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Effects of long-term treatment with ethanol on the ultrastructure of the golden hamster parathyroid gland

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Summary. The ultrastructure of the parathyroid gland in golden hamsters after long-term treatment with ethanol was studied. Male hamsters of experimental groups were given ethanol at the concentration of 7% for 3 and 5 months with food and water freely available. In the ethanol-treated hamsters, the Golgi complexes associated with many prosecretory granules were well developed and many secretory granules were located near the plasma membrane as compared with those of the control animals. Exocytotic events were observed in 5-month-treated animals. These findings suggest that the secretory activity of the parathyroid gland is stimulated after long-term treatment with ethanol.

Key words: Parathyroid gland, Ultrastructure, Exocytosis, Golden hamster, Ethanol

Introduction

The increasing incidence of osteoporosis has prompted the research for etiological factors. Among these factors, ethanol has recently been implicated in osteoporosis as a risk factor. Constant consumption of ethanol is associated with disturbance of bones and mineral metabolism (Bikle et al., 1985). The mechanism of the production of ethanol-associated bone diseases seems to be complex. There is a direct effect of ethanol on bone cells and an indirect or modulating effect through mineral regulating hormones such as vitamin D metabolites, parathyroid hormone (PTH), and calcitonin (Bikle et al., 1985; Laitinen and Välimäki, 1991). However, the possibility that ethanol affects the parathyroid gland directly has received little attention. Previously, we found morphologically that the hamster parathyroid activity was suppressed after short-term treatment with ethanol (Chen et al., 1997, 1998). In the present study, we observed the ultrastructural changes of the hamster parathyroid gland to examine whether long-term treatment with ethanol affects the parathyroid function.

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Materials and methods

Six-week-old male golden hamsters with an average body weight of 92 g were divided into 4 groups of 7 animals each. Hamsters were housed in stainless steel cages that were equipped with depressions where food pellets (CE-2, Clea Japan Inc.) were placed. Experimental animals were given tap water and 7% ethanol solutions provided in calibrated glass bottles fitted with a ball bearing tube to limit spillage. The ethanol solution intake of a hamster averaged 11.2 ml per day. Control hamsters had tap water available as the only drinking fluid. Three and 5 months later, the parathyroid glands of all groups were removed under sodium pentobarbital anesthesia. 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-800 electron microscope. Twenty micrographs at final magnifications of 22,000 were taken from different regions of the parathyroid glands of each animal from four groups. The areas of the cytoplasm, nuclei, cisternae of the granular endoplasmic reticulum, mitochondria, Golgi complexes, lysosomes, lipid droplets and large vacuolar bodies, and secretory granules were estimated with the aid of an image measuring system (Finetec).

The blood ethanol concentrations were determined by gas chromatography, and the serum calcium levels were measured using a Corning calcium analyzer 940. The bone mineral content (BMC) and the bone mineral density (BMD) of the whole body were measured by Dual Energy X-ray Absorptiometry (DXA) using a Hologic QDR type 2000.

All data are presented as mean±SEM. Statistical analysis was done using Stat View J-4.5 (Abacus Concepts) on a Macintosh computer. Group mean values were compared by one-way analysis of variance (ANOVA) and Fisher's PLSD test for multiple comparisons as the post hoc test. A p value <0.05 was considered statistically significant.

Results

Blood ethanol concentration and serum calcium level

The mean blood ethanol concentrations (mg/ml) and the serum calcium levels (mg/100 ml) of the control and experimental groups are shown in Table 1. The blood ethanol concentration of the 3- and 5-month-treated animals was higher than that of the control animals ($p<0.05$). There was no significant difference between the control and the experimental groups regarding the serum calcium level.

BMC and BMD of the whole body

The results of BMC and BMD of the whole body are also shown in Table 1. There was no significant difference between the control and 3-month-treated groups. In 5-month-treated animals, BMD seemed to decrease, but there was no significant difference.

Table 1. Blood ethanol concentration (mg/ml), serum calcium level (mg/100 ml) and BMC (gr), BMD (gr/cm^2) of the whole body.

