Changes in Somatostatin Release from Perfused Pancreas of Streptozotocin-Induced Diabetic Rats

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Abstract

To clarify the mechanism of an increased response of diabetic D-cells to arginine, we studied the changes in responsiveness of pancreatic D-cell to this agent with the passage of time after the onset of diabetes and the effect of chronic insulin substitution on the altered D-cell function utilizing the perfused pancreas of streptozotocin diabetic rats. The magnitude of somatostatin (SRIF) response to arginine (10 mM) was progressively increased with the duration of diabetes. The integrated response of SRIF during a 20 min period of an arginine infusion one week after the injection of streptozotocin was not different from that in the normals, but it was increased by 240% at 8 weeks and by 390% at 15 weeks. The exaggerated pancreatic SRIF release observed in 8 week diabetic rats was partially ameliorated with the daily insulin replacement (15.0±1.0 units/kg of B.W.). This was accompanied by a partial recovery from growth failure. The integrated amount of SRIF secreted during an arginine-stimulated period (20 min) was inversely correlated with an increase in body weight during an 8 week period (r=-0.741, p <0.001). From these results, it might be concluded that the alteration in diabetic D-cell function may be related to the metabolic and hormonal abnormalities of diabetes, rather than insulin deficiency itself.

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of time after the onset of streptozotocin-diabetes, and to elucidate the effect of chronic insulin substitution on the hyperfunction of pancreatic D-cells in streptozotocin-induced diabetic rats.

Materials and Methods

Animals and experimental procedures
Overnight-fasted male Wistar rats, weighing 140-290 g, were rendered diabetic by the administration of a single injection of streptozotocin (60 mg/kg of B.W., i.v.). Within 24 hr after injection, they had a glycosuric concentration of more than 2% which continued until sacrifice unless treated. The rats were subjected to two types of experiments. In experiment 1, one week, 8 weeks and 15 weeks after injection of streptozotocin, the pancreases were removed from a group of six diabetic rats, each remaining untreated until sacrifice, and perfused in vitro to study arginine-stimulated SRIF secretion. The normal group consisted of six intact rats, 50 to 90 days of age. Experiment 2 was designed to assess the effect of chronic insulin substitution on the pancreatic SRIF release stimulated by arginine in diabetic rats. Two days after intravenous injection of streptozotocin, ultralente insulin (NOVO) began to be administered daily in a subcutaneous injection between 14.00 and 15.00 h. A dose of 15.0 ± 1.0 units per kg of body weight a day (Mean ± SEM) was given for 8 weeks. The insulin dose was adjusted so as to maintain a urinary glucose concentration of less than 0.1% at the time of the injection. Age-matched, control animals were given an equivalent volume of 0.01 M sodium acetate buffer daily for 8 weeks. Both groups of animals were subjected to the experiment 22 h after the last insulin injection. All animals were weighed at the time of the injection of insulin or the buffer solution.

Perfusion System
The pancreas was isolated and perfused by the procedure described by Grodsky et al. (1967). The perfusate was a modified Krebs-Ringer bicarbonate buffer containing 0.1% bovine serum albumin, 5.5 mM glucose and 4.5% dextran T70, and was bubbled with 95% O2-5% CO2 prior to use. Complete portal vein effluent, after a single passage through the pancreas, was collected into a tube containing Trasylol® (500 KIU/ml) and EDTA-2Na (1 mg/ml) at 60 sec intervals; flow rate was constant at 2.2 ml/min throughout the perfusion. After a 15-minute equilibration period, the pancreas was perfused with the control perfusate for another five minute period to determine the baseline level (t−5−t0). At the end of the baseline period, a rapid switch was made to the perfusate containing 10 mM arginine. The duration of the arginine infusion period was 20 minutes (t0−5: the first phase, t5−20: the second phase), again followed by the final 10-minute baseline period.

