Quantitative aspects of response of canine pancreas to duodenal acidification

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Although stimulation of pancreatic secretion by the presence of acid in the intestine has been recognized for over a century (9), the quantitative aspects of this effect have received little attention.

This is the report of experiments in conscious dogs to delineate the pancreatic response to varying acid loads introduced into the intestine by a variety of methods.

Methods

Four mongrel dogs, weighing from 16 to 22 kg, were prepared surgically with a permanent pancreatic fistula according to a method we have described (13). At a later operation a Thomas cannula (14) was inserted into the most dependent portion of the stomach to form a gastric fistula. At least 3 weeks were allowed for recovery from these procedures before experiments were started.

One of the animals died during the course of the experiments, as will be described, and was replaced with a dog of similar weight.

After an overnight fast, both gastric and pancreatic fistulas were opened as the dogs stood quietly in the laboratory. Two 15 min collections of gastric and pancreatic juice were always made to confirm fasting levels of secretion before proceeding with any experiments. The presence of food in the stomach or duodenum or unduly high basal levels of secretion caused the experiments to be abandoned for that day.

To introduce acid into the duodenum a small plastic tube (diameter 0.5 cm) was passed distally through the Thomas cannula of the pancreatic fistula for 4-6 cm. The pancreatic drainage tube was then screwed in position, and the open base of the Thomas cannula in the duodenum occluded firmly around both tubes with wadding. Since the pancreatic fistula in these animals was positioned about 6-8 cm from the pylorus, the open end of the plastic tube lay approximately 10-14 cm from the pylorus during the experiments. In one series of experiments acid was introduced directly into the stomach via a plastic tube which fitted snugly through a hole in a cork occluding the gastric cannula.

The acid used throughout was standard hydrochloric acid (1.0 N or 4.0 N, A. C. S. grade) which was diluted with distilled water to give the desired concentration. Infusion of acid through the delivery tube was maintained by a peristaltic pump (Harvard Apparatus Co.). By using tubing of different internal diameters combined with the variable speed control of the pump, the infusion rate could be varied at increments between 6.6 and 424 ml/hr. The concentration or flow rate was adjusted so that the amount of acid infused was doubled every 60 min.

In most of the earlier experiments with the introduction of acid into the duodenum, a small amount of phenol red (100 mg/liter) was added to the acid solutions before infusion, and the gastric juice collected during the experiments was observed when titrated to pH 7.0 for the presence of red coloration. This was taken to represent escape of the infused acid solution back up through the pylorus. Only one dog intermittently showed a trace of this marker in the gastric collections, especially at the higher rates of acid infusion. However, this was not constant and did not appear to be associated with any diminution in pancreatic secretion, and has been ignored in the cal-
culation of the results. All of the animals exhibited gross discoloration of gastric contents with phenol red when they retched, and this is discussed later.

The pancreatic responses to the continuous intravenous infusion of histamine dihydrochloride and to secretin (Vitrum A. B., Stockholm, Sweden) were also measured. The dose of both histamine and secretin was doubled every 60 min.

For comparison, the pancreatic responses were also measured to a single feeding of a meat meal and to multiple feedings at 30-min intervals of the same quantity of food in divided portions (total, 450 g). The food used was a proprietary kennel ration (Friskies, Carnation Co., Los Angeles, Calif.).

In the experiments with the introduction of acid into the duodenum, and during the response to secretin, the gastric fistula was open to avoid passage of endogenous acid into the intestine. In all other experiments the gastric fistula was closed. No stimulation of gastric secretion above basal levels was observed where juice was collected from the gastric fistula, and this has been omitted from the results.

Pancreatic juice was collected every 15 min. Bicarbonate concentration was measured by adding 0.5 ml pancreatic juice to 1.0 ml 0.1 N HCl, bringing the mixture briefly to the boil, and back-titrating the residual HCl with 0.2 N NaOH in an automatic titrator (pH 7.0). The output of bicarbonate in the diagrams is given in milliequivalents secreted per 15 min. Protein concentration in the pancreatic juice was determined by diluting samples 1:100 with distilled water and reading the optical density at 280 nm in a Zeiss spectrophotometer; this was compared with a standard solution of bovine serum albumin. The protein output by the pancreas was taken as an indication of the total enzyme secretion (6) and is expressed in milligrams protein secreted per 15 min.

