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Electronmicroscopic studies of parathyroid storage granules in the mouse (I). ——Effects of hypocalcemia

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SUMMARY: In the mouse parathyroid chief cells fixed by perfusion with glutaraldehyde numerous secretory granules of various sizes, 100-600 nm, were distinguishable from lysosomal dense bodies, because the former had a halo beneath the limiting membrane. The number and size of all the secretory granules were measured and correlated with the mean serum calcium level (SCL). Small secretory granules less than 300 nm in diameter were rapidly decreased in number as soon as SCL lowered to 5.2±0.9 mg/dl at 10 min after an injection of 4% EDTA, whereas most of large secretory granules more than 300 nm in diameter did not decrease until the SCL dropped to 3.6±0.8 mg/dl at 20 min after the injection. On the basis of these data, it was concluded that in the mouse parathyroid, secretory granules are subdivided into two populations, small and large storage, and that the former population is easily released in response to the decrease in SCL, but that of the latter population remains stored in the cytoplasm until the SCL becomes reduced to a certain level. In addition, it was suggested that both prosecretory and storage granules mainly originated from the trans-Golgi network.

INTRODUCTION

Electron microscopic studies of the mouse parathyroid gland under normal and experimental conditions have been reported by several authors (Hara and Nagatsu, 1964; Stockel and Porte, 1966; Nakagami, 1967; Latta and Rutz, 1968). However, the interest of these authors was principally concentrated on the morphological change of the cell organelles and not always on the secretory granules.

Recently, in our laboratory it has been observed that in the rat parathyroid gland secretory granules are divided into two populations, i.e. small vesicular granules and large storage ones, and that each of these two kinds of granules plays an important role in parathormone secretion (Setoguti et al., 1981). Unlike the rat, the mouse parathyroid gland is known to contain many secretory granules of various sizes in the cytoplasm. The present study concerns the relationship between the size of secretory granules and serum calcium levels in the mouse parathyroid, but its major aim is to elucidate whether two populations of secretory granules as seen in the rat exist also in the mouse parathyroid gland.

MATERIALS AND METHODS

Thirty-one healthy, adult male DDK mice, weighing 40-50 g, were used for this study. The animals were divided into seven experimental groups: group 1 served as controls, being injected with 0.1 normal saline into the peritoneal
cavity; groups 2-7 were given an intraperitoneal injection of 4% EDTA (ethylenediamine tetraacetic acid) in 0.1 ml of saline solution for 10, 20, 30, 90, 270 and 360 min, respectively (Table 1).

Table 1. Experimental treatment

<table>
<thead>
<tr>
<th>Time after injection</th>
<th>Treatment</th>
<th>No. of animals</th>
<th>No. of cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>cont.</td>
<td>Solvent only</td>
<td>4</td>
<td>243</td>
</tr>
<tr>
<td>10 min</td>
<td>4% EDTA 0.1ml</td>
<td>5</td>
<td>303</td>
</tr>
<tr>
<td>20 min</td>
<td>&quot;</td>
<td>4</td>
<td>237</td>
</tr>
<tr>
<td>30 min</td>
<td>&quot;</td>
<td>5</td>
<td>315</td>
</tr>
<tr>
<td>90 min</td>
<td>&quot;</td>
<td>5</td>
<td>372</td>
</tr>
<tr>
<td>270 min</td>
<td>&quot;</td>
<td>4</td>
<td>261</td>
</tr>
<tr>
<td>360 min</td>
<td>&quot;</td>
<td>4</td>
<td>270</td>
</tr>
</tbody>
</table>

Each animal was perfused via the left ventricle under sodium pentobarbital anesthesia with 5% heparin in saline solution for 1 min, followed by a mixture of 2.5% glutaraldehyde + 2% paraformaldehyde in 0.1M phosphate buffer (1/2 Karnovsky’s fixative, pH 7.4) for 15 min at room temperature. The parathyroid glands were removed on each side and immediately immersed in the same fixative for 1h. After being rinsed in buffer solution containing 7% sucrose, the tissues were postfixed in 1% OsO4 in Millonig’s phosphate buffer for one hour, followed by dehydration in graded ethanol, and embedded in Epon 812. Ultra-microtomy was performed on a Porter-Blum M-1 ultramicrotome, and thin sections were stained with uranyl acetate and lead citrate. The sections were examined and photographed with a JEOL-100B electron microscope. The number and size of secretory granules per cell section was measured on the electron micrograph print at a final magnification of $\times$10,000. The measurement was performed in at least 10 different areas in each animal, and only in profiles of chief cells containing a nucleus. Additionally, about 1ml of blood was collected from the inferior vena cava of each animal prior to perfusion, for the measurement of serum calcium level by the OCPC (o-cresolphthalein complexon) method.

