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Short Communication

Ultrastructural Responses of Pancreatic β Cells to Metabolic Alkalosis

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Summary. The ultrastructural changes in pancreatic β cells were studied following glucose-induced insulin secretion in vitro, at two different extracellular pH (7.4 and 7.8). The pancreata perfused at pH 7.4 exhibited a biphasic insulin response to glucose challenge together with signs of increased emiocytotic activity and numerous microtubules in the β cells. Conversely, the pancreata perfused at pH 7.8 showed a significant decrease in insulin secretion, and their β cells revealed scarce emiocytotic images and a marked increase of intracellular granulolysis. These results represent the ultrastructural correlate of the reduced insulin secretion produced by metabolic alkalosis in the perfused rat pancreas.

Key words: Insulin secretion – Acid-base changes – Electron microscopy – Pancreas perfusion.

Secretion of insulin in the perfused rat pancreas is markedly decreased at pH 7.8 despite the administration of different stimulatory agents (Rebolledo et al., 1978). Therefore, the final step in the process of hormone release would seem to be affected by this extracellular pH. Insulin granules are released mainly by emiocytosis (Lacy et al., 1968). These granules appear to be transported across the cytoplasm by the action of a contractile protein, tubulin (Lacy et al., 1972). In islets, like in other tissues, the tubulin is present in two different forms, depolymerized and polymerized (Pipeleers et al., 1977). The latter represents the active form, identifyable as microtubules (Pipeleers et al., 1976). Electron microscopic studies

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were undertaken in rat pancreata perfused with pH 7.4 and pH 7.8 buffers, in order to detect possible structural correlates.

Materials and Methods

All pancreas samples were obtained 20 min after a glucose 16.6 mM stimulus had started, i.e., 40 min after the beginning of the perfusion. They were fixed for 3 h in a phosphate buffered p-formaldehyde-glutaraldehyde solution (Karnovsky, 1965) at room temperature, postfixed in 1% phosphate buffered osmium tetroxide, and embedded in araldite. Ultrathin sections were mounted on copper grids, stained with uranyl acetate and lead citrate and examined with a Siemens Elmiskop I electron microscope. Four pancreata perfused at pH 7.4 and four at pH 7.8 were used. No less than 10 islets per animal were observed and more than 15 β cells in each islet were studied with different magnifications ranging from $\times 3{,}000$ to $\times 40{,}000$.

For cytochemical studies, the pancreata were fixed for 1 h in 2% (v/v) glutaraldehyde in 0.1 M cacodylate buffer with 34 g/l sucrose, pH 7.4. Determination of acid phosphatase (Ac Pase) activity was performed according to the method of Gomori (1956) as modified by Barka and Anderson (1962). Incubations were done at 37° C for 2 h. Control batches were incubated in a substrate-free medium. After incubation, the tissues were washed several times in the corresponding buffers and postfixed for 1 h in 1% (v/v) osmium tetroxide in 0.1 M cacodylate buffer pH 7.4. All the tissues were dehydrated and processed for electron microscopy as described above.

Results and Discussion

Clear ultrastructural differences were found between the pancreatic β cells of the two groups. Morphological evidence of increased secretory activity was present in most of the β cells from pancreas perfused at pH 7.4. A great number of secretory granules were at the cell periphery, many of them being released by emiocytosis (Fig. 1). Moreover, in the areas of granular extrusion, the cell surface frequently showed cytoplasmic projections or microvilli. The Golgi complex appeared frequently hypertrophic and the rest of the organelles did not show important changes. The occurrence of a large number of microtubules crossing the cytoplasm in different directions, was another common finding in this group (Fig. 2).

Conversely, β cells from pancreas perfused at pH 7.8 did not show signs of increased secretory activity (Fig. 4). Emiocytosis seldom occurred; only a few cells showed some isolated microtubules, and cytoplasmic projections were infrequent. The Golgi complex was less well developed as compared with the pH 7.4 group. Besides, under these conditions, an increased number of lysosomes were found (Fig. 3). Based on their ultrastructural features as well as their content of acid phosphatase (Fig. 4), they were functionally identified as primary, secondary and tertiary lysosomes (De Duve and Wattiaux, 1966; Meda, 1978). These images corresponded to the process of crynophagy or granulolysis.

As it was previously shown in the isolated perfused rat pancreas, glucose-induced insulin secretion is sharply diminished when the pH of the buffer is raised from 7.4 to 7.8 (Rebolledo et al., 1978). But even under the blocking effect of high pH, the β cells were still able to switch insulin secretion on and off according to the glucose concentration in the perfused buffer (Rebolledo et al., 1978). The effect of the extracellular alkalosis was also reflected at the ultrastructural level.

Clear-cut and reproducible differences between the groups were seen. They were of such magnitude that, even though no quantitative studies were performed, the

