

Role of Intraduodenal Proteases in Plasma Cholecystokinin and Pancreaticobiliary Responses to Protein and Amino Acids

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Background & Aims: The role of small intestinal proteolytic activity in the regulation of upper gastrointestinal function in humans is poorly understood. The aim of this study was to determine the importance of proteolytic activity for protein- or amino acid-induced cholecystokinin release and pancreaticobiliary secretion. **Methods:** In 9 healthy subjects, saline was perfused intraduodenally for 3 hours either with or without the synthetic protease inhibitor camostatate. During the last hour, albumin or amino acids in the same molecular composition as albumin were also perfused. **Results:** Perfusion with camostatate, in concentrations that abolished intraduodenal proteolytic activity, had no effect on unstimulated plasma cholecystokinin concentrations or gallbladder emptying, but markedly ($P < 0.05$) increased unstimulated pancreatic enzyme output. Perfusion with protein distinctly stimulated cholecystokinin release, gallbladder emptying, and pancreatic enzyme output ($P < 0.05$). Perfusion with camostatate resulted in significantly lower protein-stimulated plasma cholecystokinin, gallbladder, and pancreatic enzyme responses ($P < 0.05$). Perfusion with amino acids also stimulated plasma cholecystokinin, gallbladder emptying, and pancreatic enzyme output ($P < 0.05$). Camostatate did not inhibit these values. **Conclusions:** This study shows that appropriate digestion of protein is required to stimulate plasma cholecystokinin release, gallbladder emptying, and pancreatic enzyme secretion in humans.

Pancreaticobiliary responses to meals, which are essential for the digestion of nutrients, can be classified into (1) cephalic responses, which are mainly activated by seeing, smelling, chewing, and swallowing of food and are primarily induced by vagal cholinergic activity¹; (2) gastric responses, which are activated when food enters the stomach and are principally dependent on local cholinergic activity²; and (3) intestinal responses, which are activated when food enters the duodenum and are not only dependent on cholinergic but also on peptidergic activity.⁴ Intestinal responses are considered to be most important for appropriate digestion of nutrients. Regulation of pancreaticobiliary secretion at the intestinal level is subjected to feedback control, in which protease activ-

ity seems to play an important role.⁴⁻⁷ However, indications accumulate that species differences in feedback regulation of pancreaticobiliary secretion complicate extrapolation of animal data to humans.⁸⁻¹² A clear comprehension of feedback regulation of pancreaticobiliary secretion in humans is of major importance for the understanding of clinical conditions such as exocrine pancreatic insufficiency and gallstone disease.

The aim of the present study was to investigate the importance of protease activity in the regulation of cholecystokinin (CCK) release, gallbladder contraction, and pancreatic exocrine secretion under both basal conditions and during stimulation with protein (albumin) or an amino acid mixture. For this purpose, protease activity in the lumen of the small intestine was inactivated in some of the experiments by perfusing the duodenum with camostatate, a synthetic protease inhibitor that inactivates proteases including trypsin, chymotrypsin, phospholipase A₂, and pancreatic kallikrein.^{8,9,13}

Materials and Methods

Subjects

Nine healthy volunteers (1 woman and 8 men; mean age, 24 ± 3 years) participated in the studies. None of the volunteers was taking any medication or had a history of gastrointestinal symptoms or surgery. The study protocol was approved by the ethical committee of the University Hospital Nijmegen, and all subjects gave their written informed consent before entering the study.

Reagents

Camostatate was a gift from Professor Guido Adler (University of Ulm, Germany). Radioiodinated porcine pancreatic polypeptide (¹²⁵I-PPP) was obtained from Novo Nordisk AS (Bagsvaerd, Denmark); synthetic CCK₃₃ from Peninsula Laboratories Europe, Ltd. (St. Helens, England); [¹²⁵I]hydroxyphenylpropionic acid-succinimide ester (Bolton-Hunter reagent) from New England Nuclear (Boston, MA); Pharmacia De-

canting Suspension #3 from Pharmacia Diagnostics (Uppsala, Sweden); human pancreatic polypeptide, L-amino acids, and albumin from Sigma Chemical Co. (St. Louis, MO); polyethylene glycol 4000 from BDH Ltd. (Poole, England); and trypsin radioimmunoassay kits from Behring Diagnostica (Amsterdam, The Netherlands). All other materials were obtained from Merck (Amsterdam, The Netherlands).

Study Protocol

After a 12-hour fast, the volunteers presented at the laboratory at 7:30 AM. In random order, 4 experiments were performed separated from each other by at least 1 week. A triple-lumen polyvinyl tube with an outer diameter of 5.7 mm was positioned under fluoroscopic control with the proximal lumen in the stomach, the middle lumen at the level of the ampulla of Vater, and the distal lumen at the ligament of Treitz. In addition, an indwelling intravenous catheter was placed in a forearm for the collection of blood samples. After an equilibration period of at least 30 minutes, the following tests were performed.

Study 1. In 9 subjects, polyethylene glycol 4000 (0.6 g/100 mL saline) was perfused through the middle opening of the tube at a rate of 5 mL/min during the entire 3-hour study period. Camostate (0.6 g/h) was added to this solution during the last 2 hours of the test. During the last hour of the test, albumin (15 g/h, 330 mosmol/L) was also perfused intraduodenally through the middle opening of the tube (Figure 1).

Study 2. The protocol of study 2 was comparable with that of study 1; however, in this experiment, 15 g of albumin without camostate was perfused intraduodenally in the 9 subjects during the last hour of the test (Figure 1).

