Canine pancreatic responses to intestinally perfused proteins and protein digests

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MEYER, J. H., AND G. A. KELLY. Canine pancreatic responses to intestinally perfused proteins and protein digests. Am. J. Physiol. 231(3): 682-691. 1976.—Pancreatic bicarbonate and protein secretory responses to intestinally perfused proteins or digests of proteins were measured in dogs with chronic gastric and pancreatic fistulas when luminal pancreatic protease concentrations were reduced to undetectable levels. Protein digests were analyzed for amino acid content, and various other indirect methods were used to assess the composition of the digest mixtures. Of five undigested proteins, none evoked more pancreatic secretion than a control perfusion with saline. Peptic digestion of the same proteins converted four of them to polypeptides that were potent stimuli of a pancreatic juice similar in HCO₃⁻/protein ratios to that evoked by luminal amino acids. Dialyzed peptic digests of one of the proteins, bovine serum albumin (BSA), retained potency. Likewise, digestion of BSA with endogenous or exogenous pancreatic proteases converted the protein to a stimulus about equipotent with the peptic digest, though the composition of the pancreatic digests differed markedly from that of the peptic digests. We conclude that a) luminal peptides of four or more amino acids can stimulate the pancreas and b) during protein alimentation a wide array of luminal protein products may evoke pancreatic secretion.

THE MECHANISMS MEDIATING pancreatic secretory responses to protein feeds remain enigmatic: previous work (18) indicated only a few luminal amino acids were potent and were effective only at luminal concentrations or loads above those probably encountered during normal alimentation. These findings raise the question as to whether oligopeptides, polypeptides, and/or even native protein in the small intestinal lumen may evoke pancreatic secretion. A preceding report (17) suggested that only luminal oligopeptides containing potent amino acids stimulated the pancreas. In the present study, protein solutions or solutions of protein digests, prepared in vitro, were instilled into the proximal small intestines of dogs with pancreatic fistulas to determine whether proteins or various protein digestive products were stimuli of pancreatic secretion.

METHODS

Mongrel dogs were prepared with chronic gastric and pancreatic fistulas of the modified Herrera type (11). At surgery, a cuff of Dacron mesh was placed around the first portion of the duodenum. Except for the use of the newer Herrera fistula, these dogs were entirely similar to those used in the preceding reports (17, 18). Likewise, methods of intestinal perfusion, collecting pancreatic secretion, and measuring pancreatic outputs of bicarbonate and protein were the same as used previously (17, 18).

In the present studies, however, no background infusions of intravenous secretin were given. Perfusions of protein or protein digest were begun only after the proximal bowel had been perfused for 1 h with 0.15 N NaCl at 25-50 ml/15 min, once all endogenous pancreatic juice had been diverted from the gut by insertion of the Herrera obdurator. After this initial hour of saline perfusion, luminal concentrations of endogenous pancreatic protease (trypsin) were negligible (Fig. 1).

As in previous studies (17, 18), dogs underwent multiple perfusions on each experimental day in random order. Results were compared between experimental and control perfusions or between two experimental perfusions with a t test for unpaired values. The results that were statistically significant by t test were further examined by analysis of variance for repeated observations, as previously described (18). In the case of multiple test perfusions (Figs. 2, 4, 5, 6, and 8), results were analyzed with an analysis of variance (which controlled for effects between dogs) followed by a multiple-range test (6).

Perfusates. Perfusates were prepared on the day of each experiment. All were instilled at pH 7 and at 25 ± 1 ml/15 min unless otherwise noted. The osmolarity of the various solutions ranged from 310 to 435 mosM, the higher tonicities resulting from 310 mosM NaCl plus small-molecular-weight protein digestive products in the various digests. All solutions contained phenol red, which served to indicate either a) reflux of perfusate from duodenum to stomach (indicator in drainage from gastric fistula) or b) leakage of perfusate into the pancreatic collecting system (indicator in collected pancreatic secretion). The former was never noted in these experiments; leakage of perfusate into the pancreatic collecting system was a common finding in these Herrera fistula dogs, usually appearing after 6-8 wk of experimentation (dogs were discarded once leakage was detected).

Bovine serum albumin (BSA) solutions were prepared from Cohn fraction V (Miles Laboratories). Other proteins used were crude egg albumin (Schwarz/Mann), bovine serum albumin; gastric fistulas; pancreatic fistulas
Pancreatin digests were prepared with crude pancreatin (Sigma Chemical Co.; 3 g of crude pancreatin/100 g wt/vol) was suspended in 113 mM NaCl + 25 mM CaCl₂, then centrifuged free of undissolved material. The supernatant was then added to various protein solutions in a ratio of 1 ml of pancreatin supernatant to 10 ml of protein solution. In control solutions, 1 ml of 113 mM NaCl + 25 mM CaCl₂ was added in place of pancreatin to every 10 ml of protein solution. Pancreatin digestion was carried out under constant agitation at 37°C. Digestion was scheduled so that the desired time of digestion was completed just prior to perfusion of the digest; during the hour of subsequent digest perfusion, the perfusate was iced, being brought back to room temperature as the perfusate traversed the infusion line. For analytical values, pancreatin digestion was terminated by a) mixing a sample of the digest with an equal volume of 7% HClO₄, or b) adding the appropriate amount of sample to biuret reagent, or c) adding formaldehyde (see below).

