Independent secretion of different digestive enzymes by the pancreas

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Rothman, S. S. Independent secretion of different digestive enzymes by the pancreas. Am. J. Physiol. 231(6): 1847–1851. 1976. — Pancreatic secretion was collected from the cannulated duct of anesthetized rabbits during the perfusion of the upper duodenum with a balanced salt solution with or without 5 mM glucose. The secretion of amylase, chymotrypsinogen, trypsinogen, and total protein was measured. While glucose did not increase overall digestive enzyme secretion, it did change the proportions of the enzymes in secretion. In addition, the following was observed: 1) non-zero intercepts when the output of one enzyme was plotted against that of another (an enzyme-pair plot), 2) changes in the variance of the slope and intercept of enzyme-pair plots, and 3) an increase in the variance around the slope of an enzyme-pair plot, concurrent with a decrease in the variance around the slope of a plot for another enzyme pair that contained a common member. These observations suggest that different digestive enzymes can be secreted independently of each other.

Methods

Animal preparation. The upper duodenum (between the pylorus and the entrance of the pancreatic duct into the duodenum) of anesthetized (Dial-urethan, CIBA Pharmaceutical Co., 0.7 ml/kg body wt sc) male New Zealand rabbits was perfused with a bicarbonate-buffered Krebs-Henseleit medium (pH 7.4) (8) with or without the addition of 5 mM glucose. The flow of the perfusion fluid through the intestine was produced by using gas pressure (95% O₂ + 5% CO₂) as the driving force. The technique has been described elsewhere (19). Pancreatic secretion was collected from the cannulated duct (polyethylene tubing, PE-10) in tared vessels (19).

Assays. Trypsinogen, chymotrypsinogen, amylase, and protein were estimated in pancreatic juice. Chymotrypsinogen and trypsinogen were activated by incubation in 40 mg purified enteropeptidase (EC 3.4.21.9) (grade B, Calbiochem) per 100 ml 0.1 M sodium phosphate buffer (pH 7.4) for 30 min at 37°C. The presence of trypsin and chymotrypsin in activated samples was determined from the esterolytic activity of each enzyme using p-toluenesulfonyl-L-arginine methyl ester·HCl as the substrate for trypsin (EC 3.4.21.4) (28) and N-acetyl-L-tyrosine ethyl ester·H₂O as the substrate for chymotrypsin (EC 3.4.21.1) (31). Both reactions were measured as initial rates.

Amylase activity was estimated by the "solubilization" of a dye (Remazol Brilliant Blue R) from an amylase substrate to which it was covalently linked (14). The details of these enzyme assays for pancreatic secretion have been described elsewhere (2, 3, 15, 18, 20). The protein content of samples was estimated using the Folin phenol reagent (10).

Results

Gross response to duodenal perfusion of glucose. The addition of 5 mM glucose to the perfusion medium for 60 min did not manifestly alter the rate of digestive en-
zyme secretion by the pancreas; that is, enzyme secretion in general decreased gradually over time in the presence of glucose at much the same rate as it did in its absence (Fig. 1). Thus, the average secretory outputs of trypsinogen, chymotrypsinogen, amylase, and total protein were unaltered by glucose (Fig. 1).

Changes in proportions of enzymes secreted in presence of glucose. As mentioned above, a portion of the variability observed in the amount of enzyme secreted by animals, ostensibly in the same physiological state, is related to differences in the general secretory activity of different glands. The averaging of enzyme outputs incorporates this source of variance into the calculated standard deviation (Fig. 1) and makes it difficult to discern changes in the proportions of the enzymes secreted because of the resultant large and often overlapping variance (Fig. 1). This source of experimental variance can be isolated from the data by plotting the individual sample points for one enzyme measure against another, and determining the relationship between the two variables (Figs. 2 and 3). When this is done, the amount of one enzyme secreted relative to another is defined by the slope of the line (b), as well as by the intercept (a) if the line does not pass through the origin and is independent of the general secretory activity of the gland. When the present data were treated in this way and the relationship between the two variables was estimated by linear regression, substantial changes in the proportions of the enzymes in secretion were disclosed as a result of the glucose perfusion (Table 1), differences that were not apparent when averages were compared (Fig. 1). There was a greater than threefold increase in trypsinogen secretion relative to chymotrypsinogen, an approximately twofold increase in amylase secretion relative to chymotrypsinogen, and a smaller, but significant, increase in trypsinogen secretion relative to amylase (Table 1).

