Raw Soybeans Stimulate Human Pancreatic Proteinase Secretion

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ABSTRACT Intraduodenal instillation of raw soybeans stimulated pancreatic proteinase secretion in humans. Raw soybeans almost abolished the activity of chymotrypsin and severely reduced (50%) the trypsin activity. Immunoreactive trypsic and chymotryptic material simultaneously appeared in amounts 2 to 4 times basal concentrations. This increase, demonstrated with rocket immunoelectrophoresis, was begun within the first 10 min of soybean instillation. The enhanced secretion also persisted throughout the succeeding saline instillation, and it is suggested that the presence of Kunitz trypsin inhibitor contributed to this postprandial stimulation. An amidase that hydrolyses low-molecular-weight substrates (i.e., benzoyl-arginine p-nitroanilide) was found in raw soybeans. Its low activity was not assumed to substantially bias standard trypsin assays. The increased proteinase secretion was, as previously published, not preceded by an elevated plasma cholecystokinin concentration. The raw soybeans also caused a nonparallel secretion of amylase and proteinases. Nervous, perhaps cholinergic, regulation mediates the inhibitor-stimulated proteinase secretion in humans. This stimulation yields both a general increase of proteinases and also a specific inhibitor-resistant trypsin. This is consistent with the physiologic need for proenzyme-activation in the presence of inhibitors and for restoration of the proteolytic capacity of the duodenal juice. J. Nutr. 122: 1407-1416, 1992.

INDEXING KEY WORDS: • cholecystokinin • feedback regulation • humans • trypsin • soybean amidase • soybean proteinase inhibitors

The feedback regulation of pancreatic secretion in humans has been debated (Adler et al. 1989, Owyang et al. 1986a). In rats, the animal most frequently used as a model, a feedback regulation seems well documented on the basis of certain levels of free trypsin in the duodenum and plasma cholecystokinin (CCK).2 Reduction of trypsin (and chymotryptic) activity by proteinase inhibitors, or by diversion of pancreatic juice, causes a prompt increase in plasma CCK that in turn stimulates pancreatic enzyme secretion (Fushi et al. 1989, Liddle et al. 1984, Miyasaka and Green 1984). Continued CCK stimulation causes hyperthropy and hyperplasia, resulting in nodules and later in neoplastic changes in the rat pancreas (Lienier et al. 1985).

In a recent study (Holm et al. 1988a) raw soybeans, a soybean protein isolate or bovine serum albumin were continuously instilled into human duodena on different days while intermittent samples of blood and duodenal juice were collected. Only when bovine serum albumin was instilled was a significant increase in plasma CCK found. Parallel to this increase in CCK, increases in trypsin (EC 3.4.21.1) and amylase (EC 3.2.1.1) activities were also found. The CCK concentrations during soybean protein isolate and raw soybean instillations were almost identical to the basal CCK levels. A considerably larger increase in proteinase activities was seen during the soybean protein isolate instillation, whereas the raw soybeans caused a substantial inhibition of proteolytic activity. Although the trypsin and the chymotryptic activities varied considerably, no significant difference could be seen in the amylase activity when any of the three test proteins was instilled. In two similar instillation experiments with proteinase inhibitors, a general stimulation

1Supported by the Anders Jahres Foundation and the Norwegian Cancer Society.
2Abbreviations used: BAPNA, Na-tol-arginine p-nitroanilide; BTEE, Na-benzyl-t-tyrosine ethyl ester; CCK, cholecystokinin; OD₄₅₀, optical density at 1 nm; S-2677, N-t-butyloxycarbonyl-L-glutamyl-[α-O-benzyl]-glycyl-arginine-p-nitroanilide-HCl; SBTI, Kunitz soybean trypsin inhibitor; TTBS, tris-tween buffered saline.
(trypsin, chymotrypsin, amylase and lipase) was obtained by the use of Bowman-Birk inhibitor (Lienert et al. 1988) and a partial stimulation (amylase and lipase) was obtained by the use of Camostate (Adler et al. 1989). The Bowman-Birk inhibitor inhibits both trypsin and chymotrypsin, whereas Camostate only inhibits trypsin. In the Bowman-Birk inhibitor experiment CCK was not determined, whereas the plasma concentrations of CCK were not altered after the Camostate instillation. It has been suggested that nonparallel secretion may take place in humans and that duodenal proteinase levels are not reflected in human plasma CCK levels as in rats (Dagorn et al. 1977, Gøke et al. 1986). Recently, we demonstrated by means of immunologic methods that duodenal juices collected during raw soybean instillation contained both free and inhibitor-complexed trypsin (Thorsen et al. 1991). Consequently the aim of the present study was to test, by immunologic techniques with commercial antibodies produced against trypsin and chymotrypsin, the hypothesis that total intraduodenal trypsin and chymotrypsin (free and inhibitor-complexed) is enhanced due to the intraduodenal instillation of raw soybeans.

