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

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Summary. Ultrastructural changes of the parathyroid glands of pinealectomized golden hamsters were investigated. The main changes in the parathyroid glands 1 hour and 1 day after pinealectomy compared with the control and sham-operated groups were an increase of the Golgi complexes, cisternae of the granular endoplasmic reticulum and large vacuolar bodies. In addition, many chief cells contained numerous prosecretory granules in the Golgi areas and many secretory granules in the peripheral cytoplasm. The morphology of the parathyroid glands 7 and 30 days after pinealectomy resembled that of the control parathyroid glands.

These results suggest that pinealectomy affects the secretory activity of the parathyroid gland.

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

Introduction

Several 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 have suggested that the pineal gland may inhibit parathyroid function (Miline and Krstic, 1966; Krstic, 1967, 1968) or stimulate parathyroid function (Kiss et al., 1969). In addition, it has been shown that pinealectomy produced changes in the morphology of the parathyroid gland (Krstic, 1967; Kiss et al., 1969). The data are, however, inconsistent with regards to the question whether the effect of pinealectomy on the parathyroid gland is a positive or a negative one.

The purpose of this study is to ultrastructurally estimate the secretory activity of the parathyroid glands in pinealectomized golden hamsters.

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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 9 groups of 5 animals each. One group served as controls. The parathyroid glands of the control group were removed under sodium pentobarbital anesthesia. In the remaining eight groups, pinealectomy (pinealectomized groups) was performed by a method of boring a hole at the junction between the occipital and parietal bones with a drilling machine and cauterizing the exposed pineal gland with hot wire, and sham-operation (sham-operated groups) by a method of incising the skin and trepanning the skull without injuring the pineal gland under sodium pentobarbital anesthesia. The golden hamsters of the experimental groups were maintained on solid chow (Clea Japan Inc., CE-2) and tap water ad libitum. The parathyroid glands of the experimental groups were removed under sodium pentobarbital anesthesia 1 hour and 1, 7 and 30 days after pinealectomy and sham-operation, respectively. 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-300 or Hitachi H-700 H electron microscope.

In each golden hamster from the 9 groups, 20 micrographs at final magnification of 14,000 were taken from different regions of the parathyroid glands. The area of cytoplasm, nuclei, the Golgi complexes, cisternae of the granular 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).

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

All data are presented at the mean ± SEM. One-way analysis of variance (ANOVA) was used to detect significant differences among the control, sham-operated and pinealectomized groups, and the 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 concentration (mg/100 ml) of the control and experimental groups are shown in Table 1. There were no significant differences among the control, sham-operated and pinealectomized groups.

Fine structure of the parathyroid gland

Control golden hamster

The chief cells were oval or polygonal in shape. The plasma membranes of adjacent chief cells pursued a tortuous course with occasional interdigitations (Fig. 1). The intercellular spaces were generally narrow and occasional enlargements had floccular or finely particulate material. The chief cells had an oval or polygonal nucleus. 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. Most Golgi complexes were relatively well developed (Fig. 1) and associated with some prosecretory granules having floccular material. Secretory granules of 150-300 nm in diameter were filled with a finely particulate material (Fig. 1). Secretory granules were located in a peripheral cytoplasm. Large secretory granules of 350-600 nm in diameter showing lower electron density than the secretory granules, large vacuolar bodies of 350-750 nm in diameter containing floccular material and/or vesicles, lysosomes and lipid droplets (Fig. 1) were sometimes observed in the cytoplasm. A few transitional forms between large secretory granules and large vacuolar bodies were present. Several vesicles were seen juxtaposed to some of the large secretory granules, large vacuolar bodies and transitional forms.

Sham-operated golden hamster

The morphology of the parathyroid glands of the golden hamsters 1 hour and 1, 7 and 30 days after sham-operation resembled that of the control animals.

