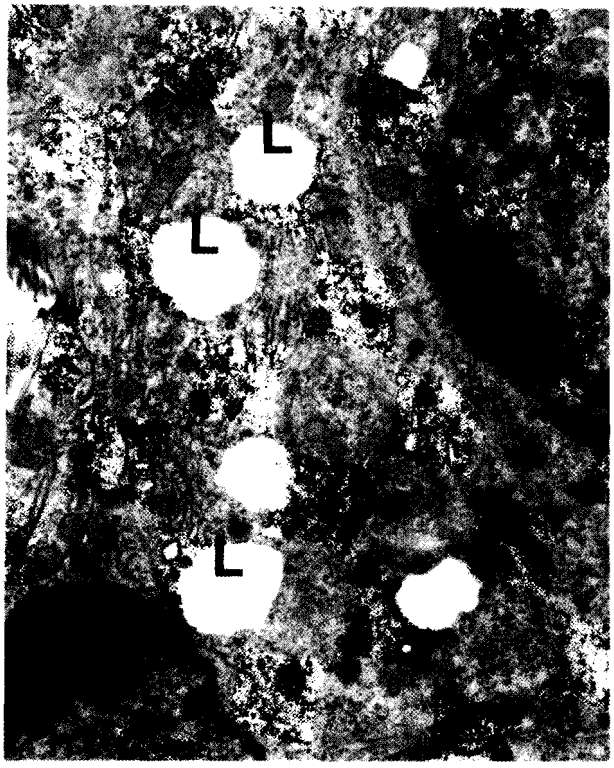

Fig. 3. Parathyroid chief cells of the 1-hour-melatonin-treated golden hamster. Many lipid droplets (L) are observed in the chief cells. × 12,000

Fig. 4. Parathyroid chief cells of the 24-hours-melatonin-treated golden hamster. Well-developed Golgi complexes (G) associated with many prosecretory granules (P) are observed. Secretory granules (arrowheads) are located in the peripheral cytoplasm. × 28,000

