ANALYSIS OF PATHWAYS OF TRANSPORT OF MATERIALS INTO GLANDULAR CELLS

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Movement of materials in the metabolic component of the microcirculatory system of the exocrine part of the pancreas in Rana temporaria takes place from the blood capillary to the pericapillary space, then into the intercellular spaces, and from them into the glandular cells. An ultrastructural study of the localization of ATPase activity in the metabolic component confirmed these data: A high concentration of lead phosphate granules was observed in the endothelium of the blood capillaries, on the fibrillary structures of the interstices of the pericapillary space, on the lateral plasma membrane of the exocrine pancreacytes, and on the cytoplasmic outgrowths forming pinocytotic vacuoles.

KEY WORDS: pancreas; microcirculation; cytoplasmic outgrowths; ATPase.

Among the unsolved problems in histophysiology of the glands a special place is occupied by the transport of original products from the blood to the glandular cells. A direct role in the relations between the cells and fluids surrounding them is played by the blood capillaries, the pericapillary space, and the intercellular spaces connected with it [6]. Several studies have been made of the morphology of these components of the microcirculatory system of the pancreas [1, 5, 7, 15, 18] and the pathways of transport of materials into the glandular cells have been analyzed [11, 13]. It has been shown that on the lateral and basal surfaces of the acinar cells of the pancreas in various vertebrates there are cytoplasmic outgrowths which take part in the regulation of entry of substances from the blood stream into the intercellular spaces [2-4]. On the basis of these findings the cytoplasmic outgrowths have been included in the metabolic component of the pancreatic microcirculatory system (capillaries, pericapillary space, intercellular fields).

It was decided to study the role of each element of the metabolic component in the transport of materials and, in particular, to study the localization of enzymes responsible for the transport of materials on the membranes.

EXPERIMENTAL METHOD

The test object was the pancreas of amphibians (Rana temporaria). Ferritin was injected into the heart of the animals under ether anesthesia in a dose of 20 mg/100 g body weight, and 20 min later material was taken intravitally for investigation. Pieces of the pancreas were fixed in 3% glutaraldehyde for 1 h, then postfixed in Millonig's osmium mixture at pH 7.3 for 3 h, dehydrated in acetone, and embedded in Epon–Araldite. The localization of ATPase in the pancreas of R. temporaria was determined by the method of Wachstein and Meisel [17] in the modification of Buchvalow (Bukhvalov) et al. [12]. Pieces of the gland were fixed in 4% paraformaldehyde by Karnovsky's method for 1 h and then washed in 0.25 M sucrose for 2 h at 4°C. Sections were cut from these blocks on a cryostat and kept for 50 min in incubation medium (pH 7.2) at room temperature. The sections were then quickly rinsed in 0.25 M sucrose and postfixed in 1% OsO_4 for 40 min at room temperature, dehydrated in alcohols and acetone, and embedded in Epon 812. Ultrathin sections were examined in the JEM-100B electron microscope. To determine the precise character of the reaction three controls were

