Comparison of the Effect of Diabetogenic Agents on the Microdissected Pancreatic Islet Tissue of the Rat[1] (36687)

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Rakieten, Rakieten, and Nadkarni (1) first reported that streptozotocin induced frank diabetes in rats and dogs. Streptozotocin has also been shown to have a cytotoxic action on the pancreatic B-cells of mice and guinea pigs (2), rabbits (3), rhesus monkeys (4), and on functioning islet cell carcinoma of humans (5). The earliest effect on the B-cells of rat islets of Langerhans was noticeable about 1 hr following an intravenous injection of streptozotocin. After 7 hr a massive necrosis of the B-cells accompanied by cellular disintegration and complete caryolysis was observed (2). Similar observations on rats were also made recently by Hoftiezer (6).

In rats, the administration of streptozotocin did not appear to affect all the B-cells simultaneously. Insulin release occurred between 6 and 10 hr after the drug administration only from the damaged B-cells resulting in their degranulation (7).

It was reported earlier from this laboratory (8) that following alloxan administration, the insulin content of the microdissected islet tissue was not affected up to 24 hr, but after 48 hr the hormone content of the islet tissue was decreased to approximately 4% of the normal value. Morgan and Lazarow (9) confirmed these observations. These workers reported that 24 hr after alloxan injection, the amount of extractable insulin from the rat pancreas was approximately the same as that of the untreated controls. The above studies thus clearly indicated that the cytotoxic action of the two diabetogenic agents, alloxan and streptozotocin, was different.

In the present study, we have determined the insulin content and peptidase enzyme activity of the islet tissue of rats at various times following streptozotocin injection and compared these values with those reported for corresponding tissues obtained from alloxan-injected animals.

Materials and methods. Six-week old male Holtzman rats, weighing approximately 180 g were fasted overnight and injected with a dose of streptozotocin (65 mg per kilogram body weight) into the tail vein. The animals were sacrified by decapitation 8, 12, 24, 48, 72, and 168 hours after the injection. Blood was collected in heparinized tubes for blood-sugar determination by a modification of the method of Hoffman (10) using the Auto Analyzer. Plasma insulin was determined by the two antibody method described by Morgan and Lazarow (11).

The animals were opened up, the pancreas was quickly excised, and a portion was immersed in liquid nitrogen (—196°). The frozen pancreas was mounted on metal blocks and sectioned at —20°; the frozen sections (20 μm thick) were lyophilized at —35° and stored in vacuum jars at —20°, as described by Lowry (12). The technic of microdissection of the pancreatic islets and their weighing on the quartz fiber balance has been described earlier (13). The insulin content of the islet was determined on a pooled sample of 15–20 microdissected islets obtained from each rat, using a fat pad assay technic as described earlier (14).

The peptidase activity of the microdissect-

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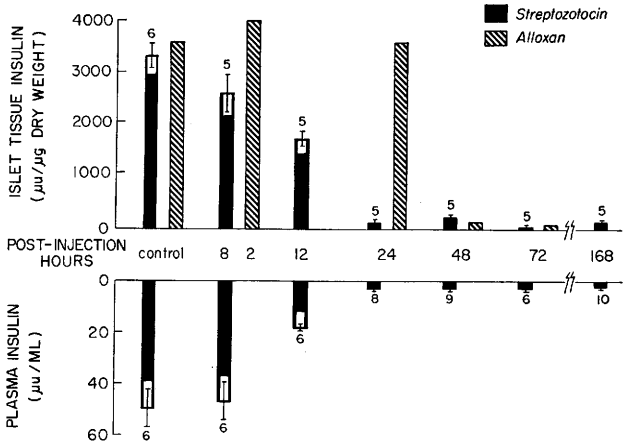

FIG. 1. Insulin content of microdissected rat islet tissue and plasma at various time periods following the administration of streptozotocin. Bars and numbers represent the mean value and the number of animals in the group, respectively. The vertical lines represent the standard error of the mean.

ed islet tissue was determined using the fluorometric method of Robins and Lowe (15), using glycyl-1-phenylalanine as a substrate. The free phenylalanine produced by the enzyme action was reacted with ninhydrin and the fluorescence was measured (16).