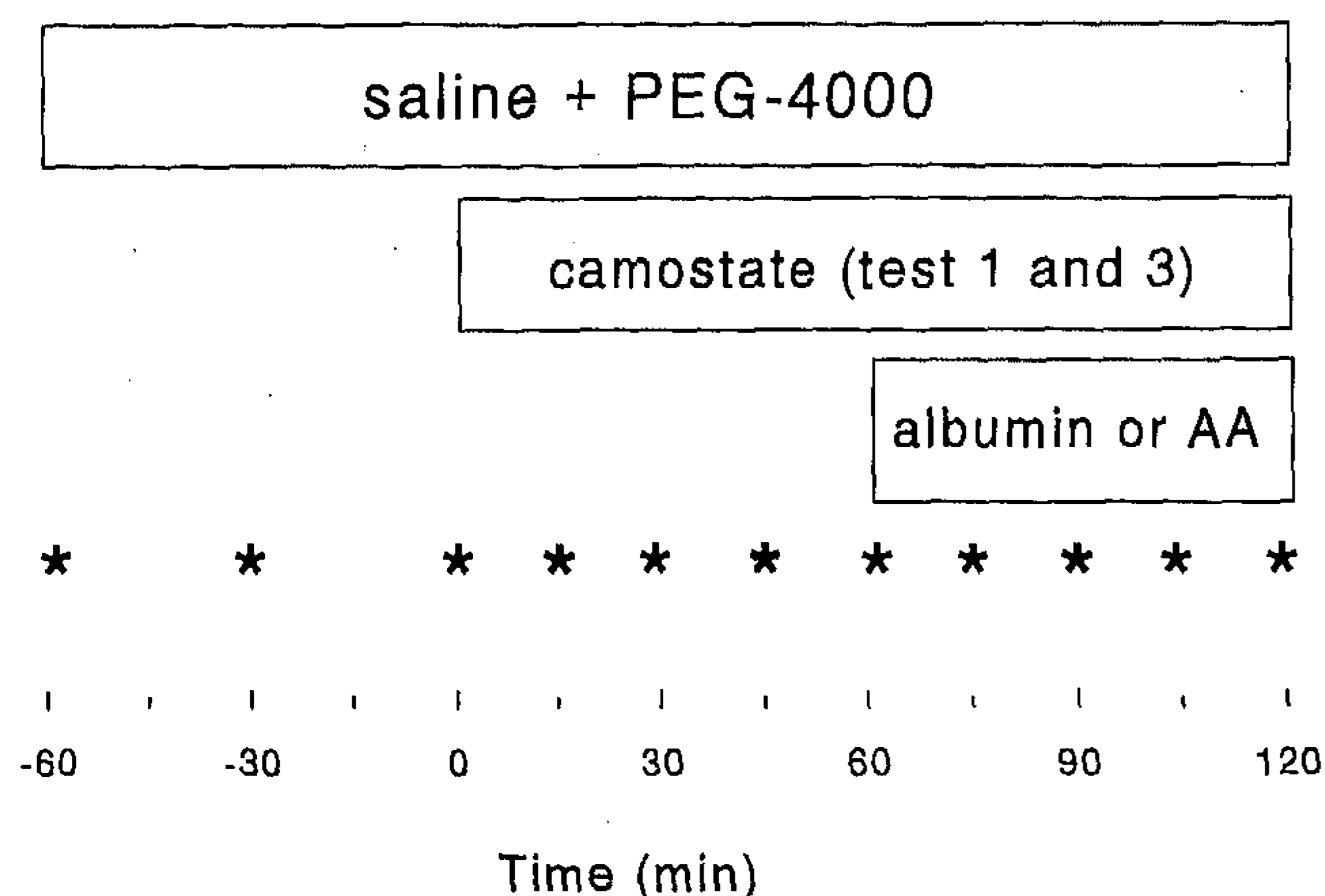


Figure 1. Study protocol for the different experiments in studies 1–4. In study 1 (9 subjects), saline (300 mL/h) was continuously perfused intraduodenally with the recovery marker polyethylene glycol 4000 (1.8 g/h) for 3 hours. During the last 2 hours of the test, the protease inhibitor camostate (0.6 g/h) was added to saline, whereas albumin (15 g) was added to saline during the last test hour. In study 2 (9 subjects), albumin was perfused during the last test hour without camostate. In studies 3 and 4 (6 subjects), the same experiments as in studies 1 and 2 were performed. However, instead of albumin, amino acids were perfused. Asterisks denote time points when blood was drawn and ultrasonography was performed. Duodenal juice was collected during each 15-minute period by spot sampling.

Table 1. Amino Acid Content of the Albumin Meal

Amino acid	Content (mmol) ^a
Alanine	8.9
Arginine	5.3
Aspartic acid	10.5
Cysteine	3.3
Glutamic acid	14.4
Glycine	3.0
Histidine	3.3
Isoleucine	2.5
Leucine	12.0
Lysine	14.0
Methionine	0.7
Phenylalanine	5.1
Proline	5.3
Serine	5.1
Threonine	6.2
Tryptophan	0.4
Tyrosine	3.6
Valine	6.4

^aAmount of amino acids (mmol) in 15 g of the albumin meal.

Study 3. The protocol of study 3 was also comparable with that of study 1; however, instead of albumin, 15 g of an L-amino acid solution (330 mosmol/L) in the same molecular composition as in albumin (Table 1) was perfused intraduodenally in 6 of the 9 subjects (6 men; 24 ± 1 years of age) during the last hour of the test (Figure 1).

Study 4. The protocol of study 4 was comparable with that of study 3 but, in this experiment, 15 g of the L-amino acid mixture without camostate was perfused intraduodenally in the 6 subjects of study 3 during the last hour of the test (Figure 1).

In all tests, gastric acid was continuously drained through the proximal opening of the tube by intermittent negative pressure.

Five-milliliter samples of duodenal contents were taken during 15-minute periods from the tip of the tube by spot sampling¹⁴ and kept on ice. Blood samples were taken every 30 minutes during the first hour and subsequently every 15 minutes until the end of the test period (Figure 1).

Blood was collected in ice-chilled glass tubes containing 2 g/L of ethylenediaminetetraacetic acid. After the experiments were performed, the blood samples were centrifuged at 4°C for 10 minutes at 3000g.

Plasma and duodenal samples were stored at -20°C until assayed for CCK, pancreatic polypeptide, or polyethylene glycol 4000, bilirubin, amylase, trypsin, and chymotrypsin. Each time a blood sample was drawn, two longitudinal and two transverse images of the gallbladder were obtained by real-time ultrasonography.

To determine if camostate affects amylase concentrations in vitro, 4 mL of duodenal juice obtained in 5 subjects during amino acid perfusion was divided into 2 samples of 2 mL each and incubated at 37°C for 30 minutes with or without 2 mg/mL of camostate. Subsequently, the samples were put on ice for another 60 minutes and frozen until assayed for amylase activity.