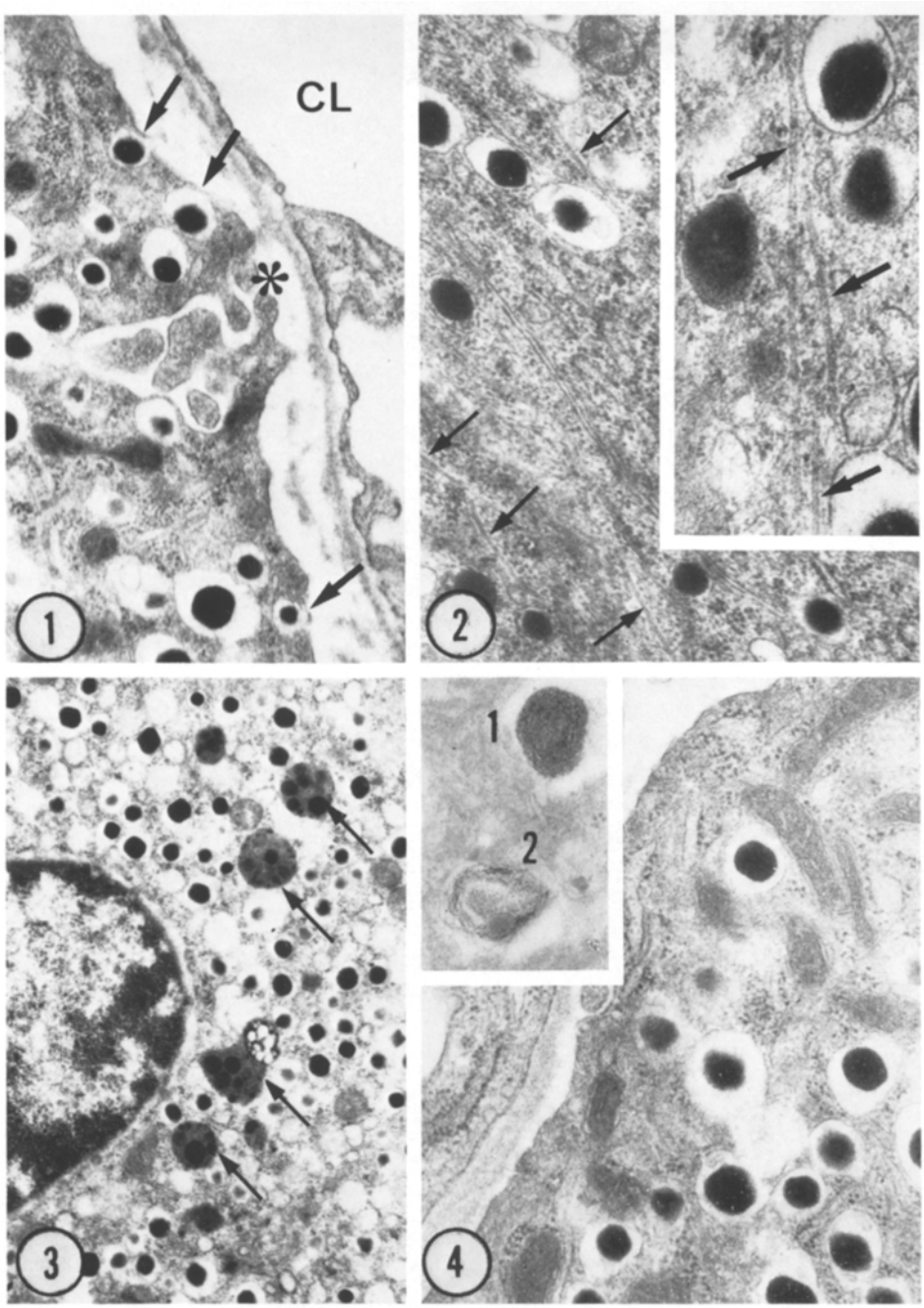

Fig. 1. β cell from pH 7.4 perfused pancreas challenged with 16.6 mM glucose. Note images of emiocytotic granule release (*arrows*) and irregular cytoplasmic projections (*asterisk*). *CL*, capillary lumen. × 18,000

Fig. 2. Microtubules (*arrows*) within cytoplasm of β cells from pH 7.4 perfused pancreas. × 20,000. *Inset:* Several microtubules (*arrows*) close to secretion granules, at higher magnification. × 48,000

Fig. 3. β cells from pH 7.8 perfused pancreas stimulated with 16.6 mM glucose. Note signs of granulolysis (*arrows*). × 10,000

Fig. 4. β cell from same group of animals as in Fig. 3. Cell border regular and secretion granules not close to plasma membrane. × 20,800. Inset: acid phosphatase in β cell (pH 7.8 group). Reaction product in a primary lysosome (*1*). A secondary lysosome (*2*) contains AcPase activity in addition to unreactive structures. × 40,000

two groups (pH 7.8 and 7.4) were easily identified under a double blind classification system.

The ultrastructural characteristics of the pH 7.8 group have been interpreted by other authors (Creutzfeldt et al., 1969; Klöppel et al., 1978) and by ourselves, as morphological evidence for an acute shutdown of insulin release. Microtubules have been suggested to effect the transport of β granules from the Golgi zone to the plasma membrane resulting in emiocytosis (Lacy et al., 1968, 1972; Malaisse-Lagae et al., 1971). The decrease in microtubules and cytoplasmic projections observed in the pH 7.8 group could be due to some interference of the extracellular alkalosis with the trigger factor for emiocytosis, or with the normal process of tubulin polymerization. The present experimental design cannot decide between these possibilities. However, these results lend support to our original suggestion (Rebolledo et al., 1978) that the mechanism hampered by extracellular alkalosis is mainly the final step in stimulus-secretion coupling.

The granulolysis phenomenon observed has been previously described both in pancreatic A (Orci et al., 1970) and B (Creutzfeldt et al., 1969; Klöppel et al., 1978; Meda, 1978; Théret, 1969) cells. Although its control remains unknown, its increase has been interpreted as a phenomenon related to modifications in hormone secretion by different means over a long period of time.

Taking into account the demonstrated capacity of β cells to recognize changes in the glucose concentration of the perfusion medium, our acute granulolysis might be interpreted as a compensatory mechanism that disposes of an excess of β cell granules that cannot undergo release (Smith and Farquhar, 1966), rather than as a final step in cellular breakdown. On the other hand, since a complete sequence of granulolysis, as demonstrated by the enzymatic studies, has been obtained in a short period, our system would represent a suitable experimental model to study the control and mechanism of this process. As a final conclusion, we believe that the ultrastructural approach, presently used, gives new insights into the mechanism of insulin release affected by high extracellular pH.

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