Another portion of the pancreas was fixed in Bouin's solution. The tissues, embedded in paraffin, were sectioned at 5 μm, and stained with a modification of aldehyde fuchsin–Ponceau method (17) for insulin granules.

Results and discussion. Insulin content of the rat islet following streptozotocin injection. The insulin content of the microdissected islets of streptozotocin treated rats was decreased to 80% of the control value by 8 hr and to 53% by 12 hr ($p = < .001$). At the end of 24 hr following the administration of streptozotocin, the islet insulin content was only about 4% of the control value (Fig. 1).

Histology. The effect of administering streptozotocin on the aldehyde–fuchsin staining of the B-granules, in general, confirmed the insulin assay results. The normal control rat islets appeared to contain a very large number of cells with full complement of aldehyde–fuchsin stained granules in the cytoplasm. Eight hr following streptozotocin administration, no marked decrease in the staining of the B-cell was discerned. This observation is in conflict with the observations of Junod and coworkers (2), who reported a massive necrosis and disintegration of B-cells within 7 hr; the surviving B-cells were largely degranulated. We noted that 12 hr after streptozotocin a large number of islets exhibited an uneven distribution of the granular material in the B-cells. In some islets, a few B-cells contained very little granular material, while in other islets approximately two-thirds of the cells contained less intensely stained but definite insulin granules. In general, a majority of the islets obtained 12 hr after streptozotocin injection exhibited decreased amounts of beta granulation. After 24 hr, the B-cells of all the islets examined were practically degranulated. An occasional cell contained very lightly stained material in the cytoplasm. At later time periods, the histologic picture was as described by others (2, 6, 18). The islets consisted of alpha cells and few hypertrophied B-cells (19).

The plasma insulin levels of the rats appeared to mimic the islet insulin content (Fig. 1). Eight hr following administration of the drug the plasma insulin levels were unchanged, but 12 and 24 hr thereafter the values were significantly decreased ($p = < .001$) to about 37 and 6% of that of the controls, respectively.

In an earlier communication (8) we had

reported that the insulin content of the microdissected rat islets 24 hr following alloxan administration was unchanged, despite highly elevated blood sugar levels. At that time, we had not determined the insulin content of the islets in the interim periods, namely at 8 and 12 hr. We had, however, found that the insulin content of the islets was slightly, though not significantly higher (approximately 113% of the normals), 2 hr after alloxan treatment. At 48 and 72 hr after administration of the drug the islet insulin levels dropped to less than 5% of the normal values. Morgan and Lazarow (9), in a later study, confirmed these findings using a two-antibody immunoassay technique for insulin determination in rat pancreatic extracts. In order to explain the high insulin content in the rat islets at 24 hr after alloxan administration even though hyperglycemia persisted, we suggested that perhaps the drug affected the mechanism of release or secretion of the hormone from the damaged B-cell. The sharp fall in the insulin content of the islet tissue 48 hr after alloxan administration was attributed to insulin destruction within the cell as a result of the release of lysosomal enzymes during autolysis. In a later study (20), we reported a significant increase in the islet peptidase activity between 24 and 48 hr after alloxan injection. The close correspondence between the disappearance of islet insulin and the rise in the peptidase activity of the islet tissue following alloxan, prompted a similar study in the streptozotocin-injected rats.