**MATERIALS AND METHODS**

**Instillation of test meals.** Detailed descriptions of the test meals, instillation and enzyme analysis were previously reported (Holm et al. 1988a, 1988b). In brief, 11 volunteers were given three iso-osmotic test meals containing bovine serum albumin, soybean protein isolate and a freeze-dried water extract of raw, finely milled soybeans [10 g/(100 mL-h)] on different days. The test meals were given as a continuous instillation through a Portex PP120 tube (Portland Plastics, Kent, U.K.) attached to a Lagerlöf tube (Rusch, Waiblingen, Germany). The Portex tube ended close to the papilla of Vater and the tip of the Lagerlöf tube 18 cm distally. Before and after each test meal, saline [100 mL/h] was infused for 60 and 30 min, respectively. The sample of duodenal juice and the blood sample drawn during the last 10 min of the first saline period were designated basal value. These samples were used as a reference value [100%] for the subsequent samples. The study was approved by the Ullevål Hospital Ethics Committee, and written consent was obtained from all participants.

**Enzyme assays.** Nα-benzoyl-DL-arginine p-nitroanilide (BAPNA) and Nα-benzoyl-L-tyrosine ethyl ester (BTEE) were used as substrates in the trypsin and chymotrypsin assays as previously described (Holm 1988a, Krogdahl and Holm 1979). In addition, BAPNA was replaced by N-t-butylxoycarbonyl-L-glutamyl-(α-O-benzyl)-glycyl-arginine-p-nitroanilide-HCl [S-2677] from Kabi Diagnostica (Nyköping, Sweden) (Thorsen et al. 1991). Total proteolytic activity was measured with casein as substrate. As a nonproteolytic enzyme, amylase was chosen and its activity measured with Phadebas Amylase test kit (Pharmacia, Uppsala, Sweden). Inhibition of trypsin activity with phenylmethylsulfonyl fluoride was conducted as previously (Holm et al. 1988b).

**Duodenal juices investigated enzymatically and immunologically.** Enzyme activities and outputs in duodenal juices from 11 persons given bovine serum albumin, soybean protein isolate and raw soybeans have recently been presented (Holm et al. 1988a). Complete series [basal, protein-stimulated and saline-terminated] were available from only five persons for the immunologic studies. Consequently, all results are presented and calculated as means ± SEM of this particular group [n = 5]. To avoid confusion with the dilution-corrected enzyme output data already given (Holm et al. 1988a), only enzyme activities and enzyme concentrations are given here.

Antiserum was produced, at the Norwegian Food Research Institute, against Kunitz soybean trypsin inhibitor (SBTI) type I-S purchased from Sigma Chemical (St. Louis, MO). Purification was performed according to the method of Harboe and Ingild (1983), and biotin labeling of the purified antibody to SBTI was performed as described by Hudson and May (1989). Antisera produced against human cationic trypsin and against human chymotrypsin were obtained from Calbiochem, Behring Diagnostics (La Jolla, CA) and antiserum to soybean proteins from Behringwerke A. G. (Marburg, Germany). Horseradish peroxidase–labeled goat anti-rabbit immunoglobulins were obtained from Southern Biotechnology (Birmingham, AL).