Chemical analysis. Total protein concentration was measured by the biuret method (8); biuret-reactive nitrogen (BRN) is a measure of nitrogen in protein and peptides longer than dipeptides (28). Free amino acids and two-member peptides produced only minimal absorbance at 540 nm over a large range of concentration, three-member peptides produced linear absorbance up to a concentration of 5 mg/ml, above which absorbance was constant and about half the maximal absorbance produced by proteins and/or longer peptides. Thus, a decrease in the BRN in various digest solutions in these experiments could be taken as evidence of conversion of protein substrate to free amino acids and/or oligopeptides of less than four amino acids in chain length.

The course of digestion was followed by measuring changes in formol titration values (ΔFo). Formol titration measures amino end groups, so that ΔFo values indicate the number of bonds split during digestion (20). The ΔFo values reported below were calculated in millimoles per liter by subtracting formol titration values of undigested protein solutions from the formol titration values of various digests of those same protein solutions.

Total free amino acid concentration (AA) was measured by the titrimetric Van Slyke method (25). This method was found specific for free amino acids (it did not measure peptide nitrogen), was precise (± 5%), and was found to be capable of detecting between 1.5 and 2 mM free amino acid in 2-ml samples of the digest solutions.

In addition to total amino acid concentration, the concentration of free aromatic amino acid (ArAA) was estimated in various digests by the method of LaDue and Michael (13). This L-amino acid oxidase method was specific for free, not peptide, aromatic amino acids, but it could not be applied with precision to analyzing concentrations of individual amino acids in mixtures of three aromatic amino acids. However, absorbance at 308 nm was found to be a reasonable measure of total ArAA (Y = 0.502X + 0.061; R = 0.99; where Y = absorbance, X = total ArAA (mM), and R = correlation coefficient) with known mixtures of the three aromatic amino acids. However, absorbance at 308 nm was found to be a reasonable measure of total ArAA (Y = 0.502X + 0.061; R = 0.99; where Y = absorbance, X = total ArAA (mM), and R = correlation coefficient) with known mixtures of the three aromatic amino acids. However, absorbance at 308 nm was found to be a reasonable measure of total ArAA (Y = 0.502X + 0.061; R = 0.99; where Y = absorbance, X = total ArAA (mM), and R = correlation coefficient) with known mixtures of the three aromatic amino acids. However, absorbance at 308 nm was found to be a reasonable measure of total ArAA (Y = 0.502X + 0.061; R = 0.99; where Y = absorbance, X = total ArAA (mM), and R = correlation coefficient) with known mixtures of the three aromatic amino acids. However, absorbance at 308 nm was found to be a reasonable measure of total ArAA (Y = 0.502X + 0.061; R = 0.99; where Y = absorbance, X = total ArAA (mM), and R = correlation coefficient) with known mixtures of the three aromatic amino acids.
Various peptic digests, as well as control solutions of undigested protein, were analyzed for NH₂-terminal amino acids. Dinitrofluorobenzene (DNFB) was conjugated to digested or undigested protein by adding an excess of DNFB to the protein solution containing 250-350 mg of protein and stirring constantly while maintaining the solution at a temperature of 40°C and a pH of 9, with a pH stat (1). The course of the reaction was followed by plotting the volume of 0.2 N NaOH added vs. time; the reaction was judged completed (usually after 80 min) when the slope of this line no longer changed and was similar to that of DNFB plus water (1). Extrapolation of this constant slope back to time zero provided quantitation of groups with which DNFB had reacted (1). Subtracting this value for undigested (control) protein solution from that of the digest thus gave an estimate (ΔDNFB) of the number of NH₂-terminal groups liberated in the course of the digestion.

The bulk dinitrophenyl (DNP)-peptides so generated were precipitated by acidifying the solutions to pH 1. The DNP-peptides were then collected by filtration over sintered glass. The ether-washed precipitate was hydrolyzed at 110°C for 16-18 h in 5.7 N HCl. After hydrolysis, free DNP-amino acids were extracted into diethyl ether. The ether was evaporated, and dinitrophenyl was removed by sublimation (1). Ultimately, material corresponding to 10-35 mg of original protein was applied to Whatman no. 1 filter paper for two-dimensional paper chromatography (14). The DNP-amino acids were identified by comparing spots with 2-6-DNP-amino acid standards (Sigma Chemical Co.) applied in both dimensions on the chromatograms. This method would not identify NH₂-terminal tryptophan (destroyed during hydrolysis) and arginine or histidine (not extracted into ether). The NH₂-terminal isoleucine could not be distinguished from leucine (14). In addition DNP-glutamate and aspartate were incompletely separated on chromatograms (14). The DNP-peptides soluble at pH 1 were not identified by comparing spots with 26-DNP-amino acid standards (Sigma Chemical Co.) applied in both dimensions on the chromatograms. This method would not identify NH₂-terminal tryptophan (destroyed during hydrolysis) and arginine or histidine (not extracted into ether). The NH₂-terminal isoleucine could not be distinguished from leucine (14). In addition DNP-glutamate and aspartate were incompletely separated on chromatograms (14). The DNP-peptides soluble at pH 1 were not routinely examined for DNP-amino acid content, except in the case of gelatin digests (see below).