RESULTS

A. Ultrastructure of the parathyroid gland of the normal mouse

The parenchyma of the mouse parathyroid gland consists of only a single cell type, the chief cell. The chief cells composed cell masses or cords. Loose connective tissues containing rich capillaries are present between these parenchymal cell clusters. The chief cells are cuboidal or polyhedral in outline, and are compactly packed in the cluster (Fig 1). Each chief cell has three surfaces, the apical, lateral and basal. The basal surface is surrounded by the basal lamina and faced to the pericapillary space. Therefore, it is believed that the parathyroid chief cell has a polarity.

The plasma membranes of the adjacent chief cells run in a slight tortuous course and in places show interdigitations. The nucleus of the chief cell is round or elliptical in shape and contains one or more nucleoli. In the cytoplasm, mitochondria are not evenly distributed, but usually located close to cisternae of the rough-surfaced endoplasmic reticulum (RER).

The Golgi apparatus consists of stacks of cisternae, dark vesicles and clear vacuoles, 100—200 nm in diameter; the latter with different amounts of finely particulate content are usually located apart from the innermost Golgi cisterna at trans side and some of which are known as prosecretory granules corresponding to immature small secretory granules. Besides, larger vacuolar secretory granules, about 300 nm in diameter, containing similar finely particulate material were frequently observed in the Golgi area. Some of both the prosecretory granules and larger vacuolar ones are seen connected with smooth-surfaced tubular network in the Golgi area (Fig 2). The ordinary secretory granules are membrane-bounded, round or ovoid bodies, 100—600 nm in diameter, and can be distinguished from lysosomes by both the finely particulate, homogeneous content and the halo of different widths beneath the limiting membrane (Fig 1, 2). Sometimes, some of the large secretory granules have been observed to have one or more coated patches and occasional stalks on their outer surface.

B. Effects of EDTA injection

In the mice at 30 min after an injection with 0.1 ml of EDTA, the apposed plasma membranes of adjacent chief cells mostly pursued a more tortuous course, and interdigitations are more
Fig 1 Cluster of parathyroid chief cells from a control mouse. Note that each chief cell shows a slightly tortuous outline. G Golgi apparatus, ly lysosomal body, L large secretory granule, S small secretory granule. × 5,600

Fig 2 Parathyroid chief cell from a control mouse, showing Golgi area. Prosecretory granules (arrowheads) and larger vacuolar secretory granules with dense content (probably immature storage granules) can be seen. Some of which are connected with a part of GERL (arrows). ly lysosomal body, S small secretory granule. × 19,000
Fig 3 Parathyroid chief cell from 30 min after EDTA-injection. More tortuous outlines and complicated interdigitations can be seen. G Golgi apparatus. × 5,600

Fig 4 Parathyroid chief cell from 30 min after EDTA-injection. The Golgi apparatus consists of elongated cisternae and numerous vesicles, accompanying a few prosecretory granules and larger secretory granules, probably developing immature storage granules. Increased free ribosomes in polysomal clusters are prominent. S small secretory granule. × 19,000
complicated (Fig 3). Free ribosomes, in polysomal clusters, were densely distributed throughout the cytoplasm, and mitochondria and cisternae of RER appeared more numerously. The Golgi apparatus consisted of dilated and elongated cisternae and numerous vesicles, accompanying a few large vacuolar secretory granules as well as prosecretory granules (Fig 4).

In the present study, we measured the number and size of all the secretory granules in cell sections containing a nucleus and examined the relationship between their size distribution and serum calcium levels, as shown in Table 2.

In the experimental animals at 10 min after the injection of EDTA, the serum calcium level (SCL) promptly lowered to 5.2 mg/dl (Table 2): the mean number of small secretory granules less than 300 nm in diameter decreased remarkably; but that of large secretory granules more than 300 nm in diameter decreased only slightly (Fig 5, 6).

Next, at 30 min after the injection, the SCL declined to a minimum (Table 3) and both the small and large secretory granules decreased significantly. Thereafter, the SCL rapidly rebounded to 8.5 mg/dl at 90 min; however, the mean number of both types of secretory granules remained almost unchanged. But, at six hrs after the injection both the small and large secretory granules recovered to the value of the control group. On the other hand, granules of 300 nm in diameter decreased in number only slightly 90 min after the injection.