**Rocket immunoelectrophoresis.** Rocket immunoelectrophoresis was performed with minor modifications in accordance with Laurell (1972) and Weeke (1973), using the *LKB 2117 Multiphor system* (Pharmacia LKB Biotechnology, Uppsla, Sweden). Agarose gels, containing the respective antiserum, consisted of 10 g/L human serum albumin agarose (Litex, Copenhagen, Denmark) in Tris-glycine buffer (38 mmol/L Tris, 100 mmol/L glycine, pH 8.7) and 20 g/L PEG-6000. The gels were cast on Gelbond *PAG film* (FCM BioProducts, Rockland, ME) with dimensions of 210 × 100 × 1.5 mm. The antisera to chymotrypsin, trypsin and SBTI were added to final concentrations of 1.23 mg/L gel, 3.4 mg/L gel and 25 mg/L gel, respectively. All samples of duodenal juices were diluted 1:10 (v/v) in Tris-glycine buffer before application in sample wells. Buffer strips [3 mm thick] consisting of 20 g/L agarose in a fivefold concentrated Tris-glycine buffer were used as buffer reservoirs during the electrophoresis (20 mA, 3–4 h). After electrophoresis the gels were washed three times in distilled water before being dried. The immunoprecipitates were stained with 1% [v/v] Coomassie Brilliant Blue R-250 in 2
mol/L acetic acid and 40% (v/v) methanol. Peak heights were measured from the center of the application well, and compared with rockets obtained with the purified standard protein [human cationic trypsin and human chymotrypsin, Calbiochem, Behring Diagnostics]. Increasing amounts (1.5, 2.5, 12.5 and 25 mg) of raw soybeans were added to 1 mL of pooled bovine serum albumin juices to study the effect of complex binding between active trypsin and soybean inhibitors on the peak heights (1.5-12.5 mg caused approximately 10 to 100% enzyme inhibition). Ten microliters of these mixtures were analyzed by rocket immunoelectrophoresis in 10 g/L agarose containing 6 mg/L anti-trypsin.

**Identification of trypsin-SBTI complexes.** The dried rocket immunoelectrophoresis gels, made with anti-trypsin, were incubated with biotin-labeled anti-SBTI (0.3 mg/L, for 4–6 h at room temperature). Excess of biotin-anti-SBTI was washed away with Tris-Tween buffered saline (TTBS) buffer [20 mmol/L Tris, 0.5 mol/L NaCl and 0.05% (v/v) Tween 20] three times, followed by 2-h incubation with Strept AB-Complex horseradish peroxidase [Dakopatts A/S, Copenhagen, Denmark]. After three times in TTBS the gels were finally washed in distilled water before substrate reaction. The 3,3’-diaminobenzidine [tetrahydrochloride]-based horseradish peroxidase reaction product was visualized with some modifications as described by Adams [1981], using a 50 mmol/L (NH₄)₂CO₃ + 0.03% (v/v) H₂O₂ + 0.3 g/L DAB and + 0.5 g/L NiCl₂. The gels were Coomassie blue–stained after this immunostaining procedure, to visualize all precipitates formed by the precipitating antibodies.

**Western immunoblotting.** To ascertain whether soybean protein still was present and possibly responsible for this post-soybean stimulation, duodenal juices from all 11 persons [Holm et al. 1988a] were studied by SDS-PAGE performed in accordance with Laemmli [1970]. Soybean protein bands were visualized after electrophoresis with antibodies to soybean protein. The stacking gel contained 40 g/L and the separating gel 150 g/L polyacrylamide. Western electrophoeto blotting [Towbin et al. 1979] of the SDS-PAGE–separated proteins onto nitrocellulose membrane was performed with transfer buffer consisting of 25 mmol/L Tris, 192 mmol/L glycine and 20% (v/v) methanol, pH 8.3. After transfer the residual binding capacity of the membrane was blocked by incubation in a solution of 30 g/L dried milk proteins for 1 h with shaking. The blocked nitrocellulose sheet was then first incubated in a 1:3000 dilution of both antiserum to SBTI and antiserum to soybean proteins and further incubated in a 1:3000 dilution of the horseradish peroxidase–labeled goat anti-rabbit immunoglobulin. Between the antibody incubations and before the final substrate reaction, the membrane was washed twice for 5 min in TTBS and once for 10 min in distilled water. The substrate reaction was visualized as described above for the immunorockets.