Dialysis. Some peptic digests were dialyzed prior to perfusion to rid them of free amino acids and smaller peptides; 100 to 150 ml volumes of digests were placed in cellulose dialysis bags 1/4 inches in diameter and dialyzed 48 h without stirring at 5°C against 8 liters of 0.15 N NaCl in four changes of 2,000 ml each. Preliminary experiments had shown that under these conditions of dialysis 98-99% of free amino acids were removed and 90% of tripeptide was removed.

Plan of experiments. On each experimental day, two to four doses of stimulant (intestinally perfused protein, amino acid, or saline; intravenously synthetic octapeptide of cholecystokinin (CCK-OP)) were administered by constant infusion for hour-long periods. Periods of stimulation were alternated with hour-long basal periods during which the Foley balloons were deflated and the gut was perfused with 0.15 N NaCl. Doses of stimuli were randomized. When perfused amino acids were compared with perfused digests or intravenous CCK-OP (Squibb Institute of Medical Research), these different stimuli were alternated among experimental days in the same group of dogs. This same general design was employed when peptic digests of BSA were compared with peptic plus pancreatin digests of BSA.

RESULTS

Effect of peptic digestion time. Undigested 3.5% BSA plus pepsin produced no more pancreatic protein output than 0.15 N NaCl (P > 0.10). On the other hand, peptic digestion of 3.5% BSA for 30, 60, 120, 240 min produced digestive products that significantly stimulated (P < 0.05 vs. NaCl) output of pancreatic protein when infused into the proximal gut (Fig. 2). Outputs of pancreatic bicarbonate paralleled outputs of protein (data not presented, see below). When 3.5% BSA was incubated for 60 min in 0.15 N HCl without pepsin, then brought to pH 7 and perfused, there was no significant stimulation of pancreatic secretion (Table 1). The various digests had similar stimulating effects even though digestion beyond 30 min produced progressively more hydrolysis of BSA, as evidenced by a) a further increase in ΔF₀ and b) conversion of more and more BSA from precipitable to nonprecipitable BRN (Fig. 3). The highest AA concentration found in any of the digests was 1.8 ± 0.8 mM (in the 120-min digests); the highest ArAA concentration was 0.35 ± 0.20 mM (in the 60 min digests). None of the values of AA or ArAA in the digests differed signifi-

![Fig. 2](http://ajplegacy.physiology.org/DownloadedFrom/...)

**TABLE 1. Effect of incubating BSA in HCl with or without added pepsin**

<table>
<thead>
<tr>
<th>Perfusate, 25 ml/15 min</th>
<th>Pancreatic Protein Output, mg/15 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.15 N NaCl (control)</td>
<td>118 ± 24</td>
</tr>
<tr>
<td>3.5% BSA incubated 60 min in 0.16 N HCl, then brought to pH 7</td>
<td>112 ± 15</td>
</tr>
<tr>
<td>3.5% BSA incubated 60 min in pepsin + HCl, then brought to pH 7</td>
<td>225 ± 25*</td>
</tr>
</tbody>
</table>

Values are means ± SE (vertical bars) from 6 observations/3 dogs. All digests of 30 min and longer stimulated more (P < 0.05, t test, multiple-range test) protein output than did saline or the 0-min digest.

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Effect of BSA concentration. Bovine serum albumin concentrations of 1.75%, 3.5%, 7%, and 14% were digested for 60 min in pepsin and then perfused after digestion was terminated. Increasing initial concentrations of BSA produced increasing outputs of both pancreatic bicarbonate (Fig. 4) and protein (Fig. 5). This pattern of increasing responses with increasing digest concentrations paralleled increasing values for total AFo and for nonprecipitable BRN, but, conversely, the more potent and more concentrated BSA digests had less nonprecipitable BRN and less AFo per gram of BSA than the less potent digests (Table 2). None of these digests contained significant concentrations of AA (Table 2) or ArAA (highest values were 0.13 ± 0.09 mM in the 7% digests).