Trunk blood (3 ml) was collected in a tube containing Trasylol® (500 KIU/ml) and EDTA-2Na (1 mg/ml). After centrifugation at 4°C, the plasma was separated and stored at −20°C until assay for glucose, IRI, GI and CPR. Plasma glucose was measured by the 0-toluidine method. Insulin was determined radioimmunochemically using rat insulin (NOVO Industries) as a standard, as described previously (Morishita et al., 1972). Rat C-peptide was measured by radioimmunoassay (Yanaihara et al., 1979). Glucagon was assayed radioimmunologically with an antibody specific for pancreatic glucagon (Shima et al., 1977). SRIF was measured by radioimmunoassay using the antisomatostatin serum kindly donated from Dr. Utsumi (Utsumi et al., 1979). This assay system could detect as little as 6.9 pg/ml of SRIF. The intra-, and inter-assay coefficients of variation were 9% and 14%, respectively.

Statistical Analysis
Statistical analysis of data was performed using the Wilcoxon unpaired rank test (Mann-Whitney) and Student’s t-test for paired samples. Results are expressed as mean ±SEM.

Results

Body weight, plasma glucose, immunoreactive insulin (IRI), C-peptide immunoactivity (CPR) and glucagon immunoreactivity (GI).

Table 1 lists mean values of the body weight, plasma glucose, IRI, CPR, and GI of control and diabetic rats at the time of the perfusion in each experiment. Figure 1 shows changes in the body weight during the experimental period. As expected (Junod et al., 1969), the diabetic rats without insulin-treatment failed to gain weight, resulting in no significant difference between the body weight between just before the injection of streptozotocin and at sacrifice for the experiment (lower panel in Fig. 1), whereas the insulin-treated diabetic rats showed a significant (p<0.001, paired
Table 1. Body weight, plasma glucose, immunoreactive insulin (IRI), C-peptide immuno-reactivity (CPR) and glucagon immunoreactivity (GI) in normal control and streptozotocin-diabetic rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>BW (g)</th>
<th>Glucose (mg/100 ml)</th>
<th>IRI (ng/ml)</th>
<th>CPR (ng/ml)</th>
<th>GI (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>6</td>
<td>308 ±31</td>
<td>251 ±24</td>
<td>8.1 ±2.9</td>
<td>32.8 ±4.0</td>
<td>204 ±33</td>
</tr>
<tr>
<td>Diabetic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Week</td>
<td>6</td>
<td>193 ± 9</td>
<td>645 ±47**</td>
<td>1.3 ±0.5*</td>
<td>3.6 ±0.8**</td>
<td>267 ±29</td>
</tr>
<tr>
<td>8 Weeks</td>
<td>6</td>
<td>275 ±22</td>
<td>894 ±50**</td>
<td>0.7 ±0.6*</td>
<td>6.0 ±3.0**</td>
<td>334 ±63</td>
</tr>
<tr>
<td>15 Weeks</td>
<td>6</td>
<td>305 ±37</td>
<td>795 ±58**</td>
<td>2.5 ±1.6*</td>
<td>4.3 ±1.1**</td>
<td>281 ±47</td>
</tr>
<tr>
<td>Exp 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>5</td>
<td>364 ±17</td>
<td>264 ±22</td>
<td>5.6 ±0.6</td>
<td>35.7 ±8.3</td>
<td>656 ±141</td>
</tr>
<tr>
<td>Diabetic, with Insulin Tx 8 Weeks</td>
<td>7</td>
<td>247 ±16**</td>
<td>727 ±83**</td>
<td>6.1 ±4.0</td>
<td>1.2 ±0.4**</td>
<td>706 ±27</td>
</tr>
</tbody>
</table>

Mean ±SEM. Normal controls in Exp 1 are not age-matched, whereas those in Exp 2 are age-matched and buffer-treated.
*p<0.05, **p<0.01: vs. normal controls in each Exp.

During streptozotocin-diabetic rats, the mean body weight was still significantly (p<0.0001) less than that of the normal rats. Plasma glucose was markedly elevated in each of the four diabetic groups in experiments 1 and 2, being approximately three times as high as in normal animals. Plasma IRI and CPR levels were significantly lower in diabetic rats at all times in experiment 1. Twenty two hours after the last insulin injection, the plasma IRI level of the insulin-treated diabetic rats in experiment 2 was comparable with that of the normals.