**Intraduodenal osmolality.** To study the degradation of exogenous [test meal] and endogenous protein [mostly enzymes], the osmolality of bovine serum albumin, soybean protein isolate and raw soybean samples was determined by freezing point depression.

**Statistical analysis.** All results given are means and SEM of observations from five persons. The Spearman rank correlation test was used when quantitation by means of rocket heights was correlated with enzyme activities. The significance of differences between groups was first assessed with the Kruskal-Wallis rank sum test, and, if significant, the Mann-Whitney rank sum test was used on each pair of data [Bradley 1968]. P values ≤ 0.05 were judged significant.

**RESULTS**

**Stimulated proteinase secretion.** Instillation of raw soybeans in human duodena stimulated the secretion of pancreatic proteinases. The concentration of total trypsin [free and inhibitor-complexed] increased two to three times and the total chymotrypsin concentration increased three to four times the basal values, (Fig. 1 and 2, upper panels). A substantial part of the proteinases appeared intraduodenally as enzyme-inhibitor-complexes, because the enzyme activities were substantially lower during the raw soybeans instillation than in basal-, bovine serum albumin- and soybean protein isolate-samples (Fig. 1 and 2, lower panels).

Anti-trypsin–precipitating material in all duodenal samples caused two peaks in rocket immunoelectrophoresis with different peak heights and staining intensity. Duodenal juices after raw soybeans instillation showed an increased height of both the higher and the lower immunorocket compared with other samples. Addition of raw soybeans to bovine serum albumin juices resulted in double rockets with similar staining intensities and with the increased height of the lower peak as seen in raw soybean juices. For the quantitation of trypsin, the highest peak was measured because the height of this rocket was unaffected by the inhibitor concentration and the amount of free vs. inhibitor-complexed trypsin (Fig. 3).

Basal trypsin concentrations in the three groups were not different and the average value (n = 15) was calculated to 5.0 ± 0.4 g/L. During the stimulation period [10–60 min], the average concentration increased significantly [1.8 ± 0.3, 3.0 ± 0.4 and 5.5 ± 0.4 g/L in bovine serum albumin, soybean protein isolate and raw soybean samples, respectively]. The changes were all significantly different from one another. The mean specific trypsic activity [optical density at 410 nm (OD₄₁₀)/µg trypsin] in basal, bovine serum albumin and soybean protein isolate samples was 4.3 ±
0.2, 4.8 ± 0.3 and 6.2 ± 0.7, respectively. Basal and soybean protein isolate samples differed significantly. In the raw soybean samples the corresponding specific activity, 2.3 ± 0.35, was significantly lower than in any other samples. A significant correlation was found between tryptic activity and immunoreactive material in all duodenal juices (Table 1).

All duodenal juices gave rise to only one chymotryptic peak. Basal chymotrypsin concentrations in the three groups were not different and the average value was calculated to 3.0 ± 0.8 g/L. The average increase of chymotryptic material, after 10–60 min instillation, was 1.8 ± 0.5, 2.4 ± 0.2 and 4.7 ± 0.9 g/L in the bovine serum albumin, soybean protein isolate and raw soybean samples, respectively. The raw soybeans and bovine serum albumin values differed significantly. The specific activity of chymotrypsin (OD$_{253}$/µg chymotrypsin) in basal, bovine serum albumin and soybean protein isolate
samples was 2.7 ± 0.3, 2.7 ± 0.2 and 2.4 ± 0.2, respectively. The specific chymotryptic activity was not calculated in the raw soybean samples owing to almost complete enzyme inactivation (Fig. 2, lower panel). Chymotrypsin inactivation was also reflected in the negative and not significant correlation of activity and immunoreactive material (Table 1). Specific enzyme activities of trypsin and chymotrypsin were not significantly different in basal or bovine serum albumin samples.