These dose-response data for intestinally perfused BSA were compared in the same dogs with dose-response data to a) intravenous infusions of CCK-OP and b) intestinal perfusion of a mixture of L-phenylalanine and L-tryptophan. The protein response curves to all three agents were approximately parallel (Fig. 5); however, in the doses given, CCK-OP produced more protein output than either intestinal stimulus and somewhat less bicarbonate output relative to protein secretion (Figs. 4 and 5). Ratios of $HCO_3^-$ output/protein output did not significantly differ between BSA vs. CCK-OP or BSA vs. phenylalanine plus tryptophan.

Effect of dialysis of digest stimulation. The 60-min peptic digests of 7% BSA were dialyzed for 48 h to rid them of traces of free amino acids and small peptides. The dialyzates were then perfused and pancreatic responses to the dialyzates compared with those to a 0.15 N NaCl control perfusion and to a second control perfusion of the same 60 min 7% digest that was not dialyzed, but stored for 48 h similarly at 5°C. Both the dialyzed and undialyzed digests stimulated about the same protein output from the pancreas, significantly more than the NaCl ($P < 0.05$) (Table 3). The dialysis removed 20% of BRN from the digest (Table 3), indicating loss of peptides longer than two or three amino acids.

Pancreatin plus peptic digestion. Bovine serum albumin at 3.5% was digested in pepsin for 0, 30, and 240 min and perfused, after dilution with NaCl plus CaCl₂ (controls). Similar digests were further digested in vitro with pancreatin (in NaCl plus CaCl₂) for 60 or 240 min and then perfused. As previously noted, the 0-min peptic digest did not stimulate more than NaCl, whereas the 30- and 240-min peptic digests did (Fig. 6). However, incubation of the 0-min peptic digest with pancreatin for either 60 or 240 min converted the digest into a significant stimulus of pancreatic protein secretion (Fig. 6). On the other hand, pancreatin digestion of the 30- or 240-min peptic digests did not significantly alter the pancreatic protein responses to the peptic digests, even though the pancreatin digestion profoundly altered the composition of the protein digests by greatly increasing the ΔFo (Fig. 7), the AA (Fig. 7), and the ArAA (increased in parallel to AA from 0 mM in peptic digests).
**TABLE 2. Analyses from 60-min peptic digests of BSA**

<table>
<thead>
<tr>
<th>Initial BSA</th>
<th>Nonprecipi-</th>
<th>Nonpreci-</th>
<th>Δ Fo, mM</th>
<th>Δ Fo, g BSA*</th>
<th>AA, mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concent, g/100 g</td>
<td>table BRN</td>
<td>table BRN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.8</td>
<td>1.62±0.05</td>
<td>1.02±0.05</td>
<td>7.9±0.6</td>
<td>0.50±0.03</td>
<td>0.02±0.6</td>
</tr>
<tr>
<td>3.5</td>
<td>2.35±0.19</td>
<td>0.77±0.06</td>
<td>13.4±1.6</td>
<td>0.42±0.04</td>
<td>0.99±0.5</td>
</tr>
<tr>
<td>7</td>
<td>3.97±0.14</td>
<td>0.57±0.06</td>
<td>20.2±1.2</td>
<td>0.30±0.02</td>
<td>1.71±0.81</td>
</tr>
<tr>
<td>14</td>
<td>4.24±0.14</td>
<td>0.33±0.01</td>
<td>13.0±1.3</td>
<td>0.10±0.01</td>
<td>1.68±0.45</td>
</tr>
</tbody>
</table>

Values are means ± SE. * Calculated by dividing Δ Fo (mM) by total BRN (g/liter).

**TABLE 3. Dialyzed vs. nondialyzed 60-min peptic digests of 7% BSA**

<table>
<thead>
<tr>
<th>Perfusate</th>
<th>Total BRN, mg/ml</th>
<th>Pancreatic Protein Output, mg/15 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.15 N NaCl (control)</td>
<td>0</td>
<td>144±31</td>
</tr>
<tr>
<td>Dialyzed digest</td>
<td>52±2</td>
<td>268±44*</td>
</tr>
<tr>
<td>Undialyzed digest</td>
<td>65±2</td>
<td>290±42†</td>
</tr>
</tbody>
</table>

Values are means ± SE (8 observations/4 dogs) for perfusion at 25 ml/25 min. *P < 0.05 vs. NaCl control. † Analyses of variance showed responses to both digests differed (P < 0.06) from response to saline but responses to digests did not differ from each other.

**FIG. 6.** Pancreatic protein responses to intestinally perfused digests of 3.5% BSA. BSA was digested in pepsin alone for 0, 30, or 240 min (notation on horizontal axis) and then mixed with saline (open bars), pancreatin in saline and further incubated 60 min (dark bars), or pancreatin in saline and further incubated 240 min (lightly hatched bars). Saline controls consisted of saline or saline + pancreatin (incubated 60 or 240 min). Data are means ± SE, 8 observations/4 dogs. Asterisk indicates P < 0.05 compared with response to corresponding peptic digest + saline, either by t test or by multiple-range test.