TIME	PROTOCOL	ETHANOL	CALCIUM	BMC	BMD
3 m	Control	<0.1	10.88±0.13	3.457±0.122	0.117±0.004
	Experiment	0.33±0.06*	11.00±0.19	3.420±0.104	0.111±0.003
5 m	Control	<0.1	10.93±0.11	3.643±0.251	0.121±0.003
	Experiment	0.33±0.06*	10.77±0.13	3.509±0.249	0.111±0.003

Values are shown as mean±SEM, *: $p<0.05$.

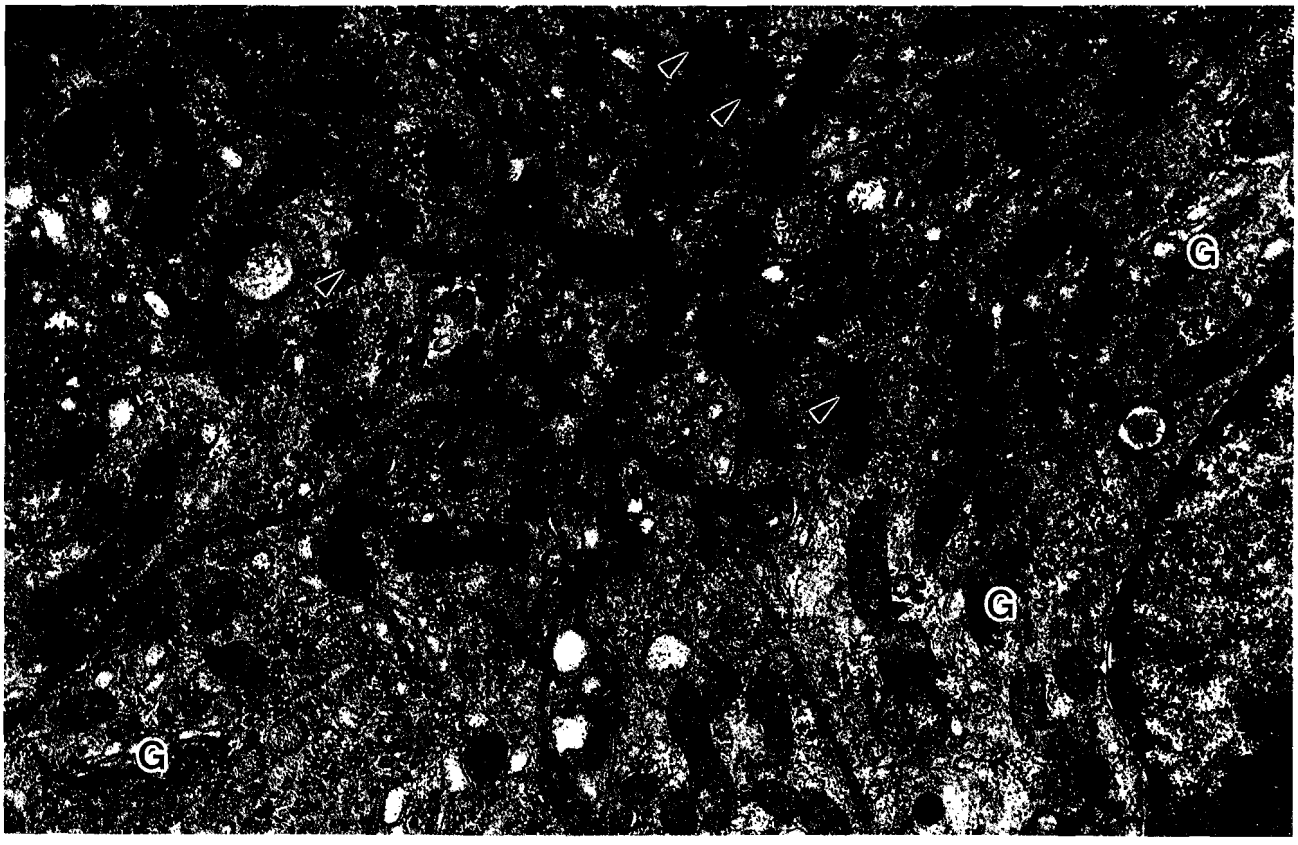

Fig. 1. Parathyroid chief cells of the 3-month control hamster. Relatively well-developed Golgi complexes (G) and secretory granules (arrowheads) are seen. x 22,000