During the terminating saline period (70–90 min) after raw soybeans instillation, an increased trypsin and chymotrypsin secretion persisted (Fig. 1, 2). A possible post-soybean stimulation was studied by SDS-PAGE. The similarity of the protein pattern in different juices was remarkable, thus enabling the presentation of a single immunoblot as representative for the combined results (Fig. 4). With the exception of the SBTI, extensive degradation of the soybean protein took place. Most of the soybean protein disappeared from the duodenum within the terminating saline period (70–90 min), again with the exception of the SBTI. Quantitative determinations of this SBTI in the raw soybean samples from the five complete series were carried out by rocket immunoelectrophoresis. The results are given in Table 2 together with calculated SBTI concentrations in accordance with the previously recorded sample dilutions [Holm et al. 1988a].

In contrast to the three- to fourfold increase in proteolytic enzymes due to raw soybeans, no such stimulatory effect could be seen for amylase in the same duodenal juices (Fig. 5).

### Table 1

**Correlation of enzyme activities and immunoreactive material in duodenal juice after instillation of raw soybean, soybean protein isolate, and bovine serum albumin**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>BSA</th>
<th>SPI</th>
<th>RS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chymotrypsin</td>
<td>0.54</td>
<td>0.84*</td>
<td>-0.64</td>
</tr>
<tr>
<td>Trypsin</td>
<td>0.64*</td>
<td>0.66*</td>
<td>0.52*</td>
</tr>
</tbody>
</table>

1. Pearson's correlation coefficient: ODx/mL vs. g/L. *P ≤ 0.05, Mann-Whitney rank sum test.
2. Abbreviations used: BSA, bovine serum albumin; SPI, soybean protein isolate; RS, raw soybean extract.

### Table 2

**Concentration of Kunitz soybean trypsin inhibitor in duodenal juice after instillation of raw soybean (RS) or saline**

<table>
<thead>
<tr>
<th>Instillation (meal/time)</th>
<th>Analyzed²</th>
<th>Calculated³</th>
</tr>
</thead>
<tbody>
<tr>
<td>RS, 10 min</td>
<td>0.56 ± 0.18</td>
<td>1.35 ± 0.20</td>
</tr>
<tr>
<td>RS, 20 min</td>
<td>1.68 ± 0.09</td>
<td>1.39 ± 0.23</td>
</tr>
<tr>
<td>RS, 30 min</td>
<td>1.70 ± 0.07</td>
<td>1.41 ± 0.21</td>
</tr>
<tr>
<td>RS, 40 min</td>
<td>1.30 ± 0.30</td>
<td>1.26 ± 0.39</td>
</tr>
<tr>
<td>RS, 50 min</td>
<td>1.66 ± 0.06</td>
<td>1.18 ± 0.38</td>
</tr>
<tr>
<td>RS, 60 min</td>
<td>1.74 ± 0.09</td>
<td>1.13 ± 0.34</td>
</tr>
<tr>
<td>Saline, 70 min</td>
<td>1.26 ± 0.17</td>
<td></td>
</tr>
<tr>
<td>Saline, 80 min</td>
<td>0.76 ± 0.18</td>
<td></td>
</tr>
<tr>
<td>Saline, 90 min</td>
<td>0.40 ± 0.11</td>
<td></td>
</tr>
</tbody>
</table>

1. Values are means ± SEM, n = 5.
2. By rocket immunoelectrophoresis.
3. According to intraduodenal dilution [Holm et al. 1988a].
Intraluminal osmolality. Extensive protein degradation took place also when bovine serum albumin and soybean protein isolate were instilled. When studied by SDS-PAGE, protein patterns (Coomassie-stained) similar to those in Figure 4 were found (not shown). Expressed as osmolality, this degradation of the three different test meals and endogenous proteins is visualized in Figure 6. The osmolality increased approximately twofold. The mean osmolality during soybean protein isolate instillation was significantly higher than during the bovine serum albumin instillation. At the end of the terminating saline period, the osmolality of all three groups returned to values not significantly different from the basal values.