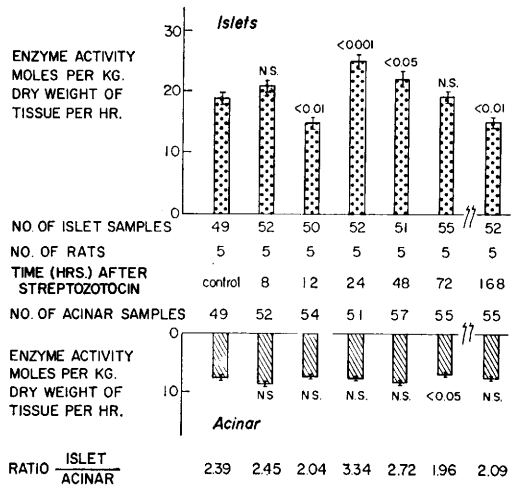

FIG. 2. Effect of streptozotocin administration on the dipeptidase (PEPT) activity of microdissected rat islet and acinar tissue. The number on the top of each bar denotes the p value.

Peptidase (PEPT), activity of the islet tissue following streptozotocin administration. The peptidase activity of the islet and acinar tissue at different time periods following streptozotocin injection is depicted in Fig. 2. The peptidase activity of the islet tissue appears to be greatly influenced by the drug administration. The islet peptidase activity was not affected 8 hr following the drug injection, but after 12 hr the enzyme activity was significantly decreased to about 79% of that of the controls. At 24 hr the islet enzyme activity was markedly elevated to 137% of that of the control value. Even at 48 hr the islet PEPT activity remained appreciably high, although there was a gradual downward trend as may be seen from the enzyme levels noted 72 and 168 hr after streptozotocin administration. In contrast to that of the islets, the peptidase activity of the acinar tissue did not exhibit significant changes except at 72 hr, clearly indicating that streptozotocin, like alloxan, specifically affected the endocrine tissue of the pancreas.

In Fig. 3, we have compared the effect of alloxan and streptozotocin on the islet peptidase activity. It is clearly evident from the data presented that the two cytotoxic drugs affect the islet tissue in a somewhat similar manner. Initially the drugs produced a decrease in the peptidase activity of the tissue which was followed by a sudden rise at a specific time period. The major difference in the activity of the two drugs was the time of the rise in the peptidase activity after administration of the drug. In the streptozotocin-injected animals this occurred after 24 hr while in the alloxan-injected animals after 48 hr. This time relationship coincided with the loss of the insulin within the islet tissue after the administration of the respective drugs. It is tempting to speculate that perhaps streptozotocin causes a more rapid disruption of the metabolism of B-cells with the consequent release of the lysosomal enzyme.

It seems unlikely that the above observa-

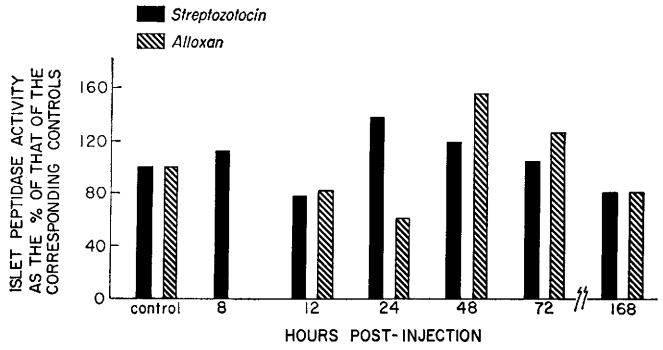

FIG. 3. Comparison of the rat islet peptidase activity at various times following the administration of the diabetogenic agents.

tions could be considered entirely fortuitous. However, it should be pointed out that the presence of the enzyme associated with the breakdown of glycyl-l-phenylalanine may not necessarily indicate the presence of a "true" peptidase in the tissue. It should also be realized that the alteration of the activity of any particular peptidase within the tissue need not necessarily reflect a commensurate change in the over all proteolytic activity of the cell or the extent of the cell degeneration. It is also possible that the enzyme acting on the glycyl-l-phenylalanine may not necessarily be a lysosomal enzyme and that changes in an appropriate lysosomal enzyme, namely β-glucuronidase or a protease, may furnish a better criterion for lysosomal breakdown as a result of the drug administration. Our choice of glycyl-l-phenylalanine enzyme activity was dictated by the availability of a simple and sensitive ultramicromethod.

