The Effect of Glucose and Tolbutamide on Immunoreactive Somatostatin Release from Perfused Pancreatic Islets of Normal and Streptozotocin Diabetic Rats

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Abstract

The patterns of immunoreactive somatostatin (IRS) responses to glucose and tolbutamide were investigated in perfused pancreatic islets of both normal and diabetic rats.

The incubation chamber of the perfusion apparatus for the islets was devised with a 2.5 ml disposable syringe. By the method of Lacy, the pancreatic islets were isolated from diabetic rats about 30 days after a single intravenous injection of streptozotocin (STZ, 60 mg/kg body weight) and from normal rats of comparable body weight. The fasting blood glucose was 437 ± 3 mg/100 ml in the diabetic rats and 120 ± 5 mg/100 ml in normal ones. The concentration of IRS in each effluent was measured by the specific double antibody radioimmunoassay for somatostatin.

During the initial 20 min incubation period with the medium containing 2.8 mM glucose, the levels of IRS were 11.3 ± 1.0 pg/100 islets/2 min in normal islets and 32.0 ± 3.4 pg/100 islets/2 min in diabetic islets. The latter value was significantly higher than the former ($p < 0.001$). IRS levels gradually rose following 16.7 mM glucose and reached the maximal values with 30.2 ± 5.7 pg/100 islets/2 min at 66 min in normal islets and with 56.8 ± 13.3 pg/100 islets/2 min at 88 min in diabetic islets. The levels of IRS in diabetic islets were significantly higher than those of normal islets ($p < 0.001$) throughout the experimental period. In the case of tolbutamide the IRS levels increased promptly to 51.9 ± 6.6 pg/100 islets/2 min in diabetic islets and to 23.7 ± 4.6 pg/100 islets/2 min in normal islets 2 min after initiation of the infusion. The former value was significantly higher than the latter ($p < 0.001$). The levels of immunoreactive glucagon (IRG) in response to tolbutamide were significantly higher in the diabetic islets than in normal islets ($p < 0.001$). These results indicate that there exists a hypersecretion of IRS from STZ-induced diabetic rats during the infusion with glucose and tolbutamide.

It is well established that immunoreactive somatostatin (IRS) is distributed widely in the extrahypothalamic tissues and organs including the pancreas and gastrointestinal tracts (Arimura *et al.*, 1975; Hök-

felt *et al.*, 1975). Immunohistochemical studies clearly demonstrated that IRS existed in D-cells of the islets of Langerhans (Dubois, 1975). Exogenously administered somatostatin inhibits the release of insulin (IRI) (Alberti *et al.*, 1973) and glucagon (IRG) (Koerker *et al.*, 1974). On the other hand, glucose (Schauder *et al.*, 1976, 1977; Ipp *et al.*, 1977a) amino acids (Ipp *et al.*,

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Fig. 1-(A). Incubation chamber. The components of units are designated as follows: (a) barrel of 2.5-ml disposable syringe; (b) connector of 18-gauge scalp vein set; (c) plunger of 2.5-ml disposable syringe; (d) rubber gasket; (e) Tygon tubing (i.d. 0.025 in.); (f) 18-gauge steel needle; (g) islets; (h) glass filter paper (Whatman GF/A); (*) It was necessary to cut away part of the plunger base to allow insertion of the needle with the attached tubing.

1977a), tolbutamide (Ipp *et al.*, 1977a) and gastrointestinal hormones (Ipp *et al.*, 1977a, b) have been reported to stimulate the release of endogenous IRS from isolated perfused pancreas, or from isolated perfused pancreatic islets. These observations led to a postulation that IRS in D-cells of the islets may play a physiological role on the regulation of pancreatic endocrine functions (Unger, 1977).

In the islets of diabetic rats and humans, the hypertrophy and hyperplasia of D-cells are observed (Orci *et al.*, 1976). These morphological changes are associated with an increase in somatostatin content in the D-cells (Patel and Weir, 1976). The secretory dynamics of somatostatin from the D-cells of diabetic islets are not fully understood. In the present study, we investigated

the responses of IRS as well as IRI and IRG in the islets of diabetic rats to glucose and tolbutamide using the *in vitro* perfusion apparatus for the pancreatic islets which had been developed in our laboratory.

Materials and Methods

Male Wistar rats weighing 250–300 g were used in this study. Rats with diabetes induced by a single intravenous injection of STZ (60 mg/kg body weight) were kept on a normal diet and water *ad lib.* for 30–40 days. The rats were anesthetized by Nembutal (50 mg/kg) and their peripheral blood was drawn from the jugular vein. Blood glucose level was determined by the glucose oxidase method using the Blood Sugar-GOD-Perid-Test (Boehringer-Mannheim Co. Ltd.). Pancreatic islets were isolated by the method of Lacy *et al.* (1967, 1972) using collagenase (Type IV, Worthington Biochemical Co., Freehold, New Jersey) at a dose of 25 mg per pancreas.