Amylase activity in raw soybeans. When duodenal juices were added to raw soybeans and analyzed by rocket immunoelectrophoresis (Fig. 3) they were also assayed for enzyme activity. In spite of a calculated surplus of inhibitors, enzyme activity was indicated because the substrate BAPNA was hydrolyzed. This activity was highly sensitive to phenylmethylsulfonyl fluoride and totally different from that of the inhibitor-resistant trypsin recently found (Holm et al. 1988b). Moderate amounts of raw soybeans (0.01-0.1 g/L) yielded a typical descending curvilinear enzyme-inhibitor curve (Fig. 7). With increasing amounts of raw soybean (0.5-10 mg) the OD_{410} increased in proportion to the added raw soybean. In the absence of any duodenal juice this soybean amylase activity was reproduced with the same linear dose/response curve as shown in Figure 7. When the BAPNA substrate was replaced with another arginine-p-nitroanilide, S-2677, the soybean amylase gave only 10% of the p-nitroaniline obtained from BAPNA. When human duodenal juice (basal and bovine serum albumin-instilled) was used as the enzyme source, 12 times more p-nitroaniline was formed from S-2677 than from BAPNA under otherwise identical conditions (data not shown). When the raw soybean amylase was tested for proteolytic activity (casein) and for esterolytic activity (BTEE), no activity was found.

**DISCUSSION**

A substantial stimulation of pancreatic proteinase secretion, two to four times basal levels, was observed when duodenal juices containing raw soybean extract were analyzed by rocket immunoelectrophoresis. This was, as previously shown, not preceded by an increase in plasma CCK (Holm et al. 1988a). The raw soybean instillation required only minutes to be reflected in increased trypsin and chymotrypsin secretion. The intraluminal proteinase inhibitors reduced the proteolytic activity significantly. A corresponding reduction in the hydrolysis of exogenous and endogenous protein was not found. A possible increased survival time of the enzymes, which could have contributed to the enhanced proteinase concentration, is therefore not likely to be of importance here. If the 1.5- to 2.5-fold increase in enzyme concentration within 10 min was entirely due to inhibited proteolysis, this would imply a normal breakdown rate in the order of 75-80%/10 min. This is not in accordance with the previously demonstrated pronounced
stability of the proteins (Layer et al. 1986, 1990). Prolonged luminal half-life of trypsin and chymotrypsin is consequently not believed to be of significance as an explanation for the enhanced trypsin and chymotrypsin concentrations found.

This is the first time raw soybeans have been shown to stimulate human pancreatic proteinase secretion. Previously, Calam et al. (1987) found that a solid meal was required in addition to raw soybeans. The stimulated secretion, seen both in the present experiment and that of Calam et al. (1987), likely was due to the presence of proteinase inhibitors present in raw soybeans. The persistent presence of SBTI (1.27–0.40 g/L) and the enhanced secretion in the terminating saline period support this assumption. Increased proteinase secretion has also been obtained by the addition of the Bowman-Birk inhibitor to pure human pancreatic juice (ensuring >90% elimination of trypsin and chymotryptic activities) before the reentry of juice into the duodenum (Liener et al. 1988).

Although these results indicate that a stimulated secretion, due to proteinase inhibitors, was operating in humans and resembled the feedback regulation found in rats, the stimulating mechanisms may be different. A CCK-mediated stimulation in response to proteinase inhibitors in humans has not been reported. In the experiments of Calam et al. (1987), no CCK increase was observed until the solid meal was given in addition to the raw soybeans. In the instillation experiment with the Bowman-Birk inhibitor, CCK was, however, not determined (Liener et al. 1988). When 380 mg/h of the Kunitz trypsin inhibitor was perfused intraduodenally in a basal (saline) situation resembling our raw soybean instillation experiment, the trypsin activity was abolished without affecting the plasma CCK level (Owyang et al. 1986b). In similar instillation experiments in which the inhibitor Camostate (FOY-305) was given, resulting in 90–99% inhibition of trypsin and chymotryptic activities, no increase in plasma CCK was found (Adler et al. 1988). This supports our findings that proteinase inhibition by raw soybeans may stimulate pancreatic proteinase secretion independent of an elevated plasma CCK level. In favor of mechanisms different from the CCK-mediated regulation seen in the rat are also the studies in dogs by Singer et al. (1980), which show that cholinergic control may be equally important.

