SECRETION IN THE B-CELLS OF ISLETS OF LANGERHANS AS DEMONSTRATED BY ZINC STAINING

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Detailed electron microscopic investigations on the islets of Langerhans of the pancreas reveal the following ultrastructure of the islet cells:

In most animal species (as in the rat), the A-cells are located in the periphery; the B-cells are localized in the central part of the islets. Compared to the B-cells, the number of A-cells is 30-40%. Under the electron microscope, the A-cells differ from the B-cells in that their cytoplasm contains numerous granules, but less endoplasmic reticulum, Golgi apparatus, mitochondria and ribosomes. The α granules are somewhat denser and smaller, have a narrow halo and show fewer differences in the density of the granule matrix than is the case for the B-cells.

The B-cells are mostly round to oval, have a somewhat larger and clearer nucleus than the A-cells and show peripheral chromatin densifications. It is assumed that the B-cells are present in two different forms: in the granular stage which has already often been observed (in this stage, the β-granules are the characteristic feature; fig. 1), and an agranular stage during which insulin synthesis takes place (fig. 2). The granular stage is characterized by a clearer cytoplasmic matrix, fewer endoplasmic reticula, few ribosomes and many β-granules. The granules are dense and surrounded by a membrane. Between the granules and membrane, there is a halo of varying width. On the other hand, the agranular B-cell is characterized by many extracellular spaces, Golgi bodies, mitochondria and ribosomes. In addition, its cytoplasm is relatively free from β-granules.

The biological role of zinc in the B-cells is not yet unequivocally elucidated. It is assumed that a complex between insulin and the zinc of the granules lowers

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the solubility of the storage form of insulin. It can be assumed that with raised glucose metabolism, substances (inorganic phosphate, oxalo-acetate, histidine, cystine, glutathione) are released which in turn dissolve out the zinc from the less soluble insulin-zinc complex, thus enabling insulin secretion. This process is dependent on the zinc concentration. LOGOTHETOPOULOS et al. were also able to show that zinc and insulin are present not only as a complex in the granules of the B-cells, but that in addition zinc holds the granules together by intramolecular forces. On the other hand, it appears to have been established that zinc does not play a crucial role in insulin synthesis.

Since it has become possible to make the granules visible under the light microscope as well as under the electron microscope by showing up the zinc in the B-cells of the islets of Langerhans, the fluctuations in zinc content under different experimental conditions and in different animal species have been the subject of heated discussion. This was based partly on the uncertainty as to whether the staining of the black granules and the visualization were really a silvering effect of the zinc and not of another metal. However, investigations by WEITZEL et al., and BERGLUND and HELLMAN clearly show that the argyrophilic granules in the islet cells are unequivocally zinc-insulin compounds. The most usual histochemical visualizations are based on staining methods with dithizone, magnesium dithizonate and silver sulfide.

Despite detailed histochemical and cytological investigations, the mechanism of stimulation of the B-cells has not yet been clarified. It is speculated that the zinc of the islet cells also plays a significant role here. Thus YOSHINAGA and YAMAMOTO showed that zinc forms a labile complex with sulfonylureas. This enables insulin in the islets to lose temporarily its firm bonding to zinc, thus making the way free for insulin secretion.

The present study was performed with the objective of carrying out electron microscopic comparisons on B-cells of the islets of Langerhans of normal and sulfonylurea-treated rats by means of vital fixation and a modified silver sulfide method. The zinc of the zinc-insulin complex shown up in the granules served as an indicator of the insulin content of the B-cells. The unequal density and the number of \( \beta \)-granules thus permit inferences as to the insulin content or insulin release.

MATERIALS AND METHODS

Normal (200-250 g) and adipose male Wistar rats with a body weight of about 400 g were used for the experiments. They were kept with full food and water ad libitum. The animals were subdivided into the following three experimental groups of 16 animals each: Group I: normal rats which were not pretreated; Group II: normal rats treated with sulfonylurea; Group III: adipose rats treated with sulfonylurea.

First of all, a vital lavage was carried out on the anesthetized rat via the abdominal aorta and the external carotid artery. It was carried out with carbogen-saturated Krebs-Ringer-Bicarbonate (KRB) buffer (pH 7.4) to which Liquemin and Trasylol were added. This buffer (4 × 20 ml) was injected with an even working pressure of 10 ml/min. After 2 min, the external carotid artery was opened to balance the pressure.