The results are shown in the form of dose-response curves where possible. The mean value of the last two 15-min collections at any one dose level of the stimulus used was taken to construct these curves.

RESULTS

Intraduodenal acid infusion. Acid was infused into the duodenum by three different methods:

1) rate constant at 7.5 ml/15 min but concentration varying from 33.3 to 1,066 mN (0.25-8 mEq infused per 15 min); 2) concentration constant (37.8 mN) at rates varying from 6.5 to 106 ml/15 min (0.25-4 mEq infused per 15 min); 3) concentration constant (151 mN) at rates varying from 1.7 to 26 ml/15 min (0.25-4 mEq infused per 15 min).

The highest concentration of acid used was 1,066 mN and the highest flow rate at which it was infused was 7.5 ml/15 min. At this dose level, all of the animals exhibited restlessness, excess salivation, and retching. Twenty-four hours after the second experiment with this concentration of acid, one of the dogs died of...
PANCREATIC RESPONSE TO DUODENAL ACIDIFICATION

TABLE 1. Peak response of pancreatic fistula to various modes of stimulation

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Infusion Rate</th>
<th>Conc</th>
<th>Dose for Peak Response</th>
<th>Peak Vol, ml/15 min</th>
<th>Peak Bicarbonate, mEq/15 min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intravenous secretin</strong></td>
<td>Constant (7.5 ml/15 min)</td>
<td>Variable</td>
<td>300 U/15 min</td>
<td>22.8±8.7</td>
<td>3.01±0.36</td>
</tr>
<tr>
<td><strong>Intraduodenal HCl</strong></td>
<td>Constant (7.5 ml/15 min)</td>
<td>Variable</td>
<td>4 mEq/15 min</td>
<td>15.9±3.1</td>
<td>1.83±0.46</td>
</tr>
<tr>
<td></td>
<td>Variable (6.5-106 ml/15 min)</td>
<td>Constant (33.3 mN-1066 mN)</td>
<td>4 mEq/15 min</td>
<td>13.6±2.4</td>
<td>1.65±0.33</td>
</tr>
<tr>
<td></td>
<td>Variable (1.7-26 ml/15 min)</td>
<td>Constant (151 mN)</td>
<td>2 mEq/15 min</td>
<td>13.1±2.8</td>
<td>1.54±0.40</td>
</tr>
<tr>
<td><strong>Intragastric HCl</strong></td>
<td>Variable (6.5-106 ml/15 min)</td>
<td>Constant (160 mN)</td>
<td>17 mEq/15 min</td>
<td>15.2±1.5</td>
<td>1.81±0.20</td>
</tr>
</tbody>
</table>
| **Histamine dihydrochloride intra-
  venously (G.F. closed)**          | Constant (7.5 ml/15 min) | Variable | 0.5 mg/15 min | 22.1±2.4 | 2.85±0.36 |
| **Meat meal**                     | Single feedings     |       | 450 g            | 13.6±2.8 | 1.58±0.47 |
|                                   | Multiple feedings   |       | 75 g/30 min      | 20.4±3.0 | 2.51±0.70 |

Values are means ± standard error of the mean. G.F. = gastric fistula. *Maximal response. The peak response is designated a maximal response in those instances where doubling the stimulus caused no further increase in the response.

peritonitis following an acute perforation of the duode-
num 12 cm from the pylorus, in the area where the end of the plastic tube used for infusion was thought to have been situated. The highest rate of acid infusion was there-
fore limited in subsequent experiments to 4 mEq/15 min, and at this level no signs of distress were observed in any dog.

Figures 1 and 2 show that the pancreatic volume flow and bicarbonate output were related to the rate of infu-
sion of acid into the duodenum. Figure 3 shows that a straight-line relationship could be obtained by probit transormation of the bicarbonate output, confirming the sigmoid nature of the dose-response curve. It is ob-
vious from these figures that the flow rate and bicarbon-
ate output of pancreatic juice were related to the amount of acid infused into the duodenum per unit time and were independent of the volume or concentration of the acid infused. It is of interest that the peak rates of flow and bicarbonate output in these studies were lower than those obtained with other methods of pancreatic stimulation (Table 1).