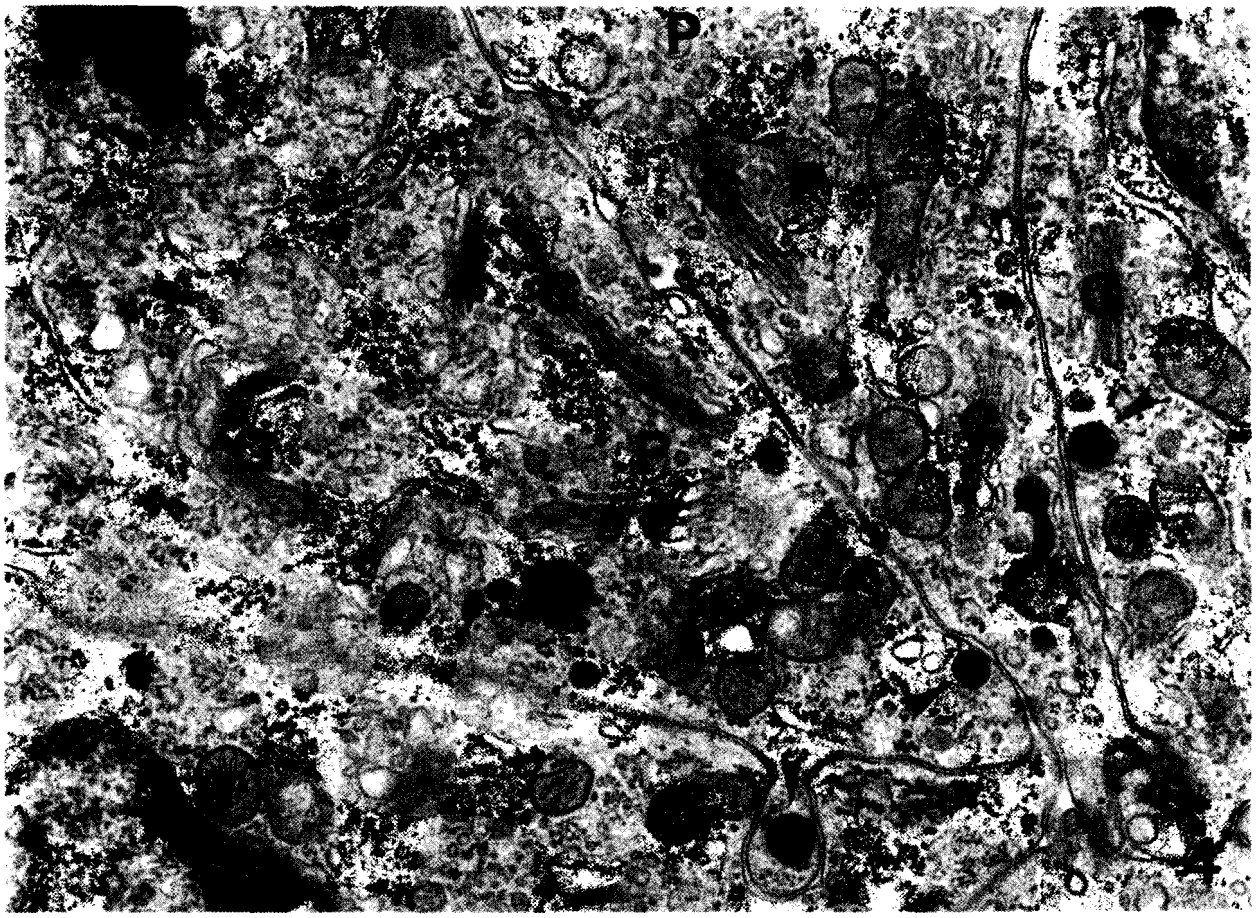

Fig. 5. Parathyroid chief cells of the 24-hour-melatonin-treated golden hamster. Showing numerous large vacuolar bodies (VB). LG = large secretory granule; T = transitional forms between the large secretory granule and large vacuolar body. × 19,000

Stereological analysis of the parathyroid gland

The results obtained from the control and experimental groups are shown in Table 2. In the parathyroid glands of the golden hamsters after 1 hour of administration of melatonin, the volume density occupied by the Golgi complexes was significantly decreased ($p < 0.05$) as compared to that of the control animals and the 24- and 48-hour-melatonin-treated animals and appeared to be decreased as compared to that of the 5-hour-melatonin-treated animals, but in the 5- and 48-hour-melatonin-treated animals the volume density occupied was almost the same as that of the control animals. In addition, in the 24-hour-melatonin-treated animals, it was significantly increased ($p < 0.05$) as compared to that of the control animals and the 1- and 5-hour-melatonin-treated animals. In the 48-hour-melatonin-treated animals the volume density occupied by cisternae of the granular endoplasmic reticulum was significantly increased ($p < 0.05$) as compared to that of the control and 5-hour-melatonin-treated animals, and in the 24-hour-melatonin-treated animals the volume density appeared to be increased as compared to that of the control animals, but in the 1-and 5-hour-melatonin-treated animals it was almost similar to that of the control animals. In the 1-hour-melatonin treated animals the volume density occupied by lipid droplets was significantly increased ($p < 0.05$) as compared to that of the control animals, but in the 5-and 48-hour-melatonin-treated animals the volume density occupied by them was significantly decreased ($p < 0.05$) as compared to that of 1-hour-melatonin-treated animals, and in the 24-hour-melatonin-treated animals the volume density was significantly decreased ($p < 0.05$) as compared to that of the control animals and the 1- and 5-hour-melatonin-treated animals. There were no significant differences among the control animals and the 5- and 48-hour-melatonin-treated animals with regard to the lipid droples. In the 24-hour-melatonin-treated animals the volume density occupied by large vacuolar bodies was significantly increased ($p < 0.05$) as compared to that of the control animals and the 1-, 5-and 48-hour-melatonin-treated animals, but there were no significant differences among the control animals and the 1-, 5-and 48-hour-melatonin-treated animals. In the 48-hour-melatonin-treated animals the volume density occupied by lysosomes was significantly increased ($p < 0.05$) as compared to that of the control animals, but there were no significant differences among the control animals and the 1-, 5-and 24-hour-melatonin-treated animals. There were no significant differences among the 5 groups with regard to the secretory granules and large secretory granules.

