ULTRASTRUCTURAL LOCALIZATION OF ACID PHOSPHATASE IN THYROCYTES OF RATS WITH THYROID HYPERFUNCTION

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Acid phosphatase was detected electron-cytochemically in lysosomes which appeared in large numbers in the follicular cells of the hyperplastic rat thyroid gland. The remaining types of granules (mature secretory granules, lipid granules), the number of which also increased appreciably during functional stress on the thyrocytes, did not contain the reaction product. By the character of distribution of acid phosphatase the lysosomes could be subdivided into three main groups: those with a dense homogeneous residue and those with residue in the form of densely or infrequently distributed circular grains. The heterogeneity of the lysosomes may be attributed to differences in their functional state. Besides in the lysosomes, the reaction product was found in pale cells as a sprinkling of widely scattered dark grains. These drops evidently appeared as the result of fusion of droplets of colloid with lysosomes. The acid phosphatase of the lysosomes in this case participates in hydrolysis of the secretion product of the cells with the formation of the active substances.

KEY WORDS: electron cytochemistry; acid phosphatase; lysosomes; hyperplasia; thyrocyte.

Several types of granules are distinguished in the cytoplasm of the follicular cells of the thyroid gland: 1) round apical secretory granules with fine-grained contents of average density; 2) pale, large, colloid drops, formed in the opinion of most workers as a result of phagocytosis of reserve colloid from the lumen of the follicle at times of intensive liberation of colloid from the cells; 3) dense osmiophilic lysosomes; 4) lipid granules. A common structural feature of all the granules is the presence of a single membrane. During hyperplasia of the follicular cells caused by prolonged administration of 6-methylthiouracil (6-MTU), a marked increase in the number and size of the apical secretory granules and lysosomes was observed in them [2]. Considering the polyfunctional nature of the lysosomes, it was decided to study their structural and functional changes during chronic functional overloading of the cells as is observed in the stage of hyperplasia. Since maturing and certain mature secretory granules and lysosomes are of common origin and are similar in external appearance, it is difficult to identify the latter purely from ultrastructural criteria. However, the lysosomes contain all the hydrolytic enzymes of the cell and also much of its acid phosphatase, which served as their marker.

The object of this investigation was the electron-cytochemical identification of lysosomes among the various granules of the thyrocytes and the elucidation of their possible role during functional overloading of thyroid gland cells.

EXPERIMENTAL METHOD

Intact thyroid glands (control) of noninbred albino rats weighing initially 200-250 g and hyperplastic thyroid glands of rats receiving 6-MTU (10 mg/100 g body weight) with their drinking water daily for 6-12 months were investigated. The thyroid glands of the control and experimental rats were removed 6, 8, 10, and 12 months after the beginning of the experiment. In the experimental animals both lobes of the gland were greatly

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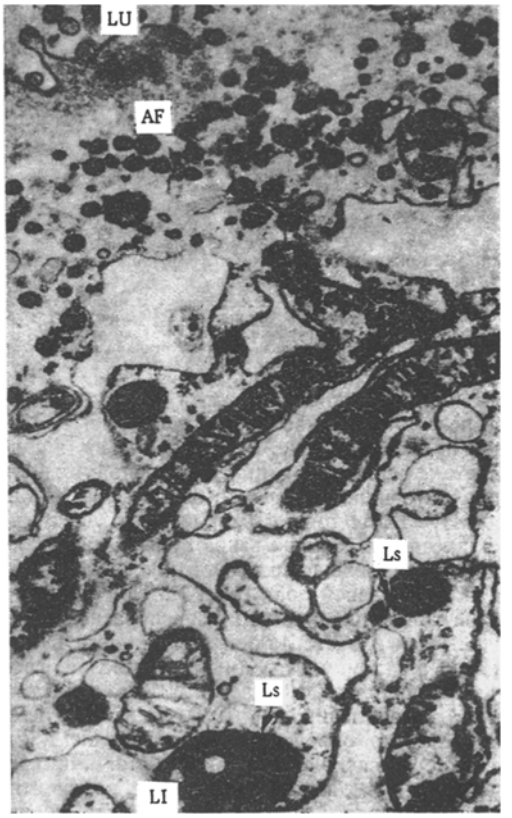

