Enhancement of pancreatic enzyme synthesis by pancreozymin

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ROTHMAN, S. S., AND HERBERT WELLS. Enhancement of pancreatic enzyme synthesis by pancreozymin. Am. J. Physiol. 213(1): 215-218. 1967.—These experiments were designed to ascertain whether pancreozymin would affect synthesis as well as secretion of exportable pancreatic enzymes. Pancreozymin (20 U/kg), secretin (20 U/kg) or methacholine (5 mg/kg) were injected subcutaneously 3 times a day for 4 days. The rats were fasted and killed 16 hr after the last injection. Pancreozymin, but not secretin or methacholine, caused a significant increase in pancreas weight attributable to hypertrophy of pancreatic acinar cells. In the enlarged pancreas, tissue concentrations of trypsinogen, chymotrypsinogen, and amylase were significantly increased. The change in enzyme concentrations was “non-parallel,” for the increase in chymotrypsinogen and amylase was more marked than that of trypsinogen. The data indicate that pancreozymin enhances the synthesis of three exportable pancreatic enzymes.

secretin; chymotrypsinogen; methacholine; trypsinogen; pancreas weight; amylase

The administration of exogenous pancreozymin or the reflex stimulation of its release produces an enzyme-rich pancreatic juice and a simultaneous diminution in the number of zymogen granules in acinar cells (1). On the basis of these observations, it is generally thought that pancreozymin stimulates transport of enzyme contained in zymogen granules out of pancreatic acinar cells. Although pancreozymin clearly enhances enzyme secretion, its effect on enzyme synthesis by the pancreas remains unclear. Since certain polypeptide hormones, ACTH and TSH, for example, appear to affect synthesis as well as “release” in their target organs, it seemed likely that duodenal hormones such as pancreozymin might also serve such a dual function. The present results suggest that this is indeed the case.

METHODS

Male and female rats (Holtzman Co.) were used in these studies. They were fed Purina laboratory chow and tap water ad lib. The animals were fasted for 16-24 hr prior to autopsy. Rats were killed by an overdose of ether. The pancreas and, in one experiment, the submandibular and parotid glands, were quickly removed, dissected free of connective tissue, blotted gently on filter paper, and wet weights determined. All dissections were carried out by one investigator and, to minimize the possibility of inconsistent dissecting technique, the treatment of the animals prior to autopsy was not known to the disector. In some experiments, small pieces of control and experimental pancreas were placed in Bouin fixative and later sectioned and stained with hematoxylin and eosin. Immediately after they were weighed, pancreata were homogenized in a 0.08 M sucrose solution (1:10 tissue to fluid). Homogenates were stored at 0 C prior to analysis for enzyme content. Tissue was thawed at 4 C overnight and then whole cells and nuclei were removed from the homogenate by centrifugation at 760 X g for 15 min. The esterase activity of trypsin and chymotrypsin were determined from the initial reaction velocities of their hydrolysis of P-toluene sulfonyl-L-arginine methyl ester-HCl (TAME) and N-acetyl-L-tyrosine ethyl ester-H$_2$O (ATEe), respectively (5, 7). The reaction mixtures were 1.0 ml phosphate buffer (pH 7.4), purified entero-kinase (minimum 0.2 mg) (3), and the sample. Sample size was 100 µl for trypsin analysis and 25 µl for chymotrypsin analysis. The reaction mixture was incubated at 37 C for 30 min. After incubation, the mixture was added to 3.0 ml 0.008 M ATEe or 0.027 M TAME. Hydrolytic activity was measured at room temperature (24-26 C) by pH-stat techniques at a pH of 7.8 for both trypsin and chymotrypsin. Amylase activity was estimated by using solubilized starch as substrate and iodine to determine substrate presence (6). For amylase determinations, 1.0 ml homogenate was used and diluted as much as 1/2000 prior to assay. Reaction mixtures were incubated at 37 C for 30 min and the reaction stopped by the addition of 1.0 N HCl. The color present was measured on a Klett colorimeter with a filter at 622.5 ma. Trypsin and chymotrypsin activities are expressed as micromoles of substrate split per min$^{-1}$ X g tissue$^{-1}$ and amylase activity is expressed as milligrams of starch hydrolyzed per min$^{-1}$ X g tissue$^{-1}$. 

