Histol Histopath (1988) 3: 93-95

Histology and Histopathology

Immunocytochemical localization of parathyroid hormone in rabbit parathyroid glands

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Summary. Immunocytochemical localization of parathyroid hormone was examined in the rabbit parathyroid gland by means of protein A-gold technique. Protein A-gold particles were observed on the secretory granules and the large secretory granules thought to be storage granules. No protein A-gold particles were observed on cisternae of the endoplasmic reticulum and the Golgi apparatus.

Key words: Immunocytochemistry - Rabbit - Electron microscopy - Parathyroid gland

Introduction

In several mammalian parathyroid glands, immunoreactivity of the parathyroid hormone (PTH) has been demonstrated in chief cell (Ravazzola et al., 1978; Futrell et al., 1979; Limacher et al., 1979; Setoguti et al., 1985; Inoue and Setoguti, 1986), and oxyphil cell (Ordoñez et al., 1982). In these studies they used mouse, rat, gerbil, dog, bovine and human parathyroid glands as the materials. In the present study, we examined the electron microscopic localization of PTH in the chief cells of the rabbit parathyroid gland using protein A-gold technique.

Materials and methods

Adult male rabbits were used for this study. They were perfused with Zamboni fixative for 15 minutes. The removed parathyroid glands were immersed in the same fixative for one hour at 4° C, postfixed with phosphate-buffered 1% osmium tetroxide for 30 min at 4° C, dehydrated in acetone, and embedded in epoxy resin. Ultrathin sections were mounted on the nickel grids, incubated with 1% bovine serum albumin for 30 min in phosphate-buffered saline (PBS), and followed by incubation with guinea pig antiserum against bovine parathormone (Eiken Immunochemical Lab.) for 15-20 hours at 4°C. Antiserum was applied at dilution of 1:1000 or 2000. The sections were rinsed in PBS and incubated in protein A-gold complex (E-Y Laboratories Inc.) for 30 min at room temperature according to the method of Roth et al. (1978). After washing with distilled water, sections were briefly stained with uranyl acetate and lead salts, and examined with a H-700H electron microscope. As immunochemical controls, antiserum to parathormone was absorved with an excess of bovine parathormone (CalBiochem) or sections were incubated with diluted normal guinea pig serum instead of antiserum before incubation with protein A-gold.

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Results

The rabbit parathyroid gland was composed of one cell type, chief cell. Secretory granules of 150-300 nm in diameter containing homogeneously dense material were observed in the cytoplasm of the chief cell. Large secretory granules of 300-500 nm in diameter and multivesicular bodies of 400-700 nm in diameter sometimes occurred in the cytoplasm. Occasional transitional forms between large secretory granules and multivesicular bodies were present. Large secretory granules showed rather lower density than secretory granules and were accompanied by small vesicles around them. Protein A-gold particles were concentrated over the secretory granules (Fig. 1), and the large secretory granules (Fig. 2). But protein A-gold particles were not observed on the multivesicular bodies (Fig. 3). Nuclei and mitochondria did not contain protein A-gold particles, and particles were not observed on cisternae of the granular endoplasmic reticulum (Fig. 1) and the Golgi apparatus (Fig. 2). Positive reactions were completely inhibited when the antisera were absorbed with the homologous antigens.

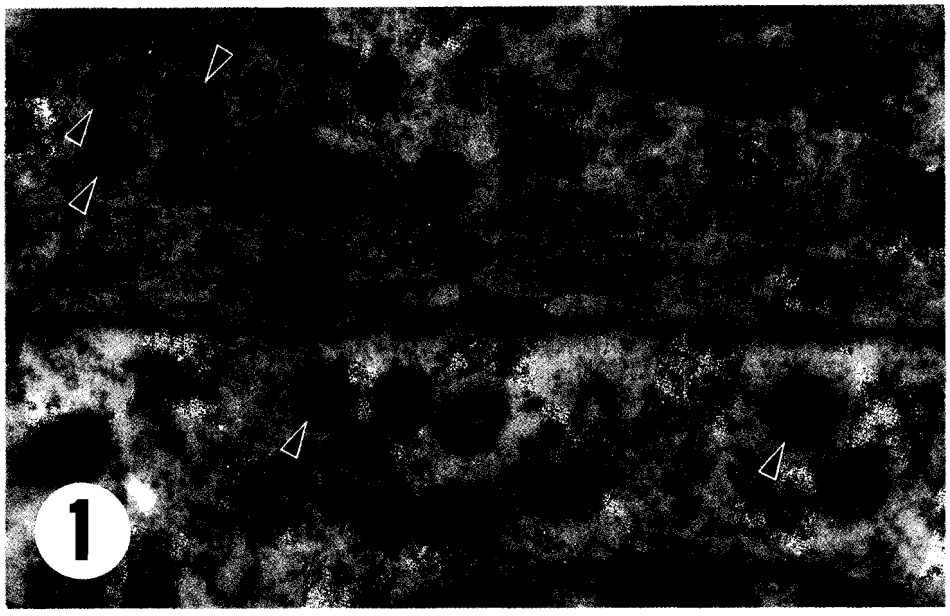

Fig. 1. Protein A-gold particles are observed on the secretory granules (arrow heads), but are not observed on cisternae of the endoplasmic retiulum (ER). × 34,000

