THE GOLGI COMPLEX AND MITOCHONDRIA IN THE SECRETORY CYCLE OF CHROMAFFIN CELLS DURING THE SECRETORY PHASE

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The participation of cellular organelles in the formation and release of secretions by glands, in particular the endocrines, is a subject which has been studied and discussed for many years [1-3, 5, 10-11]. The mechanism of hormone secretion has been closely linked with the structural components of the cytoplasm inglandular cells. Morphological variability in cell organelles—the Golgi apparatus and the mitochondria—is conditioned by the functional variability of the cell in different phases of its secretory cycle. The interpretation of changes in the organelles during the secretory cycle, the establishment of a correlation of their separate forms with definite states of functional activity in the cell is one of the ways to elucidate the mechanism of the formation, accumulation, and release of the secretory products of the endocrine cell.

In the present work we have studied the Golgi complex and mitochondria in the chromaffin cell of the cortical layer of the rat adrenal during the release phase of secretory granules of adrenaline.

The only detailed work on the cytomorphology and cytochemistry of the chromaffin cell during secretory activity [4] does not present a concept of the morphological pictures which would permit estimation of the nature of the participation of cellular organelles in the formation and release of hormones.

As it is well known, the cells of this glandular tissue function asynchronously. Unification of the secretory phase, leading to the structural unification of the gland cells, would permit the construction of a true picture of the correlation between structural and functional changes in the cell. With this goal, insulin was used in our experiments as a powerful stimulus to the release of adrenalin from chromaffin cells of the adrenal into the blood in response to insulin hypoglycemia [7].

METHODS

Male mice weighing 150 to 200 grams were used in the experiments. The animals were injected once subcutaneously in the abdomen with insulin in a dose of 15-20 units per 100 gm of live weight. After the onset of hypoglycemic shock (sweating, exophthalmos, opisthotonus, loss of pupillary reaction) the rats were decapitated. To make preparations for review the adrenals were fixed in a mixture of "suso" and Carnoy's, for bringing out catecholamines totally—in a mixture of potassium dichromate and potassium chromate (histochemical reaction with adrenalin and noradrenalin), to show noradrenalin in a 10% solution of potassium iodate [6]; in order to stain the Golgi complex the material was tested according to the method of Kopsh Kalachev, Da Fano and Aoyama. The mitochondria were brought out by the fluids of Shamni, Rego and Kol'ster followed by staining according to Al'tman and iron hematoxylin.

RESULTS

The chromaffin reaction for the total appearance of adrenalin and noradrenalin served as a control for determining the results of hypoglycemic shock produced by a massive outpouring of secretion—of adrenalin from the chromaffin cells of the adrenal.

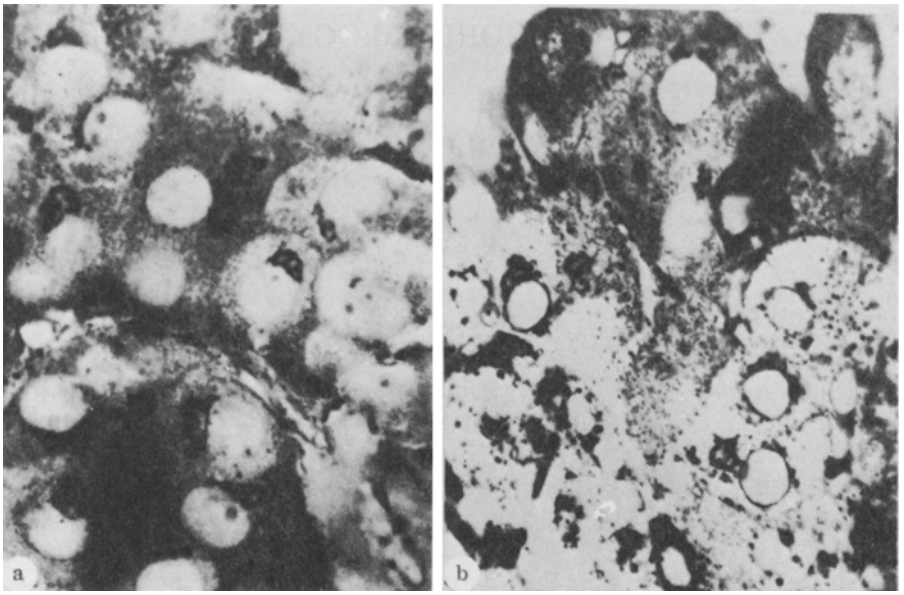

Fig. 1. Golgi complex in chromaffin cells of the rat adrenal gland. a) Control; b) phase of active secretion. Fixation: Shampi's solution. Magn. 900 x.

In control animals the entire adrenal cortex gives a positive chromaffin reaction. The majority of its cells are stained a yellow color. Against the yellow background are more brilliant, separate islands of cells (with a brown hue). This, evidently, represents islands of noradrenalin, judging from the accompanying reaction with potassium iodate (a specific reaction for noradrenalin). At a greater microscopic magnification it is evident that the cellular composition of the adrenal cortex is varied. Cells display the dynamics of secretion, expressed in a lesser or greater density of the granular contents or in the degree of intensity of homogeneous staining. This indicates that the cells containing adrenalin are found in different phases of the secretory cycle.

