CYTOCHEMICAL LOCALIZATION OF EXOGENOUS PEROXIDASE IN ADRENAL MEDULLARY CELLS OF HAMSTER

I. Benedekzy and P. Somogyi

BIOLOGICAL RESEARCH INSTITUTE OF THE HUNGARIAN ACADEMY OF SCIENCES, TIHANY, HUNGARY

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Abstract

The endocytosis in hamster adrenal medullary cells has been studied by intravenously administered horseradish peroxidase, which was then detected with the Graham–Karnovsky method. The protein uptake was investigated 30, 60, and 120 min after the peroxidase injection. The exogenous protein was accumulated mostly in the lumen of the sinusoids, around the capillaries as well as at the apical pole of chromaffin cells 30 min after the peroxidase injection. After another 30 min, a large amount of the reaction product was detectable also in the chromaffin cells. The protein uptake was very intensive beside the plasma membrane, both apically and laterally, through coated- and smooth-surfaced vesicles. Two hours after peroxidase injection, the majority of the reaction product was present in multivesicular bodies. It is supposed that the primary endocytic vesicles were taken up by multivesicular bodies and digested by lysosomal enzymes. The ultimate fate of the incorporated granule’s membranes seems to be digestion by lysosomal enzymes, thus they are not reutilized during granulogenesis in the chromaffin cells.

Introduction

It has been generally accepted that the endocrine, as well as a great number of exocrine, cells release their secretory material by exocytosis from granules [1, 2, 3, 4, 5, 8]. However, the release of secretory material by exocytosis requires secretion product present in membrane-bounded secretory granules in the cytoplasm. It is not surprising, therefore, that exocytosis has not yet been observed in steroid-producing adrenocortical cells, where cytoplasmic storage of steroids is negligible. During exocytosis, the membrane-bounded secretory granules are closely attached to the plasma membrane, thereafter the two membranes, namely the membrane of granules and the plasma membrane, fuse. After the membrane fusion, the apical part of the cell membrane opens, and thus the secretory material can diffuse into the extracellular space [1, 3, 5, 8].

The phenomena of the exocytosis have been thoroughly studied by biochemical, physiological and morphological methods. Several questions have been clarified, however, the fate of the granule’s membranes dissolved.
in the plasma membrane during exocytosis has remained unknown. It is obvious that during repeated exocytosis the plasma membrane significantly increases in volume and, owing to the incorporation of excessive amounts of granule's membrane, its chemical composition, molecular organization and physicochemical character are altered. The question arises: does the plasma membrane reorganize its fine structure after exocytosis, and if it does, what kind of mechanism can provide the process of reconstruction?

Morphological studies [1, 2, 3, 5, 8] have suggested that there exists a reorganization process in the plasma membrane after exocytosis, and this takes place by endocytosis. Unfortunately, the exocytosis–endocytosis coupling has been studied mainly in rats [1, 9] where the morphological features of the exocytosis are poor.

Since exocytosis is common in the hamster adrenal medulla and its cytochemical properties have not been studied, it was reasonable to investigate the exocytosis–endocytosis coupling in this species. Horseradish peroxidase was injected into hamsters to study the endocytosis activity of the plasma membrane in adrenomedullary cells.

Materials and Methods

Adult golden hamsters (100–120 g) of both sexes were used. Before the injection of horseradish peroxidase, the animals were anaesthetized with 1 ml 3.5% chloralhydrate by the i. p. route. Twenty mg horseradish peroxidase (Schwarz, New York) was dissolved in 0.5 m saline and was injected into the vena jugularis. Control animals were injected with 0.5 ml saline. The uptake of peroxidase was studied 30, 60 and 120 min after the injection. Before fixation, the animals were perfused with saline for 2 min to remove the red blood cells. Perfusion fluid (and later the fixative, too) was introduced via a needle into the left ventricle of the heart, and was allowed to escape through a cut in the right auricle. The fixative contained 2.5% glutaraldehyde and 4% formalin in cacodylate buffer (pH 7.4). The duration of perfusion fixation was 25 min, and 1 h more immersion in the same fixative. For cytochemical incubation 70–100 μm sections were made from the adrenal by a Smith-Farquhar tissue sectioner. These sections were thoroughly washed for 1 h in 0.05 M Tris-HCl buffer (pH 7.65). Cytochemical detection of peroxidase was carried out as described by GRAHAM and KARNOVSKY [7]. After incubation, the sections were washed in Tris-HCl buffer for 5 min and postfixed in OsO₄ for 1 h. Blocks were dehydrated in a series of alcohol and embedded in Durcupan ACM (Fluka). Ultrathin sections were cut with LKB Ultrotom and were stained with saturated aqueous solution of uranyl acetate for 30 min, followed by RENOLOD'S lead citrate for 2 min. The sections were examined in a JEM 100 B electron microscope.