Plasma Samples

Plasma CCK was measured by a sensitive and specific radioimmunoassay as described previously.^{15,16} The antibody used (T204) binds to biologically active CCK peptides containing the sulfated tyrosine region with almost equal affinity. On a molar base, sulfated gastrins cross-reacted <2% in the assay, whereas no cross-reactivity with unsulfated gastrins or structurally unrelated peptides was found. The detection limit of the assay was between 0.5 and 1.0 pmol/L CCK in plasma. The intra-assay precision ranged from 4.6% to 11.5% in the steep part of the standard curve. All measurements of plasma CCK levels were performed in the same run.

Plasma pancreatic polypeptide levels were also determined by radioimmunoassay.¹⁷ The antibody used showed no cross-reactivity with structurally related gastrointestinal regulatory peptides, such as peptide YY or neuropeptide Y, or with structurally unrelated peptides. The detection limit of the assay was 0.5 pmol/L of incubation mixture. The intra-assay variation ranged from 4% to 7% in the steep part of the standard curve. All measurements of plasma pancreatic polypeptide levels were performed in one run.

Duodenal Samples

Duodenal samples were analyzed for polyethylene glycol 4000,¹⁸ bilirubin,¹⁹ amylase activity,²⁰ and trypsin concentrations. Trypsin concentrations in the duodenal samples were measured by a commercially available radioimmunoassay kit.²¹ For measurement of trypsin concentrations, the duodenal samples were diluted with assay buffer finally containing 2 mg/L of camostate. Camostate did not displace radiolabeled trypsin from the antibody, and the addition of camostate to the standard trypsin solution resulted in an identical standard curve as without camostate (data not shown). Flow rates passing the distal duodenal sampling site were calculated based on known perfusion rates and polyethylene glycol 4000 concentrations at the perfusion and sampling ports.¹⁴ Outputs of bilirubin, trypsin, and amylase were calculated from the product of concentrations or activity and flow rates. In basal duodenal samples taken in the 15-minute period before and from 45 to 60 minutes after the start of albumin perfusion with or without camostate, trypsin and chymotrypsin activity was determined.^{22,23} In these samples, amino acid analysis was also performed by ion-exchange chromatography²⁴ on an amino acid analyzer (Sykam S432) according to the procedure from the manufacturer (Analytica bv; Rijswijk, The Netherlands). Ninhydrin reagent was used, and the ninhydrin-amino acid adduct was quantified with an absorbance detector (Uvis 205; Linear, Analytica bv, Rijswijk, The Netherlands).

Gallbladder Ultrasonography

Longitudinal and transverse images of the gallbladder were obtained by real-time ultrasonography (Sonolayer Sal 77-B; Toshiba, Tokyo, Japan) using a 3.75-MHz transducer.^{25,26} Gallbladder volume was calculated from these images by the sum of cylinders method using a computer system.²⁷ The variation of volume measurements ranged from 6% to 22%.

Statistical Analysis

All measurements were performed in duplicate, and the mean of these two measurements was used for further analysis of results. Gallbladder volume was expressed in milliliters. Integrated plasma CCK, pancreatic polypeptide, and gallbladder responses were determined by calculating the area under the CCK, pancreatic polypeptide, or gallbladder contraction time curves. Subsequently, incremental CCK, pancreatic polypeptide, and gallbladder responses to the various stimulants were calculated by subtracting the integrated response in the basal period (0–60 minutes) from the integrated response in the period of stimulation (60–120 minutes) in each experiment. Similarly, incremental bilirubin, amylase, and trypsin outputs were calculated by subtraction of the total output in the basal period (0–60 minutes) from the total output in the period of stimulation (60–120 minutes).

Results are expressed as mean \pm SEM. Statistical analysis was performed by two-way analysis of variance (trypsin output after logarithmical transformation) and subsequently by least significant difference test or by Student's *t* test for paired results. When appropriate, the method of Bonferroni was used to correct for multiple testing. Differences with a two-tailed *P* value of <0.05 were considered significant.

Results

Plasma Concentrations of CCK and Pancreatic Polypeptide

Plasma CCK and pancreatic polypeptide time curves are shown in Figures 2 and 3. Perfusion of camostate did not significantly influence basal CCK (2.3 ± 0.3 pmol/L) and pancreatic polypeptide (20 ± 3 pmol/L) levels (Table 2). Both the albumin and amino acid meal induced significant ($P < 0.05$) increases of plasma CCK to 3.0 ± 0.4 pmol/L and 4.1 ± 0.5 pmol/L (Figure 2) and of plasma pancreatic polypeptide to 30 ± 3 pmol/L and 44 ± 4 pmol/L (Figure 3), respectively. Camostate abolished ($P < 0.05$) incremental plasma CCK and pancreatic polypeptide responses to albumin perfusion but was without effect on incremental CCK and pancreatic polypeptide responses to the amino acid meal (Table 3). Incremental CCK and pancreatic polypeptide values in the amino acid experiments were higher than in the albumin experiment, but in the case of CCK, this difference failed to reach statistical significance.

Gallbladder Volume and Bilirubin Output

Camostate did not significantly influence basal gallbladder volume or bilirubin output (Figures 4 and 5 and Table 2). Perfusion of albumin or amino acids without camostate induced a statistically significant decrease of gallbladder volume ($P < 0.001$) that was accompanied by a significant ($P < 0.05$) increase of bilirubin output into the duodenum (Figures 4 and 5). Camostate com-

pletely inhibited incremental gallbladder contraction and bilirubin output in response to albumin ($P < 0.05$) but not to amino acids (Table 3). Gallbladder and bilirubin responses to amino acids with and without camostate were higher than those to albumin with and without camostate, but in the case of bilirubin output, this difference failed to reach statistical significance.

Pancreatic Enzyme Output

Perfusion of camostate markedly ($P < 0.05$) increased basal trypsin and amylase output when compared with saline (Figure 6 and Table 2). In the albumin experi-

ments, camostate significantly ($P < 0.05$) inhibited incremental trypsin output and reduced incremental amylase output when compared with albumin perfusion alone (Table 3). Incremental trypsin and amylase outputs during perfusion of amino acids were not influenced by camostate.