The perfusion apparatus for rat adenohypophysis (Sawano *et al.*, 1977) was modified as illustrated in Fig. 1-(A). Two chambers, one for normal islets (control group) and the other for diabetic islets (diabetic group), were composed of 2.5 ml disposable syringes (Termo). This system was devised for comparing simultaneously the dynamics of IRS release from diabetic islets and from normal islets. One hundred and fifty to two hundred islets from normal and STZ diabetic rats were placed in each incubation chamber of which the volume was adjusted to 0.1 ml. The medium used was Krebs-Ringer-bicarbonate buffer containing 0.1% human serum albumin (Worthington Biochemical Co., Freehold, New Jersey) and 2.8 mM glucose at pH 7.4 and was equilibrated with 95% O₂-5% CO₂ mixture before use. As illustrated in Fig. 1-(B), two incubation chambers, one medium reservoir and one sample chamber were all immersed in a water bath at 37°C. The medium was pumped from the reservoir through the incubation chamber by a two-channel peristaltic pump. Preincubation was carried out at a rate of 0.5 ml/min for 90 min, and then the effluent from each incubation chamber was collected every two min (1.0 ml per fraction) in glass test tubes immersed in ice-cold water on the fraction collectors. The following materials were dissolved in the medium saturated with 95% O₂-5% CO₂ immediately before use, and infused into the system by means of the three-way stopcocks: the final concentrations of glucose and tolbutamide (Hoechst Japan, Ltd.) were adjusted to 16.7 mM and 400 µg/ml, respectively.

Assay of IRS in the effluent was performed by radioimmunoassay (RIA) for somatostatin using anti-somatostatin serum (R501, Sawano *et al.*, 1978; Ku-

Fig. 1-(E). Diagram of the perfusion apparatus. (a) 18-gauge steel needle; (b) Tygon tubing (i.d. 0.025 in.); (c) connector (the tip of 1-ml disposable syringe barrel); (d) 18-gauge steel needle; (e) ice-cold water.

masaka et al., 1979). IRI was measured by the double antibody method using Dinabot Kits (Dinabot Radioisotope Lab., Tokyo), and IRG by the dextran coated charcoal method using 30K antiserum (Unger). The amount of hormone released was expressed as pg/100 islets/2 min for IRS and IRG, and μ U/100 islets/2 min for IRI. The results were statistically analyzed by Duncan's new multiple range test or Student's *t*-test.

Results

Blood glucose levels.

The blood glucose level in STZ-treated rats was 437 ± 3 mg/100 ml (Mean $\pm$ S.E., $N = 8$), which was significantly higher than that in control rats (120 ± 5 mg/100 ml, $N = 8$, $p < 0.001$).

Displacement curve of effluent in somatostatin RIA.

Fig. 2 shows the displacement curve of the effluent in the somatostatin RIA system. Serial dilutions of the effluent from the peri-

fusion system were proportional and their dilution slopes paralleled those of synthetic somatostatin.

IRS, IRI and IRG released by glucose.

During the initial 20-min incubation period with 2.8 mM glucose, the levels of IRS were 32.0 ± 3.4 pg/100 islets/2 min in the diabetic group and 11.3 ± 1.0 pg/100 islets/2 min in the control group. The former value was significantly higher than the latter ($p < 0.001$). A significant elevation of IRS levels was observed 56 min after the initiation of the perfusion with 16.7 mM glucose in the diabetic group. Such a delayed response of IRS was also noticed in the control group. Then, the levels further rose and reached the maximal values with 56.8 ± 13.3 pg/100 islets/2 min at 88 min in the diabetic group and with 30.2 ± 5.7 pg/100 islets/2 min at 66 min in the control group. Throughout the perfusion periods with 16.7 mM glucose, the levels of IRS in

Fig. 2. The dilution curve of effluent in the somatostatin radioimmunoassay.

Fig. 3. The effect of 16.7mM glucose on the responses of IRS, IRI and IRG in perfused rat islets *in vitro*. In one experiment, two chambers, one for normal and the other for diabetic islets, were simultaneously perfused with medium containing 2.8 mM glucose from -20 to 0 min and from 90 to 120 min. Glucose at the dose of 16.7 mM was administered from 0 to 90 min. Each point and vertical bar indicates the mean $\pm$ S.E. of four observations. N represents the response of normal islets and S represents the response of streptozotocin-induced diabetic islets. Each asterisk indicates the significance of the difference at 5% level from any of the base line values obtained from -20 to 0 min.

the diabetic group were significantly higher than those of the control group ($p < 0.001$). When the glucose concentration in the medium was decreased to 2.8 mM IRS levels rose transiently in both groups (Fig 3. lower panel).

The biphasic IRI release was observed with 16.7 mM glucose in the control group, while the secretion of IRI was negligible in the diabetic group (Fig 3. middle panel). On the other hand, IRG release was suppressed in both groups during the perfusion with 16.7 mM glucose, and the value returned to each baseline level when the glucose concentration of the medium was decreased from 16.7 mM to 2.8 mM (Fig 3. upper panel).

IRS, IRI and IRG release by tolbutamide.

