Cholecystokinin/Pancreozymin Induces the Parallel Discharge of Digestive Enzymes from the in Vitro Rabbit Pancreas*

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Considerable controversy has surrounded the question of whether the exocrine pancreas discharges digestive enzymes in a parallel or nonparallel fashion. A recent report (Rothman, S. S., and Wilking, H. (1978) J. Biol. Chem. 253, 3543-3549) claimed that the in vitro rabbit pancreas demonstrated nonparallel enzyme discharge after stimulation with cholecystokinin/pancreozymin, but that parallel discharge followed stimulation with the COOH-terminal octapeptide of cholecystokinin/pancreozymin. It was suggested that the full hormone acted to inhibit chymotrypsinogen secretion while stimulating trypsinogen secretion. Because of the fundamental importance of this question to our understanding of the exocrine secretion of exportable proteins, we have repeated these experiments using the same preparation and stimulant but have observed only parallel enzyme discharge. We conclude that it is unlikely that cholecystokinin/pancreozymin causes the selective inhibition of chymotrypsinogen secretion.

It is generally believed that digestive enzymes synthesized on the rough endoplasmic reticulum of the pancreatic acinar cell migrate through a series of membrane-bound spaces before eventually being concentrated in zymogen granules. Fusion of the zymogen granule membrane with the luminal plasma membrane allows for enzyme discharge into the ductal system, and enzyme secretion is believed to occur exclusively by this mechanism. The zymogen granules contain a mixture of the various exportable proteins, and the ratios of these proteins to one another in zymogen granules is believed to approximate that found in the ductal juice. If this concept is valid, stimulation of enzyme secretion should cause a parallel increase in the ductal content of each of the digestive enzymes, and the ratios of these enzymes to each other should remain constant. In 1966, Rothman (2) reported that an impure preparation of cholecystokinin/pancreozymin stimulated trypsinogen secretion more than chymotrypsinogen secretion, and thereby induced the nonparallel discharge of digestive enzymes from the in vitro rabbit pancreas. Subsequently, Adelson and Rothman (3) reported that an extract of duodenal chyme homogenate elicited the preferential secretion of chymotrypsinogen. These observations challenged the so-called "mass transport" theory of Palade, as Rothman (4) suggested that the digestive enzymes were present within at least two distinct intracellular pools, one of which included the zymogen granules while the other included digestive enzymes located outside the granules but within the cytoplasm. He argued that the composition of these pools might differ and that nonparallel secretion indicated that the pools could be selectively discharged following hormonal stimulation.

Considerable controversy has surrounded the question of whether nonparallel secretion occurs, and recent reports have either confirmed or refuted its existence in a variety of species (5-11). Because of the fundamental importance of this question to our understanding of the intracellular transport and secretion of exportable proteins, we chose to re-examine this issue (12). Our studies indicated that the in vitro rabbit pancreas demonstrated only parallel secretion of amylase, trypsinogen, and chymotrypsinogen after stimulation with either methacholine or the synthetic COOH-terminal octapeptide of CCK-PZ' (Sincalide or CCK-OP). A parallel response followed exposure to a low concentration of purified CCK-PZ, but this stimulus was not of sufficient magnitude to cause significant increases in protein or enzyme secretion. A subsequent report by Rothman and Wilking (13) confirmed our observation that CCK-OP causes parallel secretion, but these workers found a 4-fold difference in the relative outputs of trypsinogen and chymotrypsinogen after stimulation with the full polypeptide hormone (CCK-PZ). They suggested that some portion of the naturally occurring polypeptide caused the selective inhibition of chymotrypsinogen secretion. In this communication, we report unsuccessful attempts to confirm these latter observations.

MATERIALS AND METHODS

All studies were performed using pancreata obtained from male New Zealand rabbits (2 to 3 kg body weight) after an overnight fast. After extraduodenal cannulation of the pancreatic duct, the pancreas was removed, mounted, and maintained in organ culture as previously described (12). The gland was allowed to equilibrate for 1 h, and then pancreatic juice was collected over the three subsequent 1-h periods. After the 1st h of collection, CCK-PZ (pure 39-amino acid polypeptide kindly provided by Professor V. Mutt of the Karolinska Institutet) was added to the incubation fluid at a final concentration of 2.5 µg/100 ml. The volume of pancreatic juice secreted and the concentrations of protein, amylase, trypsinogen, and chymotrypsinogen in the juice secreted over each of the subsequent two 1-h periods were determined as previously described (12). For the statistical analysis of data, results from the 2nd and 3rd h (i.e. 1st and 2nd h after addition of CCK-PZ) were compared to those obtained during the 1st hour (i.e. secretion obtained prior to addition of CCK-PZ). The data were analyzed in the following two ways. Raw data were analyzed using the paired t-test (14). In addition, data were analyzed using the Wilcoxon matched pairs sign test (14) after the post-CCK-PZ values were calculated as a percentage of the pre-CCK-PZ value.

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The abbreviations used are: CCK-PZ, cholecystokinin/pancreozymin; CCK-OP, synthetic COOH-terminal octapeptide of cholecystokinin/pancreozymin.