Trypsin and Chymotrypsin Activity

Trypsin and chymotrypsin at the end of the last test hour (105–120 minutes) were 1.13 ± 0.62 and 0.34 ± 0.11 kU/L, respectively, during perfusion of albumin without camostate, whereas they were below the

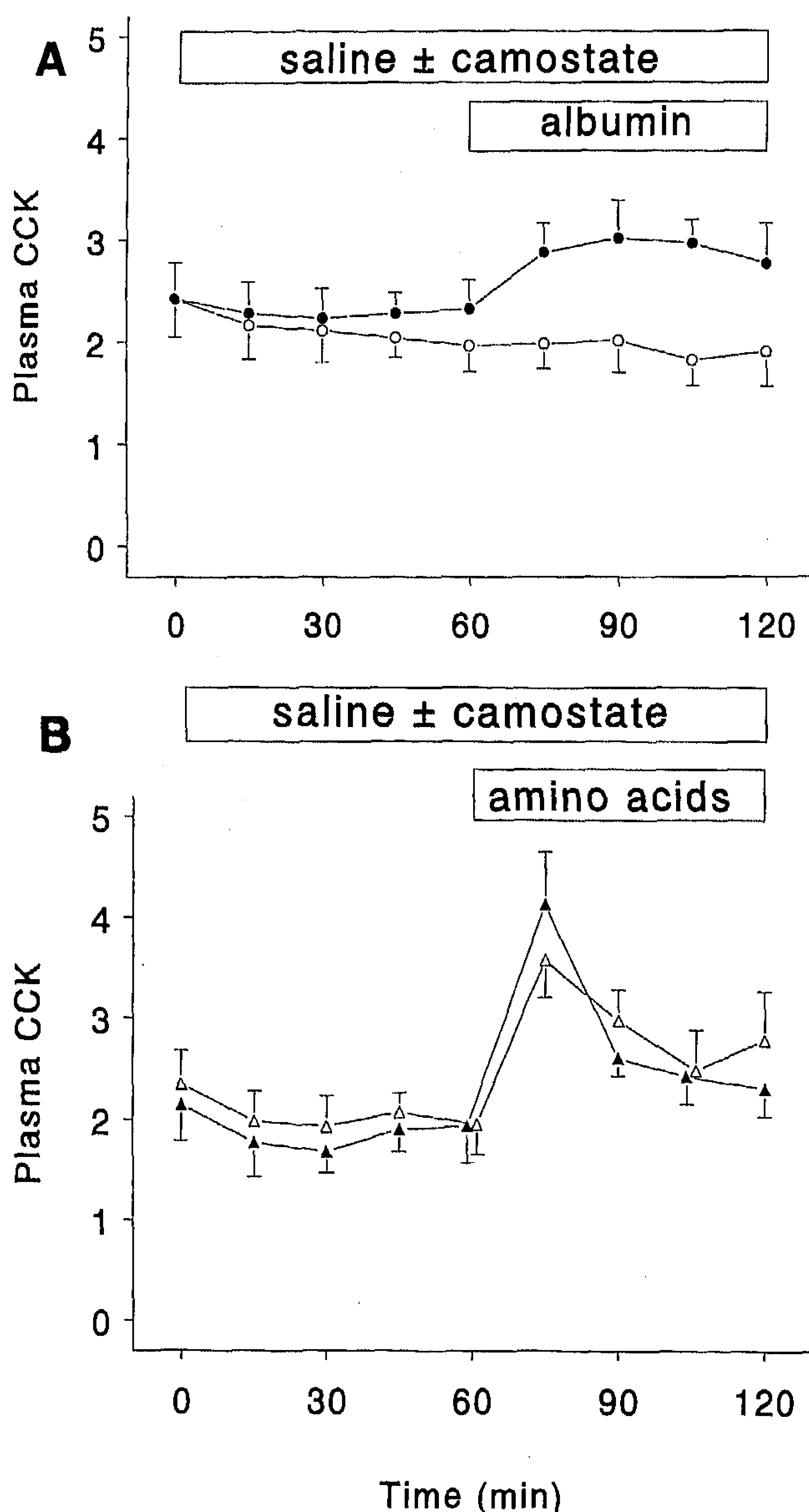


Figure 2. Plasma CCK (pmol/L) in response to intraduodenal perfusion of (A) 15 g of albumin in 9 healthy subjects or (B) 15 g of amino acids in 6 healthy subjects with (open symbols) or without (closed symbols) the protease inhibitor camostate. Results are expressed as mean \pm SEM.

