Insulin Releasing Activity of Gastrointestinal Glucagon-like Immunoreactive Materials in Perfused Rat Pancreas

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Synopsis

Insulin-releasing activity of porcine gastrointestinal glucagon-like immunoreactive materials purified by affinity chromatography was examined in the perfused rat pancreas. When glucose concentration of the perfusate was raised from 60 to 100 mg/dl, augmented insulin release was observed. The mean incremental area of immunoreactive insulin during the first 10 min thus observed was 19.07 ± 3.76 ng/10 min. Pancreatic glucagon and the extract from the gastric fundus showed the enhancement of insulin release in this system when they were added to the perfusate at the rate of 100 ng/min for 5 min; ΔIRI were 41.92 ± 8.47 and 71.70 ± 18.09 ng/10 min, respectively, which were significantly higher than that of 100 mg/dl of glucose alone. However, no significant difference in the insulinogenic activity was noticed between the extracts from the small intestine and the control.

These results suggest that the extract from the gastric fundus has insulinogenic activity similar to that of pancreatic glucagon.

The existence of hyperglycemic glyco-
genolytic factor in the pancreas and the gastric fundus was first reported by Sutherland et al. (1948 and 1949). Thereafter the existence of glucagon-like materials in the stomach and the small intestine of several species was confirmed immunologically by Unger et al. (1961 and 1966). Later on, cells to secrete glucagon-like immunoreactive materials (GLI) have been demonstrated in the canine stomach and intestine (Polak et al., 1971).

Although the biological activity of GLI in the gastrointestinal tract was still a matter of speculation, controversy continued as to whether the intestinal GLI possesses the insulinogenic activity (Buchanan et al., 1969; Moody et al., 1970; Gutman et al., 1973; Murphy et al., 1973; Tanaka et al., 1975b; Ohneda et al., 1976). Conflicting results regarding this matter were partly due to a difference in the purity of the material employed and the system to study. Therefore, a further investigation into this matter using the purified material in a more sensitive system seems to be worthwhile.

Furthermore, recent reports by Matsuyama and Foà (1974), Vranic et al. (1974) and Sasaki et al. (1975) suggest the presence of glucagon immunoreactivity, which reacted with antibody specific for pancreatic glucagon, in the gastrointestinal tract, especially abundant in the gastric fundus (Matsuyama et al., 1977). The biological activities of this material were reported to

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be close to those of pancreatic glucagon, as far as glycogenolytic and adenylate cyclase stimulating activities were concerned (Sasaki et al., 1975). However, no information regarding the insulinogenic activity of the material was available.

In order to clarify these points, the insulin-releasing activity of the gastrointestinal GLI purified by affinity chromatography was studied in the rat pancreas perfusion system.

**Materials and Methods**

The gastric fundus and the lower half of the small intestine were obtained immediately after pigs were sacrificed at a slaughter house and were brought to the laboratory chilled on ice. The mucosa of these preparations was minced and mixed in a cold room. Gastrointestinal GLIs were extracted by a slight modification of acid alcohol method (Tanaka et al., 1975a) of Kenny (1955) from the pancreas (EX-P), the mucosa of the gastric fundus (EX-F) and the lower half of the small intestine (EX-S). When applied to a Bio-Gel P-10 column, 10 x 100 cm, and eluted with 0.1N acetate solution, EX-S yielded two peaks, Peak I (EX-S1) and Peak II (EX-S2). EX-F, EX-S1 and EX-S2 were further purified by affinity chromatography (Tanaka et al., 1974) using antiglucagon rabbit serum bound to CNBr-activated Sepharose 4B and were termed EG-F, EG-S1 and EG-S2, respectively. The antibody (AGS10) employed was "non-specific" and details of its characteristics were reported elsewhere (Matsuyama and Foa, 1974). The perfused rat pancreas (Grodsky et al., 1967) was used for evaluating the insulinogenic activity of the materials thus obtained. After perfusing the pancreas with the control perfusate; Krebs Ringer bicarbonate buffer, pH 7.4, containing 4.5% dextran (m. wt. 70,000) and 60 mg/dl glucose, for 15 min, the glucose concentration of the perfusate was raised to 100 mg/dl and pancreatic glucagon (crystalline, Lilly) (PG) or the purified preparations of GLI were added simultaneously to the perfusate at the rate of 100 ng/min for 5 min. As the control normal saline was added for 5 min. The volume added was less than one tenth of the perfusate (2.2 ml/min). Oxygenation was performed by the bubbling of the mixed gas (O2 95%, CO2 5%). The sample was collected into a tube containing Trasylol (500 KIU/ml) and EDTA-2Na (1 mg/ml) in each one minute.

The IRI concentration in the perfusate was measured using rat insulin standards. Glucagon was determined by radioimmunoassay using polyethylene glycol for B, F separation (Shima et al., 1977). The two antiglucagon sera were employed, namely AGS10 which reacted with both GLI and pancreatic glucagon, the other AGS 18 that was specific for pancreatic glucagon.