Pinealectomized golden hamster

In the parathyroid glands of the golden hamsters 1 hour and 1 day after pinealectomy, many chief cells contained rich free ribosomes, abundant mitochondria, well-developed Golgi complexes associated with numerous prosecretory granules (Figs. 2, 5, 6) and cisternae of the granular endoplasmic reticulum (Figs. 3, 4), a few lipid droplets and numerous large vacuolar bodies. Many secretory granules were located in the peripheral cytoplasm adjacent to the plasma membrane (Fig. 4) and enlarged intercellular spaces containing a finely particulate material were sometimes observed. Some of large vacuolar bodies were observed near the Golgi areas (Fig. 3). Occasional lysosomes, and a few transitional forms and large secretory granules (Figs. 5, 6) were present.

The ultrastructure of the parathyroid glands of the golden hamsters 7 and 30 days after pinealectomy was almost similar to that of the control golden hamsters.

Stereological analysis of the parathyroid gland

The analytical results obtained from the control group and the experimental groups are shown in Table 2. There were no significant differences between the control and sham-operated groups with regard to all the cell organelles estimated in this study. In the parathyroid glands of the golden hamsters 1 hour after pinealectomy the volume density occupied by the Golgi complexes was significantly increased ($p < 0.05$) as compared to that of the control animals and the animals 1 hour after sham-operation. In the parathyroid glands 1 day after pinealectomy the volume density occupied by the Golgi complexes was significantly increased ($p < 0.05$) as compared to that of the animals 1 day after sham-operation and appeared to be increased as compared to that of the control animals. However 7 and 30 days after pinealectomy the volume density occupied by the Golgi complexes was almost the same as that of the control animals and the animals 7 and 30 days after sham-operation. 1 hour and 1 day after pinealectomy the volume density occupied by cisternae of the granular endoplasmic reticulum appeared to be increased as compared to that of the control animals, but 7 and 30 days after pinealectomy the volume density of cisternae of the granular endoplasmic reticulum was almost similar to that of the control animals and the animals 7 and 30 days after sham-operation. 1 hour after pinealectomy the volume density occupied by large vacuolar bodies was significantly increased ($p < 0.05$) as compared to that of the control animals and appeared to be increased as compared to that of the animals 1 hour after sham-operation, but 1, 7 and 30 days after pinealectomy the volume density occupied by large vacuolar bodies was almost the same as that of the control animals and the animals 1,7 and 30 days after sham-operation. There were no significant differences between the control animals and the animals 1 hour and 1, 7 and 30 days after pinealectomy with regard to the lipid droplets, lysosomes, secretory granules and large secretory granules.

Discussion

It has been described that pinealectomy elevates the serum calcium levels (Kristic, 1967). In the present

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

Control	10.87 ± 0.25
1-hour-sham-operated	10.65 ± 0.13
1-hour-pinealectomized	10.88 ± 0.09
1-day-sham-operated	10.62 ± 0.21
1-day-pinealectomized	10.71 ± 0.36
7-days-sham-operated	11.16 ± 0.26
7-days-pinealectomized	11.05 ± 0.19
30-days-sham-operated	10.84 ± 0.37
30-days-pinealectomized	10.61 ± 0.08

the parathyroid gland, which showed an increase in functional activity 1 hour and 1 day after pinealectomy, decreased to the levels in the control parathyroid gland 7 and 30 days after pinealectomy.

The number of secretory granules in the parathyroid gland does not appear to correlate with the functional condititon of the parathyroid gland (Roth and Raisz, 1966; Altenähr and Seifert, 1971). In our work, there were no significant differences between the control and pinealectomized groups, but many secretory granules gathered beneath the plasma membrane in the active chief cells 1 hour and 1 day after pinealectomy. A similar