Fine structure of the parathyroid gland

Control groups

In the parathyroid glands of the 3- and 5-month control hamsters, 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. Most Golgi complexes were relatively well developed and associated with some prosecretory granules (Fig. 1). Secretory granules 150-300 nm in diameter filled with a finely particulate material were scattered in the Golgi area as well as in the peripheral cytoplasm (Fig. 1). Large secretory granules, 350-600 nm in diameter, showed lower electron density than the secretory granules. Large vacuolar bodies 350-750 nm in diameter contained floccular material or vesicles. Lysosomes and lipid droplets were sometimes seen in the cytoplasm.

Ethanol-treated groups

In the parathyroid gland of the 3-month ethanol-treated hamsters, many chief cells had rich ribosomes and well-developed Golgi complexes associated with many prosecretory granules (Fig. 2). Randomly distributed cisternae of the granular endoplasmic reticulum were often arranged in parallel arrays. Secretory granules were observed in the peripheral cytoplasm. Some granules were located very near to the plasma membrane (Fig. 2). Large secretory granules, large vacuolar bodies, and lysosomes were sometimes observed in the cytoplasm.

In the parathyroid glands of the 5-month ethanol-treated hamsters, many chief cells had rich ribosomes and well-developed Golgi complexes associated with many prosecretory granules (Fig. 3). Secretory granules were observed in the peripheral cytoplasm. The membrane of some granules was fused with the plasma membrane (Figs. 3, 4). Large vacuolar bodies and lipid droplets were occasionally found in the cytoplasm. Large secretory granules and lysosomes were sometimes