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RESULTS

The administration of hormones such as ACTH and TSH result in increased size and weight of their target organs. Our first experiment was to determine whether this was also true for gastrointestinal hormones. Toward this end, pancreozymin (20 U/kg) and secretin (20 U/kg) were administered intraperitoneally three times a day for 4 days. The rats were autopsied 16 hr after the last injection, and the results (Fig. 1) show that the administration of pancreozymin, but not secretin, caused a significant (P < 0.01) increase in the wet weight of the pancreas.

Another experiment was designed to determine whether this effect of pancreozymin was accompanied by changes in pancreatic content of three enzymes, trypsinogen, chymotrypsinogen, and amylase, whose secretion is enhanced by administration of the hormone. In this experiment, methacholine, which has also been shown to strongly affect enzyme secretion, was administered as well. Because methacholine has a profound effect on salivary as well as pancreatic secretion, the weights of the submandibular and parotid glands were also obtained. Pancreozymin (20 U/kg), secretin (20 U/kg) and methacholine chloride (5 mg/kg) were administered subcutaneously 3 times a day for 4 days. The rats were killed with ether 16 hr after the last injection, during which time they had been deprived of food, but not water. The results (Table 1) again demonstrated that pancreozymin caused an increase in the mean pancreatic weight. On the other hand, only methacholine caused a significant increase in the weight of the parotid salivary glands. None of the agents had a significant effect on the weight of the submandibular glands. Pancreozymin administration resulted in a marked increase in the concentration of all three enzymes. Mean enzyme concentrations were slightly, but not significantly, elevated by administration of both secretin and methacholine. Probability values were determined from an analysis of variance.

Pancreozymin caused a greater relative increase in the concentration of chymotrypsinogen and amylase as compared to its effect on the concentration of trypsinogen (Table 2). Therefore, the ratio of chymotrypsinogen to trypsinogen increased significantly after pancreozymin (P < 0.05) whereas the amylase-to-chymotrypsinogen ratio was unaltered. Neither secretin nor methacholine significantly altered the relative enzyme concentrations.

Microscopic examination of sections taken from the pancreata of the four groups revealed a moderate enlargement of the acinar cells only in rats which had received pancreozymin. There was no observable increase in the normally low number of mitotic figures. Neither the ducts nor the islet cells appeared to be altered appreciably. The suggestion of acinar cell hypertrophy was confirmed by actual counts of acinar cell nuclei which in the enlarged glands were decreased by approximately 20%. The histological data thus suggests that the increase in pancreas weight following administration of pancreozymin was due to hypertrophy of exocrine acinar cells.

DISCUSSION

The primary action of pancreozymin is generally considered to be a stimulation of the secretion of enzymes by the pancreas. It has not been thought to influence the synthesis of these exportable proteins. The idea that pancreozymin does not promote the synthesis of proteins is based particularly on the observation that when it was added to a solution bathing slices of pigeon pancreas there was an augmentation of enzyme secretion but no enhancement of uptake of amino acids by the slices (2). The purpose of the present studies was to reexamine possible effects of pancreozymin on the synthesis of pancreatic enzymes. It seemed possible that the in vitro preparation of pancreas slices, the short incubation period used in these studies, and the indicator method for protein synthetic activity might have combined to yield a relatively insensitive measure of the effects of pancreozymin on protein synthesis. For this reason, we adopted a different experimental approach. In the present experiments relatively large amounts of the hormone were administered in vivo for several days. The synthesis of enzymes was assessed by measurement of the gland content of enzymes rather than by amino acid uptake. With this procedure we demonstrated a marked increase in the tissue content of three exportable enzymes as well as a moderate increase in organ weight. Recently, Webster and Tyor (8) demonstrated an increased uptake of L-phenylalanine-14C by pigeon pancreas using a in vitro technique (viz., injecting the hormone into the animal and measuring amino acid uptake subsequently in vitro). Their results and ours suggest that, in at least two species, rats and pigeons, pancreozymin can augment the synthesis of exportable protein.