In experimental animals killed in a state of shock, attention is focused on the absence of the chromaffin reaction in the main bulk of cells in the adrenal cortex, which indicates the exodus of the secretory products and the devastation of the majority of the cells. The presence in such preparations of transitional forms of cells with a weakly expressed reaction or complete lack of it demonstrates the dynamics of the secretory process. Noradrenalin remains "untouched" by the action of insulin,and the islands of these cells are morphologically of one type in the control and in the experimental animals (with a histochemical reaction using potassium iodate).

The Golgi complex in chromaffin cells of the adrenals in control animals (Fig. 1a) is represented by rather uniform structures. In the shape of balls, rings, baskets,and composed of osmophilic bars, it is located near the nucleus. In addition to such structures another type of organization of the complex is encountered—as dissociated fragments of a network placed at some distance from the nucleus or scattered in the cytoplasm. In preparations fixed in Da Fano and Aoyama fluid, orange granules are often distributed more thickly in the region of the Golgi apparatus. Some granules are surrounded by black wheels and half-rings (silver impregnation of the Golgi complex components), but it is difficult to say if they are granules of adrenalin.

In experimental animals (Fig. 1b) the cytoplasm of the adrenal chromaffin cell is more friable than in control animals. The Golgi complex in the main cell mass of the experimental gland is altered and loosened. Fine strands, wheels,and half-rings are distributed in different parts of the cytoplasm or in the perinuclear zone. In Fig. 1b is seen a lobule of chromaffin tissue from the adrenal of an experimental animal; the majority of cells in it have extruded their secretion (clear cells). The Golgi complex in them is scattered in the cytoplasm as separate granules or fragments of strands. In addition to light cells come cells with more dense and darker cytoplasm, filled with secretory granules, are seen in the lobule. The Golgi complex in these was noted to be unchanged and preserves its netlike

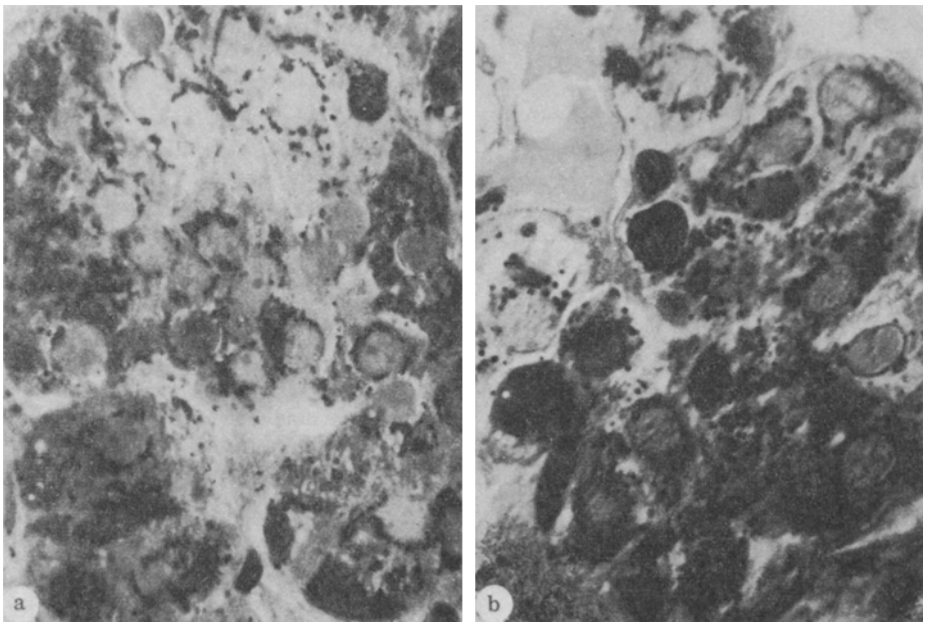

Fig. 2. Mitochondria in the chromaffin cells of the rat adrenal gland. a) Control; b) phase of active secretion. Fixation: Rego's fluid. Staining: method of Aoyama. Magn. 900 ×.

complex dispersed its material completely (in invertebrates) or partially (in vertebrates) at the formation of secretions, or different substances were adsorbed on its surface (lipoids, yolk, hormones, enzymes, etc.) [1, 2]. form and perinuclear location. In this case we also are dealing with the dynamics of the process of release of the secretion. Lobules are seen, in the cells of which the Golgi complex is reduced to a structure barely perceived in the light microscope.