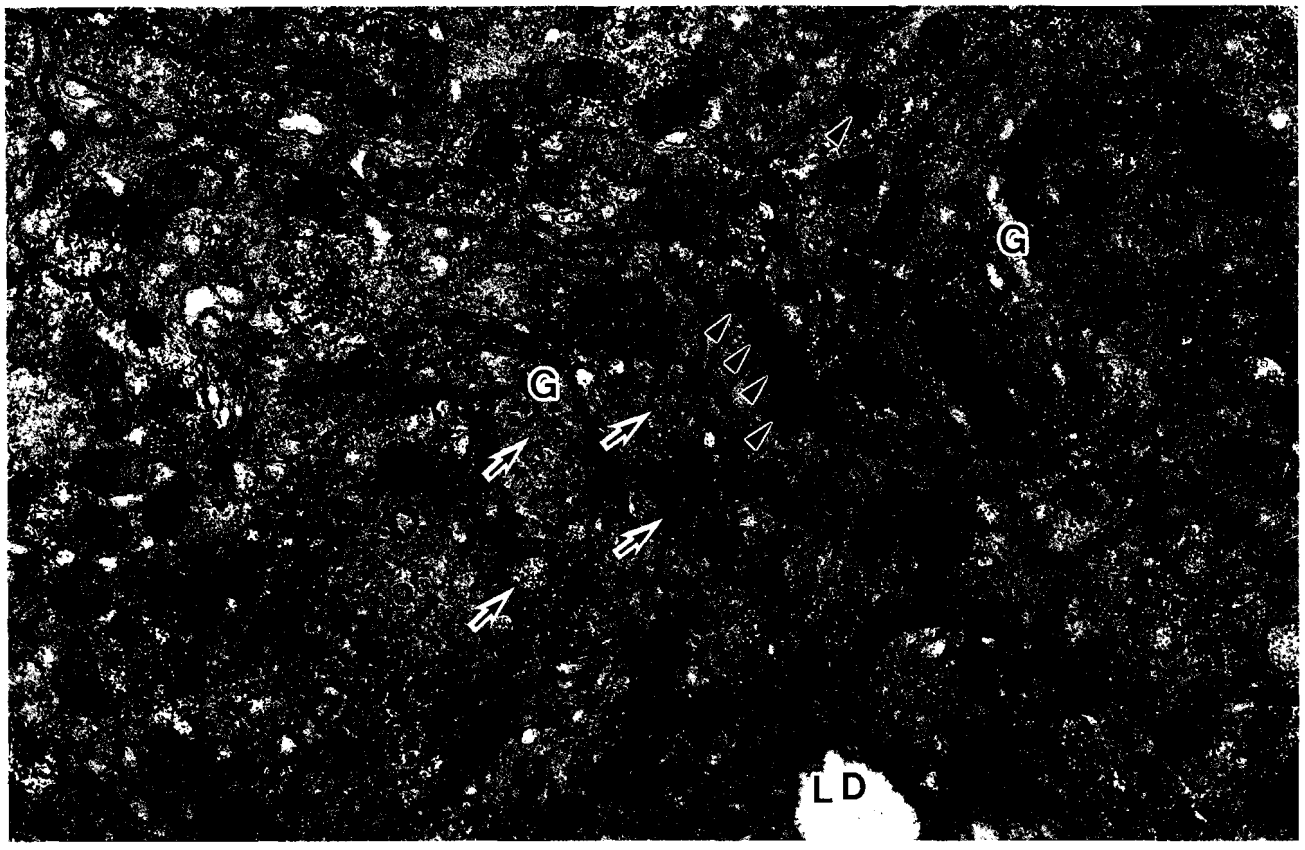

Fig. 2. Parathyroid chief cells of the 3-month ethanol-treated hamster. Well-developed Golgi complexes (G) associated with many prosecretory granules (arrows) are observed. Secretory granules (arrowheads) are seen at the lateral surface of the cell. LD: lipid droplet. x 22,000

observed in the cytoplasm.

In the parathyroid chief cells of the 5-month ethanol-treated hamsters, the exocytotic events were observed in the apicolateral domain of the plasma membrane of the chief cells (Fig. 4). Many secretory granules were observed in the peripheral cytoplasm. Some granules were situated close to the plasma membrane. Some secretory granules were found near to the enlarged intercellular spaces (Fig. 4). Some intercellular spaces contained floccular or finely particulate materials (Fig. 4).

The exocytotic events also frequently occurred in the basal domain of the plasma membrane (Fig. 5). There were many secretory granules near the plasma membrane facing the blood capillaries via the basal lamina (Fig. 5). Although the basal lamina surrounded the plasma membrane of the chief cell, exocytotic events were observed in portions both with and without the basal lamina.

Stereological analysis

The results obtained from the control and ethanol-treated groups are shown in Table 2. In the parathyroid glands of the 3- and 5-month ethanol-treated hamsters, the volume density occupied by the Golgi complexes

Table 2. Volume density (%) of cell components.

TIME	PROTOCOL	M	G	LY	LD	VB	SG
3 m	Control	8.72±0.18	6.82±0.18	0.51±0.03	0.15±0.02	0.24±0.02	0.58±0.04
	Experiment	8.84±0.29	7.47±0.18*	0.47±0.05	0.11±0.01	0.18±0.01	0.55±0.03
5 m	Control	8.54±0.20	6.73±0.13	0.47±0.04	0.12±0.03	0.26±0.03	0.68±0.07
	Experiment	8.86±0.17	8.34±0.20*	0.41±0.02	0.12±0.02	0.20±0.03	0.63±0.08

M: mitochondria; G: Golgi complex; LY: lysosome; LD: lipid droplet; VB: large vacuolar body; SG: secretory granule. Values are shown as mean±SEM, *: $p<0.05$.