### Table 2. Number of secretory granules and serum calcium concentration after EDTA injection

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of secretory granules</th>
<th>Serum calcium concentration (mg/dl)</th>
<th>Concentration of Ca++</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time after EDTA injection (min)</td>
<td>100 150 200 250 300 350 400 450 500 550</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solvent only</td>
<td>8.50 7.50 3.54 1.63 0.78 0.76 0.39 0.17 0.07 0.02</td>
<td>9.9±1.0</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>5.50 5.10 3.30 1.50 0.70 0.56 0.34 0.19 0.08 0.04</td>
<td>5.2±0.9</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>2.80 3.10 1.50 1.10 0.64 0.44 0.14 0.03 0.03 0.00</td>
<td>3.6±0.8</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>2.00 3.00 2.20 1.20 0.60 0.27 0.08 0.05 0.02 0.00</td>
<td>3.5±1.2</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>2.20 3.20 2.20 1.67 0.52 0.29 0.06 0.00 0.00 0.00</td>
<td>8.5±0.6</td>
<td></td>
</tr>
<tr>
<td>270</td>
<td>3.00 3.90 3.10 1.66 0.83 0.51 0.19 0.05 0.00 0.00</td>
<td>8.8±0.7</td>
<td></td>
</tr>
<tr>
<td>360</td>
<td>9.40 9.10 3.14 1.38 0.57 0.76 0.38 0.29 0.09 0.09</td>
<td>9.2±0.3</td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION

In the cytoplasm of mammalian parathyroid chief cells, membrane-limited, electron dense granules, measuring about 200—500 nm in diameter, have been described by several authors (DAVIS & ENDERS 1961, ROTH and MUNGER 1962, MUNGER and ROTH 1963, HARA and NAGATSU-ISHIBASHI 1964, ROTH and RAISZ 1964, 1966, CAPEN et al. 1965, ALTENÄHR 1970, NAKAGAMI et al. 1971, STOECKEL et Porte 1973, HASSE 1978). Furthermore, some of these authors admitted that the granules may be derived from the prosecretory granules of the Golgi area. In addition, such granules have been demonstrated to increase in the parenchymal cells stimulated by low calcium conditions (DAVIS & ENDERS 1961, ROTH and RAISZ 1964, 1966, CAPEN et al. 1965) and to decrease in high calcium medium in vitro (ROTH and RAISZ 1964, 1966). However, most of these authors considered that only the small vesicular granules located in or around the Golgi area are secretory granules (DAVIS and ENDERS 1961, HARA and NAGATSU-ISHIBASHI 1964, NAKAGAMI et al. 1971, STOECKEL et Porte 1973), while only a few studies have described large membrane-bounded granules with a dense homogeneous, fine particulate texture, as mature secretory or storage granules (ROTH and RAISZ 1964, ALTENÄHR 1970, HASSE 1978).

In the present study, small secretory granules less than 300 nm in diameter were rapidly decreased in number as soon as the SCL lowered to 5.2±0.9 mg/dl at 10 min after the injection of EDTA, whereas most of large secretory granules more than 300 nm in diameter did not decrease until the SCL dropped to 3.6±0.8 mg/dl at 20 min after the injection. Since such decreases in number of secretory granules are thought to be due to their discharge from the cytoplasm by exocytosis, these results may suggest that the population of the small secretory granules is easily released in response to the decrease of serum calcium concentration, but that of large secretory granules remains stored in the cytoplasm until the SCL becomes reduced to a certain lower level (Table 3, Fig 6).

Recent biochemical studies have suggested that there may be two functional pools of parathormone in the parathyroid; one, a newly formed parathormone pool with a steady secretion responds to basal small variation of serum calcium level; and the other, a pool that is quickly discharged in response to extreme and rapid hypocalcemic stress (MACGEGOR and COHN 1978).

Morphologically, the former pool has been reported to correspond to the small secretory granules, and the latter, the large storage granules (SETOGUTI et al. 1981). Furthermore, these two kinds of granules have recently been demonstrated immunocytochemically to store parathormone in their core (INOUE and STOGUTI 1986). Therefore, we can conclude that in the mouse parathyroid gland there are also two populations of secretory granules, small and large storage. Probably, the latter are thought to be discharged as an emergency supply of parathormone in the case of rapid hypocalcemia, as suggested in the rat parathyroid (SETOGUTI et al. 1981).
In the present work, the number of secretory granules, about 300 nm in diameter, showed only slight variations at any time after the injection. This may be explained by the following reason: most of these granules were distributed in the Golgi area and some of them were also connected with the smooth-surfaced tubular network in the Golgi area as were prosecretory granules. Such an agranular tubular network in the Golgi area is thought to correspond to a part of GERL (Novikoff, 1964) or the trans Golgi network (Griffiths and Simons, 1986). Therefore, these granules may be mainly originated from the trans—Golgi network and may be immature, developing storage granules because they are apparently larger in size than prosecretory granules located in the Golgi area. Accordingly, we included them in the category of storage granules. However, such developing granules may not be discharged even under extremely low concentrations of calcium because of their connection with the trans—Golgi network.

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