To achieve high, low and no inhibitor test meals (10 g/h), raw soybeans, soybean protein isolate and bovine serum albumin were given. This led to a difference in protein intake (6, 8 and 10 g, respectively). Only the bovine serum albumin meal caused a significant increase in plasma CCK. That CCK did not increase when the soy products were given may have been due to the smaller amounts (6–8 g) of protein (Holm et al. 1988a), which is consistent with the results of Calam et al. (1987). Not until a solid meal (scrambled eggs with milk, providing another 15 g of protein) was given together with the raw soybean was an increase in CCK found. Volume- and osmotic-receptors in the duodenum are known to elicit pancreatic secretion without raising the plasma CCK.

**FIGURE 6** Osmolality of duodenal juices after instillation of saline (B = basal value), bovine serum albumin [BSA], soybean protein isolate [SPI], or raw soybean extract [RS] (10–60 min), and saline (70–90 min). Values are means ± SEM, n = 5.

**FIGURE 7** Inhibition of tryptic activity in duodenal juice (left part) and amidase activity in raw soybean meal (right part) visualized by p-nitroaniline release from Nα-benzoyl-DL-arginine p-nitroanilide (BAPNA), [optical density at 410 nm (OD410)]. Standard trypsin/inhibitor assays (2.5 mL) with graded amounts of raw soybean (0–0.3 mg, and 0.5–10 mg).
levels (Owyang et al. 1986a). In our previous work (Holm et al. 1988a) volume-receptors were assumed to be of little importance owing to the very similar dilution of the three test meals. Effects due to different osmolalities seem also less obvious, because no direct relationship could be found between osmolality and amounts of enzymes in the raw soybean and soybean protein isolate samples.

Parallel increases in amylase and lipase secretion due to intake of Camostate have been considered an indication of a general parallel secretion of the pancreatic enzymes (Adler et al., 1988, Adler et al. 1989). In the study of Liener et al. (1988), in which proteinase inhibition was caused by Bowman-Birk inhibitor, a two- to threefold increase in amylase as well as in proteinases may also indicate parallel secretion. However, when raw soybeans were instilled in this study, no significant changes in amylase output could be found. This does not support the concept of parallel stimulation. Similarly, when the purified Kunitz trypsin inhibitor was perfused no evidence was found that indicated a parallel secretion (Owyang et al. 1986b). The apparently conflicting results may, however, be correct and may well be due to the different experimental designs, because the enzyme regulation is sensitive to nutritional, endocrine and cholinergic factors (Singer et al. 1980, Støkmann and Søling 1981).

In raw soybean samples low specific activity (OD140/µg trypsin) as well as poor correlation of enzyme activity and immunoreactive material is consistent with the presence of trypsin-inhibitor complexes. Assuming that the specific activity of the residual trypsin activity is similar to that of normal trypsin, the amount of complexed enzyme will be approximately 50–80% of the total trypsin concentration. The total amount of inhibitor-complexed enzymes will then be in the order of 6 g trypsin and 8 g chymotrypsin/L duodenal juice. In animal studies these enzyme-inhibitor complexes are recovered as increased fecal nitrogen. However, the high proportion of sulfur-containing amino acids lost in this way may be more important from a nutritional point of view (Holm et al. 1973). The demonstration of an unspecific amidase in raw soybean introduced some uncertainty with regard to the use of BAPNA as substrate in trypsin assays. However, duodenal juices containing substantial amounts of raw soybeans, as in the present experiments (Holm et al. 1988a), will most probably give only a marginal effect: that is, on an average 30 µL raw soybean juice, containing ~2 mg of the instilled raw soybean, will in the standard trypsin assay give an “amidase” reading of OD410 = 0.1 or less.