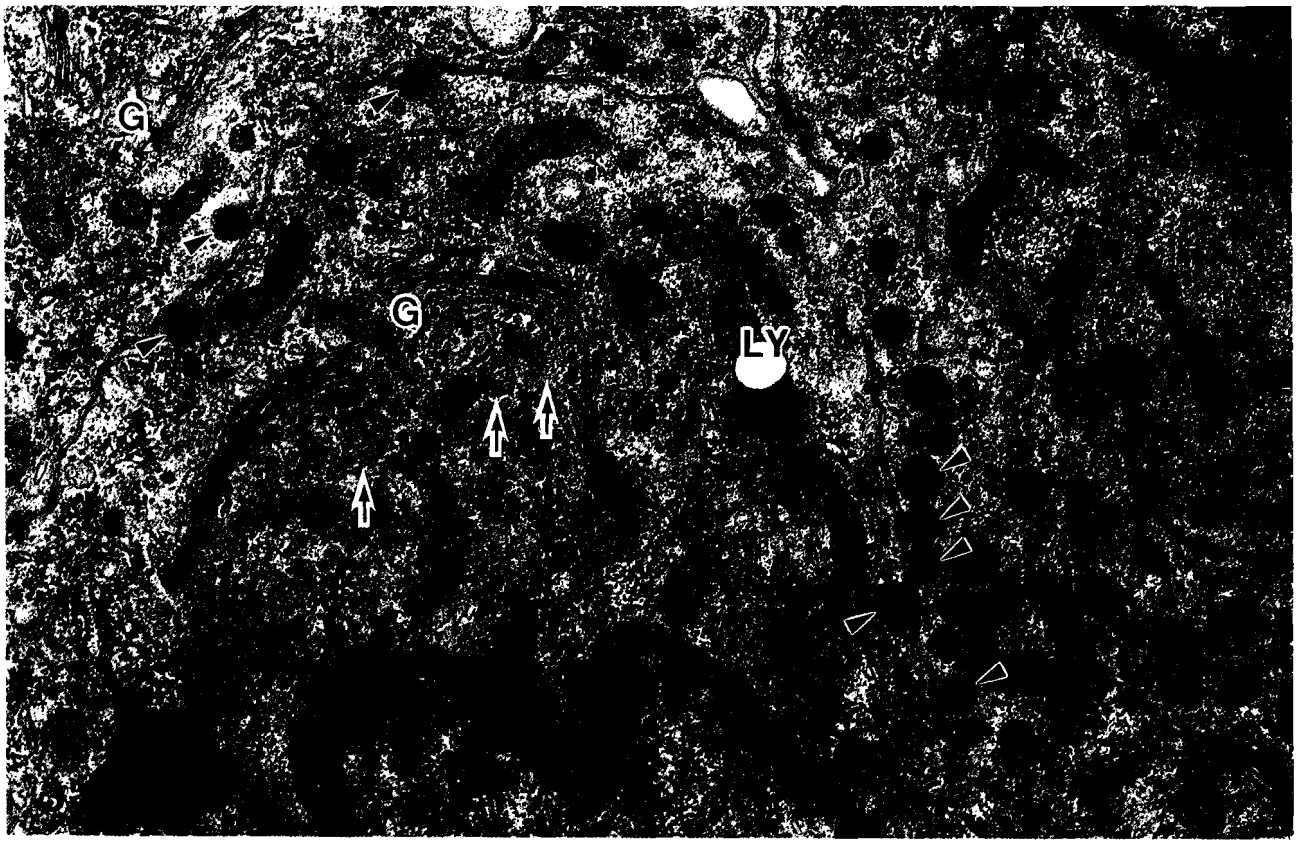

Fig. 3. Parathyroid chief cells of the 5-month ethanol-treated hamster. Well-developed Golgi complexes (G) associated with many prosecretory granules (arrows) are observed. Secretory granules (arrowheads) are seen very near the plasma membrane. LY: lysosome. x 22,000

Fig. 4. Parathyroid chief cells of the 5-month ethanol-treated hamster. Secretory granules (arrowheads) are located at the periphery of the enlarged intercellular spaces. x 30,000