**Results**

**Glucagon contents of the extracts from the pancreas and the gastrointestinal tract**

Total glucagon-like immunoreactivity and glucagon immunoreactivity were measured using nonspecific antibody (AGS10) and pancreas-specific antibody (AGS18), respectively. As shown in Table 1, there was no difference between both values in the extract from the pancreas (EX-P). In the crude fundic extract (EX-F) and further purified preparation by affinity chromatography (EG-F), glucagon immunoreactivity was about half of total glucagon-like immunoreactivity, which was less than those of the small intestine. Glucagon immunoreactivity of the small intestine was 5-13% of total glucagon-like immunoreactivity. By means of affinity chromatography GLIs were purified by 2 to 8 times.

**Insulin-releasing activity of pancreatic glucagon and gastrointestinal GLIs in the perfused rat pancreas**

Table 2 shows the insulin-releasing activity of the test agents. The amount of insulin released was significantly enhanced 7 min after the commencement of the perfusion with 100 ng/min of PG compared with the perfusion with control solution, which contained 100 mg/dl of glucose alone. A significantly elevated insulin release was observed in 5-min perfusion with 100 ng/min of EG-F at 5-, 7- and 10-min points. However, 5-min perfusion with 50 ng/min of PG or EG-F yielded no significant increase in insulin output over the control. No discernible insulin releasing activity was found in EG-S1 and EG-S2 from the small intestine.
Table 1. Contents of glucagon-like immunoreactivity in extracts from pancreas and gastrointestinal tract

<table>
<thead>
<tr>
<th>extract</th>
<th>total glucagon-like immunoreactivity* (ng/mg)</th>
<th>glucagon immunoreactivity** (ng/mg)</th>
<th>glucagon immunoreactivity</th>
<th>total glucagon-like immunoreactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>EX-P</td>
<td>195.0</td>
<td>208.0</td>
<td></td>
<td>1.07</td>
</tr>
<tr>
<td>EX-F</td>
<td>11.4</td>
<td>7.6</td>
<td></td>
<td>0.67</td>
</tr>
<tr>
<td>EX-S</td>
<td>66.5</td>
<td>3.6</td>
<td></td>
<td>0.05</td>
</tr>
<tr>
<td>EX-S₁</td>
<td>156.3</td>
<td>6.9</td>
<td></td>
<td>0.04</td>
</tr>
<tr>
<td>EX-S₂</td>
<td>61.4</td>
<td>7.9</td>
<td></td>
<td>0.13</td>
</tr>
<tr>
<td>EG-F</td>
<td>70.3</td>
<td>37.2</td>
<td></td>
<td>0.53</td>
</tr>
<tr>
<td>EG-S₁</td>
<td>283.5</td>
<td>29.2</td>
<td></td>
<td>0.10</td>
</tr>
<tr>
<td>EG-S₂</td>
<td>487.0</td>
<td>65.3</td>
<td></td>
<td>0.13</td>
</tr>
</tbody>
</table>

* total glucagon-like immunoreactivity; measured using non-specific antiglucagon serum (AGS 10)
** glucagon immunoreactivity; measured using specific antiglucagon serum (AGS 18)

Table 2. Insulin-releasing activity of pancreatic glucagon (PG) and the gastrointestinal GLIs in the perfused rat pancreas

<table>
<thead>
<tr>
<th>sample</th>
<th>control</th>
<th>PG</th>
<th>PG</th>
<th>EG-F</th>
<th>EG-F</th>
<th>EG-S₁</th>
<th>EG-S₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>concentration (ng/min, 5 min)</td>
<td>0</td>
<td>50</td>
<td>100</td>
<td>50</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>number</td>
<td>6</td>
<td>4</td>
<td>8</td>
<td>4</td>
<td>6</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>ΔIRI (ng/ml)</td>
<td>1 min.</td>
<td>1.40±1.35</td>
<td>0.79±0.35</td>
<td>4.66±0.84</td>
<td>2.09±2.17</td>
<td>3.59±1.04</td>
<td>4.89±2.54</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6.26±3.00</td>
<td>2.85±1.85</td>
<td>6.93±2.05</td>
<td>5.62±2.33</td>
<td>9.01±2.57</td>
<td>7.16±2.71</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5.77±1.82</td>
<td>5.37±1.91</td>
<td>6.43±1.47</td>
<td>4.29±1.12</td>
<td>9.05±2.47</td>
<td>6.90±1.85</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1.10±0.57</td>
<td>1.18±0.84</td>
<td>2.66±0.51</td>
<td>2.11±0.99</td>
<td>9.67±1.93***</td>
<td>8.78±4.53</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>0.67±0.66</td>
<td>1.32±0.81</td>
<td>5.63±1.60***</td>
<td>2.88±1.68</td>
<td>7.64±1.92***</td>
<td>0.08±6.43</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>-0.87±0.89</td>
<td>-0.27±0.61</td>
<td>0.88±0.61</td>
<td>0.31±0.50</td>
<td>4.57±2.12*</td>
<td>-4.31±4.15</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>-2.18±1.12</td>
<td>-0.68±0.66</td>
<td>0.81±0.65</td>
<td>-0.36±0.55</td>
<td>0.97±2.05</td>
<td>-5.49±2.74</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>-2.14±1.20</td>
<td>-0.98±0.77</td>
<td>0.56±0.43</td>
<td>-0.28±0.45</td>
<td>0.49±2.56</td>
<td>-4.94±2.75</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>-1.75±1.42</td>
<td>-0.75±0.56</td>
<td>1.09±0.97</td>
<td>-0.38±0.42</td>
<td>-0.53±2.40</td>
<td>-2.85±3.19</td>
</tr>
<tr>
<td>ΔΣIRI</td>
<td>19.07±3.76</td>
<td>16.96±3.40</td>
<td>41.92±8.47*</td>
<td>26.04±9.47</td>
<td>71.70±18.09**</td>
<td>32.54±36.68</td>
<td>8.06±6.03</td>
</tr>
</tbody>
</table>