Pancreatic juice to the lumen converted the perfused 0-min peptic digest into a stimulus of pancreatic protein secretion, but did not affect pancreatic responses to the 30- and 240-min peptic digests (Fig. 8).

**Comparison of BSA digest with mixture of component amino acids.** In these experiments, a mixture of amino acids was prepared that contained the individual l-amino acids in BSA corresponding to the reported composition of BSA (28). Cysteine was excluded because it could not be dissolved in the required concentrations. Moreover, tyrosine could not be dissolved at a concentration more than that contained in 2% BSA, and the toxicity of the amino acid mixture exceeded 320 mosM if the mixture was made up to contain amino acids in more than 2.2% BSA. For this reason, a 60-min digest of 1.75% BSA was perfused at varying rates of flow and pancreatic responses were compared to similar responses on perfusion of a mixture of amino acids in 1.75% BSA at similar flow rates. Pancreatic bicarbonate and protein responses were the same (P > 0.20) whether BSA was perfused as a 60-min peptic digest or as a complete hydrolysate (amino acid mixture) at comparable flow rates (Table 4).

**Other protein digests.** Casein, hemoglobin, gelatin, or egg albumin at 1.75% were perfused either undigested with pepsin or after 60 minutes of peptic digestion. Responses were compared with those from control perfusions of 0.15 N NaCl. All perfusions were at 100 ml/15 min. None of these proteins was as soluble as BSA; hence, lower concentrations were used at higher rates of flow. Digests of egg albumin, hemoglobin, and casein, digests to 16 mM in 240-min pancreatin digests), and by reducing the total BRN (from 31.4 ± 0.1 to 21.0 ± 0.7 mg/ml).

An analogous experiment was performed with endogenous pancreatic juice. In these experiments, the proximal gut was perfused with 0, 30, or 240-min 3.5% peptic digests of BSA for 75 min. All but 0.5 ml of pancreatic juice secreted in each preceding 15-min period was returned to the gut lumen at a uniform rate over each subsequent collection period. In control experiments, similar volumes of 43 mM NaHCO₃ (the approximate concentration in secreted juice) were injected into the proximal gut, in lieu of pancreatic juice. Returning the
but not gelatin, each stimulated more bicarbonate and protein output than did saline (P < 0.05) (Table 5). None of the undigested proteins significantly stimulated pancreatic secretion (P > 0.05 vs. NaCl control). As with BSA, peptic digestion of these proteins produced a ∆Fo, but did not liberate significant amounts of AA (Table 5).

**NH$_2$-terminal amino acids in peptic digests.** The NH$_2$-terminal amino acids analysis (Table 6) of peptic digests revealed that all peptic digests that stimulated pancreatic protein secretion contained the NH$_2$-terminal amino acids phenylalanine (Phe), valine (Val), leucine (Leu), and aspartate (Asp) in appreciable quantities. Gelatin digests (which did not stimulate) contained smaller quantities of NH$_2$-terminal valine and aspartate.

**DISCUSSION**

Thomas (23) reported that undigested egg albumin injected into the duodenum had no effect on pancreatic secretion, whereas peptic or peptic-tryptic digests of casein stimulated a juice high in protein and low in bicarbonate concentration (24). The present studies have extended these earlier observations. Other undigested proteins were shown to be without demonstrable effect even when perfused into the gut at appreciable loads (Table 5). Most proteins of dietary origin probably do not have solubilities exceeding those of egg albumin, gelatin, or hemoglobin. Even when a more soluble protein, BSA, was perfused at higher concentrations, no stimulation was observed: 3.5% BSA had no effect (Table 1) and neither did undigested 7% or 14% BSA (studied in separate experiments not reported here). Based on these findings, we surmise that undigested protein entering the gut lumen has little effect on pancreatic secretion.

By contrast, all digests of BSA stimulated pancreatic secretion—whether BSA was digested in peptic, pancreatic, or endogenous pancreatic juice. Similarly, peptic digests of hemoglobin, egg albumin, and casein evoked pancreatic secretory responses when instilled into the gut. Peptic digests of gelatin were anomalous in this regard, as they did not stimulate. Nevertheless, it appears that with most proteins digestion converts native protein from a nonstimulus to a stimulant of pancreatic secretion.