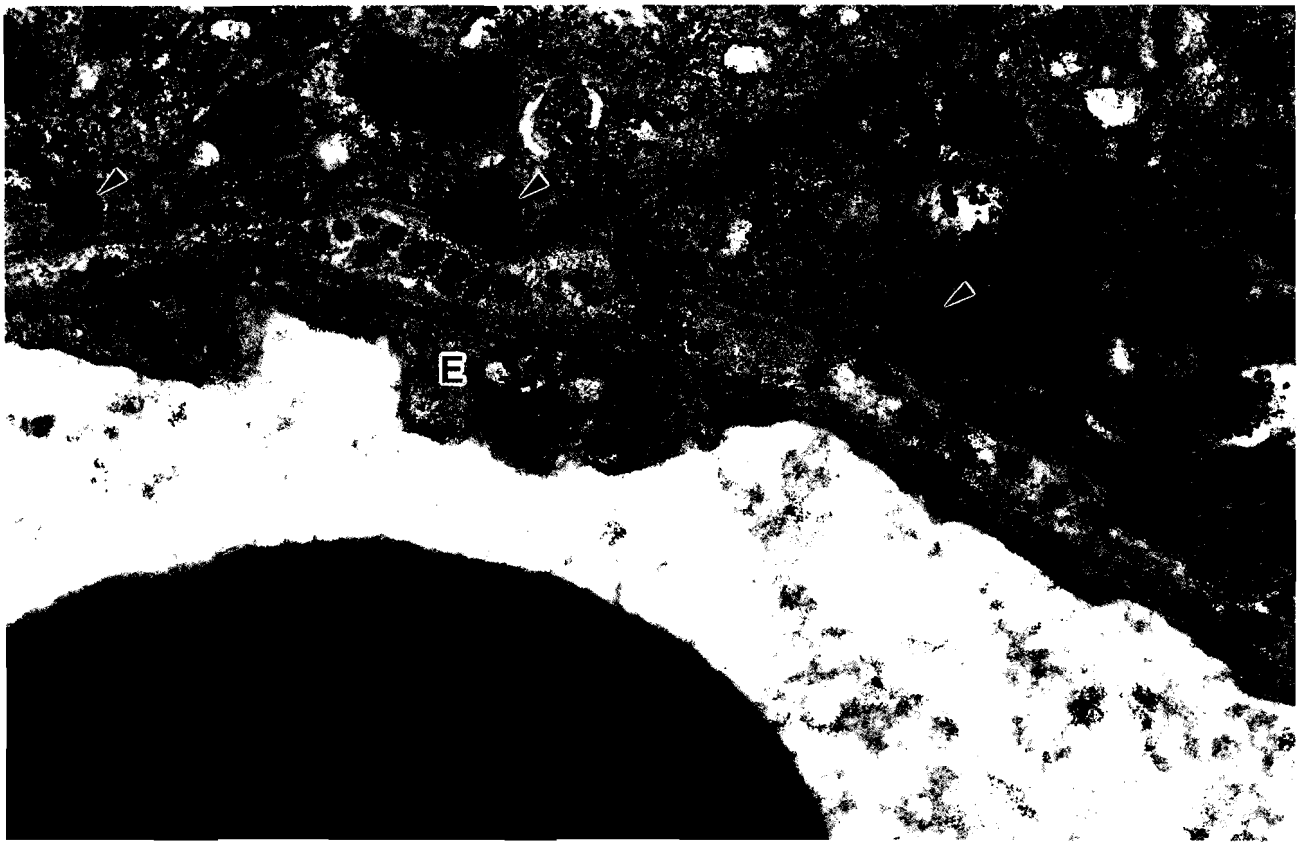

Fig. 5. Parathyroid chief cells of the 5-month ethanol-treated hamster. Secretory granules (arrowheads) are very close to the plasma membrane facing the basal lamina. E: endothelium. x 37,000

was significantly higher ($p<0.05$) as compared to that of the control groups. There was no significant difference between the controls and the ethanol-treated groups concerning other cell components.

Discussion

Baran et al. (1980) reported that constant ethanol ingestion decreased trabecular bone volume in rats. Peng et al. (1988) found that the trabeculae of the femur became thinner, more columnar and more extensive in rats, after 4 and 6 weeks of ethanol treatment. In the present study, there were no marked changes in BMC. The BMD seemed to decrease in the 5-month ethanol-treated hamsters. Unfortunately, the bone morphology was not observed at this time. We found that there were no marked changes in the livers of the 3- and 5-month ethanol-treated animals. Similar results were reported previously (Harris et al., 1979).