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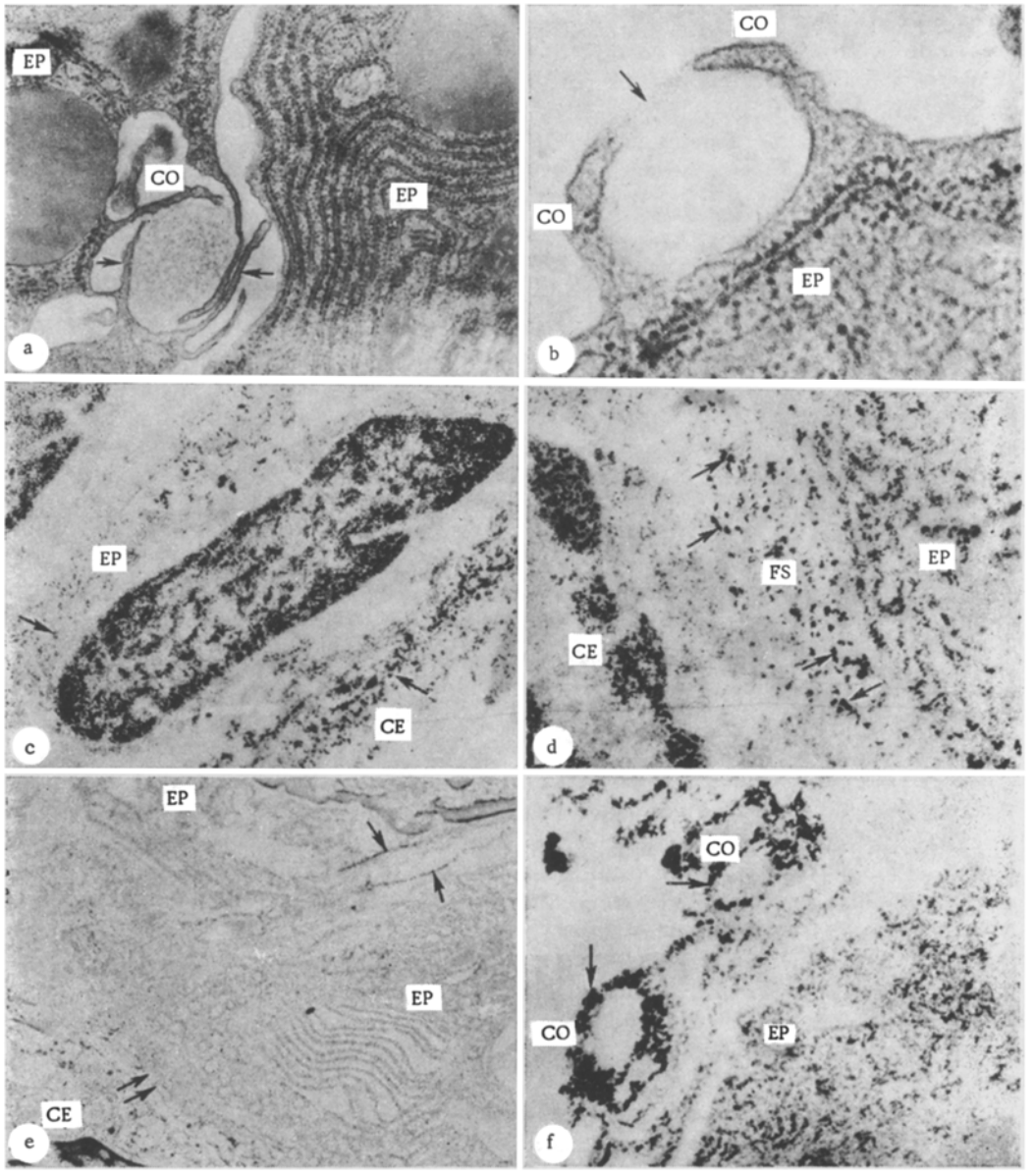

Fig. 1. Morphological and histochemical analysis of pathways of transport of materials into pancreatic acinar cells of Rana temporaria. a) Cytoplasmic outgrowths (arrow) of exocrine pancreacytes surround intercellular contents (12,000×); b) formation of pinocytotic vacuole by cytoplasmic outgrowths (arrow) on lateral surface of exocrine pancreacytes (70,000×); c) localization of enzyme reaction product for ATPase in capillary endothelium (arrow) in exocrine part of pancreas (20,000×); d) localization of lead phosphate in fibrillary structures (arrow) in interstices of pericapillary space of exocrine part of pancreas (40,000×); e) distribution of end product of reaction for ATPase on lateral and basal surfaces of plasma membrane (arrow) of exocrine pancreacytes (20,000×); f) high concentration of lead phosphate granules (arrow) on cytoplasmic outgrowths forming pinocytotic vacuoles on lateral surface of exocrine pancreacytes (10,000×). CO) Cytoplasmic outgrowths; EP) exocrine pancreacyte; CE) capillary endothelium; FS) fibrillary structures.

set up: 1) incubation medium without substrate; 2) preliminary fixation in 70° alcohol; 3) incubation medium with AMP. The controls demonstrated the specificity of the reactions.

EXPERIMENTAL RESULTS

Ferritin was detected 20 min after its injection in the endothelium of the blood capillaries and in the pericapillary space. Further movement of the marker was determined by the cytoplasmic outgrowths of the exocrine pancreacytes: In some cases they retained ferritin, whereas in others its particles penetrated freely into the intercellular spaces. Neighboring outgrowths on the lateral surfaces of the acinar cells, by approximation of their apical regions, formed a barrier around the contents of the intercellular spaces and then joined together to form pinocytotic vacuoles (Fig. 1a, b).

