Contents and Secretion of Glucagon and Insulin in Rat Pancreatic Islets from the Viewpoint of Their Localization in Pancreas

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Regional difference in secretion of glucagon and insulin from the rat pancreatic islets and their contents in pancreatic tissue and islets were studied. The glucagon content in the normal rat pancreas was the highest at the splenic part followed in order by the gastric, choledochal and duodenal parts. The effect of alloxan on the glucagon content was stronger in the dorsal lobe (combined gastric and splenic parts) than in the ventral lobe (combined duodenal and choledochal parts), and more increase of glucagon was found. The size of pancreatic islets was similar between the dorsal and the ventral lobe. The glucagon content in the islets was significantly higher in the dorsal lobe than in the ventral lobe, but the insulin content in the islets was similar in both lobes. The release of insulin from cultured pancreatic islets from the dorsal lobe was similar to that from the ventral lobe, but the release of glucagon was significantly high from the cultured islets of the former in the presence of glucose or arginine compared with that from the latter. Also in isolated pancreatic islets the release of glucagon was significantly more marked in the islets from the dorsal lobe by arginine administration. These findings show that the islets from the dorsal lobe secrete and contain more glucagon than those from the ventral lobe in contrast to there being a similar amount of release of insulin between them. IRG: IRI: localization of pancreatic islet

The contents of insulin and glucagon in the pancreas have been reported to be rich in the dorsal lobe compared with those in the ventral lobe (Wrenshall et al. 1952; Orci and Perrelet 1981; Trimble et al. 1982; Tasaka et al. 1984). On the contrary, pancreatic polypeptide is abundant in the ventral lobe of the pancreas (Gingerich et al. 1978; Gersell et al. 1979; Orci and Perrelet 1981; Tasaka et al. 1984).

These findings suggest that the pancreatic islets may be different in their hormonal contents and secretory capacities depending upon their localization within the pancreas. So far there has been only one paper regarding this problem (Trimble et al. 1982). Trimble et al. (1982) reported the high amount of glucagon

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content in the dorsal islet compared with that in the ventral lobe and it's effect on the biosynthesis of insulin and secretion, but no comparative study of hormonal secretion was done using pancreatic islets.

In this investigation, we studied and compared the secretion and content of insulin and glucagon from different parts of the pancreas and the effects of alloxan-induced diabetes on them.

**Materials and Methods**

**Animals**

Male Wistar rats (Animal Breeding Laboratories, Ohmiya, Saitama) weighing 250-300 g were used for all experiments. Rats were fed standard rat chow ad libitum, and were maintained on a 12 hr light-dark (8 a.m./8 p.m.) cycle. In the alloxan-induced diabetic rat, alloxan (alloxan monohydrate, Nakarai Chemicals Ltd., Kyoto) 60 mg/kg was injected i.v. and the pancreas was excised after 3 weeks. The rat pancreas consists anatomically of three parts: the gastrosplenic, choledochal and duodenal parts (Lambert 1965). In some specimens, the gastrosplenic part was further divided into the gastric and splenic parts.

**Isolation of islets**

The fed rats were anesthetized by i.p. injection of sodium pentobarbital and pancreatic islets were isolated by a modification of the method of Lacy and Kostianovsky (1967). For each preparation, the pancreas was inflated via the bile duct with Hanks salt solution. The distended pancreas was minced and incubated with collagenase (CLS-IV, Worthington Biochemical Corp., NJ, USA) with vigorous shaking. After several washes, the sediment was examined under a dissecting microscope and individual islets were transferred to small beakers containing incubation medium in a randomized fashion. In the experiments using pancreatic islets, the islets from duodenal and choledochal parts, or those from splenic and gastric parts were combined.

**Islet culture experiment**

In the islet organ culture experiment, a batch of five islets was placed in a 35 x 10 mm tissue culture dish (Corning 35 x 10 mm, Iwaki Glass, Tokyo) with 2 ml medium 199 (Gibco Laboratories, Chagrin Falls, OH, USA) containing 10% heat-inactivated newborn calf serum, 5,000 IU./100 ml penicillin and 5,000 μg/100 ml streptomycin. The dish was incubated at 37°C in a humidified atmosphere containing 5% carbon dioxide and 95% air. Medium samples were stored at -20°C prior to assay of immunoreactive insulin (IRI) and glucagon (IRG).

**Islet incubation experiment**

In the experiment on secretion of IRI and IRG from isolated pancreatic islets, a batch of 5 islets was used. The islets were first preincubated in 0.5 ml of Krebs-Ringer bicarbonate buffer (pH 7.4) containing 2 mg/ml bovine plasma albumin (BPA) and 0.6 mg/ml glucose for 30 min at 37°C in a shaking water bath with a gas phase of 95% O₂-5% CO₂. At the end of the preincubation period, the medium was changed, and the islets were washed once with the incubation medium. The islets were further incubated (95% O₂,5% CO₂) for 90 min at 37°C in 0.5 ml of the medium. At the end of the experiment, the medium was transferred and was stored at -20°C.

