Immunohistochemical Changes of Somatostatin Cells in the Pancreatic Islets of Rats after Streptozotocin Administration

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Synopsis

By the enzyme-labeled antibody method, cells containing somatostatin (SRIF) as well as insulin or glucagon were identified in pancreatic islets of the rat. SRIF antiserum was raised in rabbits following immunization with synthetic SRIF coupled with human serum alpha-globulin and did not cross-react with hypothalamic, pituitary or gastrointestinal hormones using our immunoassay method.

In the control rats, SRIF-containing cells were scattered in the periphery of the islets in close proximity to the outer glucagon containing cells. These latter cells were distributed in the outermost periphery of the islets. Insulin-containing cells were located in the central portion of the islets and dominantly occupied most of the islet.

In the streptozotocin-diabetic rat, SRIF-containing cells were significantly increased in number whereas insulin-containing cells were markedly reduced.

It is suggested from these findings that the number as well as the distribution of SRIF-, insulin- and glucagon-containing cells was important to the physiological and pathophysiological functioning of the islet.

Somatostatin (SRIF), cyclic tetradecapeptide, has been identified as a hypothalamic factor to inhibit the release of growth hormone from the pituitary (Brazeau et al., 1973). It has been shown that SRIF has a large extrahypothalamic distribution by radioimmunoassay measurement (Arimura et al., 1975). Furthermore, by the immunohistochemical technique SRIF-containing cells has been demonstrated in the median eminence (Pelletier et al., 1974) as well as in the D cells of the Langerhans islets of several species (Luft et al., 1974; Dubois et al., 1975). More recent data using immunohistochemical techniques suggested that streptozotocin induced diabetes could result in the hypertrophy and hyperplasia of the D cells (Orci et al., 1976).

The pathophysiological significance of these findings is yet uncertain.

On the other hand, the enzyme-labeled antibody method developed by Nakane and Pierce (1966) is as specific as an immunofluorescent method, and it is widely applied to the immunohistochemical study because it has a number of advantages. However, as for immunohistochemistry of SRIF, most investigators have been using the immunofluorescent method.

The present study was undertaken to examine the distribution of SRIF-, glucagon- and insulin-containing cells in the pancreatic islets of normal and streptozotocin-diabetic rats using the enzyme labeled antibody method.

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Materials and Methods

Male Sprague-Dawley rats weighing 200-220 g were used in the present study. Six rats were given a single injection of 65 mg/kg of streptozotocin intravenously (lot No. 60140, donated from the Upjohn International Inc., Kalamazoo, Mich., U.S.A.), and six control rats were intravenously given 0.2 ml of 0.1 M citrate buffer solution (pH4.5), which was a vehicle of streptozocin. The rats were maintained at 24°C on a schedule of 12 hr light/12 hr darkness. They were given free access to a laboratory feed (purchased from Oriental Yeast Co., Tokyo) and tap water throughout the experiment.

Four weeks following the injection, the rats were weighed and decapitated. Trunk blood was collected for glucose measurement (Technicon glucose analyzer). The pancreas was quickly removed, fixed in Bouin's solution for 2 hr, dehydrated, embedded in paraffin, serially sectioned at 5 μm and mounted on glass slides. The sections of pancreas were deparaffinized in xylene, hydrated in gradually decreasing concentrations of alcohol and washed in 0.01 M phosphate-buffered saline pH7.2 (PBS).

The enzyme labelled antibody method of Nakane and Pierce (1966) was employed in this study. The specific antibody to SRIF was raised in rabbits with synthetic SRIF (Peptide Institute, Osaka) which had been conjugated with human serum α-globulin using glutaraldehyde prior to immunization. This antiserum was checked by radioimmunoassay and proved to be specific for SRIF with no cross-reactivity to hypothalamic, pituitary or gastrointestinal hormones (Makino et al., 1977). Highly specific rabbit anti-glucagon serum was obtained following immunization with highly purified pork glucagon (Novo) which also had been conjugated with human serum α-globulin using glutaraldehyde. Guinea pig anti-insulin serum was a donation from Dr. A. Koyama. Horse radish peroxidase-labeled goat anti-rabbit or anti-guinea pig γ-globulin were purchased from Japan Immunoindustries Co., Ltd., Takasaki, Japan. Tissue sections were incubated with SRIF antiserum pretreated with human serum α-globulin diluted 1:16 for 30 min., with glucagon antiserum pretreated with human serum α-globulin diluted 1:4 for 30 min. or with insulin antiserum diluted 1:50 for 30 min. They were rinsed in PBS, incubated with peroxidase-labeled goat anti-rabbit or anti-guinea pig γ-globulin, diluted 1:10 for 15 min., rinsed in PBS and stained for peroxidase using 3,3′-diaminobenzidine (Wako Chemicals Ltd., Tokyo) as substrate as described by Graham and Karnovsky (1966). And then they were mounted by Canada Balsam (Wako Chemical Ltd., Tokyo). Control experiments were performed using the specific antiserum absorbed with the corresponding antigen, synthetic SRIF, highly purified pork glucagon or monocomponent pork insulin. Following the staining of sections for somatostatin, the number of SRIF staining cells in pancreatic islets was counted in sections of three pancreases chosen at random. The area of each islet was roughly calculated supposing the islet to be a circle. The number of SRIF staining cells was expressed per islet and per square millimeter of islet.

