INTERMEDIATE CELLS OF THE PANCREAS

III. SELECTIVE AUTOPHAGY AND DESTRUCTION OF β-GRAINULES IN INTERMEDIATE CELLS OF THE RAT PANCREAS INDUCED BY ALLOXAN AND STREPTOZOTOCIN

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SUMMARY

In addition to causing necrosis of β-cells, diabetogenic doses of alloxan or streptozotocin cause selective autophagy and destruction of β-granules in intermediate cells in the rat pancreas, thus providing evidence that these granules and those in the β-cells of the islet are identical. In acinar-β cells the autophagic vacuoles also contain small mitochondria similar to those that occur in islet endocrine cells. Apart from these effects, the cells remain structurally intact. These observations suggest that alloxan exerts a direct effect on the β-components of the β-granule-containing intermediate cells.

The destruction of the β-granules in the acinar-β cell is sometimes accompanied by the appearance of α-granules in the same cells, thus presumably reflecting the increased demand for glucagon that occurs in experimental diabetes. Although intermediate cells containing α-granules are very uncommon in the normal rat pancreas, acinar-α and α-acinar cells occurred much more frequently after alloxan treatment.

The presence of intermediate cells of the acinar-β type in normal rats and the observed change of acinar-β to acinar-α cells, or from α- to α-acinar cells after alloxan, indicates that some pancreatic cells possess a variable functional potentiality. The appearance of these cells after alloxan presumably reflects the altered metabolic state of the animal, and is not a manifestation of the transformation of one cell type into another.

INTRODUCTION

The previous 2 papers in this series have provided morphological evidence for the occurrence of intermediate cells in the normal pancreas (Melmed, Benitez & Holt, 1972) and for the greater prominence of acinar-β cells in the pancreas of rats fed on a diet containing soybean trypsin inhibitor (STI) (Melmed, Turner & Holt, 1973).

We have now made use of this greater prominence of acinar-β cells in STI-fed rats to study the effects of the selective β-cytopathic agents, alloxan and streptozotocin, on these cells as well as on other β-granule-containing intermediate cells. This study was undertaken as a possible means of further characterizing such cells, of elucidating their response to the diabetes induced by these agents and perhaps to assist in elucidating the mechanism whereby these agents exert their selective toxicity on insulin-producing cells.
All materials for electron microscopy were obtained from TAAB Laboratories, 52 Kidmore End Road, Emmer Green, Reading, U.K. Alloxan was obtained from British Drug Houses Ltd. and the streptozotocin was a gift from Dr W. E. Dulin of The Upjohn Company, Kalamazoo, Michigan, U.S.A.

All studies were made on 200-250 g adult male albino Wistar rats of the Courtauld Institute inbred strain. The details of the raw soybean-containing diet have already been described (Melmed et al. 1973). The rats were fed this diet for 6-10 weeks before injection of the diabeticogenic agents, or were fed the diet immediately after injection of alloxan. The alloxan was administered subcutaneously, freshly dissolved in 0.15 M acetate buffer, pH 5.4, at a dose of 20 mg/100 g body weight, the rats having been starved for 24 h before injection. Pancreatic tissue for electron microscopy was taken at 6 and 12 h and at 2 and 3 days after the alloxan injection and at least 2 rats were killed at each time point.

Observations were also made on the pancreas of rats which had been on a control diet containing heated soybean meal before the alloxan injection but which were then fed the raw soybean-containing diet for 8 or 10 days.

The effects of an intraperitoneal dose (10 mg/100 g body weight) of streptozotocin dissolved in 0.15 M acetate buffer, pH 5.4, were studied on pancreas removed 6 h after the injection. These observations were made on rats which had been on a diet containing raw soybean for 6-10 weeks.

Acid phosphatase cytochemistry

Staining for acid phosphatase was done on pancreatic tissue which had been fixed in 3% glutaraldehyde in 67 mM cacodylate buffer, pH 7.2, at 0-4 °C. The tissue was then rinsed in the same buffer and 40-50 μm sections were cut on a Leitz freezing microtome, Model 1310, fitted with a thermoelectric freezing stage. The sections were incubated at 22 °C for 20 min in a reaction medium containing 25 mM acetate buffer, pH 5.0, 3.75 mM lead nitrate, 5 mM manganese chloride, 0.2 M sucrose and 22 mM cytidine monophosphate (Sigma London Chemical Co. Ltd.) (after Novikoff, 1963). The sections were then washed twice for 5 min in 50 mM acetate buffer, pH 5.0, and then postfixed for 1 h in 1 % osmium tetroxide buffered at pH 7.2 with 0.1 M phosphate.