As shown in the lower panel of Fig. 4, the perfusion with 400 $\mu\text{g/ml}$ of tolbutamide caused an abrupt IRS release in both groups. The patterns of IRS response to tolbutamide were similar to each other, although the absolute value at each observation period was higher in the diabetic group than in the normal one. The maximal level of

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Fig. 4. The effect of 400 $\mu\text{g/ml}$ of tolbutamide on the response of IRS, IRI and IRG in perfused rat islets *in vitro*. In one experiment, two chambers, one for normal islets and the other for diabetic islets, were perfused simultaneously with medium containing 2.8 mM glucose and infused from -20 to 0 min and from 40 to 50 min. Tolbutamide at a concentration of 400 $\mu\text{g/ml}$ was prepared with the medium containing 2.8 mM glucose and infused from 0 to 40 min. Each point and vertical bar indicates the mean $\pm$ S.E. of four observations. N represents the response of normal islets and S represents the response of streptozotocin diabetic islets. Each asterisk indicates the significance of the difference at 5% level from any of the base line values obtained from -20 to 0 min.

IRS during the stimulation was 59.8 ± 3.7 pg/100 islets/2 min in the diabetic group and 24.0 ± 3.7 pg/100 islets/2 min in the control group. The former was significantly higher than the latter ($p < 0.001$).

Tolbutamide stimulated a rapid IRI secretion in the control group but not in the diabetic group. IRI response to tolbutamide was more marked in the diabetic group than in the normal one.

Discussion

IRI has been located in the D-cells in the pancreas and gastrointestinal tracts (Hökfelt *et al.*, 1975; Dubois, 1975). Considering such localization of somatostatin and its pharmacological action in inhibiting pancreatic and gastrointestinal hormones, it is strongly felt that somatostatin regulates the hormone release from the adjacent endocrine cells as a local hormone and plays an important physiological role on the glucose and nutrient homeostasis. In order to prove this theory, many investigations have focused on the relationship among A-, B- and D-cells in the pancreatic islets which is postulated as the "paracrine" hypothesis (Unger, 1977). According to the "paracrine" hypothesis, one of the three secretory products of these three cell types influences the hormonal secretion from its neighboring cells. The abnormal A-, B- and D-cell functions could explain many abnormal pancreatic endocrine states seen in diabetes (Unger and Orci, 1977). However secretory dynamics of somatostatin from D-cells of diabetic islets remains obscure.

In the present study, we found out that basal levels of IRS in the effluent of diabetic islets were three times as high as those of normal ones and that IRS responded remarkably to the high concentration of glucose and tolbutamide. These results would reflect the hypertrophy and hyperplasia of D-cells observed in the dia-

betic rats (Orci *et al.*, 1976). Such pathological changes in D-cells in the islets might be caused by the prolonged hyperglycemic stimulation secondary to diabetes or by the direct effect of STZ on D-cells.

In our perfusion system IRS was increased 58 min after exposure to the high concentration of glucose in normal islets. This delayed response was different from the results reported by Ipp *et al.* (1977) and Schauder *et al.* (1977), who found an immediate elevation of IRS levels after the high concentration of glucose. On the other hand, Barden *et al.* (1976) and Weir *et al.* (1979) did not observe any increase in IRS release following 300 mg/100 ml glucose. The different assay system and the different dose of glucose utilized might account for such discrepancies. The delayed response of IRS to high concentration of glucose was similarly observed in the diabetic group on our study. The significance of such responses to glucose in normal and diabetic islets remains unclear.

It is of interest to note that despite an increase in somatostatin levels in the diabetic islets, glucagon release was twice as high as the normal level. If somatostatin had inhibited glucagon secretion as a local hormone, the glucagon level would be lower in the effluent of the diabetic group than in the control group. Otherwise, D-cells would secrete a large amount of somatostatin in order to restore excessive glucagon secretion in diabetes mellitus as described by Buchanan *et al.* (1973) and Weir *et al.* (1976).

In the present study, both somatostatin and glucagon were released transiently from islets in response to an abrupt fall in the glucose concentration from 16.7 mM to 2.8 mM. A possible role of this somatostatin secretion may be related to the prevention of endogenous insulin overshoot in order to avoid further hypoglycemia. It could be also explained by the fact that increased glucagon release in response to hypogly-

cemia (Patton *et al.*, 1977; Unger *et al.*, 1962) had a stimulatory effect on D-cells within the islets. This may cause a transient increase in somatostatin secretion, since a high concentration of exogenous and presumably endogenous glucagon (Patton *et al.*, 1976) stimulated the release of somatostatin from rat pancreas.

Also noteworthy is the present finding that the D-cells in STZ diabetic islets which secreted little endogenous insulin responded in a normal fashion to glucose and tolbutamide. In other words, glucose- and tolbutamide-induced endogenous insulin did not exert a stimulatory action on somatostatin release in normal islets through the "paracrine" mechanism. Patton *et al.* (1977) showed that exogenous insulin did not augment somatostatin release from dog pancreas.

In the present study we showed the secretory patterns of somatostatin, glucagon and insulin responses to glucose and tolbutamide in normal and diabetic islets. However, it was not possible to obtain direct evidence indicating the possible interaction of the secretion of these three hormones. Further investigation will be necessary to elucidate the "paracrine" regulation of islet hormone secretion.

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