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 100㎛^{2} in the cytoplasm

	G	ER	LD	VB	LY	SG	LG
Control	5.44 ± 0.13	7.94 ± 0.12	0.17 ± 0.03	0.27 ± 0.03	0.30 ± 0.04	5.89 ± 0.21	0.23 ± 0.06
1-hour-sham-operated	5.40 ± 0.21	8.30 ± 0.24	0.22 ± 0.02	0.31 ± 0.02	0.35 ± 0.03	5.76 ± 0.23	0.28 ± 0.06
1-hour-pinealectomized	6.42 ± 0.14[a,b]	8.40 ± 0.17	0.17 ± 0.01	0.41 ± 0.04[a]	0.29 ± 0.02	5.62 ± 0.12	0.19 ± 0.02
1-day-sham-operated	5.34 ± 0.38	7.82 ± 0.26	0.20 ± 0.01	0.31 ± 0.01	0.43 ± 0.05	5.46 ± 0.18	0.23 ± 0.07
1-day-pinealectomized	6.46 ± 0.28[b]	8.80 ± 0.47	0.19 ± 0.02	0.36 ± 0.02	0.34 ± 0.02	5.27 ± 0.32	0.25 ± 0.05
7-days-sham-operated	5.54 ± 0.30	8.46 ± 0.22	0.19 ± 0.03	0.33 ± 0.02	0.32 ± 0.04	5.74 ± 0.22	0.20 ± 0.06
7-days-pinealectomized	5.28 ± 0.19	7.69 ± 0.29	0.17 ± 0.04	0.30 ± 0.02	0.32 ± 0.04	5.81 ± 0.12	0.26 ± 0.07
30-days-sham-operated	5.32 ± 0.15	8.29 ± 0.34	0.19 ± 0.04	0.30 ± 0.03	0.33 ± 0.03	5.53 ± 0.20	0.19 ± 0.03
30-days-pinealectomized	5.21 ± 0.27	8.27 ± 0.07	0.14 ± 0.02	0.33 ± 0.02	0.28 ± 0.03	5.73 ± 0.19	0.15 ± 0.05

Values are mean ± SEM.
[a]$p < 0.05$ vs. control; [b]$p < 0.05$ vs. sham-operated (ANOVA and Duncan's multiple comparison test).

study, however, there were no significant differences among the control and experimental groups.

In the present study, the main changes in the parathyroid glands of the golden hamsters 1 hour and 1 day after pinealectomy compared with the control and sham-operated groups were an increase of the Golgi complexes, the cisternae of the granular endoplasmic reticulum and the large vacuolar bodies. In addition, many chief cells contained numerous prosecretory granules in the Golgi areas and many secretory granules in the peripheral cytoplasm. 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). Similar results have also been observed in the parathyroid glands of pinealectomized rats (Krstic, 1967). Therefore, although it has been reported that pinealectomy caused a decrease in functional activity of the parathyroid gland (Kiss et al., 1969), we consider that the synthesis and release of parathyroid hormone may be stimulated in the parathyroid glands 1 hour and 1 day after pinealectomy, as described by Krstic (1967).

In this study, the morphology of the parathyroid glands 7 and 30 days after pinealectomy resembled that of the control parathyroid glands. It is conceivable that finding has also been observed in the parathyroid gland showing an increase in functional activity (Fujii and Isono, 1972; Isono and Shoumura, 1980; Emura et al., 1982, 1984). In the present study, the enlarged intercellular spaces contained a finely particulate material similar to the contents of the secretory granule. These observations suggest the possibility of exocytosis of the secretory granule.

It is demonstrated that the large secretory granules, large vacuolar bodies and transitional forms were observed in the parathyroid glands of the control and experimental groups. The contents of the large secretory granules were similar to those of the secretory granules. Protein A-gold particles are detected over the secretory granules and the large secretory granules (Inoue and Setoguti, 1986; Shoumura et al., 1988c,d). It is thought that both granule types have parathyroid hormone, as reported by Inoue and Setoguti (1986) and Shoumura et al. (1988c,d) and that the large secretory granules are storage granules, as described previously (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 particles are noted over transitional forms, and particles are absent over large vacuolar bodies (Shoumura et al., 1988d). It is supposed that some of the large secretory granules may be changed into the large vacuolar bodies through transitional forms and that such