Results

Since 30 min after peroxidase injection, we found only small amounts of the peroxidase reaction product in chromaffin cells, micrographs were taken from blocks which had been incubated for 1 and 2 h after injection. A large amount of the electron-dense precipitate (which represents the presence and roughly the amount) of the injected horseradish peroxidase was
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found at the apical pole of the chromaffin cells (Figs 1, 2) around the capillaries (Fig. 2) as well as between the lateral plasma membranes (Fig. 1). Besides the extracellular horseradish peroxidase, a considerable amount of peroxidase reaction product was present intracellularly. Peroxidase activity was associated with cell organelles only in the cytoplasm of chromaffin cells (Figs 1, 3, 4, 5).

The distribution of peroxidase-containing cell organelles is rather homogeneous in the adrenomedullary cells; peroxidase reaction product appeared beside the nucleus, in the Golgi area, as well as at the peripheral part of the cytoplasm (Fig. 5). The uptake of horseradish peroxidase took place anywhere on the cell surface, most frequently at the apical poles of chromaffin cells, near or beside the capillaries (Fig. 2). A great number of deeply-invaginated plasma membrane portions were found at the apical poles of glandular cells (Fig. 2) rich in the reaction product; moreover, at the inner surface of these invaginations coated pits and vesicles with electron-dense reaction product were often seen. Exogenous peroxidase-containing coated vesicles occurred not only beside the apical plasma membrane, but also in the deeper part of the cytoplasm (Fig. 4) as well as near the lateral plasma membrane (Figs 2, 3).

Peroxidase was taken up not only by coated vesicles, but frequently by smooth-surface vesicles and vacuoles, too (Figs 2, 4). These organelles can be found near the plasma membrane, sometimes also in the Golgi apparatus (Fig. 5).

The electron-dense reaction product of peroxidase was most frequently found in the multivesicular bodies of chromaffin cells. These bodies may be present anywhere in the cytoplasm (Fig. 5), most often near the plasma membrane (Figs 2, 4). The multivesicular bodies may be filled with the electron-dense reaction product (Fig. 5), but sometimes it is possible to identify the individual peroxidase-containing vesicles in them (Fig. 1). In a few cases, smooth- and coated-surface vesicles and saccules with peroxidase reaction product in their lumina were present beside the multivesicular bodies (Fig. 5). We have never observed electron-dense reaction product in the lamellae of the Golgi apparatus (Fig. 4), in mitochondria, in secretory granules and in the rough-surfaced endoplasmic reticulum. However, Golgi vesicles (both coated and uncoated) occasionally accumulated the injected horseradish peroxidase (Fig. 5).
Fig. 1. Chromaffin cells, 1 h after horseradish peroxidase injection. A large amount electron-dense reaction product is seen at the cell surface both apically (I) and laterally (L). Multivesicular bodies (MB) and vacuoles (V) also contain peroxidase reaction product. In multivesicular bodies small vesicles are conspicuous. × 24,000

Fig. 2. Reaction product is detectable neither in the capillary (C) nor in the endothelial cell (E) two hours after peroxidase injection. Note the peroxidase containing coated pits (cp) and vesicles (cv) beneath the cell membrane (Cm) of chromaffin cell. × 36,000
Fig. 3. Large vacuoles (V) often contain electron-dense reaction product 2 h after the peroxidase injection in chromaffin cell (Ch). I = Intercellular space. × 36,000
Fig. 4. Two hours after horseradish peroxidase injection, the majority of the enzyme was present in multivesicular bodies (MB) and vacuoles (V). Golgi apparatus (G) is free from reaction product. × 24,000
The occurrence of peroxidase in cell organelles may be nearly homogeneous in the chromaffin cell. Apically, a multivesicular body (MB) can be seen, which is full with enzymes and beside it occurs a peroxidase containing vesicle (v) and a sacculus (S) in close contact with the multivesicular body. Note the "cup-like" body (CL) at the left side of the micrograph and the peroxidase-containing multivesicular body (MB) and vesicle (v) in the Golgi apparatus (G). Right, not far from the Golgi area, there are several peroxidase-containing vacuoles (V) and vesicles (v) in close morphological contact to each other.

