Effects of Glucocorticoid on the Ultrastructure of the Mouse Parathyroid Gland*

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Summary. Ultrastructural changes in the parathyroid glands of dexamethasone-treated mice were examined. Many chief cells of the treated mice contained a decreased number of prosecretory granules, secretory granules and storage granules, and an increased number of lipid droplets, compared with the control mice. In addition, myelin-like structures associated with cisternae of the granular endoplasmic reticulum were observed in the parathyroid glands of the treated mice.

These findings suggest that cellular activity of the parathyroid gland may be suppressed by dexamethasone.

It is widely accepted that treatment with glucocorticoids affects calcium metabolism. The effects of glucocorticoids on the parathyroid gland function have been described in vivo and in vitro (Williams et al., 1974; Fucik et al., 1975; Au, 1976; Kukreja et al., 1976; Lukert and Adams, 1976; Suzuki et al., 1980; Izawa et al., 1981). However, there have been only a few morphological studies of the parathyroid gland after administration of glucocorticoids (Coleman and Silbermann, 1978; Coleman et al., 1980).

The purpose of this study is to evaluate the ultrastructural characteristics of the parathyroid glands of mice following treatment with a glucocorticoid.

MATERIALS AND METHODS

Twenty-four 2- to 3-month-old female mice of the ddY strain, weighing approximately 30 g, were divided into four groups. One group (6 mice) served as controls. The remaining groups were given synthetic glucocorticoid, dexamethasone (Takeda Chem. Ind. Ltd), intramuscularly once daily at a dose of 10 mg/kg body weight in olive oil for 1, 2 and 3 weeks. The control mice received the solvent alone. The parathyroid glands of the dexamethasone-treated mice and the control mice were removed under ether anesthesia 24 hrs after the last administration, immersed in 1% cold OsO4 in Millonig’s buffer at pH 7.2 for 1 hr, dehydrated through ascending concentrations of acetone, and embedded in Epon 812. Thin sections were cut on a LKB ultramicrotome, stained

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with uranyl acetate and lead salts, and examined with a JEM 100U electron microscope.

The stereological study of electron micrographs was performed on the parathyroid glands of 6 mice from each group. Ten micrographs were taken from different regions of the parathyroid glands of each mouse from each group at a primary magnification of 5,000 times, then photographically enlarged, which resulted in a final magnification of 11,000 times. Estimation of the volume percent of each compartment compared to the total cytoplasmic volume was calculated by the point counting stereological method of Weibel (1969). A transparent plastic sheet with a 1 cm lattice and 221 crossing points was used to estimate the volume of cytoplasm, nuclei, Golgi complexes, cisternae of the granular endoplasmic reticulum and mitochondria, and a transparent plastic sheet with a 2 mm lattice and 5,525 crossing points was used to estimate the volume of secretory granules, large secretory granules, heterogeneous dense bodies, multivesicular bodies and lipid droplets. A statistical analysis of data was performed by the t-test.

The serum calcium levels of all mice were measured by Corning calcium analyser 940.

RESULTS

1. Calcium concentration in serum
The mean serum calcium concentrations (mg/100 ml) were 10.91±0.30 (SEM) in the control mice, and 10.77±0.45 after 1 week, 11.84±0.40 after 2 weeks and 11.51±0.32 after 3 weeks of daily administration of dexamethasone. There were no significant differences between serum calcium concentrations of the control and treated animals.

2. Fine structure of the parathyroid gland
The morphology of the parathyroid gland of the control mice treated with olive oil resembled that of normal mice as reported earlier (Stockel and Porte, 1966; Isono et al., 1977). The chief cells possessed an oval or polygonal nucleus, free rich ribosomes, abundant mitochondria, well-developed Golgi complexes containing a few coated vesicles and many prosecretory granules, and numerous secretory granules of 150-200 nm in diameter (Fig. 1, 2). Cisternae of the granular endoplasmic reticulum were randomly distributed or arranged in parallel arrays (Fig. 1, 2). The secretory granules were sometimes situated close to the plasma membrane (Fig. 1, 2). Furthermore, the cytoplasm sometimes contained large secretory granules of 300-600 nm in diameter, multivesicular bodies and oval to irregularly shaped heterogeneously dense bodies containing osmiophilic and/or lipid-like material (Fig. 1-3, 3 inset). Some multivesicular bodies contained materials similar to the contents of the secretory granule (Fig. 3). Lipid droplets occasionally occurred. Numerous vesicles of 50 nm in diameter surrounded many large secretory granules and some multivesicular bodies (Fig. 3, 3 inset).