Under control conditions (no glucose in the perfusion medium), there was a positive intercept for trypsinogen secretion relative to both amylase and chymotrypsinogen (Table 1). This could mean either that trypsinogen secretion occurs in the complete absence of secretion of the other two enzymes (i.e., trypsinogen secretion can be completely independent of their secretion), or that the relationship between the respective outputs was actually nonlinear and the true intercept was still at the origin even though fitting the data to a linear regression erroneously indicated a non-zero intercept. There is evidence for both. The control values (×) in Fig. 2 (chymotrypsinogen vs. trypsinogen) can be fit about as well to a linear or nonlinear function (dashed line) (linear least-squares, r = 0.89; nonlinear least-squares, r = 0.84), and although we cannot distinguish between them statistically with the present data (N.B., a linear function is assumed in Table 1), in another study a statistically distinguishable nonlinear relationship has been observed between the secretion of these two enzymes by rabbit pancreas (19). Thus, an observed change in the proportions of trypsinogen and chymotrypsinogen in secretion might be the result of the displacement of data points toward the origin of a curvilinear relationship, as well as the result of a change in the slope of a function, or both may occur. On the other hand, the "+ glucose" data in Fig. 3 (amylase vs. chymotrypsinogen) are
clearly linear over the observed range and have a positive intercept (Table 1). In any event, both a positive intercept and a nonlinear function indicate that the proportions of the particular enzymes in secretion are not fixed or invariant.

Furthermore, the addition of glucose to the perfusion medium altered the apparent intercept. A positive trypsinogen intercept was no longer calculated relative to amylase (Fig. 2 and Table I), but still was relative to amylase (Table 1). Also, the slight positive chymotrypsinogen intercept (relative to amylase) that was statistically indistinguishable from the origin in the controls (Table 1) now appeared as a true positive intercept by statistical criteria, because the standard deviation of the intercept was sufficiently reduced by treatment (Table 1).

Sources of variance. If the general level of secretory activity were the sole source of variance in the measurement of enzyme secretion, then plotting the output of one enzyme against another should take that variance into account and produce a calculated regression line that fits the data points perfectly, i.e., the correlation coefficient should be 1. Although the data fit a regression line for the three enzyme pairs within 1% error for all of the conditions studied (Table 1), a substantial variance around the calculated functions was still observed (Figs. 2 and 3). Therefore, a portion of the variance is attributable to sources other than the general level of secretory activity, that is, it is either of biological origin or due to measurement error (predominantly assay error), or both. We can estimate the variance due to measurement error if we assume that the variance of the best-fit regression line for a given enzyme pair reflects measurement error alone, that is, no natural or biological variance exists in this case. This estimate gives a maximum value for this error. Since the estimate of error is derived from a plot of two enzyme activities, it is the sum of both assay errors. The square of the correlation coefficient can be used as an index of this error (30). If $r^2 = 1$, then there is no error. If $r^2 = 0.9$, then we estimate the error to be 10%, etc. When calculated in this way the error for trypsinogen ($E_{Tg}$) plus chymotrypsinogen ($E_{Cht}$) was 21%. The error for trypsinogen plus amylose ($E_{Amylase}$) was 17% and the error for chymotrypsinogen plus amylose was 8%. Since there are three equations (the sum of the individual errors equals the error of the pair for the three pairs) and three unknowns (the individual assay errors: $E_{Tg}$, $E_{Cht}$, $E_{Amylase}$), each individual maximum error can be calculated algebraically: $E_{Amylase} = 2\%$, $E_{Cht} = 6\%$, $E_{Tg} = 15\%$.

When the three enzymes were regressed together by multiple linear regression, the best fit gave an error of 24% ($r = 0.87$), which is in good agreement with the combined additive error of 23% for the enzymes calculated individually above. In other experiments, using a similar chymotrypsinogen assay (2), the maximum combined random assay error for chymotrypsinogen plus lipase was approximately 8%, as compared to $E_{Cht} = 6\%$ for the present data. Any other assay error would have to be systematic or nonvariable and would alter an observed slope in a constant manner and, thus, not affect comparisons between slopes.

If there were no additional variance beyond that due to the general level of secretory activity and measurement error, then the variance observed around the calculated line should have been roughly equivalent from time to time and under different experimental conditions, at least within the limits of sampling variability. This was not the case and a considerable, stimulus-induced variation in the standard deviation around the slope was observed (Fig. 4). Such differences can only be attributed to real differences in the proportions of the different enzymes secreted (see refs. 2 and 19 for other examples of the same situation). Put another way, the degree of relatedness or correlation between the secretion of different enzymes varies, as does the slope, as a result of experimental conditions (Figs. 2 and 3). If we compare the variances around the calculated slopes, we

**TABLE 1. Effect of duodenal perfusion of 5 mM glucose on proportions of three enzymes in secretion as determined by linear regression of enzyme pairs**

<table>
<thead>
<tr>
<th>Source of variance</th>
<th>Slope, b</th>
<th>Intercept, a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-Glucose</td>
<td>+Glucose</td>
</tr>
<tr>
<td>Trypsinogen/chymotrypsinogen</td>
<td>1.8 0.89 5.6 0.83 208%</td>
<td>5.1 0.3</td>
</tr>
<tr>
<td>Chymotrypsinogen/amylase</td>
<td>2.0 0.86 0.9 0.96 117%</td>
<td>0.6 0.6</td>
</tr>
<tr>
<td>Trypsinogen/amylase</td>
<td>0.4 0.1 0.2 0.2 11%</td>
<td>0.6 0.6</td>
</tr>
</tbody>
</table>