The fact that following the administration of streptozotocin or alloxan the rat islet insulin content was in the normal range for 8 or 24 hr respectively, needed explaining. It was pointed out earlier (8) that this may be attributed to the deleterious effect of alloxan on the secretory mechanism of the damaged B-cells. Secretion is a function of a viable cell and requires energy. Perhaps, despite the elevated blood sugar levels, the dying B-cell is unable to respond to the hyperglycemic stimulus. The hypothesis was tested by carrying out the following experiment.

Effect of glucose load on the plasma insulin levels in animals treated with the diabetogenic agents. In this experiment, the rats were fasted overnight and bled under light ether anesthesia from the retro-orbital vein plexus (21), for the determination of basal values of blood glucose and insulin levels. The animals were then injected with saline, alloxan, or streptozotocin by the tail vein as described above. One hour later, the animals were given a glucose load (500 mg per rat) intraperitoneally and were bled again after 20 min.

As shown in Fig. 4, there was a significant increase in the plasma insulin levels over the basal values in the saline-injected animals following the glucose load. No such change was observed in the plasma insulin levels of the animals treated with the two diabetogenic agents, although the blood sugar levels were markedly elevated. This experiment clearly indicated that both diabetogenic agents produced certain alterations in the islet B-cells which in some unknown manner precluded their responding to the hyperglycemia brought about by glucose injection. These observations are in agreement with those of Creutzfeldt *et al.* (22) who reported that one hour after the injection of streptozotocin the islet B-cells failed to secrete insulin *in vitro* medium in response to stimulation by glucose.

There is considerable evidence to indicate that alloxan acts by altering the permeability of the B-cell membrane (23, 24). This in turn may block the entrance of sugar in the B-cell and thus block the mechanism of insulin release. It is possible that streptozotocin may likewise affect the permeability of the B-cells.

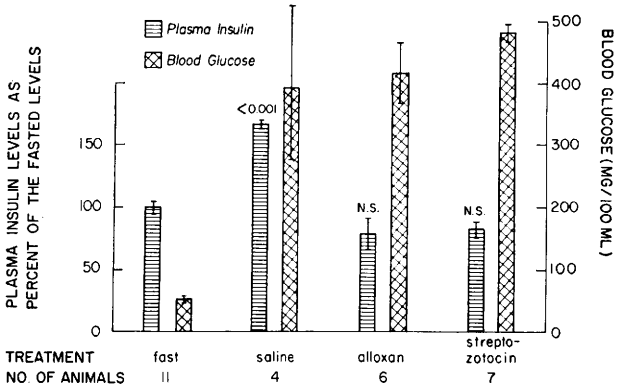

FIG. 4. Effect of glucose load on the plasma insulin levels of alloxan and streptozotocin-treated rats.

Summary. Insulin content and peptidase activity were determined in microdissected rat islet tissue at various intervals following a single diabetogenic dose of streptozotocin. The islet insulin content was decreased to approximately 53% of the normal control value at 12 hr and to 4% at 24 hr after the administration of the drug. The plasma insulin levels in general paralleled the islet insulin content.

Islet peptidase activity showed significant changes following streptozotocin administration. After 12 hr the enzyme activity was considerably decreased, but after 24 hr there was a sharp increase in the peptidase activity. When the values for insulin content and peptidase activity of islet tissue obtained from rats made diabetic with alloxan and streptozotocin were compared, it was evident that the proteolytic enzyme was elevated at about the same time that islet insulin had completely disappeared.

When either drug was injected in the rats and an hour later a load of glucose was administered intraperitoneally, the plasma insulin levels were not altered. By contrast, the saline-injected animals responded to glucose administration with a significant elevation of plasma insulin. This indicated that the two diabetogenic agents affected the sensitivity of the B-cells to hyperglycemia. Since alloxan is reported to alter the permeability of B-cell membrane, it is concluded that streptozotocin may also affect the B-cell permeability.

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