In the parathyroid glands of the dexamethasone-treated mice, many chief cells had abundant free ribosomes and mitochondria; relatively well-developed Golgi complexes contained a few prosecretory granules (Fig. 4, 5). Cisternae of the granular endoplasmic reticulum were frequently arranged in circular or parallel arrays (Fig. 5). Secretory granules were frequently found (Fig. 4) and occasionally accumulated in the cytoplasm (Fig. 6). Few secretory granules were present in the peripheral cytoplasm (Fig. 4-6). Large secretory granules and oval to irregularly shaped heterogeneously
Fig. 1. Parathyroid chief cells in a control mouse. Well-developed Golgi complex (G) and numerous secretory granules (arrows) are seen. The cisternae of granular endoplasmic reticulum (er) are distributed randomly or in parallel arrays. S large secretory granules, D heterogeneously dense body, M multivesicular body. ×9,900
dense bodies were occasionally observed, while lipid droplets and multivesicular bodies were frequently seen in the cytoplasm (Fig. 4, 5, 7, 7 inset). Numerous vesicles were found juxtaposed to many large secretory granules and some multivesicular bodies (Fig. 7, 7 inset). Myelin-like structures associated with cisternae of the granular endoplasmic reticulum were infrequently observed 2 and 3 weeks after administration of dexamethasone (Fig. 8, 9). Many of these structures contained mitochondria and membranous structures (Fig. 8).

**Table 1.** Volume percents (mean ± SEM) of cytoplasmic compartments in total parathyroid chief

| Weeks after administration | Mitochondria     | Cisternae of granular endoplasmic reticulum | Golgi complexes | Secretory granules |
|----------------------------|------------------|--------------------------------------------|-----------------|--------------------|
| Control                    | 6.06±0.42        | 7.30±0.41                                  | 11.09±0.50      | 0.188±0.010        |
| 1 week                     | 6.70±0.24        | 8.89±0.68                                  | 11.36±1.02      | 0.083±0.033*       |
| 2 weeks                    | 5.68±0.31        | 6.80±0.52                                  | 11.28±0.86      | 0.078±0.015**      |
| 3 weeks                    | 6.69±0.34        | 7.95±0.86                                  | 12.82±0.68      | 0.075±0.010**      |

Six mice are used in each group. Significance p is calculated in test group as against control group.
3. Stereological analysis of the parathyroid gland

The analytical results obtained from the control and dexamethasone-treated mice are shown on Table 1. In the parathyroid glands 1 week after administration of dexamethasone, the volume percent of secretory granules and heterogeneously dense bodies had decreased significantly (p<0.05) and the volume percent of lipid droplets had significantly increased (p<0.05), compared with that of the control mice. Two weeks after administration, the volume percent of secretory granules and large secretory granules in the gland had decreased significantly (p<0.01) and the volume percent of multivesicular bodies had significantly increased (p<0.05) as compared with that of the control mice. Three weeks after administration, the gland’s volume percent of secretory granules and large secretory granules had decreased significantly (p<0.01) and the volume percent of lipid droplets had significantly increased (p<0.05) as compared with that of the control mice.

|                       | Large secretory granules | Heterogeneously dense bodies | Multivesicular bodies | Lipid droplets    |
|-----------------------|--------------------------|-----------------------------|-----------------------|-------------------|
| 0.080±0.014           | 0.279±0.038              | 0.080±0.014                 | 0.027±0.018           |
| 0.037±0.024           | 0.162±0.025*             | 0.092±0.018                 | 0.574±0.204*          |
| 0.020±0.008**         | 0.219±0.016              | 0.143±0.021*                | 0.489±0.273           |
| 0.014±0.005**         | 0.258±0.035              | 0.129±0.029                 | 0.349±0.107*          |

*p<0.05, **p<0.01
Fig. 4. Parathyroid chief cells 2 weeks after dexamethasone administration. Many lipid droplets (\(L\)), secretory granules (arrows) and relatively well-developed Golgi complex (\(G\)) are seen. \(er\) Cisternae of granular endoplasmic reticulum, \(S\) large secretory granules, \(D\) heterogeneously dense body, \(M\) multivesicular body. \(\times 9,000\)
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There were no significant differences between the control and dexamethasone-treated mice with regard to mitochondria, cisternae of the granular endoplasmic reticulum and the Golgi complexes.