Number is 12 for each function. Goodness of fit, $P < 0.01$ for all functions; $r$, correlation coefficient; --glucose is data from periods 1 (20 min) and 2 (40 min) in Fig. 1; +glucose is data from periods 5 and 6 in Fig. 1; no changes in $b$ or $a$ were seen in time-paired controls. * Standard error of the estimate. \* Difference (– vs. + glucose) in all slopes significant at $P < 0.005$. \(8\)
can distinguish two types of apparently stimulus-induced changes in the correlation of the secretion of enzyme pairs. The first was an initial increase in the variance, followed by a decrease toward prestimulus control levels (Fig. 4A) for trypsinogen vs. chymotrypsinogen, $F = 6.3$, $P < 0.05$). This was also seen for trypsinogen vs. amylase, where $F$ approached significance at the 5% level. The second was a maintained decrease in the variance (chymotrypsinogen vs. amylase, $F = 4.0$, $P < 0.05$) (Fig. 4B).

**DISCUSSION**

A differential or nonparallel secretion of digestive enzyme was elicited by a stimulus (intestinal perfusion of glucose) that did not increase the overall secretion of digestive enzyme above “basal” levels. Since, in the present experiments, the acinar cells were filled with secretory protein and enzyme-containing zymogen granules as the result of an overnight fast, the amount of protein secreted in response to glucose was only a small fraction of the available digestive enzyme in the cell (20, 24). Thus, the differential secretion was in all likelihood the result of selective enzyme transport out of the cell and not the result of a turnover in the enzyme content of the tissue, as would be required if secretion occurred by a random or nonselective transport mechanism, which has been proposed for some years now (13).

Even in the absence of a substantial turnover in tissue enzyme content, new protein might nevertheless account for the response if it could bypass the major storage pools (presumably the zymogen granules in particular) on its way out of the cell. Indeed, such a bypass system has been demonstrated in this tissue (22, 24). If this occurred, then the effect would still be due to the differential transport of protein as well as its differential synthesis, since the secretion of new enzyme would have been favored over stored enzyme.

Whichever intracellular enzyme is secreted (new or old, or from one storage pool or another) and by whatever mechanism (exocytosis or membrane transport), the present observations indicate the rate of secretion of one digestive enzyme can be independent of another. That is, the selective secretion appears to involve, at least in part, the independent transport of different enzymes. This is suggested by, among other findings, the presence of non-0.0 or positive intercepts when the output of an enzyme is plotted against that of another (an enzyme-pair plot) (Table 1). One enzyme may be secreted in the absence of others. Furthermore, glucose perfusion increased the variance around the slope of one enzyme pair plot, while simultaneously decreasing the variance around the slope of a plot of another enzyme pair that contained a common member (Fig. 4). This indicates that the secretion of at least two of the three enzymes was independently determined. The independent secretion of different enzymes by the rabbit pancreas is also suggested by our recent reports (1, 2) that chymodenin, a peptide purified from hog duodenum, substantially augments the secretion of chymotrypsinogen, whereas it only modestly, if at all, increases the secretion of other digestive enzymes in this species.

These observations are consistent with the “equilibrium” or membrane transport hypothesis for protein secretion, which proposes that enzyme is secreted as the result of its variable and differential release from storage pools into the cytoplasm and its subsequent transport out of the cell across the luminal plasma membrane by independent equilibrium processes for each enzyme (2). The present observations are also consistent with an exocytosis hypothesis if zymogen granules contain single enzyme species (and, in addition, if the exocytosis of these granules were independently regulated). This does not seem likely on the basis of current knowledge, all of which supports the view that the enzyme contents of the zymogen granule are mixed (7, 12, 23, 37), although this view cannot be rigorously excluded. It is also conceivable that certain enzymes are excluded from specific zymogen granules in all cells or in certain groups of cells in such a way as to permit the independent secretion of enzyme by the preferential exocytosis of granules that are of mixed content but lack a specific enzyme or group of enzymes. Furthermore, a recent report (11) shows differences in the enzyme content of acinar cells from different parts of the pancreas, and thereby suggests that regional differences could be another way in which nonparallel transport could be accounted for by exocytosis. However, such differences by themselves could only account for the present observations if specific enzymes were wholly excluded from distinct regions of the gland. There is no evidence that this is the case. In fact, while the recent study cited above demonstrated that the proportions of certain enzymes were different in acinar cells in close proximity to the islets of Langerhans, large differences were not
observed, and neither were specific enzymes found to be wholly lacking from one area or the other. It should also be appreciated that perinsular cells represent only a minute fraction of the mass of secretory cells (of the order of the islets themselves) and there is no indication that other regional differences exist which could account for the variety of selective effects that have been observed.

Finally, in the absence of convincing evidence that exocytosis occurs in this system at all, no less than it occurs in a form consistent with the constraints that observations such as these would place on such a process, we should apply the law of parsimony in constructing hypotheses for protein secretion. That is, if different enzyme species can be secreted at rates that are independent of each other, as the present observations suggest, then a diffusional process should be the first considered, because diffusional processes account for this behavior the most simply.

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