Experiment 1:

Figure 2 shows SRIF, GI and IRI releases from normal and streptozotocin-diabetic rat pancreas perfused in vitro during the stimulation with 10 mM arginine. In Table 2, the integrated amounts of SRIF, GI and IRI secreted during the basal state, the first and the second phases of the arginine stimulated period are shown. Basal IRI levels in the diabetic rats were not significantly different from those in the normal rats, irrespective of the duration of diabetes, but perfusate IRI responses from the diabetic pancreas were markedly lowered during either the first, t=5 or the second phase, t>20. Particularly in one-week diabetic rats, there was no significant elevation of IRI from the base line during 10 mM arginine stimulation. Infusion of arginine stimulated biphasic GI release in normal and three diabetic groups (Fig. 2). There was no significant difference between normal and any of three diabetic groups in the first and the second phases of arginine stimulated GI release (Table 2). Basal SRIF release from the 8-, and 15-week diabetic animals showed an exaggerated response of SRIF release to 10 mM arginine compared with 1-
Fig. 1. Percent changes in the body weight of rats during the experimental period. The body weight just before the injection of streptozotocin was assigned 100%. Open bar: body weight at the injection of streptozotocin. Dotted bar: body weight at perfusion. Vertical bar indicates SEM. Statistical significance: *p < 0.001, **p < 0.0001, ***p < 0.00001.
week diabetic rats and the normal controls: 2458 $\pm$ 182 pg/20 min, and 3993 $\pm$ 628 pg/20 min in the 8- and 15-week diabetics, respectively. The SRIF area during the second phase from the 15-week diabetic pancreas was significantly greater than that from the 8-week diabetic pancreas, whereas those during the first phase did not differ from each other.

Experiment 2:

Table 3 shows SRIF, IRI and GI secretion from normal and insulin treated diabetic rat pancreas in experiment 2. Basal and arginine-stimulated IRI secretions were significantly lower in diabetic rats than in normal controls. GI secretion was similar in both normal and diabetic groups during the basal and arginine-stimulated periods. Basal and arginine-stimulated SRIF release from the diabetic pancreas was twice as great as the corresponding values for the normals. Figure 3 shows a comparison of IRI, GI and SRIF secretions from the insulin treated (from experiment 2) and untreated (from experiment 1) diabetic rat pancreas of the same affected period (8 weeks). In the basal and the arginine-stimulated state, IRI and GI secretions were similar in both diabetic groups, except for the lower basal GI output from the insulin-treated diabetic pancreas. Both diabetic groups showed similar basal SRIF secretion. There was, however, a significant difference in the arginine stimulated SRIF response between diabetics with
and without insulin substitution (Fig. 3), the amount of arginine stimulated SRIF secretion being significantly smaller in the insulin treated diabetics than in the untreated diabetics (1568 ± 101 pg/20 min vs. 2458 ± 182 pg/20 min; p < 0.002)

### Discussion

One of the aims of the present study was to examine changes in pancreatic SRIF response to arginine in relation to those in glucagon and insulin secretion over a 15-week period after the induction of streptozotocin diabetes in rats. The present study confirms the previous reports (Hara et al., 1979; Kodowaki et al., 1979b) showing that arginine-stimulated SRIF secretion was enhanced in diabetic rats. In addition, we showed that the magnitude of SRIF response to arginine was related to the duration of diabetes; it was progressively increased with the duration of the disease. The changes in SRIF secretion observed in this study are in good agreement with those in islet immunoreactive SRIF content reported by Patel et al (1978). There was no change in islet SRIF content at 2 days after the administration of streptozotocin, but thereafter, SRIF progressively increased in the diabetic animals by 45% at 2 weeks, 230% at 6 weeks, and 500% by 6 months. Coinciding with these observations, no change in pancreatic SRIF release was observed at one week after the administration of streptozotocin, whereas thereafter, SRIF secretion progressively increased in the diabetic animals by 240% at 8 weeks and 390% by 15 weeks in the present study.