Tissue blocks and cytochemically stained sections were dehydrated in graded-strength ethanols and passed via propylene oxide into Epon 812 (Luft, 1961). After polymerization at 60 °C for 12 h islets and acinar-β intermediate cell areas were located as previously described (Melmed et al. 1972) and ultrathin sections were cut from these areas for electron microscopy.

RESULTS

Alloxan effects

The administration of alloxan resulted not only in the expected necrosis of β-cells in the islets but also of the β-granule component of the acinar-β cells in the exocrine pancreas and in the much less frequent α-β and α-β, acinar cells in the islets.

Islet cells

The ultrastructural changes in the islets of the rat were similar to those observed in the rabbit after the administration of alloxan (Williamson & Lacy, 1959; Wellman, Volk & Lazarus, 1967). Widespread damage to β-cells was seen at 6 h after the injection and was more marked by 12 and 24 h. By 48 h after the alloxan injection the islets were much reduced in size and, as in the rabbit, composed almost exclusively of intact α-cells and some δ-cells. In some islets, macrophages with ingested cell debris containing recognizable β-granules were seen from 6 h after injection (Fig. 1).
Intermediate cells

Six hours after the injection of alloxan or streptozotocin, \( \beta \)-granules were present in vacuoles in the cytoplasm of acinar-\( \beta \) cells (Fig. 2). These autophagic vacuoles were distinguished from the crinophagic vacuoles previously observed in the acinar-\( \beta \) intermediate cell (Melmed et al. 1972) by the fact that the whole granule with its surrounding membrane was present in the former. By 12 h after alloxan there was apparently complete sequestration of \( \beta \)-granules in some cells (Fig. 3) although other cells showed less extensive changes. By 24 h the autophagic process had progressed, although \( \beta \)-granules could still be recognized in some of the vacuoles (Fig. 4). Other probably endocrine cytoplasmic organelles were also present in some of the autophagic vacuoles, e.g. small mitochondria (Fig. 4, inset). \( \beta \)-Granules in islet intermediate cells (Melmed et al. 1972) were also susceptible to the action of alloxan and their selective destruction and autophagy was observed in both \( \alpha-\beta \) (Fig. 4) and \( \alpha-\beta, \acinar \) (Fig. 5) intermediate cells. On the other hand, the structural integrity of the acinar or \( \alpha \)-components of intermediate cells was well preserved.

By 48 h autophagic vacuoles were numerous in acinar cells adjacent to the islets. Characterization of some of these cells as former acinar-\( \beta \) cells was not possible as the contents of the autophagic vacuoles could no longer be identified (Fig. 6). Many of these vacuoles have a characteristic elongated shape.

Granules indistinguishable from \( \alpha \)-granules were seen in some of the acinar-\( \beta \) cells from 6 h after the injection of alloxan. By 48 h, acinar cells containing \( \alpha \)-granules were frequently observed adjacent to the islets (Fig. 7). Cells of this type were more frequently seen than in the normal rat pancreas (Melmed et al. 1972). Some acinar cells in these areas also contained an abnormally large number of autophagic vacuoles, which were as numerous as those occurring in the \( \beta \)-granule-containing intermediate cells.

Cytochemical staining showed the presence of acid phosphatase from about 24 h after alloxan injection in the numerous autophagic vacuoles produced in the exocrine pancreas (Fig. 8).

No acinar-\( \beta \) cells were seen in the pancreas of rats that received an alloxan injection before being put on the diet containing raw soybean, but in specimens taken 8–10 days after the alloxan injection, acinar-\( \alpha \) cells (Fig. 9) and \( \alpha \)-acinar cells (Fig. 9, inset) were the only intermediate cells observed. The prominence of these kinds of intermediate cells was in contrast to their rare occurrence in the normal rat.