Parathyroid of pinealectomized hamster

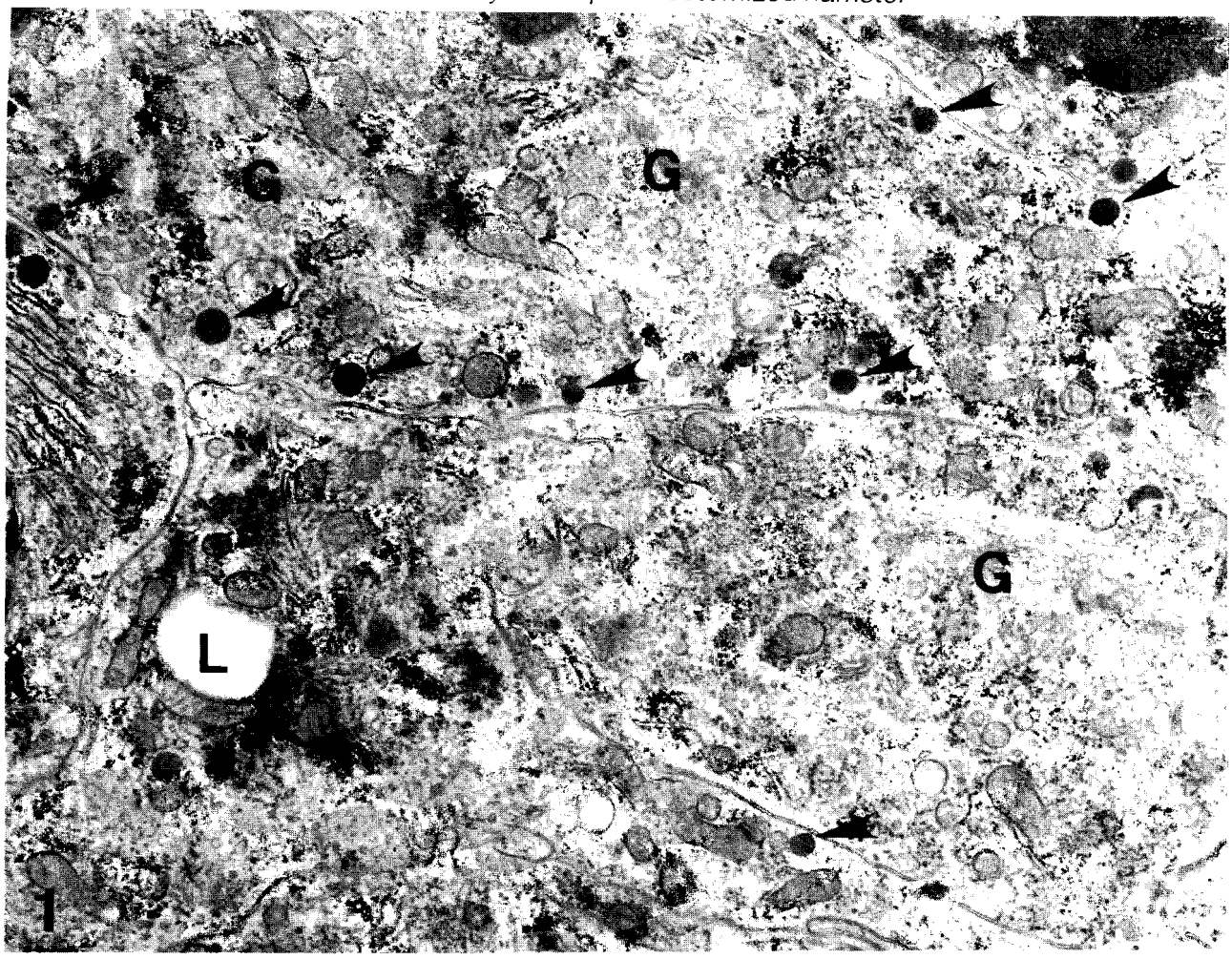

Fig. 1. Parathyroid chief cells of a control golden hamster. The Golgi complexes (G) are relatively well developed. Secretory granules (arrowheads) and occasional lipid droplets (L) are seen. × 19,000