The effect of short-term treatment with ethanol on the parathyroid gland was opposite to that of long-term treatment. Our previous observation and other studies showed that acute ethanol ingestion reduced the parathyroid function (Laitinen and Välimäki, 1991; García-Sanchez et al., 1995; Chen et al., 1997, 1998). The mechanism remains unknown, but could be due to a direct effect of ethanol on PTH secretion as shown by the in vitro study of Magliola et al. (1986). It was reported that alcoholics had low serum calcium levels. This could explain the compensatorily elevated function of the parathyroid gland found in alcoholics (Bikle et al., 1985; Laitinen and Välimäki, 1991). In the present study, there were no marked changes in serum calcium levels after ethanol treatment.

Bikle et al. (1985) reported that humans who abused of alcohol ingestion for 10 years or more had a higher level of serum PTH and lower level of serum calcium. There were no morphological reports of the parathyroid gland after long-term treatment with ethanol. The present study demonstrated that many chief cells in hamster parathyroid glands after ethanol ingestion had well-developed Golgi complexes associated with many prosecretory granules. Numerous secretory granules were located very near the plasma membrane as compared with those of the control animals. These changes are considered to be induced by stimulation of the synthesis and secretion of PTH in hamster parathyroid chief cells. These results are fairly consistent with the findings which indicate an increase in functional activity of the parathyroid gland (Roth and Schiller, 1976; Isono et al., 1977, 1979a,b, 1986, 1990; Wild and Becker, 1980; Hayashi et al., 1980; Emura et al., 1984, 1995; Shoumura et al., 1988a,b, 1989a,b, 1990, 1991a,b, 1992; Ishizaki et al., 1989; Chen et al., 1990). Accordingly, it is suggested that the cellular activity of the parathyroid gland is stimulated in the 3- and 5-month ethanol-treated hamsters.

Being of ectodermal origin, parathyroid parenchymal cells are polar cells, as clearly demonstrated by cytochemical localization (Wild and Setoguti, 1995). The basal domain of the plasma membrane has a straight course and lies on the basal lamina. The lateral and apical domains of the plasma membrane have many folds and interdigitations with neighboring parenchymal cells. It is believed that in the parathyroid cell, the secretory product is released into the intercellular space by exocytosis at the apicolateral domain of the plasma membrane (Fujii and Isono, 1972; Isono and Shoumura, 1980; Setoguti et al., 1995; Wild and Schraner, 1990; Wild and Setoguti, 1995). After release from the plasma membrane, secretory materials are transported through the intercellular space, reach the perivascular space via the basal lamina, pass through the fenestrae of the capillaries and are incorporated into the blood vessel.

Exocytosis is rarely seen in hamster parathyroid chief cells. It was reported that in the parathyroid gland of some animals, the secretory granules in the exocytotic passage were located at the apicolateral surface of the chief cells (Fujii and Isono, 1972; Isono and Shoumura, 1980, Setoguti et al., 1987, 1995; Isono et al., 1990; Wild and Schraner, 1990). Several observations supporting this notion have been published. Cell organelles involving PTH synthesis and secretion are found mainly at the apical pole of the cell, exocytotic and endocytotic processes have been observed in the apicolateral domain of the plasma membrane (Setoguti et al., 1987) and 5'-nucleotidase is localized exclusively at the apicolateral domain (Wild and Schraner, 1990). In the present study, we demonstrated the ultrastructural feature of hormone release from the basal plasma membrane of the chief cell facing the basal lamina. Similar results have been observed in rat pituitary somatotrophs (Shimada and Tosaka-Shimada, 1989). We think that the exocytosis takes place at any domain of the plasma membrane, although it does occur in the apicolateral domain more than in the basal one. These findings will be useful for clarifying the mechanism of hormone release from endocrine cells.

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