**DISCUSSION**

It has been biochemically reported that excessive parathyroid hormone secretion is induced in rat and man in response to treatment with glucocorticoid, and hyperparathyroidism accompanies (Williams et al., 1974; Fucik et al., 1975; AU, 1976; Kukreja et al., 1976; Lukert and Adams, 1976; Suzuki et al., 1980). On the other hand, a recent study has shown that serum parathyroid hormone levels are not raised in the dog after administration of hydrocortisone (Izawa et al., 1981).

Recent morphological reports have indicated that in mouse and baboon triamcinolone-induced parathyroid hyperactivity is not necessarily accompanied by ultrastructural changes in the chief cells (Coleman and Silbermann, 1978; Coleman et al., 1980). In the present study, the principal alterations of the parathyroid glands of mice given dexamethasone as compared with the control mice were a decreased number of prosecretory granules, secretory granules and large secretory granules, and an increased number of lipid droplets. These observations are fairly consistent with the findings.

Fig. 5. Parathyroid chief cells 1 week after dexamethasone administration. Cisternae of granular endoplasmic reticulum (er) are arranged in circular or parallel arrays and the Golgi complexes (G) are relatively well developed. Note secretory granules (arrow) located in the peripheral cytoplasm. M multivesicular body. ×30,000
of decreased secretory activity of the chief cells in the parathyroid gland (STOECKEL and PORTE, 1966; ISONO and SHOUMURA-SAKURAI, 1973; YOUNG et al., 1973; ROTH and SCHILLER, 1976; ISONO et al., 1977, 1978, 1980, 1981, 1982; HAYASHI et al., 1981). Our study suggests that the cellular activity of the mouse parathyroid gland may be suppressed in response to the treatment with dexamethasone. In addition, it is found here that there were no significant differences between the control and dexamethasone-treated mice with regard to the serum calcium concentrations. Similar observations have been reported in the rat, dog and man after administration of glucocorticoid (WILLIAMS et al., 1974; FUCIK et al., 1975; KUKREJA et al., 1976; COLEMAN et al., 1980; SUZUKI et al., 1980; IZAWA et al., 1981).

In the dexamethasone-treated mice, a myelin-like structure which is contiguous with cisternae of the granular endoplasmic reticulum was observed in the chief cells. A similar structure has been reported in the parathyroid glands of vitamin D- and calcium-treated mice (STOECKEL and PORTE, 1966), Ehrlich’s ascites tumor-bearing mice (LATT A and RUTZ, 1968) and reserpine-treated mice (ISONO et al., 1981), and in the parathyroid adenoma (BOQUIST et al., 1971). FAWCETT (1961) suggested that whorls of cisternae of the granular endoplasmic reticulum lose their ribosomes, and the lipoprotein membranes undergo further change, leading to the appearance of a dense myeloid ring. We consider that the myelin-like structure is associated with a degenerative process, as previously described in the parathyroid gland (LATT A and RUTZ, 1968; BOQUIST et al., 1971; ISONO et al., 1981).

Several studies have described large secretory granules with a homogeneously
dense appearance as being storage granules in the parathyroid glands (Alténaehr, 1970; Haase, 1978; Isono et al., 1978, 1980, 1981, 1982; Hayashi et al., 1980, 1981; Isono and Shoumura, 1980; Setoguti et al., 1981; Emura et al., 1982). This interpretation is supported by the finding that large secretory granules are acid phosphatase-negative.

