Freeze-fracture study of the rat parathyroid gland under hypo- and hypercalcemic conditions, with special reference to secretory granules

Takao Setoguti and Yasuhisa Inoue
Department of Anatomy, Nagasaki University School of Medicine, Nagasaki, Japan

Summary. Freeze-fracture images of the parenchymal cells in the parathyroid gland of rats were observed after vitamin D₂ plus calcium chloride-suppression and EGTA-activation of secretion. In cells of the suppressed glands, large bulges protruded from the Golgi cisternae, and large granules with a stalk, which are identified as storage granules, suggest that, during maturation, some storage granules may be connected by long tubules with the Golgi cisternae and supplied with secretory products from the Golgi cisternae via these tubules.

In the activated glands, presumptive exocytotic and endocytotic specializations of intramembranous particles of the parenchymal cell plasma membrane were frequently observed. In addition, elevations and complementary shallow depressions of various shape and extent were occasionally encountered in the intercellular space. From their morphological characteristics it was concluded that these originated from secretory granule cores, which are discharged from the parenchymal cells into the intercellular space by exocytosis, and it was suggested that discharged granule cores may retain their spherical shape until they fuse to form a flat conglomerate.

Key words: Rat – Parathyroid – Freeze-fracture – Storage granule – Exocytosis – Endocytosis – Discharged secretory granule

It is widely accepted that secretion from the parathyroid gland is regulated principally by serum calcium concentrations: a low serum calcium concentration causes an increase in parathormone secretion, whereas a high serum calcium concentration causes a decrease in parathormone secretion. Therefore, many correlative studies of the ultrastructural appearance of the rat parathyroid gland with its function have used experimentally or pathologically induced hypo- and hypercalcemia (Roth and Raisz 1964, 1966; Mazzocchi et al. 1967; Hara and Nagatsu 1968; Rohr and Krässig 1968; Roth et al. 1968; Murakami 1970; Stoeckel
and Porte 1973; Krstić et al. 1974; Lindgren and Boquist 1976; Thliveris 1976; Ream and Principato 1981a, b). In addition, recent freeze-fracture electron microscopy has revealed exocytosis in this gland in man (Thiele and Wermter 1974), as well as intercellular junctions between the parenchymal cells in rat (Ravazzola and Orci 1977) and hen (Setoguti et al. 1982).

We present freeze-fracture observations of the rat parathyroid gland under hypo- and hypercalcemic conditions which concern the development of storage granules and the morphological changes of discharged secretory granules after exocytosis.

Materials and methods

Forty, healthy, adult male rats of Wistar strain, weighing 200-300 g, were used for this study. For the purpose of correlating observation on the fine structure of the rat parathyroid with serum calcium levels, we have used our previous method (Setoguti et al. 1981) as described below.

The animals were separated into six groups of six to seven each. To induce hypercalcemia, five groups of animals were daily given for 3 days, by intragastric tube, 5 μg/kg body weight of vitamin D₂ in 0.5 ml of corn oil, followed 1 h later by 1 ml of 4% calcium chloride saline solution. Of these groups, the animals of one group were sacrificed 1 h after administration. To induce hypocalcemia successively, the animals of the remaining four groups were further injected with 4% ethylene glycol bis (β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), a chelating agent for calcium ions, in 1 ml of saline solution 1 h after the last treatment with calcium chloride, and sacrificed after 5, 10, 15, and 20 min, respectively. Another group of animals served as normal controls.

The parathyroid glands of each animal were fixed in 2.5% glutaraldehyde in cacodylate buffer either by perfusion via the left ventricle or by immersion for 3-4 h.

To prepare freeze-fracture replicas, the glands were washed in buffer overnight, soaked in 30% cacodylate-buffered glycerol solution for 3 h, and fractured in a EE-FED-B freeze-fracture apparatus (JEOL, Japan), at −130 ~ −140°C under 2 × 10⁻⁶ Torr. Additionally, for comparative observation, part of the fixed glands was processed for conventional thin section electron microscopy. Replicas and thin sections were examined in a JEOL-100B or JEM-T8 electron microscope.

Results

The parenchyma of the rat parathyroid gland is composed of clusters of densely packed chief cells. The chief cells have two types of hormone-containing granules: small secretory, or prosecretory granules, ranging from 100 to 250 nm in diameter, and large mature, or storage granules, ranging from 300 to 600 nm in diameter (Fig. 1). The characteristics of this gland under the present experimental conditions as revealed by thin-section electron microscopy have already been described in our previous report (Setoguti et al. 1981): in the animals given vitamin D₂ plus calcium chloride, secretion of the chief cells is suppressed and storage granules increase in number significantly, whereas in those injected with EGTA, the secretory activity of the chief cells is stimulated and storage granules decrease in number first gradually and then rapidly by 20 min after the injection.

Cross-fractured faces of the parenchymal cell cluster of both control and experimental animals provide the images confirming those obtained by thin sections. At higher magnification, cell organelles such as mitochondria, cisternae of the endoplasmic reticulum, the Golgi apparatus and centrioles, as well as numerous