Results

Despite being of comparable age, the diabetic rats weighed less than the controlled rats (218±10 g vs 356±15 g, mean ±SEM, p<0.01). As for the mean fasting blood glucose level, the diabetic rats showed 207±40 mg/dl, p<0.02 compared to the control level of 81±7 mg/dl. In the control rat, the cells specifically stained with SRIF antiserum were located mainly in the periphery of the pancreatic islets (Fig. 1a) and less numerous than glucagon-containing cells, which were positively stained by glucagon antiserum and distributed in the outermost periphery of the islets as a single cell layer (Fig. 1b). The former cells were in close proximity to, but less numerous than, the latter outer periphery cell. Fig. 1c shows the endocrine pancreatic cells reacting positively with insulin antiserum. They were located mainly in the central portion of the islets and dominantly occupied most of them.

On the other hand, in the streptozotocin-diabetic rats, the SRIF-positive cells were markedly increased in number and scattered into the central portion as in the periphery (Fig. 2a). Calculating the number of SRIF-containing cells roughly, the number in diabetic rats had increased compared with that of the controls (16.2±1.5/islet vs 12.0±1.0/islet, p<0.05), and expressed on the basis of per square millimeter of islet, the number of SRIF-containing cells had also increased in diabetic rats (1441.6±112.9/mm² of islet vs 780.4±79.2/mm² of islet, p<0.001) (Table 1). The glucagon-positive cells were also abundant and occupied most
Fig. 1. Serial sections of the pancreatic islets in the control rat pancreas stained with somatostatin (a), glucagon (b) and insulin antiserum (c). (×100)

Fig. 2. Serial sections of the pancreatic islets in the diabetic rat pancreas stained with somatostatin (a), glucagon (b) and insulin antiserum (c). (×100)
Table 1. The number of SRIF-containing cells in the pancreatic islet from control and diabetic rat pancreas.

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<thead>
<tr>
<th>Control rat (n=3)</th>
<th>Diabetic rat (n=3)</th>
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<tbody>
<tr>
<td>Number of islets</td>
<td>33</td>
</tr>
<tr>
<td>Langerhans islet</td>
<td>0.0221±0.0035(^1)</td>
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<tr>
<td>mm(^2)</td>
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<tr>
<td>Number of somatostatin containing cells per islet</td>
<td>12.0±1.0</td>
</tr>
<tr>
<td>per mm(^2) of islet</td>
<td>780.4±79.2</td>
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<tr>
<td>1) Mean±SE</td>
<td></td>
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<tr>
<td>* p&lt;0.05</td>
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<tr>
<td>** p&lt;0.001</td>
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parts of the islets, whereas insulin-positive cells were located in the central portion, but markedly reduced in number (Fig. 2b and c).

Incubation of sections with SRIF or glucagon antiserum without pretreatment with human serum α-globulin resulted in the non-specific staining of all tissue elements. No staining was obtained when SRIF, glucagon or insulin antiserum absorbed by corresponding antigen was used, thus indicating the specificity of the reaction.

Discussion

It has been reported by several investigators that specific population of cells was stained with SRIF antiserum in the pancreatic islets. Most of the methods utilized were of the immunofluorescent technique and only a few authors used the peroxidase anti-peroxidase technique by Sternberger et al. (1970) (Goldsmith et al., 1975; Pelletier et al., 1976). In this paper it was shown that by the enzyme-labeled antibody method SRIF cells as well as insulin or glucagon cells were stained specifically. Therefore, this method is suitable for identifying the specific cell population in the islet. There are several advantages of this method over the immunofluorescent one. The preparations are permanent, require no special equipment and are available for the electronmicroscopic study.

It has been suggested that SRIF plays an important role in the local regulation of insulin and glucagon secretion (Alberti et al., 1973; Koerker et al., 1974). The present results suggest on anatomical findings that SRIF-containing cells had a more local effect on insulin cells than on glucagon cells, because it has been reported that the blood of the islet flows from the periphery to the central portion (Fujita, 1973). However, it is possible that cell-to-cell communication may help to coordinate their hormonal output.

On the other hand, in the streptozotocin-diabetic rat the arrangement of these cells in the islets becomes completely different from the control rat and the number of SRIF increased markedly. These findings correlate closely with recent data using the immunofluorescent technique (Orci et al., 1976) as well as the radioimmunoassay technique (Patel et al., 1976; Makino et al., 1977). It was suggested that hyperglucagonemia thus produced exerted compensatory responses on SRIF-containing cells, but other possibilities may also exist and further work is called for.

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References