In some acinar-\( \alpha \) cells seen in alloxan-treated rats 2 types of Golgi complex appeared to be present, one characteristic of that normally found in islet \( \alpha \)-cells and a second, associated with condensing vacuoles, typical of the acinar cell, having stacked cisternae (Fig. 9).

Streptozotocin effects

The effects of streptozotocin on islet \( \beta \)-cells and acinar-\( \beta \) intermediate cells of the rat pancreas 6 h after the injection were similar in all details to those observed after alloxan injection at the same time point.
DISCUSSION

Response of β-granule-containing pancreatic intermediate cells to alloxan and streptozotocin

Further evidence for the identity in the rat of the endocrine component of the β-granule-containing pancreatic intermediate cells and those in the β-cells of the islets is provided by its selective autophagy and destruction in acinar-β, α-β and α-β,acinar intermediate cells after the administration of alloxan and streptozotocin. The cells in which this process occurs are readily distinguished from typical macrophages containing β-cell components, which appear to be largely responsible for the removal of the debris of β-cells following the administration of these agents. The remarkable specificity of the response to alloxan and streptozotocin, which leaves intact all but the β-components of intermediate cells, is the most striking consequence of the administration of these β-cytotoxic drugs. Only β-granules, with limited autophagy of other organelles, some of which are recognizable in the early stages as small mitochondria or rough endoplasmic reticulum, are included in the destructive process. It is known that acinar-β cells of some species contain other organelles typical of their β-cells in addition to β-granules. For example, small mitochondria typical of islet cells are present in acinar-β cells of the rat (Herman, Sato & Fitzgerald, 1964; Melmed et al. 1972), and together with glycogen, in those of the diabetes-prone mouse Acomys cahirinus (Orci et al. 1970). It is therefore quite possible that many of the other cytoplasmic organelles seen in the autophagic vacuoles of acinar-β cells are, in fact, β-cell organelles. Furthermore, the fact that some acinar cells, with no obvious endocrine character, adjacent to the islets also show autophagy of some cytoplasmic organelles after injection of alloxan or streptozotocin suggests that these cells may, because of this response, be part of the exocrine intermediate cell system. That almost only β-components in intermediate cells of the pancreas of rats treated with alloxan or streptozotocin appear to be destroyed by autophagy provides evidence that recognition of the altered organelles and their sequestration within autophagic vacuoles is a selective process, and that few, if any, of the organelles in the autophagic vacuoles are randomly included.

The early appearance in some of the alloxan-treated acinar-β cells of granules indistinguishable from the glucagon storage granules normally found in the α-cell of the islet is also of interest. It has been observed that in experimental diabetes in rats (Katsilambros et al. 1970) and dogs (Müller, Falloon & Unger, 1971) and in human diabetic subjects (Unger, Aguilar-Parada, Müller & Eisentraut, 1970), the normal suppression of glucagon secretion by experimentally elevated blood glucose levels does not operate and that serum glucagon levels are inappropriately high. The occurrence of zymogen granules in some islet α-cells after alloxan injection suggests that the α-cell function may be stimulated by the diabetic state to manifest a potentiality for the synthesis of zymogen not usually apparent under normal conditions. Furthermore, the presence of α-granules in the acinar-β cell after alloxan or streptozotocin injections presumably reflects the increased production of glucagon associated with the diabetic state.
Intermediate cells of pancreas. III

The preponderance of intermediate cells of the acinar-β type in normal rats (Melmed et al. 1972) and the observed change of acinar-β to acinar-α cells, or from α- to α-acinar cells after alloxan, emphasizes that some pancreatic cells possess a variable functional potentiality. The morphological appearances of these cells after alloxan seem to reflect the altered metabolic state of the animal, and not the manifestations of a process of transformation of one cell type into another (cf. Faller, 1966). The alternative possibility that the acinar-α and α-acinar intermediate cells arise through cell fusion after destruction of the β-cells is most unlikely as all these α-intermediate cells were always mononuclear. Moreover, the fact that acinar-α cells were seen as early as 6 h after alloxan excludes the possibility that a second nucleus had been lost as the result of mitotic activity or nuclear fusion as occurs in artificial hybrid cells (Harris, Watkins, Ford & Schoefl, 1966).