In the peptic digests, stimulants were almost certainly polypeptides. Analyses of all digests (Tables 2 and 5) showed that the concentrations of AA or ArAA in the peptic digests were at the limits of sensitivity of the analytical methods (2.0 mM for AA and 0.3 mM for ArAA). From such results, it cannot be stated whether the digests contained as much as or less than 2 mM AA or 0.3 mM ArAA; in any case, the maximal potential load of AA or ArAA in those perfusates was far below the loads of phenylalanine known to be required to stimulate secretion of this magnitude (18) (see also Fig. 5). Since phenylalanine is one of the two most potent

**TABLE 4. Pancreatic responses to peptic or complete digest of 1.8% BSA**

<table>
<thead>
<tr>
<th>Peptic Digest</th>
<th>Flow Rate, ml/15 min</th>
<th>HCO$_3^-$ output, meq/15 min</th>
<th>Protein output, mg/15 min</th>
<th>∆Fo, mM</th>
<th>AA, mM</th>
<th>ArAA, mM</th>
<th>Total BUN, mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>60-min Peptic Digest</td>
<td>25</td>
<td>0.06</td>
<td>203</td>
<td>±0.01</td>
<td>286</td>
<td>0.01</td>
<td>16.9</td>
</tr>
<tr>
<td>Complete hydrolysate (AA mixture)</td>
<td>25</td>
<td>0.07</td>
<td>195</td>
<td>±0.01</td>
<td>18</td>
<td>0.01</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Values are means ± SD (9 observations/3 dogs). Mean pancreatic protein response to 0.15 M NaCl in the same dogs on other days were 123 ± 20 mg/15 min (9 observations/3 dogs).

*Compared with 0.15 M NaCl as a control solution.

**TABLE 5. Effect of 60-min peptic digestion of 4 dissimilar proteins in 1.8 g/100 g solutions**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Saline (control)</th>
<th>Undigested protein</th>
<th>Peptic digest</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCO$_3^-$ output, meq/15 min</td>
<td>Protein output, mg/15 min</td>
<td>HCO$_3^-$ output, meq/15 min</td>
<td>Protein output, mg/15 min</td>
</tr>
<tr>
<td>Gelatin</td>
<td>0.06</td>
<td>196</td>
<td>0.07</td>
</tr>
<tr>
<td>Egg albumin</td>
<td>0.03</td>
<td>115</td>
<td>0.06</td>
</tr>
<tr>
<td>Casein</td>
<td>0.06</td>
<td>86</td>
<td>0.08</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>0.06</td>
<td>96</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Values are means ± SD (9 observations/3 dogs) for perfusions at 100 ml/15 min. *Negative value indicates titration value less than a water blank. **P < 0.05 vs. NaCl control response, t test. **P < 0.05 vs. control, analysis of variance (performed on all pancreatic responses in table).
amino acid stimulants (18), it is very unlikely that the peptic digests stimulated by virtue of their amino acid content. Furthermore, dialysis of peptic digests of BSA did not alter the potency of the digests (Table 3), even though the dialysis removed virtually all amino acids and the bulk of oligopeptides (2-3 amino acids in length). Likewise, the dialysis experiment indicated that oligopeptides in the BSA digests probably did not account for potency. On the other hand, pancreatic digestion liberated significant quantities of AA, ArAA (Fig. 7), and probably oligopeptides (17) that could well have accounted for the potencies of these digests.

**Dose-response studies.** Based on the results from 60-min peptic digests of BSA, it appears that intestinally perfused peptic digests stimulate pancreatic secretion in a dose-related fashion. Graded pancreatic bicarbonate and protein responses were observed when graded concentrations of BSA were submitted to peptic digestion (Figs. 4 and 5). Thus, 14% BSA digests evoked significantly (P < 0.05, multiple-range test) higher protein outputs than 1.8% BSA, and 14% BSA stimulated significantly more bicarbonate than 3.5% BSA. However, variations in outputs among and within dogs from day to day were substantial, and it therefore was impossible to transform the dose-response curves into statistically meaningful estimates of D50 and Vmax.

From visual inspection of the dose-response curves obtained (Figs. 4 and 5), it was apparent that the character of the responses to peptic digests of BSA was closely similar to that from a) a mixture of the amino acids phenylalanine and tryptophan or b) intravenous CCK-OP. All three stimuli evoked a juice high in protein and low in bicarbonate concentration, and bicarbonate/protein output ratios (4, 16) did not appreciably differ among the three stimuli. These results with BSA were entirely similar to those of Thomas and Crider (24) with digests of casein (see above).

Increasing evidence has accumulated (15) that free amino acids in the bowel lumen stimulate pancreatic secretion, at least in part, by releasing endogenous CCK. Since the peptic digests produced responses similar to those of CCK-OP or amino acids, it is possible, though unproven, that the digests may have released endogenous CCK. Certainly the digests must have acted locally on the gut mucosa, as such peptide products are broken down to amino acids before entering the portal blood (9) and circulating amino acids do not stimulate pancreatic secretion (7, 19, 26).

**Time course.** Neither undigested BSA nor BSA incubated 60 min in HCl without pepsin (Table 1) was potent, yet as little as 30 min of peptic digestion converted the protein to a stimulus that was fully potent, as longer digestion (up to 240 min) did not produce products with more potency (Fig. 2). It was not simply that 30-min digests of 3.5% were supramaximal stimuli, so that further gain in potency would not have been observed as digestion time was prolonged: dose-response studies (above) with 60-min digests of BSA had demonstrated that the 3.5% BSA was a submaximal stimulus. Rather, the results support the view that full potency was developed after a short period of peptic digestion.