In basal, bovine serum albumin, and soybean protein isolate samples lacking the large amounts of enzyme-inhibitor complexes, the specific activities of trypsin and chymotrypsin were as expected higher than in raw soybean samples. However, not all enzyme activities and corresponding immunoreactive material gave significant coefficients of correlation, i.e., chymotrypsin in bovine serum albumin samples. This can be explained by the variable degradation of pancreatic enzymes demonstrated by Layer et al. (1986). In some cases proteolytic degradation may lead to a higher loss of immunoreactivity than enzyme activity and in other cases higher loss of enzyme activity than immunoreactivity [Layer et al. 1986].

Anti-trypsin precipitation of basal, bovine serum albumin, soybean protein isolate and raw soybean samples gave rockets with two peaks. Visualization of SBTL by biotinylated anti-SBTL revealed staining of the lower peak only and in raw soybean and soybean protein isolate samples exclusively. Anti-SBTI does not bind to the heat-inactivated SBTI (DiPietro and Liener 1989) and thus confirms the finding of active inhibitors in the isolated soybean protein, about 3% of that present in the raw soybeans (Holm et al. 1988a). Controls, conducted with trypsin/anti-trypsin precipitates obtained from bovine serum albumin juices and chymotrypsin/anti-chymotrypsin precipitates obtained from both bovine serum albumin and raw soybean juices, gave no reaction with this technique. The low rocket present in the basal and bovine serum albumin-stimulated samples may be complexes of enzyme and inhibitor of endogenous character, i.e., trypsin bound to the pancreatic secretory trypsin inhibitor [Pubols et al. 1974]. Rinderknecht et al. (1984), using trypsin RIA, demonstrated that anti-trypsin antibody bound to trypsin and trypsinogen as well as to an inhibitor-trypsin-complex. The double trypsin peaks were also in accordance with the different immuno-precipitates obtained by Thorsen et al. (1991) when basal and raw soybean-stimulated juices were analyzed by crossed immuno-electroophoresis.

The question of parallel or nonparallel secretion becomes crucial when conclusions about feedback regulation are based on a limited number of enzymes analyzed or only enzyme activities. Dlugosz et al. (1983) concluded that a negative feedback control could not be demonstrated in humans: “A significant augmentation of amylase and lipase did not occur.” Aprotinin was instilled intraduodenally and caused a 95% inhibition of trypctic activity and a 50% inhibition of chymotryptic activity. Inhibitor-proteinase complexes were not measured, making exact quantitation of the proteinases impossible. Cannulation of the pancreatic duct and reinstillation of the pancreatic juice (Liener et al. 1988) may, in addition to immunodetection, enable quantitative determinations. Cannulation and deviation of pancreatic juice as a means to obtain a low level of enzyme activities have also been an alternative to addition of inhibitors, when studying the regulation of pancreatic secretion [Osnes et al. 1978]. This may have introduced a bias.
A putative signal-substance as the monitor peptide present in rat pancreatic juice may then be removed before it can mediate its signal as under normal conditions (Fushiki et al. 1989). In a similar way, rapid flushing with saline (3 mL/min) has been shown to remove another possible signal-substance, the CCK-releasing peptide from rat duodenum (Lu et al. 1989). The instillation rate in the present human experiments (Holm et al. 1988a) was 1.6 mL/min and cannot be regarded as flushing. The possibility that protease-sensitive signal substances are present in the human duodenum thus cannot be excluded.

We therefore may conclude that in humans, a proteinase-specific feedback regulation exists that is operative under basal and slightly stimulated conditions. This regulation does not seem to be mediated by CCK, secretin or somatostatin (Holm et al. 1988a). An alternative nervous, perhaps cholinergic, regulation is suggested. In addition to the general proteinase stimulation found here, a specific stimulation of an inhibitor-resistant trypsin due to raw soybean instillation, has recently been documented (Holm et al. 1988b). These effects on human pancreatic secretion are consistent with the suggestion that the physiological role of the resistant trypsin is to assure activation of other pancreatic proenzymes. This will allow for digestion to take place in spite of a surplus of proteinase inhibitors from ingested food.

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