**Extraction of IRI and IRG**

Extraction of IRI and IRG from the pancreatic tissue was done using acid alcohol (Hayashi et al. 1977). The islets were sonicated in acid alcohol and extracted. IRI and
IRG were determined directly in appropriated dilution.

Determination of islet volume

The spherical volume of the isolated islet was obtained from the formula \(4/3 \pi r^3\) (\(r=\)mean islet radius), based on the assumption that islets are essentially spherical in shape (Hellman 1959; Reaven et al. 1979). The X and Y diameter of each islet was measured with the use of an eye-piece micrometer on a stereoscopic dissecting microscope, and a mean diameter was calculated.

Analysis

IRI in the extract or in the medium was determined by the two-antibody immunoassay of Morgan and Lazarow (1963) with rat standard insulin. IRG was determined by the polyethylene glycol method (Henquin et al. 1974) using 30K antiglucagon serum.

Statistical difference was determined with unpaired Student's \(t\)-test and was considered significant when \(p<0.05\).

RESULTS

IRI and IRG contents in different parts of pancreatic tissue of normal and alloxan diabetes rats

Rat pancreas was divided into four regions: duodenal, choledochal, gastric and splenic parts. The IRI content in rat pancreas showed a higher value in order from duodenal to splenic parts, but not significantly; in the duodenal part it was \(2.16 \pm 0.69\) U/g (86.4 ± 27.6 \(\mu\)g/g, mean ± s.e.) and in the splenic was \(3.29 \pm 0.76\) U/g (131.6 ± 30.4 \(\mu\)g/g) (Fig. 1). On the contrary, the significant high con-
tent of IRG was found in the splenic part (9.58±1.35 μg/g) compared with that in the duodenal part (4.88±1.98 μg/g, p<0.01).

The IRI content was decreased to around one tenth of the control value 3 weeks after alloxan injection. In contrast the IRG content was increased in all four regions, especially significantly in the gastric or splenic part (p<0.01 or 0.05, respectively).

Size of pancreatic islet at different regions of pancreas

The volume of pancreatic islet was calculated. Two different parts were compared: combined duodenal plus choledochal, or combined splenic plus gastric, respectively. The former corresponds to the ventral lobe and the latter to the dorsal lobe. The islet volume in the ventral or in the dorsal lobe was not significantly different (21.1±2.3×10^6 μm^3 (n=81) vs. 22.66±5.5×10^6 μm^3 (n=75).

IRI and IRG contents in pancreatic islet

IRI content in the ventral lobe was 90.8±5.7 ng/islet and that in the dorsal lobe was 94.2±6.2 ng/islet (Table 1). On the other hand, glucagon content in the islet was higher in the dorsal lobe than in the ventral lobe (p<0.05, 4.30±0.55 ng/islet versus 2.77±0.48 ng/islet).

Release of IRI and IRG from cultured pancreatic islet

Release of IRG and IRI from cultured pancreatic islets was investigated in the presence of glucose or arginine (Fig. 2). At a high concentration of glucose (16.7 mM), the releases of IRI was increased significantly both in the combined duodenal plus choledochal parts (ventral lobe) and the combined splenic plus gastric parts (dorsal lobe) compared with those in the basal conditions (glucose 3.3 mM): in the ventral lobe it was increased from the basal of 25.7±4.5 ng/islet to the stimulated conditions of 79.3±8.3 ng/islet (p<0.01) and in the dorsal lobe from 38.1±6.3 ng/islet to 79.6±4.4 ng/islet (p<0.01). The amount of released IRI was not significantly different between ventral and dorsal lobes. On the

<table>
<thead>
<tr>
<th>Table 1. IRI and IRG contents in pancreatic islet</th>
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<td>Hormones</td>
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<tr>
<td>IRI</td>
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<td>IRG</td>
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Pancreatic islets from ventral and dorsal lobes were sonicated and extracted (See Materials and Methods). The number of the bracket indicates number of islets.

Values are expressed as mean±s.e.

*p<0.05 vs. values in the ventral lobe.
other hand, IRG release was significantly higher in the dorsal lobe compared with those in the ventral lobe in the presence of low or high concentrations of glucose or arginine (20 mM). In the low concentration of glucose, each value was 1.18±0.14 ng/islet or 0.63±0.09 ng/islet (p<0.01), respectively, and in the high concentration the value was 1.32±0.15 ng/islet or 0.66±0.10 ng/islet (p<0.01) and it was 1.61±0.17 ng/islet versus 1.01±0.12 ng/islet (p<0.05) in the presence of arginine. In the presence of arginine, a significant increase of IRG was found at both lobes compared with that in the basal state (each p<0.05).