Because of practical limitations, shorter digestion times were not tested, so it is unknown whether full potency was reached even earlier. Furthermore, it is unknown how closely the in vitro digestion mimicked in vivo conditions in the stomach. The digestion medium used contained both HCl and pepsin in concentrations approaching those in maximally stimulated gastric juice from dogs with Heidenhain pouches (assayed by us). Despite these unknowns, we think it reasonable to conclude that conversion of dietary protein to pancreatic stimulants may occur fairly early after intake, as the result of peptic digestion in the stomach.

**Specificity of peptic products.** Much of the data indicate that peptic digestion cleaves protein in such a way as to produce a mixture of peptide products in which some, but not all, peptides are luminal stimulants of pancreatic secretion. The 3.5% BSA digested for only 30 min in the peptic mixture was as potent as digests obtained after longer periods of digestion (Fig. 2). However, peptic digestion of 3.5% BSA resulted in the cleavage of more peptide bonds (increasing ΔFo) with the concomitant formation of more and more HClO4-soluble BRN (Fig. 3) as digestion time was prolonged. The findings indicate that, whatever changes took place in the first 30 min of peptic digestion, the most potent mix of products had been formed from the substrate in this time, and potency of this mixture was not affected by other changes that took place during longer periods of peptic digestion.

Likewise, the findings with peptic digests of other proteins support the conclusion that some specifically potent peptides were formed in the mix of products. Thus, the hemoglobin digest produced no more pancreatic response than the egg albumin digest in the same set of dogs (Table 5), despite a much higher ΔFo (Table 5). Also, though gelatin was cleaved by pepsin (Table 5), its digests were not potent.

All of the above point to the production of some specific products that stimulate pancreatic secretion. There are three possibilities: a) that peptide products of a certain size range stimulate regardless of their chemical composition, b) that formation of peptide fragments with specific amino acid end groups at the COOH- or NH₂-termini are responsible, or c) that peptide fragments are formed with common amino acid sequences to which gut receptors are responsive. Of the three possibilities, we consider the first least likely, as earlier work with synthetic peptides (17) has indicated that chemical composition, rather than peptide size, accounts for pancreatic stimulation. Data that bear on the two other possibilities are discussed below.

**NH₂-terminal amino acids liberated by peptic digestion.** Four amino acids (phenylalanine, leucine, valine, and aspartate) appeared consistently as NH₂-terminal amino acids in nearly every peptic digest (Table 6). All digests that stimulated (3.5% BSA, dialyzed 7% BSA, egg albumin, hemoglobin, and casein) contained in large amounts the NH₂-terminal amino acids phenylalanine and leucine, which were not found in the impotent gelatin digests. 1 Although the analytical methods used did not allow identification of every NH₂-terminal

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1 Analyses of acid-soluble filtrates of DNP-gelatin conjugates also failed to reveal the presence of NH₂-terminal phenylalanine or leucine (data not shown).
TABLE 6. NH₂-terminal amino acids liberated by peptic digestion

<table>
<thead>
<tr>
<th>Peptic Digest</th>
<th>Undigested Protein (Control)</th>
<th>Protein Digest</th>
<th>( [\text{DNP}]_2 ) mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5% BSA digested 30 min</td>
<td>Asp-L; Val-H; Leu-M; Ala-M</td>
<td>Asp-H</td>
<td>5.5</td>
</tr>
<tr>
<td>7% BSA digested 60 min and dialyzed</td>
<td>Val-H; Val-L; Leu-M; Ala-M</td>
<td>12.3</td>
<td></td>
</tr>
<tr>
<td>1.8% Hemoglobin digested 60 min</td>
<td>Phe-L*; Val-H; Leu-M; Ala-M</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>1.8% Casein digested 60 min</td>
<td>Phe-H; Val-M; Leu-H; Ala-M</td>
<td>5.3</td>
<td></td>
</tr>
<tr>
<td>1.8% Egg albumin digested 60 min</td>
<td>None*; Phe-H; Val-H; Leu-H; Ala-M</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>1.8% Gelatin digested 60 min</td>
<td>Gly-L; Val-M; Val-L; Gly-L</td>
<td>5.5</td>
<td></td>
</tr>
</tbody>
</table>

Suffixes H, M, and L following the amino acids refer to the intensity (color and area) of the DNP-amino acid spot on visual inspection. H, heavy; M, medium; L, light. This intensity has been shown (14) to be proportional to the quantity of DNP-amino acid chromatographed. * Reported NH₂-terminal amino acid for that protein (28).

amino acid that appeared in the digests, it nevertheless was apparent (Tables 5 and 6) that a wide variation in the number of bonds broken (reflected by \( [\text{DNP}]_2 \)) was apparent (Tables 5 and 6) that a wide variation in digestive products formed in the various in vitro digests. Unlike peptic digestion, pancreatic digestion (i.e., digestion with pancreatic proteases) converted native BSA to a stimulus of about equal potency to the peptic digests, pancreatic digestion of previously formed peptic digests of submaximal potency did not alter the potencies of the peptic digests (Figs. 6 and 8). Likewise, Thomas and Crider (24) observed that peptic-tryptic digests of casein were about equipotent with peptic casein digests.