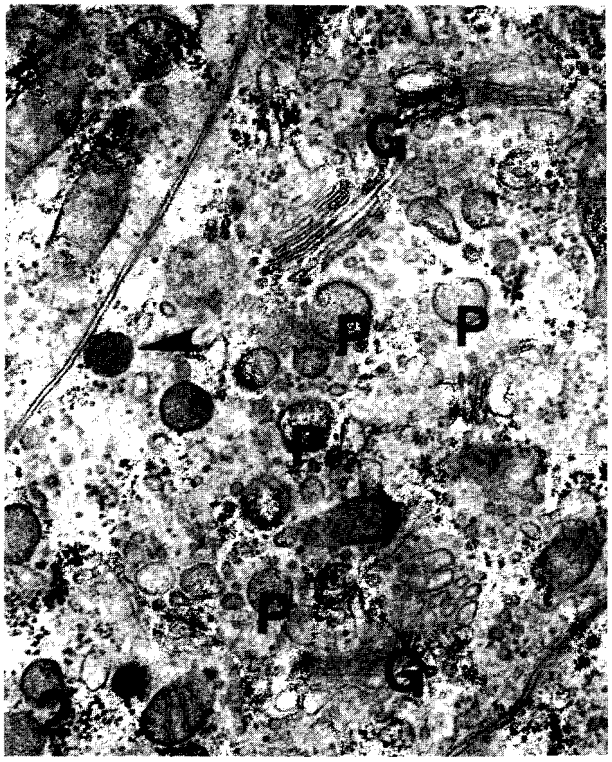

Fig. 2. Parathyroid chief cells of the 1-hour-pinealectomized golden hamster. Well-developed Golgi complexes (G) associated with many prosecretory granules (P) are observed. Arrowhead: secretory granule. × 25,000