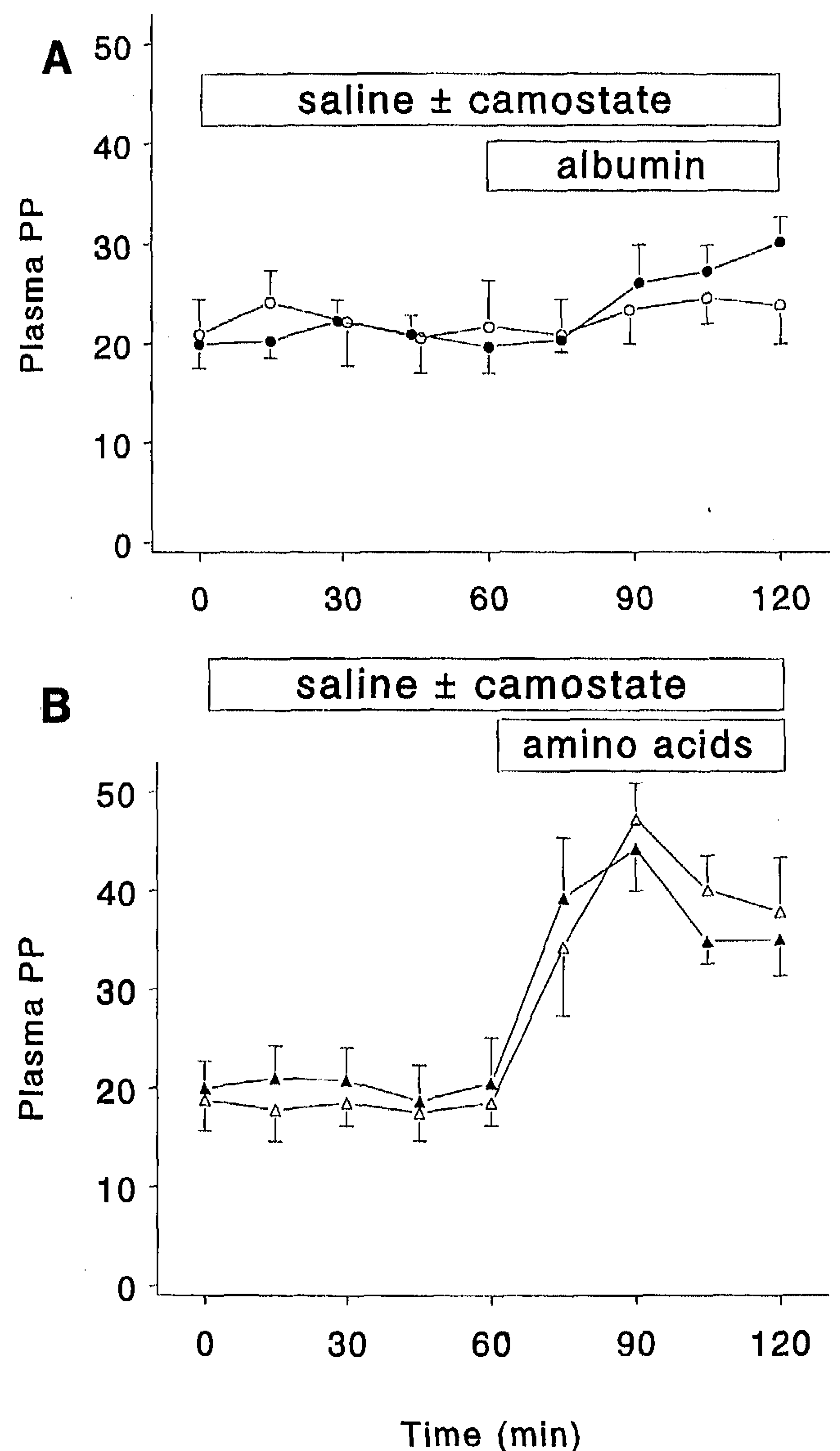


Figure 3. Plasma pancreatic polypeptide (pmol/L) in response to intraduodenal perfusion of (A) 15 g of albumin in 9 healthy subjects or (B) 15 g of amino acids in 6 healthy subjects with (open symbols) or without (closed symbols) the protease inhibitor camostate. Results are expressed as mean \pm SEM.

Table 2. Integrated Responses in the Basal Period (0–60 Minutes)

	Without camostate	With camostate
CCK (<i>pmol/L · 60 min</i>)	137 ± 16	128 ± 16
Pancreatic polypeptide (<i>pmol/L · 60 min</i>)	1246 ± 104	1318 ± 214
Gallbladder contraction (<i>mL · 60 min</i>)	-34 ± 51	18 ± 55
Bilirubin (<i>μmol</i>)	15.2 ± 4.8	16.3 ± 5.0
Trypsin (<i>μg</i>)	216 ± 43 ^a	633 ± 128
Amylase (<i>kU</i>)	5.1 ± 0.8 ^a	7.2 ± 1.2

NOTE. Integrated responses to intraduodenal perfusion of saline with or without camostate in the basal period (0–60 minutes) in 9 subjects. Results are expressed as mean ± SEM.

^aSignificantly different ($P < 0.05$) from camostate.

detection limit (<0.01 kU/L) of the assays ($P < 0.01$) when albumin was perfused with camostate.

Amino Acid Analysis

Before and at the end of albumin perfusion experiments, an additional duodenal juice sample was obtained for amino acid analysis. During albumin perfusion, high amounts of free amino acids were detectable, whereas during addition of camostate to the albumin solution, almost no free amino acids were present ($P < 0.001$ for all amino acids measured; Table 4).

In Vitro Amylase Activity

Amylase activity in the 5 duodenal samples obtained during perfusion of the duodenum with amino acids was 38.4 ± 8.4 kU/L. Addition of camostate to these samples did not significantly affect amylase activity (41.0 ± 8.8 kU/L).

Discussion

In the present study, we have shown that the synthetic protease inhibitor camostate inhibits CCK re-

lease, pancreatic polypeptide release, gallbladder motility, and pancreatic enzyme secretion in response to intraduodenally perfused albumin. Camostate by itself is not responsible for the observed effects because camostate did not affect these parameters in response to an amino acid mixture in the same molecular composition as in albumin. Camostate almost abolished trypsin and chymotrypsin activity in the lumen of the small intestine not only under basal conditions but also during perfusion of albumin. The very low concentrations of free amino acids measured in the duodenal samples during perfusion of albumin with camostate are thus explained by impaired proteolysis of albumin. We therefore conclude that digestion of protein is of crucial importance for stimulation of CCK release, pancreatic polypeptide release, gallbladder contraction, and pancreatic enzyme secretion in humans.

These findings are in line with the observation that inactivation of lipase activity in the lumen of the small intestine by the synthetic lipase inhibitor tetrahydrolipostatin inhibits CCK release in response to unhydrolysed fat and alters biological functions of the hormone, such as biliary output, pancreatic enzyme secretion, and gastric emptying.²⁸ In dogs, amino acids or fatty acids were also found to be more effective than intact proteins or fat in stimulating pancreatic exocrine secretion.^{29,30} This mechanism is different in rats, in which only intact proteins, but not amino acids, stimulate the release of CCK and pancreatic exocrine secretion.^{12,31}

Inactivation of protease activity in the lumen of the human small intestine by camostate under basal conditions resulted in a significant increase of amylase and trypsin output but did not stimulate gallbladder contraction and plasma CCK release. Addition of camostate to duodenal samples did not significantly influence amylase concentrations. This excludes the possibility that the increase of amylase concentrations during camostate perfusion was the result of impaired autodigestion of amylase