These findings were truly remarkable considering the wide variation in digestive products formed in the various in vitro digests. Unlike peptic digestion, pancreatic digestion of BSA liberated a significant quantity of amino acid and aromatic amino acid (Fig. 7), yet both digests had similar potencies (Fig. 6). There was an even more dramatic change in the mix of products when either a 30-min or a 240-min peptic digest of BSA was digested in pancreatin: considerably more amino acids were liberated, including up to 90% of the total assayable aromatic amino acids in the BSA, and, correspondingly, there was a pronounced drop in the total BRN, reflecting conversion of substantial amounts of polypeptide to free amino acid and/or oligopeptides. Nevertheless, potency was unaffected (Figs. 6 and 7).

In the final set of experiments, a complete hydrolysate (amino acid mixture) was compared with a peptic digest. Here the differences in composition between the two mixtures varied even more widely (Table 4), yet the potencies of the two solutions were about the same. These results differed from those of Greer et al. (10), who found that in the rat either acid hydrolysates (75% nitrogen as amino acids) or commercial enzymatic hydrolysates (35% amino acid) of casein evoked less pancreatic response on bolus injection into the gut than did...
similarly injected casein. Since a different protein, a different species, and a different method of administration (bolus injection vs. steady-state perfusion) were used, there are many possible explanations for this discrepancy, though we believe the use of bolus injection may introduce nonquantifiable variables in such studies that involve a variety of interrelated time-dependent processes (amino acid absorptive rates, digestion rates, transit times, lag times in pancreatic response, etc.).

The remarkably constant potencies found despite wide variations in chemical composition of digests might be the consequence of varying absorption rates among the digest components, in turn producing varying distributions of these components along the gut in relation to the distribution of gut receptors. Receptors to amino acids are known to be distributed along at least 90 cm of proximal bowel (18). It is not known, but probable, that receptors to oligopeptides and polypeptides have a similar distribution, since in a few experiments (unpublished observations) we found that pancreatic responses to peptic digests of BSA were mediated by lengths of proximal gut in excess of 45 cm (responses to perfusion of 45 cm segments with peptic digests were less than responses to perfusion of the entire small bowel). In contrast to amino acids and oligopeptides, polypeptides are not absorbed by gut mucosa (9). Therefore, relatively small quantities of polypeptides entering the gut free of pancreatic proteases might readily reach receptors along the entire receptor-bearing area, whereas only larger molar quantities of stimulating amino acids or oligopeptides (enough to overcome rapid absorption) could reach such lengths when infused at the pylorus. On the other hand, when proteins or polypeptides enter the gut containing pancreatic proteases, there would be an expected conversion to oligopeptides and free amino acids, greatly increasing the molar quantities of digestive products. Nevertheless, all these smaller products are readily absorbed, and only some appear to have potency in stimulating pancreatic secretion (17, 18). The net result might be that over a wide range of luminal conditions the entire receptor-bearing area of the gut would be exposed to stimulating products; although the composition of these products might vary widely (more amino acids and/or oligopeptides when luminal pancreatic proteases abounded, more polypeptides in the absence of luminal proteases), observed pancreatic response would not reflect such variation.

Though such an explanation is entirely theoretical, the actual observation—that digests widely varied in composition evoke about the same pancreatic secretory outputs—indicates that pancreatic secretory responses to protein entering the gut after peptic digestion in the stomach will be similar despite wide variations in luminal protease concentrations in the gut. Only when protein escapes peptic digestion in the stomach and enters a gut devoid of pancreatic protease is pancreatic response likely to be diminished.

Summary. Five soluble proteins were infused into the small intestine of dogs with pancreatic fistulas, either as native proteins or peptic digests. Only one of the five native proteins stimulated pancreatic secretion, whereas four of the five peptic digests of these same proteins were potent. Detailed studies with one of the proteins, bovine serum albumin, strongly indicated 1) that potency of the peptic digest arose from its content of polypeptides and 2) that pancreatic response was related to the dose of these products. Moreover, the findings with all the peptic digests suggested that the liberation of specific peptides accounted for potency.

Digestion of bovine albumin with pancreatic proteases produced a much different mix of products than did peptic digestion, yet these products were about equipotent with those of the peptic digests in stimulating pancreatic secretion. Moreover, a mixture of amino acids constituted from the amino acids in bovine albumin was equipotent with the peptic digest of the protein.

Each type of digest, peptic digest, pancreatic digest, or amino acid mixture, stimulated a pancreatic juice low in volume and bicarbonate concentration but high in protein concentration—a juice of the same character that secreted in response to intravenous CCK-OP.

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