The mitochondria in the adrenal cortical cells of the rat normally are rather sparse (Fig. 2a). They have varied shapes: threadlike (the most variable configuration), rodlike, granular, and rarely ringlike. In one and the same cell, mitochondria of different shapes are met, localized mainly around the nucleus. But they also have another distribution in the cell cytoplasm—between the secretory granules in some parts of the cytoplasm, free and separated from the secretory granules. In an experiment (Fig. 2b) the most distinctive feature of the mitochondria is the uniformity of shape and conversion into rounded or spheroidal granules, scattered in the cytoplasm, which is related to the release of secretory granules from the cell. Rodlike and filamentous mitochondria are rarely found. The size of the mitochondria is increased and their number grows.

The data obtained shows first that the cellular organelles of secretory cells in the rat adrenal cortex are not static structures: they change shape and location within the cell corresponding to the shape of granule-formation and release of secretion into the blood. In control animals, as histochemical reactions demonstrate, the chromaffin cells are filled with secretory granules having different shades of a yellow-brown color. The Golgi complex in the majority of cells has a compact structure of spheres and is limited in distribution near the nucleus. In a smaller number of cells the compactness is disrupted and separate fragments of filaments and spheres are distributed throughout the cytoplasm as short rods, granules, and partial rings. Between these two forms are transitional stages which are characterized by different degrees of dispersion in the cytoplasm. Under the conditions of insulin shock a unification of the secretory phase of the adrenal cortex occurs, beginning with a massive outpouring of granules of adrenalin into the bloodstream, and the cytoplasm of the chromaffin cells becomes empty, which is confirmed by the negative histochemical reaction for adrenalin. The predominant structural organization of the Golgi complex is fragmented and disrupted in form. The Golgi complex remains almost unchanged only in cells from the noradrenalin-producing islands (insulin does not show a stimulatory effect on noradrenalin).

In the literature there is not one description of the structural dynamics of the Golgi complex in relation to changes in the functional activity of glandular tissue. Upon stimulation of the process the Golgi complex hypertrophies, and with hypofunction it atrophies [2, 5]. The question of the role of the Golgi complex in the secretory process has been discussed for many years [1-3, 5, 10, 11]. Until recently two hypotheses existed: either the Golgi

The results of electron microscopic investigations permit us to obtain a new concept of the role of the Golgi apparatus in secretion. According to this concept, the secretory products in the structural elements of the Golgi complex which have been synthesized in other parts of the cytoplasm, lose water, become denser, and acquire a lipid envelope, becoming transformed into mature secretory granules [2, 3]. In this connection, that the literature is unclear on the question of the site of catecholamine synthesis in the cell and, in addition, catecholamines are not protein substances, one can hardly connect their synthesis with granules of the endoplasmic reticulum. Whether their synthesis occurs inside the vesicles of the Golgi complex or in the cytoplasmic matrix is unclear. The distribution of elements of the Golgi complex in the cytoplasm of the chromaffin cell during the period of increased secretory release, in our opinion, may be related to the preparation for the process of synthesis which follows after the secretion and occurs together with it. Whether it makes for a wide distribution of Golgi elements in the cytoplasm by approaching the site of catecholamine synthesis or by mechanical means, related to the secretion of granules from the cell and more rarely from the cytoplasm as a consequence of this process, is impossible to confirm.

In the rat adrenal cortex the mitochondria, normally few in number and varied in shape and size, lie in parts of the cell which are free from secretory granules but most often are around the nucleus. From time to time cells are seen which are rich in rounder mitochondria which are more evenly distributed throughout the cell. Under conditions of insulin shock the picture is sharply changed: the number and volume of the mitochondria is increased in cells which have released their adrenalin supply. The noradrenalin producing islands are noted to be unaltered. The alteration of the mitochondria in the secretory process (for example, their decrease during the period of secretory accumulation, fragmentation, swelling) have been described in the literature, and several investigations have related it with the direct formation of secretory granules from material from the mitochondria or from substances produced by the mitochondria [3, 8, 9]. We think that the significant increase in mitochondrial mass by increase in their number and volume in rat adrenal chromaffin cells depleted by insulin shock is related mainly to the increased requirement of these cells for energy material, necessary for the new, massive synthesis of adrenalin. In addition, the wide distribution of mitochondria throughout the entire cytoplasm may be called forth for mechanical reasons, as in the case of the Golgi complex.

Thus, it has been shown in our work that in the rat an increased secretory output from the adrenal chromaffin cells stimulated by the injection of insulin into the animal, is accompanied by definite changes in the structure and localization of the Golgi complex and the mitochondria, and also quantitative changes in the latter, also indicating the active participation of these organelles in the secretory cycle of the chromaffin cells of the rat adrenal cortex.

SUMMARY

The object of study was the organelles of the rat adrenal chromaffin cell under normal conditions and in hypoglycemic shock caused by insulin injection in the animals, resulting in the active secretion of catecholamines by the cortex of the adrenal gland. The organelles of these cells underwent considerable changes. The Golgi complex became loose and was distributed throughout the cell. Mitochondria, normally few and of granular, rod- and filamentous forms, usually localized around the nucleus, became uniformly distributed in the cell, increased in number, and enlarged in size, and were mainly granular in form.

The characteristic changes in the Golgi complex and mitochondria during the phase of active secretion are evidence for their active participation in the secretory process.

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