Table 3. Incremental Integrated Responses

	Albumin	Albumin + camostate	Amino acids	Amino acids + camostate
CCK (<i>pmol/L · 60 min</i>)	27.0 ± 6.3 ^a	18.4 ± 10.0	58.1 ± 17.6 ^a	49.0 ± 6.8 ^a
Pancreatic polypeptide (<i>pmol/L · 60 min</i>)	245 ± 71 ^a	29 ± 66	978 ± 131 ^{a,b}	1155 ± 115 ^{a,b}
Gallbladder contraction (<i>mL · 60 min</i>)	504 ± 116 ^a	110 ± 50	871 ± 123 ^{a,b}	744 ± 81 ^{a,b}
Bilirubin (<i>μmol</i>)	32.3 ± 9.4 ^a	-1.3 ± 4.6	45.2 ± 17.7 ^a	61.6 ± 12.9 ^a
Trypsin (<i>μg</i>)	903 ± 192 ^a	383 ± 123	836 ± 166 ^a	876 ± 304
Amylase (<i>kU</i>)	3.7 ± 1.7	1.3 ± 0.8	2.9 ± 0.6	3.9 ± 0.8

NOTE. Incremental responses to intraduodenal perfusion of albumin or amino acids with or without camostate in 6 subjects. Incremental responses were obtained after subtraction of integrated values in the basal period (0–60 minutes) from integrated values in the period of stimulation (60–120 minutes). Results are expressed as mean ± SEM.

^aSignificantly different ($P < 0.05$) from albumin with camostate.

^bSignificantly different ($P < 0.05$) from albumin without camostate.

by inhibition of protease activity.^{9,32} This supports the hypothesis that at least under unstimulated conditions, feedback regulation between pancreatic enzyme secretion and protease activity in the lumen of the small intestine is operative in humans^{8-10,33} as well as in other species such as rats.^{7,34,35} However, in contrast with rats,^{7,35} protease-dependent feedback regulation of pancreatic exocrine secretion under basal conditions in humans is probably not mediated by CCK but by cholinergic activity.¹⁰

CCK-dependent negative feedback regulation between intraluminal contents and pancreatic exocrine secretion

has not only been shown under basal conditions but also after stimulation with a meal.^{4,7,35} However, the present study is inconsistent with this hypothesis because camostate did not enhance albumin- or amino acid-stimulated CCK release. Methodological differences between previous studies and the present study may have contributed to these conflicting results concerning the role of protease activity in the lumen of the human small intestine and CCK release. In contrast with our study, CCK was not measured³¹ or measured by different techniques^{4,35} or less specific antibodies³² and endogenous protease activity was not inactivated by camostate^{33,34} or suppressed by perfusing bovine trypsin into the duodenum.⁴ The latter study⁴ shows that proteases can inhibit

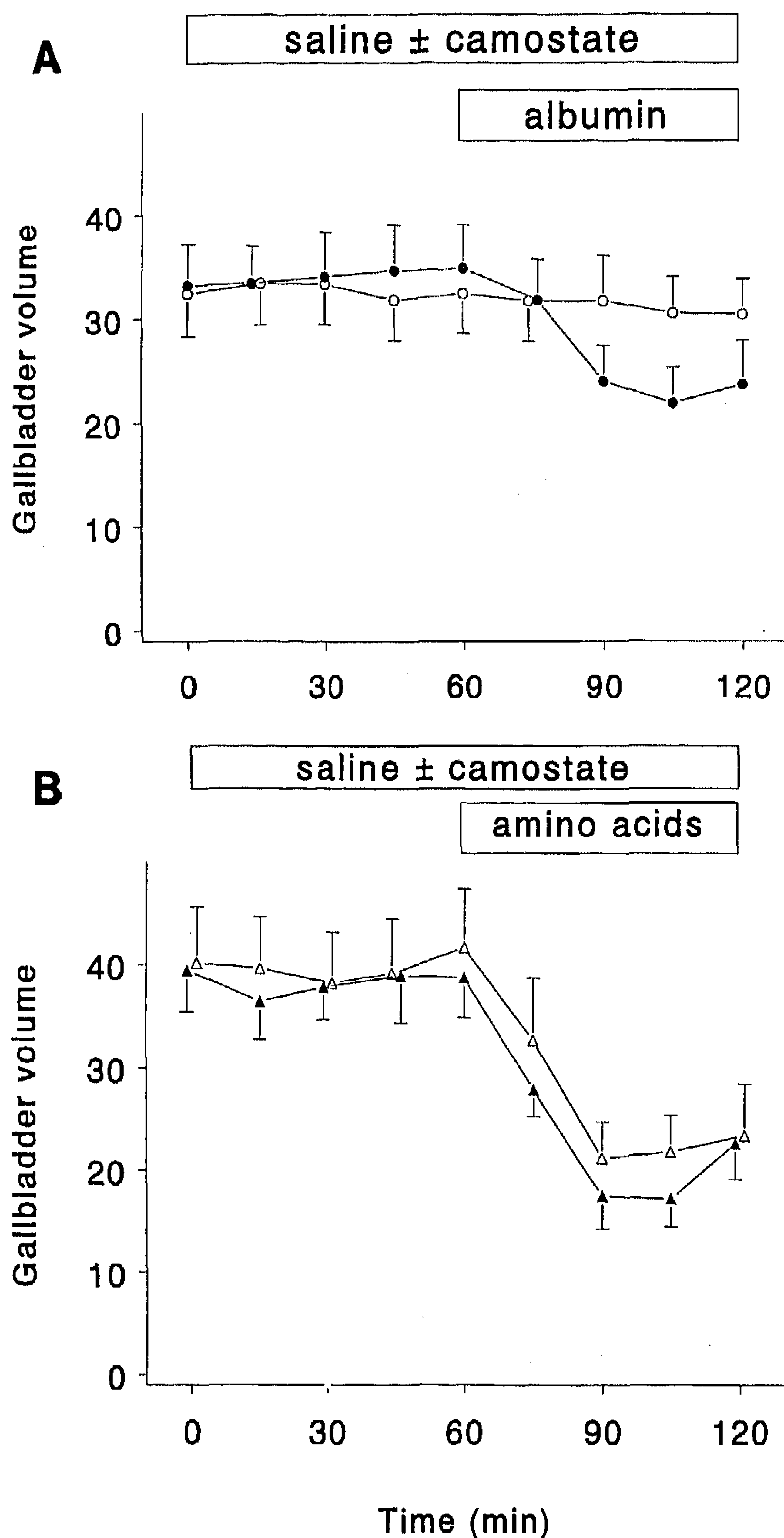


Figure 4. Gallbladder volumes (mL) in response to intraduodenal perfusion of (A) 15 g of albumin in 9 healthy subjects or (B) 15 g of amino acids in 6 healthy subjects with (open symbols) or without (closed symbols) the protease inhibitor camostate. Results are expressed as mean \pm SEM.