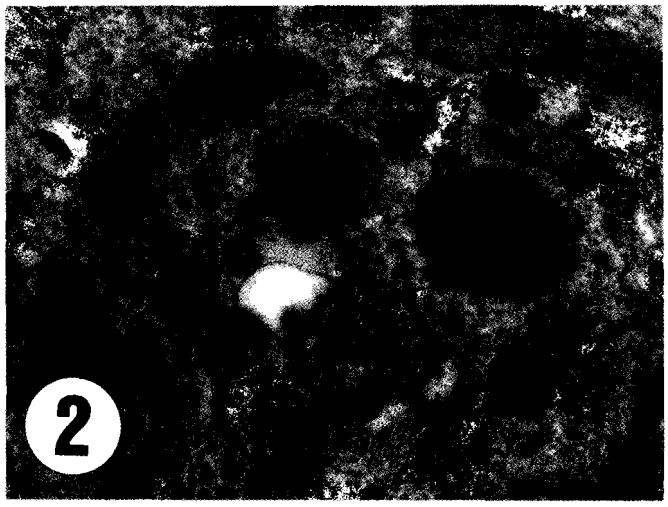

Fig. 2. Protein A-gold particles are observed on the large secretory granule (arrow) but not on the Golgi apparatus (G). × 34,000

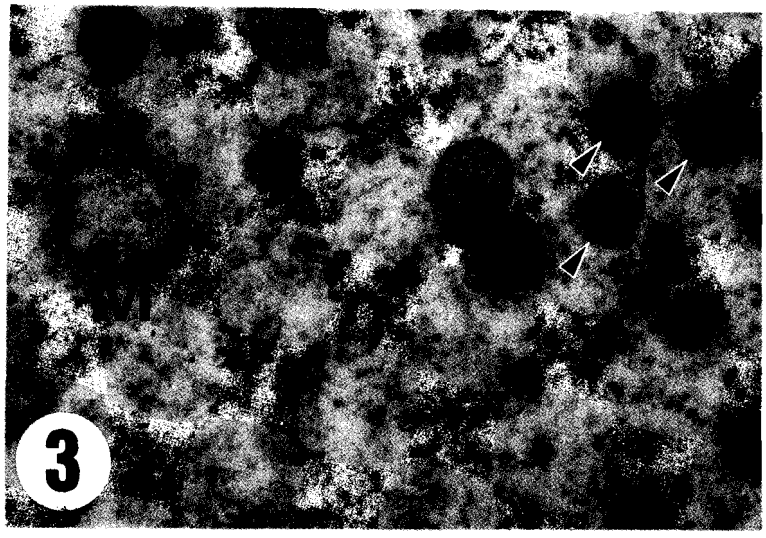

Fig. 3. Protein A-gold particles are observed on the secretory granules (arrow heads) but not on the multivesicular body (M). × 45,000

Discussion

In the present study, the contents of large secretory granules were similar to those of secretory granules except for difference of their size and electron density, and numerous vesicles similar to those inside multivesicular bodies surrounded large secretory granules. Most large secretory granules are thought to be storage granules which remain undischarged in the chief cells as reported by Isono and Shoumura (1980), and Isono et al. (1980, 1981). In the present study, protein A-gold particles were observed on the secretory granules and the large secretory granules, but were not seen on the multivesicular bodies. Accordingly, both granules showing homogeneous density are thought to include PTH as reported by Setoguti et al. (1985), and Inoue and Setoguti (1986). We suggest that some vesicles surrounding the large secretory granules may be incorporated into the large secretory granules, and the latter may be converted into some of multivesicular bodies. Those changes might indicate the process of the lysosomal digestion. We think that the fact that protein A-gold particles were not seen on the Golgi apparatus and cisternae of the endoplasmic reticulum may show a possibility that the antigenic sites on the hormone may not be reactive when the hormone is in the form of prepro-PTH and pro-PTH as described by Futrell et al. (1978).

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Accepted September 2, 1987