7228
ratios could be detected (Fig. 1).

Poststimulation result as a percentage of the prestimulation effect of animal variation was eliminated by expressing the value for each pancreas, no significant changes in the enzyme level, and the ratios of these enzymes to each other were not altered by addition of CCK-PZ (Table I). Even when the output followed addition of CCK-PZ. The outputs of amylase, trypsinogen, and chymotrypsinogen are expressed as output/hour.

The results of these studies are shown in Fig. 1 and Table I. A 3- to 4-fold increase in protein and digestive enzyme output followed addition of CCK-PZ. The outputs of amylase, trypsinogen, and chymotrypsinogen all rose to the same degree, and the ratios of these enzymes to each other were not altered by addition of CCK-PZ (Table I). Even when the effect of animal variation was eliminated by expressing the poststimulation result as a percentage of the prestimulation value for each pancreas, no significant changes in the enzyme ratios could be detected (Fig. 1).

These findings indicate that CCK-PZ induces the parallel discharge of digestive enzymes from the in vitro rabbit pancreas. Although the degree of protein and digestive enzyme secretion induced by CCK-PZ varied considerably among the various pancreata (Fig. 1), the amylase/trypsinogen, amylase/chymotrypsinogen, and trypsinogen/chymotrypsinogen ratios in the pancreatic juice remained remarkably constant.

In the past, our failure to demonstrate nonparallel discharge with CCK-PZ was attributed to our use of nonstimulating concentrations of the secretagogue (13). In this communication, we report results of studies which employed CCK-PZ concentrations sufficient to induce mean increases in protein and digestive enzyme outputs which were 3- to 4-fold higher than the unstimulated values, and in some preparations 6- to 7-fold increases were noted. Thus, even with significant CCK-PZ stimulation, only a parallel discharge pattern was observed.

We are unable to explain our failure to confirm the findings of Rothman and Wilking (13). Our model is virtually identical to their model, and we have used the same secretagogue, at the same concentration, that they have employed. In addition, our assay conditions were studied by Rothman and Wilking and found to give results similar to those obtained using their assay conditions (13). Although they noted a greater peak response to CCK-PZ, nonparallel discharge was also observed when responses comparable to those reported in this communication were elicited.

Our failure to observe CCK-PZ-induced nonparallel discharge of digestive enzymes argues strongly against the hypothesis (13) that CCK-PZ binds to receptors mediating inhibition of chymotrypsinogen secretion. On the other hand, the finding of parallel discharge after CCK-PZ stimulation does not necessarily mean that digestive enzymes are discharged from only one intracellular pool. If, for example, the digestive enzyme composition of the zymogen granule and purported cytoplasmic pools were identical, parallel increases in the secretion of each enzyme would follow discharge from either or both pools. Similarly, nonparallel discharge, if observed, would merely indicate that the composition of these pools was not identical.

### RESULTS AND DISCUSSION

The results of these studies are shown in Fig. 1 and Table I. A 3- to 4-fold increase in protein and digestive enzyme output followed addition of CCK-PZ. The outputs of amylase, trypsinogen, and chymotrypsinogen all rose to the same degree, and the ratios of these enzymes to each other were not altered by addition of CCK-PZ (Table I). Even when the effect of animal variation was eliminated by expressing the poststimulation result as a percentage of the prestimulation value for each pancreas, no significant changes in the enzyme ratios could be detected (Fig. 1).

### TABLE I

**Response of in vitro rabbit pancreas to CCK-PZ**

Results represent mean ± S.E. of 12 experiments. The stimulant (CCK-PZ) was added after the Hour 1 collection. Protein, amylase, trypsinogen, and chymotrypsinogen are expressed as output/hour.

|          | Hour 1       | Hour 2       | Hour 3       | Significance |
|----------|--------------|--------------|--------------|--------------|
| Volume (µl/h) | 240 ± 30    | 220 ± 20    | 170 ± 20    | N.S.         |
| Protein (mg/h) | 0.76 ± 0.14 | 2.17 ± 0.27 | 1.57 ± 0.47 | p < 0.001    |
| Amylase (units/h) | 69.8 ± 16.5 | 185.5 ± 42.6 | 130.6 ± 31.9 | p < 0.01     |
| Trypsinogen (units/h) | 36.0 ± 8.0 | 110.1 ± 17.8 | 74.0 ± 26.4 | p < 0.006    |
| Chymotrypsinogen (units/h) | 2.04 ± 0.52 | 6.47 ± 1.30 | 3.73 ± 0.93 | p < 0.005    |
| Amylase/trypsinogen | 1.90 ± 0.24 | 1.76 ± 0.25 | 1.91 ± 0.29 | N.S.         |
| Amylase/chymotrypsinogen | 35.3 ± 4.5 | 31.1 ± 4.0 | 34.4 ± 4.6 | N.S.         |
| Trypsinogen/chymotrypsinogen | 18.8 ± 1.1 | 18.1 ± 1.2 | 19.0 ± 1.8 | N.S.         |

*Hour 2 and 3 values were compared to Hour 1 value using a paired t-test. N.S. indicates p values of >0.05.

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