![Figure 1](http://ajplegacy.physiology.org/DownloadedFrom/10.1152/ajplegacy.10.3.31.1.216.1207)
PANCREOZYMIN-ENHANCED Pancreatic Enzyme Synthesis

TABLE 1. Effect of pancreozymin, secretin, and methacholine on parotid and pancreas weight and pancreatic concentrations of trypsinogen, chymotrypsinogen, and amylase

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Final Body Wt. g</th>
<th>Parotid Gland Wt. mg</th>
<th>Weight, mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control injection (6)</td>
<td>223±3</td>
<td>353±34</td>
<td>872±24</td>
</tr>
<tr>
<td>Pancreozymin (5)</td>
<td>234±4</td>
<td>390±30</td>
<td>1,065±89</td>
</tr>
<tr>
<td>Secretin (6)</td>
<td>226±3</td>
<td>324±34</td>
<td>814±26</td>
</tr>
<tr>
<td>Methacholine (7)</td>
<td>215±3</td>
<td>339±34†</td>
<td>1,187±84</td>
</tr>
</tbody>
</table>

Values are means ± se. No. in parentheses = no. of rats. P values compared with control injections. * = P < .05. † = P < .001.

TABLE 2. Effect of pancreozymin, secretin, and methacholine on pancreatic enzyme ratios

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ratio of enzyme activities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trypsinogen</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Trypsinogen</th>
<th>Chymotrypsinogen</th>
<th>Amylase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (6)</td>
<td>9.6 ± 1</td>
<td>10.4 ± 1</td>
<td></td>
</tr>
<tr>
<td>Pancreozymin (5)</td>
<td>13.8 ± 1</td>
<td>9.0 ± 1</td>
<td></td>
</tr>
<tr>
<td>Secretin (6)</td>
<td>10.5 ± 1</td>
<td>8.9 ± 2</td>
<td></td>
</tr>
<tr>
<td>Methacholine (7)</td>
<td>9.2 ± 2</td>
<td>8.3 ± 2</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± se. No. in parentheses = no. of rats. * = P < .05, as compared with controls.

The increased rate of protein synthesis after pancreozymin administration could result from increased enzyme secretion or transport which in some manner produces a secretory “work” enhancement of synthetic activities. That is, the process of enzyme transport may be a stimulus for elevated production of the enzymes to be secreted. Pancreozymin might, however, act on both the transport and synthetic mechanisms directly. If stimulation of enzyme secretion is the stimulus for enhancement of synthetic activity, then any substance that promotes secretion of enzymes should yield pancreata containing increased amounts of enzyme. However, methacholine chloride, which produces a large protein secretory response by the rat pancreas, had little effect on enzyme content or gland weight (Table 1). Although the size of the pancreas was not increased by the dose of methacholine administered, there was considerable enlargement of the amylase-secreting rat parotid gland (Table 1). Of course, a different regimen of methacholine chloride injection at a higher dose or for a longer duration might have resulted in enhancement of pancreatic protein synthesis. Nevertheless, these results suggest that secretion (protein transport) per se by the pancreas does not necessarily produce compensatory enhancement in the synthesis of the proteins being exported.

Secretin (Vitrum) administration produced a slight increase in pancreas weight and enzyme content. Since the Vitrum preparation of secretin possesses some pancreozymin-like activity, it is likely that pancreozymin contamination was responsible for its effect.

Pancreozymin did not cause a uniform increase in the three pancreatic hydrolases assayed. The concentrations of chymotrypsinogen and amylase rose in a roughly parallel manner, whereas the concentration of trypsinogen increased but to a lesser degree (Table 2). After the administration of secretin or methacholine chloride there was no change in the ratio of enzyme concentrations. “Nonparallel” enhancement in the concentration of enzymes in pancreozymin-treated rats may result from either a preferential synthesis of the various enzymes or a preferential transport of one enzyme out of the acinar cells, producing a qualitative change in enzyme storage. Of course, a combination of the two effects is also possible. Recent studies by one of us (2X) demonstrated a nonparallel secretion of enzymes (trypsinogen favored over chymotrypsinogen) when pancreozymin was added to the solution bathing an in vitro preparation of rabbit pancreas (4). If the resultant change in secretory pattern were due to a differential effect of pancreozymin on the synthesis of one of the enzymes, an increase in the trypsinogen-to-chymotrypsinogen ratio in pancreatic tissue as well as in the juice of pancreozymin-treated rats should have been observed. Contrary to this expectation there was a significant decline in the tissue ratio. The nonparallel increase in tissue concentration of these two enzymes may, therefore, be the result of a nonparallel transport of enzymes.

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REFERENCES