Effects of Oral Administration of Trypsin Inhibitor and Repeated Injections of Pancreozymin on the Insulin and Glucagon Contents of Rat Pancreas

TSUNE FUJITA, YOKO MATSUNARI, KOJI SATO, MUTSKU HAYASHI and YUKICHI KOGA

1Department of Anatomy, and 21st Department of Internal Medicine, Niigata University School of Medicine, Niigata 951, and 3Shimizu-Seiyaku Pharmaceutical Company, Shimizu 424, Japan

Synopsis

Rats were given soybean trypsin inhibitor or repeatedly injected with pancreozymin (daily 40 I.D.U./kg) for 7 days, and the insulin and glucagon contents of the pancreas were measured. The insulin and glucagon contents were markedly increased after these treatments and this effect was especially conspicuous after injections of large doses (daily 120 I.D.U.) of depot-type pancreozymin. Insulin content thus reached 1.9 times, and glucagon content 2.4 times as much in control values. This result is compatible with our previous histological finding that not only the exocrine pancreas but also islet cells undergo the trophic effect of endogenous and exogenous pancreozymin.

It has long been known that feeding rats with raw soybeans causes enlargement of the pancreas (Booth et al., 1960) and this effect has been ascribed to a trypsin inhibitor contained in soybeans (Haines and Lyman, 1961). Trypsin inhibitors, when infused into the rat small intestine, cause an increased enzyme output from the pancreas (Lyman and Lepkovsky, 1957; Geratz, 1968; Green and Lyman, 1972; Imai, 1974). Data have accumulated, except for evidence by a radioimmunoassay as yet, supporting that trypsin inhibitors cause the release of pancreozymin from the gut mucosa (Khayambashi and Lyman, 1969; Mainz et al., 1973). The enlarged pancreas caused by trypsin inhibitors thus seems to be accounted for by the chronic secretagogous stimulus by endogenous pancreozymin. This view is supported by a long term administration of pancreozymin causing hypertrophy of the pancreas in the rat (Rothman and Wells, 1967; Mainz et al., 1973).

Our previous studies (Fujita et al., 1976; Yanatori and Fujita, 1976) revealed that either oral administration of soybean or trypsin inhibitor or injections of pancreozymin (or its mimic substance, caerulein) in the rat over a week caused conspicuous enlargement of the pancreas, in which the exocrine cells underwent both hypertrophy and hyperplasia, whereas the endocrine cells were hyperplastic showing enlarged islets and increased mitotic figures. This result prompted us to investigate as to whether the hormone contents of the pancreas might be increased after the continued stimulation by endogenous or exogenous pancreozymin.
Materials and Methods

Using young adult male rats of the Wistar strain, two series of experiments were performed.

Series I
Rats weighing 180 g were grouped into three. In the first group, the animals received subcutaneous injections of pancreozymin (Eisai Co., purified from hog intestine according to the method by Tachibana, 1973) for 7 days: 40 Ivy Dog Units (I.D.U.)/kg were given daily, divided into four doses (at 7:00, 12:00, 17:00 and 21:00). In the second group, the rats were given for 7 days only a solution of trypsin inhibitor as drinking water. “Trypsin inhibitor soybean” (Miles), 400 mg, was dissolved in 100 ml water and the pH value was adjusted to 7.0 with dilute NaOH. The third group comprised control animals with no treatment.

Series II
Rats weighing about 180 g were grouped into three. The first group was treated with the same pancreozymin administration as in the first group of Series I. The second group received for 7 days an intensive treatment with pancreozymin: every day, depot-type pancreozymin, made by emulsification in sesame oil, was subcutaneously injected in the morning (7:00) and in the evening (21:00), each dose 50 I.D.U., while in the daytime 10 I.D.U. of normal pancreozymin was injected twice (12:00 and 17:00). The third group was control animals given sham injections of saline on the same time schedule as the first group.

All the animals in the two series were fed with commercial rat meals. On the 7th day of each treatment, they were fasted for 20 hrs and killed in the evening (17:00) by decapitation.

The pancreas was carefully and totally removed and weighed. In Series II larger lymph nodes embedded in the pancreas were excluded; thus the organ weight in this series was smaller than in Series I. In either method, weighing the pancreas with or without the embedded lymph nodes, the pancreas weight in each group of animals was satisfactorily constant (Table I, Fig. 1).

The pancreas, after being weighed, was frozen in dry ice. The organ of each animal was extracted by the acid-alcohol method and made into acetone powder. In each pancreas of Series I and II, insulin content was measured by a radioimmunoassay, using an insulin RIA kit (Dinabot Co., Tokyo). Glucagon content was radioimmunoassayed in each pancreas of Series II, using specific antisera to pancreatic glucagon, G-8 (Ito et al., 1976).