Is this alteration in islet D-cell function caused directly by insulin deficiency per se or a secondary consequence of the metabolic and hormonal abnormalities of diabetes (e.g. hyperglycemia, hyperglucagonemia, increased food intake, dehydration)
Fig. 3. Comparison of immunoreactive insulin (IRI), glucagon (GI) and somatostatin (SRIF) secretions from the insulin treated (from Exp 2) and untreated (from Exp 1) diabetic rat pancreas of the same 8-week affected period. Normal, buffer-treated rats from Exp 2 are shown as control. (a) represents the basal secretions for 5 minutes. (b) indicates 10 mM arginine-stimulated secretions for 20 minutes. Open bar: normal controls. Shaded bar: untreated diabetic rats. Dotted bar: insulin-treated diabetic rats. Mean ±SEM. *P <0.01 vs. normal controls. ++P <0.01 vs. untreated diabetic rats.

It has been reported that no acute effect of insulin on islet D-cell function was found (Kadowaki et al., 1979a). The fact that there was no change in arginine-stimulated SRIF secretion one week after the administration of streptozotocin in spite of the presence of a severe reduction in insulin release, together with the result that the hyperfunction of the pancreatic D-cells was enhanced by the prolongation of the diabetic period, suggests that the alteration in diabetic D-cell function may not be due to intra-islet insulin deficiency per se, but to a secondary consequence of the continued metabolic abnormalities of some duration in diabetes. This probability is also supported by the findings revealed in experiment 2; that is, hyperfunction in pancreatic D-cell in diabetic rats was partially corrected when they had been treated with insulin. It has been reasonably presumed that their diabetes was fairly controlled by this regimen, judging from the fact that the treated rats gained weight significantly though not to a normal level, while no increase in body weight was observed in the untreated ones for an 8 week period. The incomplete correction of the metabolic and hormonal abnormalities of the diabetes in the insulin treated rats as expressed in partial recovery from growth failure may in turn be related with the partial reduction in the augmented SRIF secretion from the perfused pancreas. When the integrated amounts of SRIF secreted during an arginine-stimulated period (20 min) are plotted against the increase in body weight during an 8 week period, these two parameters are inversely correlated ($\gamma = -0.741, p < 0.001; \text{Fig. 4}$). These results provided additional evidence for the theory that the change in diabetic D-cell functions is a secondary consequence of metabolic disturbance rather than insulin deficiency itself, supposing that body weight increase was one of the indicies for amelioration of the metabolic disturbance in diabetes.

The high glucose concentration which has been reported to stimulate SRIF sec-
Fig. 4. Correlation between the increase in body weight and the amount of pancreatic arginine-stimulated somatostatin secretion ($\Delta$SRIF). The increase in body weight is expressed as gain in weight during the experimental period (8 weeks). $\Sigma$SRIF is calculated by the integration of the area under the curve during the 20-min arginine-stimulated period. ◊: normal control rats. □: diabetic rats, insulin-treated. ■: diabetic rats, untreated.

Retention (Schauder et al., 1976; Weir et al., 1977) may account for the increased pancreatic SRIF secretion in streptozotocin diabetic rats.

Finally, the possibility that streptozotocin exerts a direct effect on the D-cells has to be considered. This seems unlikely, however, judging from the present finding that hyperfunction in pancreatic D-cells has been corrected with insulin therapy and from the fact that alloxan-induced diabetic rats showed a similar alteration in D-cell function (Hara et al., 1979; Patel et al., 1978). The present observation show that the magnitude of SRIF response to arginine is progressively increased with the duration of diabetes, and the exaggerated SRIF response to arginine is partially corrected with insulin therapy which concomitantly ameliorates growth failure in the streptozotocin-induced diabetic rats. These results suggest that an alteration in the diabetic D-cell function may be related to the metabolic and hormonal abnormalities of diabetes, but not insulin deficiency itself.

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References