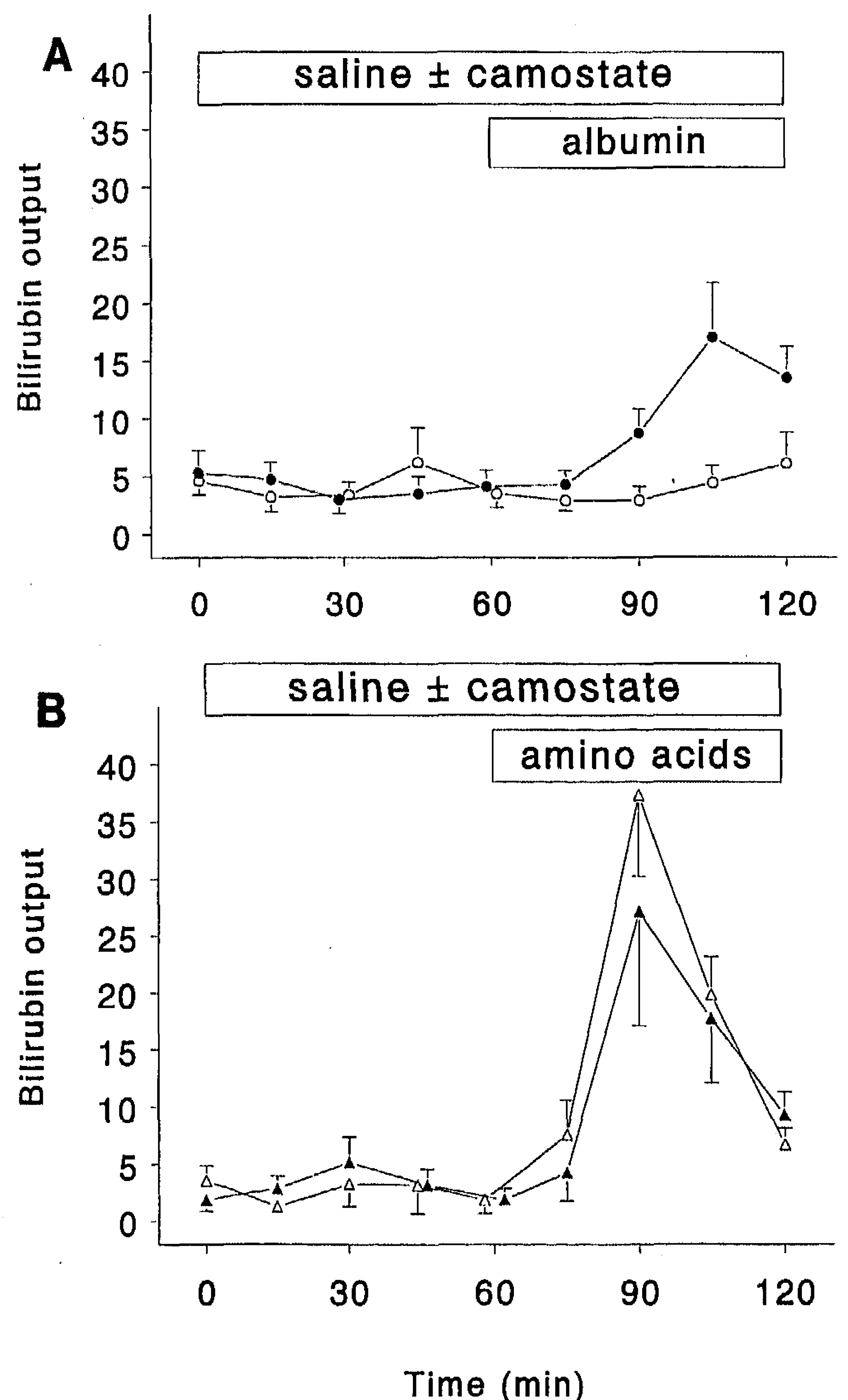


Figure 5. Bilirubin output ($\mu\text{mol}/15$ min) in response to intraduodenal perfusion of (A) 15 g of albumin in 9 healthy subjects or (B) 15 g of amino acids in 6 healthy subjects with (open symbols) or without (closed symbols) the protease inhibitor camostate. Results are expressed as mean \pm SEM.