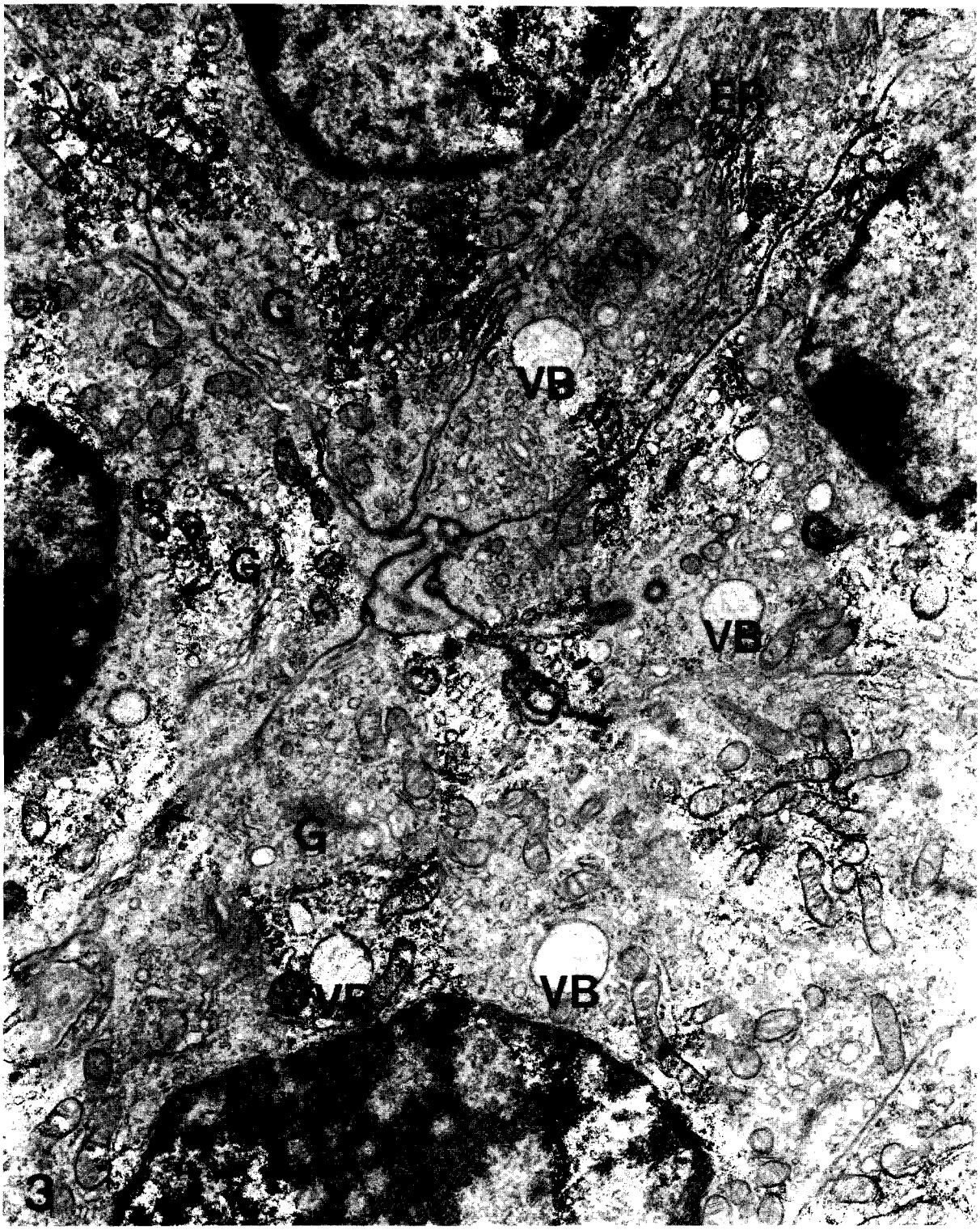

Fig. 3. Parathyroid chief cells of the 1-hour-pinealectomized golden hamster. Showing numerous large vacuolar bodies (VB) near the Golgi areas (G). ER: cisternae of the granular endoplasmic reticulum. × 17,000