Fig. 1. Ultrastructure of follicular cell of rat thyroid gland during hyperplasia. Collections of apical secretory granules (AF) and larger lysosomes (Ls), some of them containing lipid inclusions (LI), can be seen. Lu) Lumen of follicle; 20,000×.

hyperemic and enlarged; they weighed 240-360 mg compared with 20-30 mg in control rats of the same age. Pieces of tissue for cytological investigation were fixed in a 1% buffered solution of osmium tetroxide, pH 7.4, dehydrated in alcohols, and embedded in Araldite. To detect acid phosphatase other pieces of tissue were fixed in 1.5% glutaraldehyde in cacodylate buffer with the addition of 1% sucrose at 4°C for 1 h. After rinsing in buffer with 7.5% sucrose for 1 h the pieces of gland were incubated for 30 min in Gomori's medium [9] at 37°C. The specificity of the reaction was established by incubating the pieces in medium without substrate. After incubation the pieces were rinsed in phosphate buffer and postfixed in a 1% solution of osmium tetroxide in phosphate buffer (pH 7.4) for 1 h. The material was dehydrated and embedded in Araldite in the usual way. Ultrathin sections were cut on the LKB ultratome and examined in the JEM-7A electron microscope without preliminary staining and after brief staining with lead citrate. The sections were photographed under a magnification of 10,000-15,000 times; enlarged prints are shown in the illustrations.

EXPERIMENTAL RESULTS

In cells of the intact rat thyroid gland the number of the various granules and, in particular, of lysosomes, is very small. In individual cells lysosomes were found singly or sometimes in groups of one to three in the apical zone. Administration of 6-MTU in these experiments, like brief stimulation of the thyroid gland in experiments by other workers [1, 13, 15], led to a sharp increase in the number and size of the secretory granules, colloid drops, and lysosomes in the cells, especially in their apical zone (Fig. 1). The lysosomes were distinguished by extreme diversity of shape and size and often by the presence of lipid inclusions. Acid phosphatase was detected electron-cytochemically in the lysosomes of both intact and hyperplastic cells. The reaction product was clearly distinguished in the organelle as a dark black residue. Lipid inclusions in lysosomes, and also other types of cytoplasmic granules did not contain the reaction product. The specificity of the reaction was confirmed by its absence in lysosomes of thyrocytes from control pieces of the thyroid gland (Fig. 2c). Acid phosphatase was found as separate, small, scattered black spots in several of the large, pale colloid drops which appeared in large numbers in the apical zone of the hyperplastic cells (Fig. 2d).