mean±SEM
control vs sample  * p<0.05  ** p<0.025  *** p<0.01
ΔΣIRI is the increment of IR-I release above the basal line in which 60 mg/dl of glucose alone is used.

In Table 2, the sum of incremental insulin release (ΔΣIRI) from the perfused pancreas over 10 min is shown, when various preparations of gastrointestinal GLIs and PG were added to the perfusate. The values of 5-min perfusion with 100 ng/min of PG and EG-F were significantly greater than that of the control, but those of the other preparations (EG-S₁, EG-S₂) did not differ from the control.

Discussion

The existence of glucagon-like immunoreactive materials (GLI) in the gastrointestinal tract, distributed from the stomach to the rectum, is now a well documented fact. However, the composition of GLI seems to be different in various portions of the alimentary tract. A ratio of glucagon immunoreactivity to total glucagon-like immunoreactivity was 1.07 in the extract of porcine
pancreas (EX-P) by the acid alcohol extraction method, 0.53–0.67 in that of the gastric fundus (EX-F, EG-F), 0.04–0.13 in that of the small intestine (EX-S1, EX-S2, EG-S1, EG-S2). Glucagon immunoreactivity was abundant in the extract of the gastric fundus comparing with that of the small intestine. But total glucagon-like immunoreactivity in the extract of the gastric fundus was less than that of the small intestine. Similar findings were reported by others (Sasaki et al., 1975). These findings together with the morphological resemblance between the secretory granules of the pancreatic and fundic A cell in dogs (Sasaki et al., 1975) suggest that the mucosa of the gastric fundus contains the material similar or close to pancreatic glucagon, but the materials extracted from the mucosa of the lower half of the small intestine is different physico-chemically from pancreatic glucagon.

In this study, after the extraction of acid alcohol extraction method, the extract of the small intestine was divided into two peaks (EX-S1, EX-S2) by the gel filtration. Furthermore, these substance together with the acid alcohol extract of the gastric fundus (EX-F) were further purified by affinity chromatography, by which GLIs were purified 2 to 8 times per mg weight and about 170 times per mg protein (Tanaka et al., 1975b).

Using the rat pancreas perfusion system, the insulin-releasing activity of these purified gastrointestinal GLIs was studied in comparison with that of pancreatic glucagon (PG). The method is more sensitive and more physiological than our previous system in which pancreas pieces were employed (Tanaka et al., 1975b). EG-F promoted the insulin-releasing activity similarly to PG, but neither EG-S1 nor EG-S2 showed insulinogenic activities. Various enteric hormones have been reported to stimulate insulin release. Therefore, the insulin-releasing activity of EG-F might be attributed to these hormones. However, no measurable amount of secretin and CCK-PZ was found in our materials (Tanaka et al., 1975b). Furthermore, judging from specificity of affinity chromatography and scarcity of another insulinogenic hormone, GIP, in the gastric fundus, it seems unlikely that the insulin-releasing activity of EG-F might be due to these hormones contaminated in this component. Ohneda et al. (1976) reported the insulinogenic activity of the canine intestinal GLI purified by affinity chromatography. The discrepancy between the results of the present study and Ohneda et al. might be attributed to differences in the method, species and systems to study. The insulinogenic activity of their material is not due to the contaminated glucagon immunoreactivity since they failed to measure the glucagon immunoreactivity in the peak II GLI. Anyway, it is apparent that the extract from the gastric fundus possesses the insulinogenic activity. However, on the basis of the study on the secretory mechanism of gastric glucagon immunoreactivity in dogs (Blazquez et al., 1976), it is so far unclear what physiological role the insulinogenic activity of glucagon immunoreactivity plays in man.

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References


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