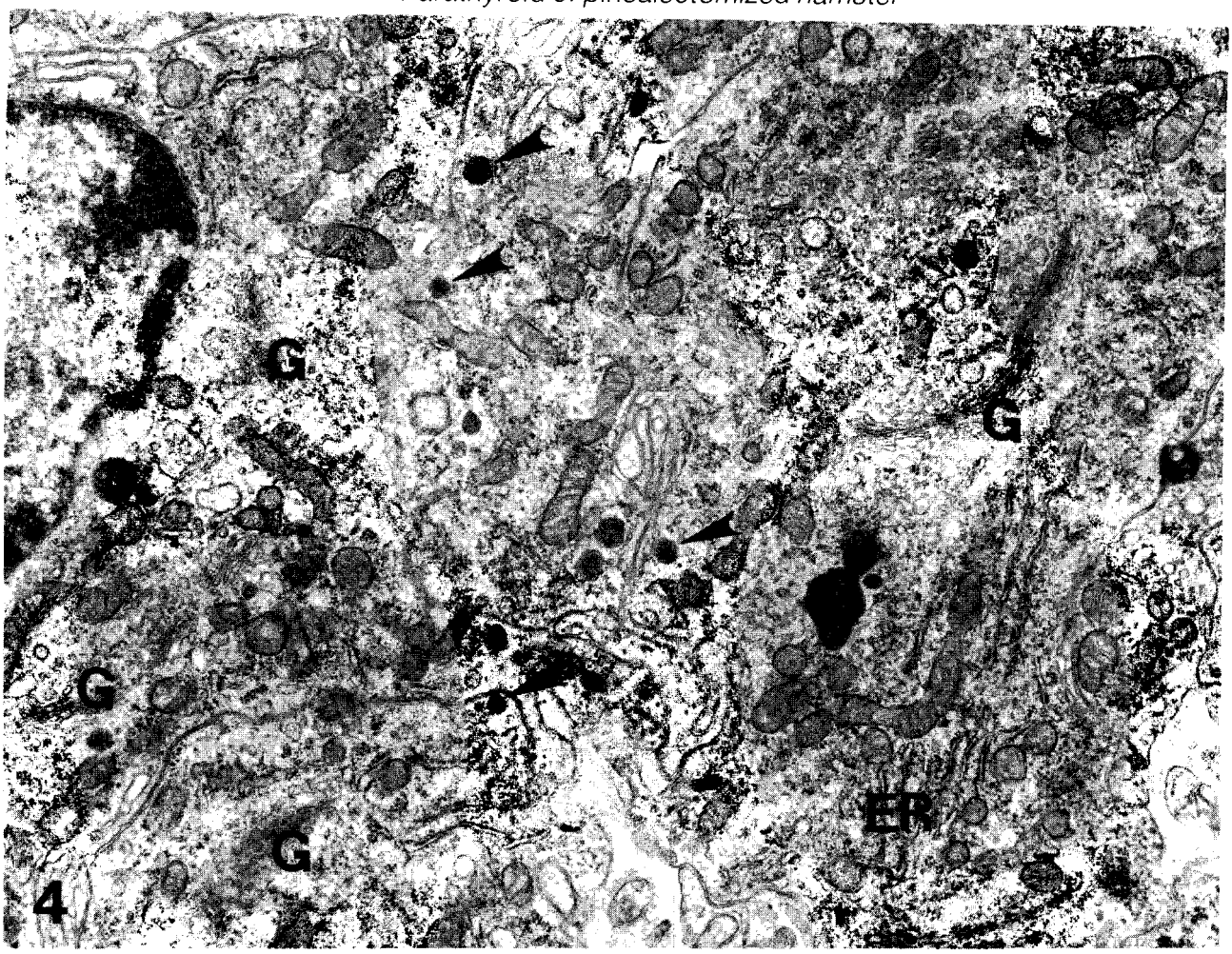

Fig. 4. Parathyroid chief cells of the 1-hour-pinealectomized golden hamster. The Golgi complexes (G) are well developed and cisternae of the granular endoplasmic reticulum (ER) are arranged in parallel arrays. Secretory granules (arrowheads) are located in the peripheral cytoplasm. × 19,000

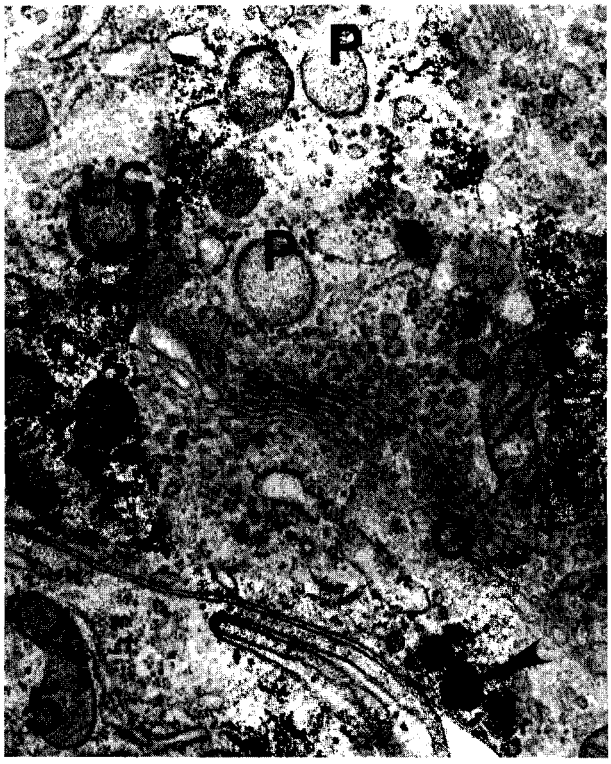

Fig. 5. Parathyroid chief cells of the 1-day-pinealectomized golden hamster. Well-developed Golgi complexes (G) associated with many prosecretory granules (P) are observed. Secretory granule (arrowheads) and large secretory granule (LG) are seen. × 19,000