---

### Table 1

<table>
<thead>
<tr>
<th>Series</th>
<th>PZ 40 I.D.U/d</th>
<th>Trypsin Inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>102.0 ± 15.7</td>
<td>183.4 ± 12.7</td>
</tr>
<tr>
<td>7 days</td>
<td>216.3 ± 5.0</td>
<td>244.3 ± 2.8</td>
</tr>
<tr>
<td>38 days</td>
<td>301.6 ± 13.0</td>
<td>364.9 ± 12.0</td>
</tr>
<tr>
<td></td>
<td>660 ± 25</td>
<td>600 ± 15</td>
</tr>
</tbody>
</table>

**Body wt (g)**
- Control: 261 ± 9.6
- 7 days: 285 ± 11.7
- 38 days: 312 ± 13.0

**Pancreas wt (mg)**
- Control: 64 ± 6.0
- 7 days: 86 ± 6.2
- 38 days: 102 ± 6.5

**Insulin contents (mU)**
- Control: 205 ± 10.2
- 7 days: 245 ± 10.3
- 38 days: 295 ± 10.4

**Glucagon contents (ng)**
- Control: 412 ± 24
- 7 days: 721 ± 111
- 38 days: 1021 ± 24

Figure represents mean ± S.E. of 5 animals. a, b, c, d, e; Statistically different from control value at the level of P < 0.001, 0.05, 0.01.
Results

Table 1 gives the results obtained in the two series of experiments. In the rats repeatedly injected with pancreozymin for 7 days, the pancreatic weight was increased in control value by 1.1 times in Series I and by 1.2 times (p<0.005) in Series II. The insulin content of the pancreas was increased to 1.5 times (p<0.02) as much as that of the controls in Series I, while to 1.25 times in Series II (Fig. 1). Not only the insulin-body weight ratio, but also the insulin-pancreatic weight ratio was raised. The glucagon content assayed in Series II was also increased but statistically insignificantly.

In the rats given drinking water containing trypsin inhibitor for 7 days, the pancreatic weight was increased more conspicuously (1.25 times as much in control

Fig. 1. Effects of 7 day administration of pancreozymin and trypsin inhibitor upon the pancreatic weight and insulin and glucagon contents. Striped bar: repeated subcutaneous injections of pancreozymin, 40 IDU/day. Dotted bar: oral administration of trypsin inhibitor. Dark bar: intensive treatment with depot type pancreozymin, 120 IDU/day. Clear bar: controls.
value, \( p < 0.01 \) than after the pancreozymin injections. The insulin content of the pancreas was increased to 1.7 times as much in control value (\( p < 0.005 \)) (Fig. 1). Its ratios to body weight and to pancreatic weight were both greater than after the pancreozymin injections.

The rats intensively treated with depot-type pancreozymin showed the most marked changes. The pancreas weighed 1.9 times (\( p < 0.001 \)) as much as that of the control animals. The insulin content of the pancreas, together with its ratio to body weight, was 1.9 times as much in control value (Fig. 1) but its ratio to pancreatic weight was not significantly increased. The glucagon content of the pancreas also was markedly increased (2.4 times, \( p < 0.001 \)). Its ratio not only to body weight (\( p < 0.005 \)), but also to pancreatic weight (\( p < 0.05 \)) was significantly increased.

**Discussion**

The trophic effect of pancreozymin has been understood to be linked with the secretagogous effect of this hormone upon the acinar cells. Little attention has been paid to a possible trophic effect upon the endocrine pancreas, although the insulin and glucagon release from islet cells has been known to be stimulated by pancreozymin (Unger et al., 1967; Pfeiffer et al., 1973). The present result reveals that the insulin and glucagon contents of the pancreas treated with pancreozymin were increased in parallel with the increase in pancreatic weight or even more intensely than the latter. This seems to be compatible with our morphological observations (Fujita et al., 1976; Yanatori and Fujita, 1976) that the endocrine cells of the pancreas undergo mitotic proliferation in rats treated with trypsin inhibitor or with pancreozymin. Although we reported in those papers that only B cells were found in mitosis, we later confirmed that also A cells were involved in mitotic division in those rats.

With regard to a possible argument that the insulotrophic effects of pancreozymin might be due to other agents, such as GIP, contaminating the commercial hormone or released together with the hormone, it is worthy to mention that administration of caerulein, a pancreozymin-mimicking synthetic was found to cause insulin and glucagon release in the dog on one hand, and hyperplasia of islet cells in the rat on the other hand (Fujita et al., 1976).

Swedish researchers (Ihse et al., 1976a; Lundquist et al., 1976) recently reported that the oral administration of trypsin inhibitor to alloxan diabetic rats markedly improved the exocrine and endocrine functions of the pancreas. Amylase contents in the pancreas decreased in alloxan diabetes were recovered by trypsin inhibitor. The elevated blood glucose levels were lowered, while the lowered values of the plasma insulin-blood glucose ratio were elevated indicating improved insulin secretion. Moreover, the low insulin content of the diabetic pancreas was significantly elevated. On the basis of these findings, Ihse et al. (1976a) suggested that the oral administration of trypsin inhibitor induced a trophic effect on the remaining insulin-secreting cells of the diabetic rat. Ihse et al. (1976a and b) examined the effects of trypsin inhibitor feeding and pancreozymin injections also in normal rats but failed to demonstrate positive results such as elevated insulin contents of the pancreas.

The important suggestion of the present result in the normal rat, together with the reports by Ihse et al. (1976a) in diabetic rats, is that either oral administration of proteinase inhibitors causing release of endogenous pancreozymin, or repeated injections of this hormone will exert a cura-
tive effect on diseases with a degenerative or defective endocrine pancreas, especially certain types of diabetes mellitus. As mentioned above, our previous study using caerulein (Fujita et al., 1976) suggests that this substance mimicking pancreozymin will be effective as well. As shown in the present study, depot forms of the hormones deserve consideration for practical use.

Acknowledgements

Pancreozymin and its depot form were kindly provided by Dr. Shinro Tachibana of the Eisai Co. Ltd. Sincere thanks are also due to Dr. Tomio Kanno, Professor of Physiology at the Hokkaido University School of Veterinary Medicine for his valuable suggestions. The authors also cordially thank Dr. Seiki Ito, First Department of Internal Medicine, Niigata University School of Medicine, for his kind cooperation and valuable advice.

References