Figure 4 shows that in these experiments there was an increase in the amount of protein secreted by the pan-
creas as the amount of acid infused into the duodenum was progressively increased. To compare the pancreatic protein response to duodenal acidification with that seen after secretin, a further series of experiments were per-
formed (Fig. 5). The response to a constant intraduoden-
al acid infusion (160 mN HCl at a rate of 7.5 ml/15 min) was compared to the response to a constant intra-
venous infusion of secretin (50 U/15 min). During the infusion of secretin a control infusion was made into the duodenum of 0.15 m NaCl at a rate of 7.5 ml/15 min. It can be seen that both these methods of stimulation caused comparable rates of flow of pancreatic juice and of bi-
carbonate output. However, the pancreatic protein out-
put to secretin had returned to basal levels of secretion within 45 min of the start of the infusion, whereas the protein response to duodenal acidification remained elevated above basal levels for 75 min after the start of the infusion. Yet Fig. 5 shows that with both of these methods of stimulation, protein output eventually fell to basal levels, although both were equally effective in maintaining increased rates of flow and bicarbonate out-
put.

**Intragastric infusion of acid.** Acid was infused into the stomach at a constant concentration (160 mN) at flow rates from 6.5 to 106 ml/15 min, to give 1.1-17 mEq in-
fused per 15 min. Figure 6 shows the pancreatic response in these experiments. The highest rate of infusion caused some retching in two animals. Comparison with the re-
results of intraduodenal acid infusion shows that more acid had to be introduced into the stomach to produce comparable pancreatic responses than into the duodenum. The peak rates of pancreatic secretion and bicarbonate output in this experiment were approximately the same
as those achieved with intraduodenal infusion of acid (Table 1).

Histamine (gastric fistula closed). It has previously been reported that the pancreatic response to histamine is small and transient when gastric juice does not enter the intestine (13). Figure 7 shows that closing the gastric fistula caused a large pancreatic response comparable to the peak rates of secretion observed with secretin or with multiple feedings (Table 1). It is of interest that higher rates of secretion were obtained with this method of stimulation of the pancreas than were seen when exogenous acid was introduced into the stomach.

Secretin. The pancreatic dose-response curve to secretin given by constant intravenous infusion is shown in Fig. 8. Pancreatic protein output was not measured in this experiment. The peak bicarbonate response to secretin (3.01 mEq/15 min) is similar to the maximal response described by Baron et al. (2) for conscious dogs. In the present study we were unable to establish this as a maximal response because of difficulty in obtaining a potent preparation of secretin. In fact, comparison between the responses seen in the present study and those previously described for similar dogs (13) suggests that the batch of secretin used was less than 50% of its indicated potency.

Meal responses. Figures 9 and 10 show the pancreatic responses to a single feeding and to multiple feedings of divided portions of the same amount of food (450 g). The peak rates of flow and of bicarbonate output after a single feeding were smaller than the peak rates after multiple feedings (Table 1). However, the peak rate of protein secretion after a single feeding (mean, 499 mg/15 min) was similar to the peak rate after multiple feedings (mean, 501 mg/15 min).

DISCUSSION

In 1902 Bayliss and Starling (3) demonstrated that acid in the intestinal lumen stimulated pancreatic secretion by a hormone. The method of release of this hormone, secretin, has been extensively studied over the ensuing years, but there have been relatively few reports of the quantitative aspects of the pancreatic response to intestinal acidification. Popielecki (12) recognized that the pancreatic response was larger when more acid was introduced into the intestine, but concluded that the main factor involved was probably the concentration of acid.
present in the lumen. The effect of varying the pH in the duodenum by a variety of methods was studied by Thomas and his co-workers (11, 15). They established that the threshold for stimulation of pancreatic secretion was not above pH 5 for acid solutions of various kinds, but were unable to correlate the rate of pancreatic secretion with pH levels in the duodenum during the response to a meal. This made them assert that the acidity of the intestinal contents during digestion of food plays a relatively minor role in determining the volume of pancreatic juice secreted.

Lagerlöf, Rudewald, and Perman (8) investigated the duodenal neutralization of acid introduced into the stomach of man, and were able to show that the rate of bicarbonate secretion into the duodenum was linearly related to the amount of acid entering the intestine from the stomach.

In the present study we have confirmed that the pancreatic response to duodenal acidification is related to the amount of acid delivered into the intestine. We have shown that the relationship between the rate of pancreatic secretion or bicarbonate output and the amount of acid infused was best expressed as a sigmoid-shaped dose-response curve (Figs. 1 and 2).