Injection of ferritin thus revealed movement of materials from the capillaries into the pericapillary space, then into the intercellular spaces, followed by their active transport into the glandular cells.

The results of a study of the localization of activity of the carrier enzyme (ATPase) in the metabolic component confirmed the above findings. A high concentration of the enzyme reaction product was observed in the capillary endothelium (Fig. 1c) and in the pericapillary space. The localization of lead phosphate granules on the fibrillary structures of the interstices in the pericapillary space will be noted (Fig. 1d). Increased ATPase activity also was found on the plasma membranes of the lateral surface of the acinar cells, whereas the basal part contained only a small amount of enzyme reaction product (Fig. 1e). The cytoplasmic outgrowths forming pinocytotic vacuoles on the lateral surface of the pancreacytes also were abundantly sprinkled with lead phosphate granules (Fig. 1f).

The results of the histochemical investigation thus also point to a role of all structures of the metabolic component in the movement of original materials and in their active transport from the intercellular spaces through the lateral surface into the glandular cells.

Enzyme activity in the capillary endothelium has frequently been described [9, 14, 16]. Considerable enzyme activity on the fibrillary structures of the pericapillary space was probably connected with the role of the interstitial tissue in perivascular transport of materials [8, 10]. When studying the submicroscopic organization of the exocrine part of the rat pancreas and the localization of ATPase at the light-optical level, Baradi and Brandis [11] also found that the basal part of the plasmalemma of the acinar cells is free from carrier enzyme, whereas ATPase activity is present on the lateral surface. On this basis they consider that the original materials enter from the intercellular spaces through the lateral surface of the plasmalemma. However, these workers do not explain the reason for the uneven distribution of materials between the glandular cells. Geuse and Poort [13] also studied the exocrine part of the pancreas, but after incubation in vitro with the ferritin marker. They found that ferritin particles enter the cytoplasm of the acinar cells by endocytosis; endocytotic vesicles are formed by the pinching off of projections of the lateral plasmalemma of the pancreacytes, whereas projections from the basal part form phagocytotic vacuoles containing ferritin. We studied the ultrastructure of the gland under more physiological conditions (injection of ferritin in vivo) and traced the process of formation of pinocytotic vacuoles through fusion of the apical regions of the cytoplasmic outgrowths of the plasmalemma on the lateral surface of the cell, the membranous component of which possesses increased ATPase activity. This would thus appear to be a more likely mechanism for the entry of materials into the cell.

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ULTRASTRUCTURAL ANALYSIS OF THE EFFECT OF ALLOXAN ON REPTILIAN PANCREATIC CELLS

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Intraperitoneal injection of alloxan into terrapins Testudo horsfieldi and Clemmys caspica in a dose of 300 mg/kg caused destructive and metabolic changes in cells of both the endocrine and exocrine parts of the pancreas. The granular cytoplasmic reticulum in the acinar cells and the mitochondria in the centroacinar cells underwent focal destruction. Hydropic degeneration developed in the B cells. Deposition of glycogen was found in all cells and nerve fibers and was abundant in the centroacinar and mucoid cells.

KEY WORDS: pancreas; alloxan; morphological and metabolic changes.

Development of the sensitivity of the insular system of the vertebrate pancreas to diabetogenic substances is a problem in comparative endocrinology that has received little study. A previous investigation [1] showed that injection of alloxan into amphibians leads to a series of morphological changes in the pancreas (degranulation, edema of the secretory granules, activation of the lysosomes) not only in the B cells, but also in the acinar and acino-islet cells. The action of alloxan on the reptilian pancreas, however, has been incompletely studied. Most attention has been paid so far to the B cells [4-6].

The object of this investigation was to continue the study of the effect of alloxan on pancreatic structures.

EXPERIMENTAL METHOD

A 3% solution of alloxan was injected intraperitoneally in a dose of 300 mg/kg into the terrapins Testudo horsfieldi and Clemmys caspica. The pancreas was investigated 1, 2, 3, 5, and 7 days after its injection. Pieces of pancreas for electron microscopy were fixed in 3% gluteraldehyde solution and then postfixed in Millonig's mixture at pH 7.4, dehydrated in alcohols of increasing concentration, and embedded in Durcupan. Sections stained by Reynolds' method were examined in the UÉMV-100K electron microscope.

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