Fig. 7. Parathyroid chief cell 3 weeks after dexamethasone administration. Multivesicular bodies (M) and heterogeneously dense bodies (D) are seen. Vesicles are juxtaposed to multivesicular bodies. ×52,000. Inset: Large secretory granule (S) in parathyroid chief cell 1 week after dexamethasone administration. ×49,000
In the present study, large secretory granules of 300-600 nm in diameter were also present in the chief cells of the control and dexamethasone-treated mice. Such granules are thought to be storage granules which remain in the cells without being released. The storage granules, secretory granules and prosecretory granules in the chief cells of the treated mice had decreased in number, as compared with the control animals. In addition, numerous vesicles similar to those inside and outside multivesicular bodies surrounded many storage granules, and multivesicular bodies contained materials similar to the contents of the secretory granule. The multivesicular bodies in the parathyroid gland are described to be acid phosphatase-negative (Setoguti and Goto, 1974; Hayashi et al., 1980; Setoguti et al., 1980). It may be possible that some of these vesicles lying adjacent to the storage granule are incorporated into the latter, forming some multivesicular bodies, as previously suggested in the studies using mice (Hayashi et al., 1980, 1981; Isono et al., 1980, 1981) and rabbits (Isono and Shoumura, 1980; Isono et al., 1982). However, further investigations should be undertaken to clarify the formation and release of storage granules.
Fig. 9. Parathyroid chief cells 3 weeks after dexamethasone administration. Myelin-like structures are present. \textit{er} Cisternae of granular endoplasmic reticulum. \times 20,000.

REFERENCES

Altenahr, E.: Zur Ultrastruktur der Rattenepitelkörperchen bei Normo-, Hyper- und Hypocalämie. Applikation von Parathormon, Thyreoalcitonin, Dihydrotachysterin, Glycerophosphate und verschiedener Diät. Virchows Arch. Abt. A Pathol. Anat. 351: 122-141 (1970).

Au, W. Y. W.: Cortisol stimulation of parathyroid hormone secretion by rat parathyroid glands in organ culture. Science 193: 1015-1017 (1976).

Boquist, L., L. Bergdahl and A. Andersson: Parathyroid adenoma complicated by acute hyperparathyroidism: Report of a case with particular regard to ultrastructural findings. Ann. Surg. 173: 593-603 (1971).

Coleman, R. and M. Silbermann: Ultrastructure of parathyroid glands in triamcinolone-treated mice. J. Anat. 126: 181-192 (1978).

Coleman, R., M. Silbermann and J. Bernheim: Fine structure of the parathyroid glands in baboons, \textit{Papio hamadryas} in response to experimental hypercorticoidism. Acta anat. 106: 424-433 (1980).

Emura, S., S. Shoumura and H. Isono: Ultrastructural changes of the parathyroid gland of the estrogen-treated golden hamster. (In Japanese). J. clin. Electron Microsc. 15: 113-126 (1982).

Fawcett, D. W.: The membranes of the cytoplasm. Lab. Invest. 10: 1162-1178 (1961).

Fucik, R. F., S. C. Kukreja, G. K. Hargis, E. N. Bowser, W. J. Henderson and G. A. Williams: Effect of glucocorticoids on function of the parathyroid glands in man. J. clin. Endocrinol. Metab. 40: 152-155 (1975).

Haase, P.: Parathyroid stimulation in phosphate-induced nephrocalcinosis. J. Anat. 125: 299-311 (1978).
Hayashi, K., S. Shoumura and H. Isono: Experimental study of the mouse parathyroid gland I. Quantitative electron microscopy after calcitonin administration and acid phosphatase activity. (In Japanese). J. clin. Electron Microsc. 13: 45–53 (1980).

Hayashi, K., S. Shoumura and H. Isono: Experimental study of the mouse parathyroid gland II. Qualitative and quantitative electron microscopy after cold exposure. J. clin. Electron Microsc. 14: 43–54 (1981).

Isono, H., K. Miyake, S. Shoumura and R. J. Barnett: Electron microscopic study on the postnatal development of the mouse parathyroid gland. Arch. histol. jap. 40: 367–380 (1977).

Isono, H. and S. Shoumura: Effects of vagotomy on the ultrastructure of the parathyroid gland of the rabbit. Acta anat. 108: 273–280 (1980).

Isono, H. and S. Shoumura-Sakurai: Electron microscopic study on the parathyroid gland of the parathormone-injected newt, Triturus pyrrhogaster (Boie). Okajimas Fol. anat. jap. 50: 9–26 (1973).

Isono, H., S. Shoumura, K. Hayashi, N. Ishizaki and S. Emura: Electron microscopic study of the parathyroid gland of the acetazolamide-treated mouse. Acta anat. 107: 8–17 (1980).