Discussion

Application of pineal extract causes a decrease in parathyroid activity (Miline and Krstic, 1966; Krstic, 1968), while in the parathyroid gland after pinealectomy the symptoms of hyperfunction, accompanied by elevated serum calcium levels, are observed, (Krstic, 1967). On the other hand, parathyroid activity is suppressed in response to pinealectomy (Kiss et al., 1969).

The present study demonstrated that many chief cells in the parathyroid glands of golden hamsters after 1 hour of administration of melatonin had poorly-developed Golgi complexes associated with a few prosecretory granules and numerous lipid droplets as compared with those of the control animals. These changes, together with a decrease of serum calcium level, are considered to be induced by suppression of the synthesis of parathyroid hormone in many chief cells in 1-hour-melatonin-treated animals. These results are fairly consistent with the findings which indicate 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).

In this study, the morphology of the parathyroid glands after 5 hours of administration of melatonin resembled that of the control golden hamsters. It is conceivable that the parathyroid gland which showed a decrease in functional activity after 1 hour of administration of melatonin increased to the levels in the control parathyroid gland after 5 hours.

Our results demonstrated that many chief cells in the

parathyroid glands of golden hamsters after 24 hours of administration of melatonin contained well-developed Golgi complexes and cisternae of the granular endoplasmic reticulum, numerous prosecretory granules, a few lipid droplets, and many secretory granules in the peripheral cytoplasm as compared with those of the control animals. These findings are essentially similar to the observations of an increase in functional activity of the parathyroid gland (Roth and Schiller, 1976; Isono et al., 1977, 1979a,b, 1986, 1990; Hayashi et al., 1980; Isono and Shoumura, 1980; Wild, 1980; Emura et al., 1982, 1984; Wild and Manser, 1986; Shoumura et al., 1988a,b, 1989, 1990; Ishizaki et al., 1989). Therefore, it is possible to speculate that the synthesis and release of parathyroid hormone may be stimulated in the parathyroid glands after 24 hours.

In the present study, the ultrastructure of the parathyroid glands after 48 hours of administration of melatonin was almost similar to that of the control animals except for well-developed cisternae of the granular endoplasmic reticulum. It is supposed that the parathyroid gland which indicated an increase in function of the parathyroid gland after 24 hours decreased to the level in the control parathyroid gland after 48 hours.

In this work, large secretory granules, large vacuolar bodies and transitional forms were present in the control and melatonin-treated golden hamsters. The contents of the large secretory granules were similar to those of the secretory granules, although there was a difference in their size and electron density. Protein A-gold particles are detected over the secretory granules and the large secretory granules (Inoue and Setoguti, 1986; Shoumura et al., 1988c,d). We think that both granule types include parathyroid hormone, as previously described (Inoue and Setoguti, 1986; Shoumura et al., 1988c,d), and that the large secretory granules are storage granules, as previously reported (Isono and Shoumura, 1980; Isono et al., 1980, 1981, 1982, 1985, 1990; Setoguti et al., 1981; Shoumura et al., 1988a,b,c,d, 1989, 1990). Very few protein A-gold particles are noted over transitional forms, but particles are absent over large vacuolar bodies (Shoumura et al., 1988d). It is thought that some of the large secretory granules may be changed into the large vacuolar bodies through transitional forms and that such transformation may involve lysosomal digestion of the storage granules.

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