**Release of IRG and IRI from isolated pancreatic islet**

Release of IRG and IRI from isolated pancreatic islet was studied (Table 2). Significant increases of IRG and IRI were found in the islets from the dorsal lobe in the presence of arginine. A greater release of IRG was observed in the islets from the dorsal lobe than in those from the ventral one; the former being 163.3±19.0 pg/islet and the latter being 99.3±18.1 pg/islet (p<0.05).
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TABLE 2. *Secretion of IRG and IRI from isolated pancreatic islet*

<table>
<thead>
<tr>
<th>Hormones</th>
<th>Ventral lobe (pg/islet)</th>
<th>Dorsal lobe (pg/islet)</th>
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<tbody>
<tr>
<td>IRG</td>
<td>Basal 93.2 ± 21.2 (10)</td>
<td>101.7 ± 20.5 (13)</td>
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<tr>
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<td>Arginine 99.3 ± 18.1 (15)</td>
<td>163.3 ± 19.0 (15)</td>
</tr>
<tr>
<td>IRI</td>
<td>Basal 4.41 ± 0.52 (11)</td>
<td>3.21 ± 0.65 (12)</td>
</tr>
<tr>
<td></td>
<td>Arginine 3.92 ± 0.54 (15)</td>
<td>5.43 ± 0.76 (14)</td>
</tr>
</tbody>
</table>

Pancreatic islets were freshly preincubated for 30 min and then incubated for 90 min at 37°C in a Krebs Ringer bicarbonate medium.

The number of the bracket indicates number of experiments.

Values are expressed as mean±s.e.

a: p<0.05 vs. basal values (Dorsal lobe), b: p<0.05 vs. basal values (Dorsal lobe), c: p<0.05 vs. values in the ventral lobe

**DISCUSSION**

In this study the IRI content in the pancreas was the highest in the splenic, followed in order by the gastric, choledochal and duodenal parts. The IRG content was high in the gastric and splenic parts, and very low in the duodenal part. Similar findings were also reported in the canine pancreas (Gingerich et al. 1978; Tasaka et al. 1984). Orci et al. (1976) and Baetens et al. (1976) found morphologically using the immunofluorescence technique that glucagon-containing cells were abundant in the body and tail of the pancreas, and that, on the other hand, PP-containing cells were abundant in the head and scarce in the body and tail of the rat pancreas. Bencosme and Liepa (1955) demonstrated that histologically. In alloxan-induced diabetic rats, a more remarkable increase in IRG was found in the dorsal lobe compared with that in the ventral lobe in this investigation. These findings might be due to the possibility that the islets in the dorsal lobe may be more abundant in glucagon-containing cells than those in the ventral lobe.

In the present experiments, the IRG content of the dorsal lobe was higher, confirming the report by Hellman (1959). Although the amount of released IRI from cultured pancreatic islets was similar in the ventral lobe and the dorsal one, the amount of released IRG in the presence of glucose or arginine was significantly higher in the dorsal lobe than in the ventral lobe. Similar results were also obtained from this experiment in which isolated pancreatic islets were incubated in the presence of arginine. Thus we confirmed that the islets in the dorsal lobe have more hormonal secretory capacity than those in the ventral lobe. In the experiment using cultured pancreatic islets, the amount of released insulin was not significantly different between the basal and arginine-containing medium. This reason is not clear. It might be due to the relatively low potency of secreting insulin by arginine.
In the present experiment the size of pancreatic islets did not differ between the dorsal lobe and the ventral lobe, and the IRG content in the islets was significantly high in the former. Trimble et al. (1982) mentioned that dorsal lobe islets contain more IRG than do ventral lobe islets, and IRI secretion and proinsulin biosynthesis were greater in the dorsal lobe islets under the condition of glucose stimulation. Hayek and Guardian (1986) reported that IRG release by alanine was higher in the dorsal islets of fetal or neonatal rats than in the ventral islets. Kaihoh et al. (1986) reported that the smaller the islet, the smaller the percentage of islets containing A cells with the vast majority of cells in the smallest islets being B cells. Conversely, the proportion of B cells to non-B cells was found not to change with size by Colella et al. (1985), thus showing inconsistent results.

Already Orci and Perrelet (1981) have reported that insulin and somatostatin cells are present in approximately the same numbers, but that glucagon cells are abundant in the dorsal islets and rare in the ventral islets, by specific numerical and topographic analysis. Therefore, the greater release of IRG from the dorsal islets and the high content of IRG in them might mainly be due to the abundance of glucagon-producing cells and there is no report that the glucagon cell in the dorsal lobe has stronger secretory capacity than that in the ventral lobe.

In the region of the superior mesenteric artery which mainly supplies the ventral lobe, the percentage of blood supply to the islets in relation to the acinar part, in spite of there being no difference in total blood flow within the pancreas or in the islet blood flow, was somewhat higher than that in the region of the coeliac artery which chiefly perfuses the dorsal lobe (Jansson and Hellerström 1987). Therefore, the effect of alloxan administration on the glucagon-containing cells was thought not to be due to an overflow of chemicals to the dorsal lobe.

References