It is of interest that two other physiological responses to duodenal acidification also appear to depend on a receptor or receptors sensitive to the amount of acid presented to the intestine. Hunt and Knox (5) measured gastric emptying of test meals containing various acid solutions in man, and suggested that the results were consistent with the concept of control of gastric emptying somehow related to the amount of titratable acid transferred to the duodenum. Recently the inhibitory effect of duodenal acidification on gastric secretion has been found to show a linear relationship between rate of absorption of acid from the duodenum and degree of inhibition (7).

We have shown (Fig. 3) that the amount of bicarbonate secreted by the pancreas is much less than enough to neutralize the amount of acid required in the duodenum to elicit that response. For example, when HCl was infused into the duodenum at a rate of 1 mEq/15 min, the pancreatic bicarbonate response was 0.4 mEq/15 min. This suggests that the contribution of pancreatic juice to duodenal neutralization is considerably less than previously estimated. It is in agreement with the demonstration that the maximal secretory capacity of the canine stomach for acid secretion is about four times greater than the maximal secretory capacity of the pancreas for bicarbonate (13).

In the present study all the pancreatic juice secreted was continuously drained to the exterior, and no attempt was made to return the secretions to the duodenum. Aniss and Hallenbeck (1) have shown that the pancreatic response to a meal is significantly smaller when the secretion collected is immediately returned to the duodenum; they suggested that this effect is due to more complete neutralization of gastric acid when pancreatic bicarbonate is replaced in the duodenum, with a consequent decrease in secretin release. It would appear probable that a similar effect was operating in our experiments, and hence it is likely that the true pancreatic response to duodenal acidification in the intact animal is smaller than we have observed. This again suggests that
there are other important factors beside pancreatic secretion in the control of duodenal acidity. The peak rates of pancreatic secretion in response to the various methods of duodenal acidification by exogenous acid (Table 1) did not reach the peak rates observed on stimulation with exogenous secretin. A possible explanation of the low rates obtained during the introduction of acid into the duodenum (peak bicarbonate, 1.83 mEq/15 min) is that the infusion was introduced by a tube placed toward the lower end of the duodenum, since it has been shown that the secretin content of the intestinal mucosa declines as it is followed distally (10). This explanation is supported by the finding that higher rates could be achieved when gastric acid secretion was stimulated with histamine and allowed to enter the duodenum (peak bicarbonate, 2.85 mEq/15 min); the response in these experiments was presumed to be due to acidification of the upper duodenum, as histamine alone (in the absence of duodenal acidification) has been shown to have little effect on pancreatic secretion (13). The peak rates seen when exogenous acid was introduced into the stomach were smaller than those observed during histamine stimulation. No explanation can be offered for this finding, and it suggests that perhaps the stomach empties endogenous acid more readily than acid introduced by tube.

Wang and Grossman (16), in a study of the responses of the transplanted pancreas to the introduction of acid and other substances into the duodenum, found that acid caused a higher output of enzymes than observed after intravenous secretin, and suggested that acid causes the release of both secretin and pancreozymin. The pancreatic protein responses observed in the present study support this suggestion, except for the experiment shown in Fig. 5. This shows that the protein response to a moderate amount of acid infused into the duodenum (1.2 mEq HCl/15 min) was not maintained. Similarly the protein response to a constant intravenous infusion of secretin, after a brief increase, returned to basal levels of secretion. This latter finding is in agreement with the hypothesis that secretin is not a true stimulant of pancreatic enzyme secretion but only causes a “washout” of preformed enzymes present in the pancreatic ductular system (17). By analogy it can be argued that the protein response to acid infusion shown in Fig. 5 is also indicative that acid is not a true stimulant of pancreatic enzyme output when infused into the duodenum at the rate employed in this experiment. However, the protein responses observed in other experiments described in this report can hardly be explained on this basis, and it would appear that high rates of acid introduction into the duodenum exerted a direct stimulatory effect on the output of protein by the pancreas (e.g., Fig. 4).

The pancreatic responses to a single feeding of 450 g meat meal (Fig. 9) were considerably smaller than the rates of secretion obtained when the same amount of food was given in 75 g portions at intervals of 30 min (Fig. 10). In fact, the peak rates of flow and bicarbonate output after multiple feeding approached the highest rates obtained with intravenous secretin stimulation (Table 1). This finding is similar to the demonstration that a Haldenhain pouch of the stomach responds to multiple feedings with a greater output than seen after a single feeding (4).

REFERENCES