X 36,000
Discussion

The endocytosis of rat adrenal medulla was studied as early as in 1968 by Holtzman and Dominitz [9] using horseradish peroxidase as tracer molecule. These authors established that injected horseradish peroxidase was taken up first of all by vesicles, tubules and "cup-like" bodies, which immediately were transformed to multivesicular bodies. In that paper [9], Holtzman and Dominitz did not associate the endocytotic activity of the gland with exocytosis since the existence of exocytosis was not unambiguously accepted at that time. The connection between exo- and endocytosis was re-investigated by Abrahams and Holtzman [1] in 1973, after insulin treatment. Although their observations concerning exocytosis are not convincing, they succeeded in corroborating their previous results, among them, the intensive peroxidase uptake by chromaffin cells after insulin treatment. According to Abrahams and Holtzman [1], one group of the peroxidase-containing structures proved to be lysosome, the others after the uptake fuse with lysosome, in which the exogenous protein is digested by lysosomal enzymes. It was suggested [1] that the membrane of chromaffin granules, which has been recaptured by coated vesicles from the plasma membrane, will be eliminated by a similar process, after the hormone has been released.

As regards the connection between exo- and endocytosis several authors supposed a close coupling between them. First, Palade [10] supposed that the intact membrane segments of the extruded zymogen granules can be reutilized in the course of granulogenesis in the Golgi apparatus. In contrast to this, Fawcett [6] stated that the membrane of the secretory granules degrades after the secretory material has been released. The hormone liberation process of adrenal medulla had been explained similarly to other endocrine glands until 1967, when Diner [4] proposed a new hormone extrusion process, called exocytosis. On the omega-shaped membrane invaginations of the exocytosis Diner [4] often observed coated pits and vesicles, but then, she did not explain their role in the secretion. On the basis of our electron microscopic studies [2, 3] we supposed that the coated vesicles might play a role in the retrieval of the granule's membranes, which dissolved in the plasma membrane during exocytosis. The thorotrast uptake, which reflects the endocytotic activity of coated vesicles, was proved by Douglas et al. [5] both in the hypophysis and in the rat adrenal medulla. Not much later, Abrahams and Holtzman [1] published cytochemical observations concerning the peroxidase uptake by rat adrenal medulla, however, the endocytotic activity of the hamster adrenal medulla remained still obscure. Since the adrenal medulla of the hamster has been accepted as the classical test object of exocytosis, we supposed it is basically important to study, the morphological response of the chromaffin cells on the injected horseradish.
peroxidase. We found that both coated- and smooth-surfaced vesicles are able to take up and store the exogenous protein. Since the number of peroxidase-containing cell organelles was rather high in the adrenomedullary cell of the hamster, it can be stated that the endocytotic capacity of the chromaffin cells is rather high. In the chromaffin cells not only the endocytotic capacity is high, but the protein transport, too; 1 h after the peroxidase injection, the protein was present everywhere in the cytoplasm. The fact on the other hand, that 2 h after peroxidase injection, the majority of the protein was found in the multivesicular bodies suggests that the ultimate fate of exogenous protein is lysosomal digestion in chromaffin cells. Based on this experiment we suppose that the fate of granule’s membrane is similar during catecholamine secretion, namely it will be digested by lysosomal enzymes. This is supported by our negative cytochemical results. We have never found electron-dense reaction product in the Golgi’s lamellae; this means that the elements of the degraded granule’s membranes are not reutilized during de novo granulogenesis.

Since the results of our experiment are consistent with those of Abrahams and Holtzman [1], we can state that though exocytosis is different in many aspects in the adrenal medulla of hamster and rat, the morphological features of endocytosis are practically the same in the two species.

REFERENCES


István Benedeczky
Péter Somogyi

H-8237 Tihany, Fürdőtelep 56.
H-1094 Budapest, Tűzoltó u. 58.