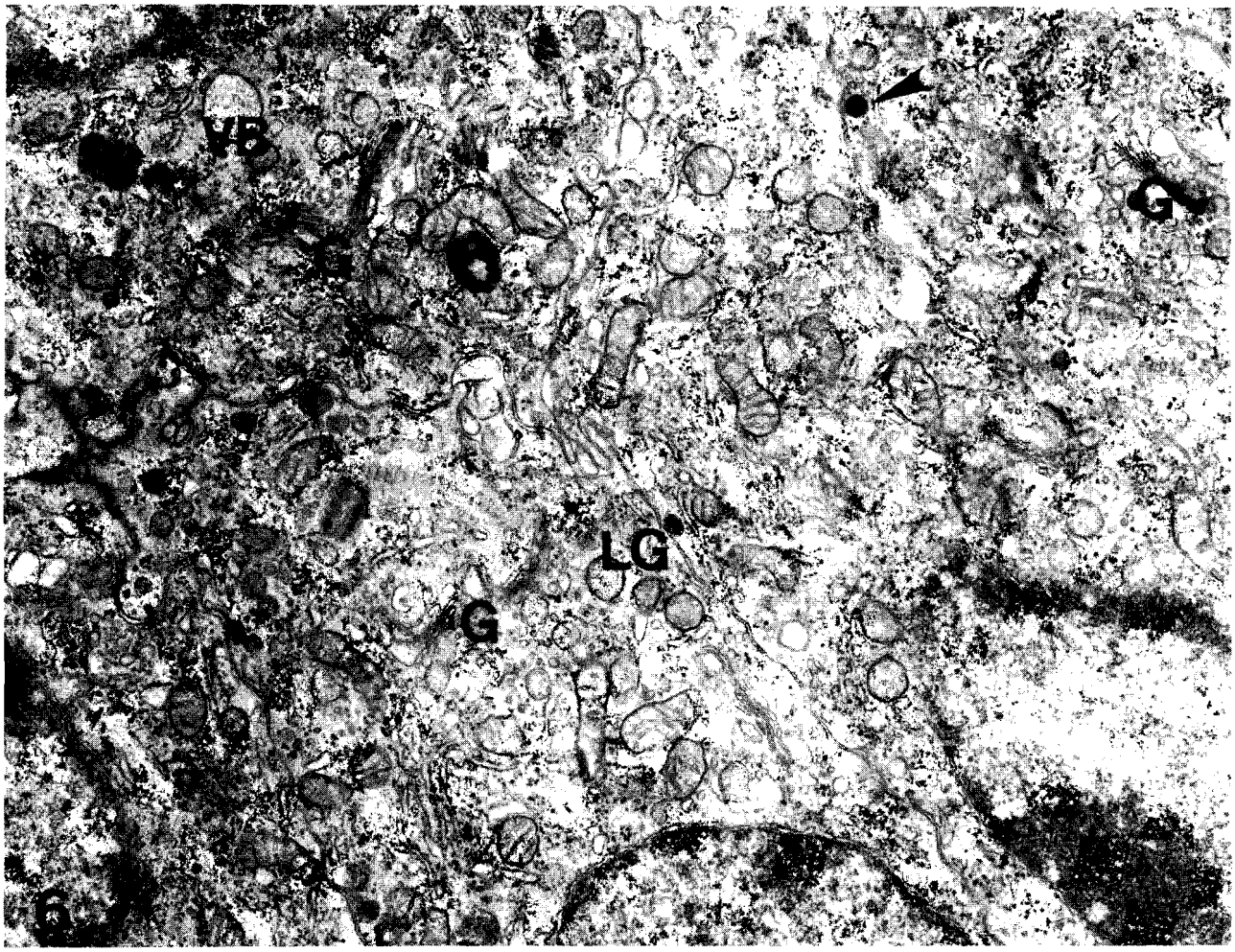

Fig. 6. Parathyroid chief cells of the 1-day-pinealectomized golden hamster. The Golgi complexes (G) are well developed. Secretory granules (arrowheads), large secretory granule (LG) and large vacuolar body (VB) are observed. × 25,000

transformation may involve lysosomal digestion of the storage granules. In addition, our study demonstrated that large vacuolar bodies were also observed near the Golgi areas associated with numerous prosecretory granules in the parathyroid glands 1 hour and 1 day after pinealectomy. The contents of some of the large vacuolar bodies were similar to those of the prosecretory granules, although there was a difference in their size. We think that some of the prosecretory granules may be changed into the large vacuolar bodies and that such transformation may indicate a lysosomal digestion in the regulation of overproduction of the prosecretory granules. However, additonal investigations are required to clarify the origin of the large vacuolar bodies.

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