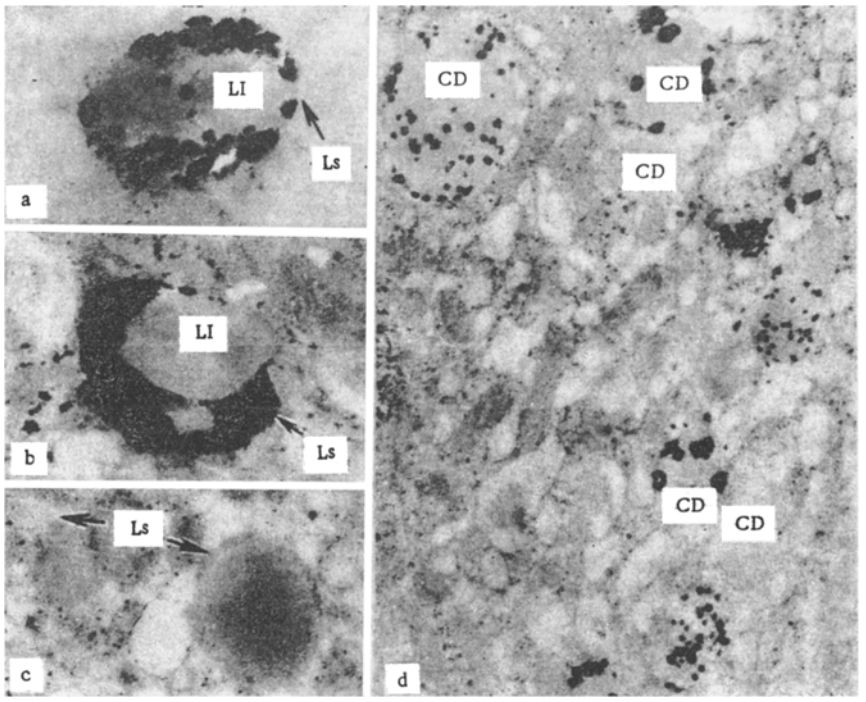

Fig. 2. Localization of acid phosphatase in follicular cells of hyperplastic rat thyroid gland: a, b) acid phosphatase in the form of large grains and a compact homogeneous black residue, respectively, in lysosomes (Ls); lipid inclusions (LI) do not contain acid phosphatase (50,000×); c) control; no reaction product visible in lysosomes (35,000×); d) colloid drops (CD) in apical zone of cell containing reaction product in the form of many tiny or few large black grains (20,000×).

Thyrocytes thus contain fewer lysosomes than other granules [11]. Usually these organelles are larger and more polymorphic than secretory granules. Lysosomes often contain cytoplasmic elements and lipid inclusions, which are never found in secretory granules. Finally, lysosomes contain acid phosphatase, which disappears from mature secretory granules that are morphologically similar to single lysosomes. However, in the small immature secretory granules located in the zone of the Golgi apparatus this enzyme is present [5, 6, 12]. Another enzyme, peroxidase, was found by the writers previously [3, 4] in most of the mature secretory granules of hyperplastic cells in the rat thyroid gland.

By the character of distribution of the reaction product the lysosomes were conventionally subdivided into three groups: 1) those with a dense homogeneous residue (Fig. 2b), 2) those containing residue consisting of separate round grains (Fig. 2a), and 3) those with a residue consisting of a few infrequent medium-sized and small grains. In the writers' view the heterogeneity of the lysosomes is attributable to differences in their functional state.

Most workers associate the appearance of lysosomes in thyrocytes with disintegration of colloid drops phagocytosed from the cavity of the follicle. If these drops merge with lysosomes, active hormones of the thyroid gland are liberated from the thyroglobulin of the colloid drops with the aid of the lysosomal enzymes [8, 13-16]. Although no direct contacts between lysosomes and colloid drops were found, the pale drops containing spots of reaction product are, in the writer's view, the result of fusion of these formations. The role of lysosomes in the secretory processes of cells of various glands (pituitary and others) has recently been reported by several workers [7, 10, 14].

Lysosomes thus participate not only in intracellular digestion, but also in the regulation of the secretory process. In the writers' view, the dominant function of the lysosomes in hyperplastic thyroid gland cells is their participation in the process of rapid liberation of large quantities of hormone from the cell.

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CONNECTION BETWEEN MOSAIC PATTERN OF MYOCARDIAL LESIONS AND METABOLIC HETEROGENEITY OF THE MYOCARDIOCYTES

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Uridine-^{3}H (2 μCi/g) was injected intraperitoneally into albino mice followed 1 h later by isopropylnoradrenalin (0.1 mg/g), and after an interval of 10 min the animals were killed. Autoradiographic analysis and polarization-microscopic investigation of sections through the myocardium showed that primarily myocardiocytes with a lower level of RNA synthesis developed contracture lesions.

KEY WORDS: heterogeneity of myocardiocyte nuclei; RNA synthesis; mosaic pattern of myocardial lesions.

Myocardial lesions as a rule are mosaic in pattern. This is true both of metabolic lesions, affecting single cells or groups of cells [2, 5], and also of myocardial infarcts [1], when cells are damaged at different times and the type of lesion varies [6]. The causes of this heterogeneity are not clear. It has been suggested that at each given moment different muscle cells of the heart are in different states, possibly on account of some cyclic pattern of their vital activity [4]. Sarkisov and Vtyurin [3] proved that renewal of the intracellular structures of the myocardial cells takes place in a cyclic manner. It is therefore natural to suppose that the heterogeneity of lesions to myocardiocytes may reflect their metabolic heterogeneity [5].

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