Isono, H., S. Shoumura, N. Ishizaki, S. Emura, K. Hayashi, Y. Iwasaki and Y. Kitamura: Effects of electrical stimulation of the vagus nerve on the ultrastructure of the rabbit parathyroid gland. Okajimas Fol. anat. jap. 58: 453–466 (1982).

Isono, H., S. Shoumura, N. Ishizaki, S. Emura, K. Hayashi, T. Yamahira and Y. Iwasaki: Electron microscopic study of the parathyroid gland of the reserpine-treated mouse. J. clin. Electron Microsc. 14: 113–120 (1981).

Isono, H., S. Shoumura, K. Miyake, N. Ishizaki and K. Hayashi: Effects of artificial hibernation on the ultrastructure of the parathyroid glands of the summer frog, Rana catesbiana. Okajimas Fol. anat. jap. 55: 41–56 (1978).

Izawa, Y., T. Makita, S. Hino, K. Sagara, N. Ohnuma, Y. Hashimoto, Y. Kawaguchi, I. Rsukui, Y. Ogura, T. Miyahara, K. Tasaka and H. Ichiki: Studies on bone disorders in dogs caused by hydrocortisone. (In Japanese). Bone Metab. 14: 45–54 (1981).

Kukreja, S. C., E. N. Bowser, G. K. Hargis, W. J. Henderson and G. A. Williams: Mechanisms of glucocorticoid-induced osteopenia: Role of parathyroid glands. Proc. Soc. Exp. Biol. Med. 152: 358–361 (1976).

Latta, J. S. and T. J. Rutz: Special ultrastructural features of parathyroid cells from Swive mice bearing Ehrlich’s ascites tumor. Anat. Rec. 160: 255–260 (1968).

Lukert, B. P. and J. S. Adams: Calcium and phosphorus homeostasis in man. Effect of corticosteroids. Arch. intern. Med. 136: 1249–1253 (1976).

Roth, S. I. and A. L. Schiller: Comparative anatomy of the parathyroid glands. In: (ed. by) R. O. Greep and E. B. Astwood: Handbook of physiology. Sec. 7/Vol. VII. American Physiological Society, Washington, D. C., 1976 (p. 281–311).

Setoguti, T. and Y. Goto: Electron microscopic studies on the identification of secretory granules in the parathyroid gland of several animals by means of acid phosphatase reaction. Arch. histol. jap. 36: 379–389 (1974).

Setoguti, T., Y. Inoue and K. Kato: Electron-microscopic studies on the relationship between the frequency of parathyroid storage granules and serum calcium levels in the rat. Cell Tiss. Res. 219: 457–467 (1981).

Setoguti, T., M. Takagi and K. Kato: Ultrastructural localization in the rat parathyroid gland. Arch. histol. jap. 43: 45–56 (1980).

Shannon, W. A. and S. I. Roth: An ultrastructural study of acid phosphatase activity in normal, adenomatous and hyperplastic (chief cell type) human parathyroid glands. Amer. J. Pathol. 77: 493–506 (1974).

Stoeckel, M. E. and A. Porte: Observations ultrastructurales sur la parathyroide de souris. II. Etude experimentale. Z. Zellforsch. 73: 503–520 (1966).

Suzuki, Y., E. Saito, Y. Ichikawa, K. Arikawa, Y. Abe, T. Shinozawa, S. Kawai, H. Oshima and M. Homma: Parathyroid function and calcium metabolism in patients under glucocorticoid therapy. (In Japanese). Bone Metab. 13 534–540 (1980).
Weibel, E. R.: Stereological principles for morphometry in electron microscopic cytology. Int. Rev. Cytol. 26: 235-302 (1969).

Williams, G. A., W. C. Peterson, E. N. Bowser, W. J. Henderson, G. K. Hargis and N. J. Martinez: Interrelationship of parathyroid and adrenocortical function in calcium homeostasis in the rat. Endocrinol. 95: 707-712 (1974).

Young, D. M., H. M. Olson, D. J. Prieur, D. A. Cooney and R. L. Reagan: Clinicopathologic and ultrastructural studies of L-asparaginase-induced hypocalemia in rabbits. An experimental animal model of acute hypoparathyroidism. Lab. Invest. 29: 374-386 (1973).

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