Mechanism of action of alloxan and streptozotocin

The precise mechanism of the selective β-cytotoxicity of alloxan and streptozotocin is not properly understood. Cooperstein, Watkins & Lazarow (1964) have shown that alloxan probably exerts its toxic effect on the β-cell by selectively interacting with certain components of the plasma membrane. This results in an altered membrane permeability which permits the diffusion of extracellular fluid markers such as D-mannitol and inulin into the β-cell and the leakage of cell protein into the surrounding incubation medium. However, the fact that only β-cell components of intermediate cells are affected by alloxan suggests that there is no comparable damage to their plasma membrane as occurs in the β-cell (Cooperstein et al. 1964), but is consistent with the possibility that alloxan interacts with the membranes of the β-cell cytoplasmic organelles. In intermediate cells this initiates the remarkably selective recognition and autophagy of these organelles. The same considerations probably apply to the effects of streptozotocin on the intermediate cells.

Whatever the correct explanation for the effects of alloxan on the rat acinar-β cell may be, whether it is administered before or after dietary STI, they result in an apparent inability of the cell to form new β-granules in spite of the demand created by the presence of diabetes and the fact that the cells otherwise appear to remain structurally intact.

We thank the Wellcome Trust for supporting this work.

REFERENCES


(Received 11 October 1972)

Fig. 1. Typical macrophage in an islet of an alloxan-treated rat showing numerous large phagocytic vacuoles (v) containing β-cell debris. × 18000.

Fig. 2. Six hours after streptozotocin, 2 small autophagic vacuoles (av) each containing an intact β-granule (b), are present in the cytoplasm of the acinar-β cell in the centre of the field. Note that the extensive acinar-type rough endoplasmic reticulum of this cell and the cytoplasmic organelles of the α-cell on the left and of the acinar cell on the right are unaffected. × 26250.
Intermediate cells of pancreas. III
Fig. 3. A large autophagic vacuole showing apparently complete sequestration of the \( \beta \)-granules in the otherwise intact cytoplasm of an acinar-\( \beta \) cell 12 h after the administration of alloxan. \( \times 14750 \).
Fig. 4. Twenty-four hours after the administration of alloxan, β-granules (b) are still recognizable in the autophagic vacuole (av) in the acinar-β cell in the lower left of the field. Other autophagic vacuoles (av') contain debris in a more advanced stage of digestion. These autophagic vacuoles and that in the inset appear to contain small mitochondria (m). The α-β intermediate cell in the top left of the field contains a compact collection of damaged β-granules (b), whereas the α-granules (a) and the rest of the cytoplasm are apparently unaffected. × 22 500. Inset, × 18 000.
Intermediate cells of pancreas. III
Fig. 5. This α−β, acinar cell shows selective autophagy (αv) of β-granules at the top of the field 24 h after the administration of alloxan. Note that neither zymogen (z) nor α-granules (α) are included in this process. Also present are a large intact acinar cell type mitochondrion (m) and several small damaged mitochondria (m') of the endocrine type, some of which contain an array of small tubular elements (m''). × 30000.
Intermediate cells of pancreas. III
Fig. 6. Forty-eight hours after alloxan administration the cytoplasm of this acinar cell, which is adjacent to an islet, contains several autophagic vacuoles (av), the contents of which can no longer be identified. \( \times 22700 \).

Fig. 7. Alpha granules (a) and autophagic vacuoles (av) are present in the cytoplasm of this acinar cell 48 h after the administration of alloxan. The papillary type rough endoplasmic reticulum (p), together with zymogen granules (z) and large mitochondria (m), are typical of acinar intermediate cells. \( \times 22700 \).
Intermediate cells of pancreas, III
Fig. 8. This shows the reaction product of acid phosphatase staining localized in autophagic vacuoles (av) 48 h after alloxan administration. The condensing vacuoles (cv) in the lower half of the field also contain small amounts of the reaction product. × 27700.
Intermediate cells of pancreas. III
Fig. 9. Only acinar-α and α-acinar (inset) intermediate cells are observed in the pancreas of rats fed STI for 8–10 days after the administration of a diabetogenic dose of alloxan. In Fig. 9, in addition to α-granules (a) and zymogen granules (c), the cell contains both exocrine (g) and endocrine (g') types of Golgi complex on either side of an intracellular membranous partition (mp). ×30000. The inset shows a typical α-acinar cell in the islet containing both α-granules (a) and zymogen granules (c). ×10400.