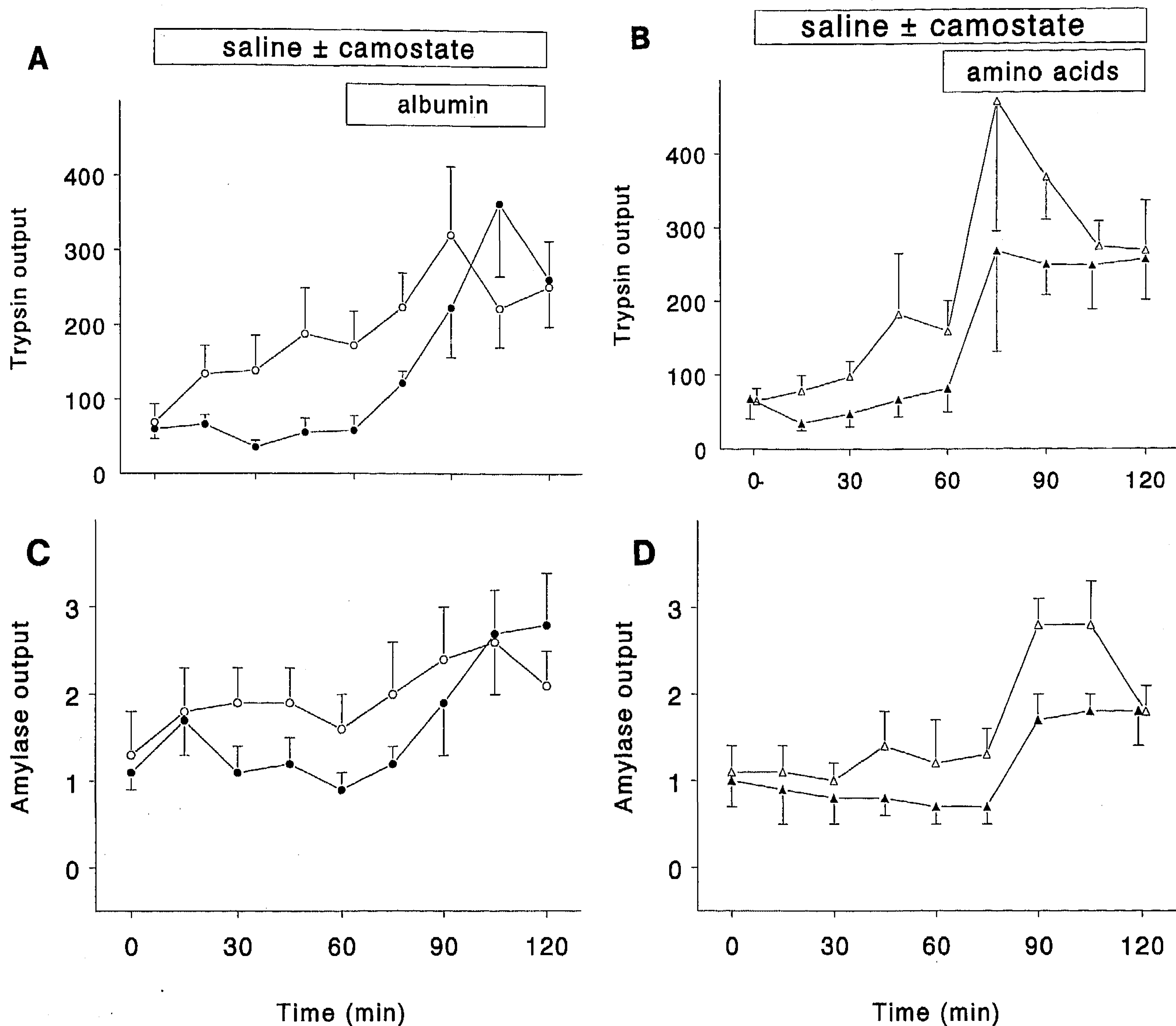


Figure 6. (A and B) Trypsin output ($\mu\text{g}/15 \text{ min}$) and (C and D) amylase output ($\text{kU}/15 \text{ min}$) in response to intraduodenal perfusion of (A and C) 15 g of albumin in 9 healthy subjects or (B and D) 15 g of amino acids in 6 healthy subjects with (*open symbols*) or without (*closed symbols*) the protease inhibitor camostatate. Results are expressed as mean \pm SEM.

CCK release, whereas the present study shows that inhibition of proteases does not stimulate CCK release.

Because we and others have shown that CCK release and gallbladder contraction in response to intraduodenal administration of a meal or bombesin are augmented by sequestration of bile acids^{11,36-39} but not by protease inactivation, we believe that CCK-mediated feedback control of pancreaticobiliary secretion in humans is principally regulated by bile acids and not by protease activity.^{10,36}

Protein and fat are the major constituents of a meal that stimulate CCK release, pancreatic exocrine secretion, and gallbladder contraction.³⁹⁻⁴¹ In a meal that was isonitrogenous to 18 g of beef hydrolysate, the amount of the individual amino acids tryptophan, methionine,

phenylalanine, and valine, which were potent stimuli of exocrine pancreatic secretion,^{42,43} was 0.8, 3.1, 4.0, and 7.7 mmol/h, respectively. The amounts of the individual amino acids present in our albumin and amino acid meal (0.4, 0.7, 5.1, and 6.4 mmol/h, respectively) were comparable. These results confirm that CCK is important for stimulating pancreaticobiliary secretion in humans at nutrient quantities comparable with a regular meal.^{39,43}

The present findings, together with the report that lipolysis is essential to stimulate CCK release and gallbladder contraction in humans,²⁸ provide an explanation for our findings in patients with exocrine pancreatic insufficiency in which impaired postprandial release of CCK and gallbladder motility are restored by supplementation of pancreatic enzymes.⁴⁴ The present data also

Table 4. Increments (Mean \pm SEM) of Intraduodenal Free Amino Acid Concentrations in Response to an Albumin Meal With or Without Camostate

Amino acid	Albumin	Albumin + camostate
Threonine	1963 \pm 414	163 \pm 62
Serine	1420 \pm 303	144 \pm 46
Aspartic acid	708 \pm 135	33 \pm 20
Glutamic acid	1599 \pm 334	82 \pm 36
Proline	579 \pm 145	150 \pm 68
Glycine	226 \pm 86	115 \pm 41
Alanine	2836 \pm 635	278 \pm 64
Valine	3397 \pm 744	192 \pm 42
Cysteine	391 \pm 79	12 \pm 6
Methionine	612 \pm 93	37 \pm 12
Isoleucine	1189 \pm 231	61 \pm 18
Leucine	6073 \pm 1186	302 \pm 69
Tyrosine	2727 \pm 809	78 \pm 23
Phenylalanine	4420 \pm 858	154 \pm 42
Lysine	7108 \pm 1426	173 \pm 32
Histidine	1248 \pm 246	54 \pm 16
Arginine	3664 \pm 721	90 \pm 20
Tryptophan ^a	++++	++

NOTE. Values expressed as μ mol/L.

^aNot quantitatively tested.

provide an explanation for our experiences in patients with celiac disease and a flat jejunal mucosa in which CCK release and gallbladder contraction are also impaired in response to an ordinary meal but not to a predigested meal.^{45,46}

In conclusion, the present study shows that intraduodenal stimulation of CCK release, gallbladder contraction, and pancreatic enzyme secretion is dependent on digestion of protein. Undigested protein only weakly stimulates pancreatic enzyme output and does not significantly stimulate CCK release and gallbladder contraction, whereas digested protein markedly stimulates the release of CCK, gallbladder contraction, and pancreatic enzyme output.

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