The gene of a novel chymotrypsin-like serine protease has been cloned from human pancreas. The chymotrypsin-like enzyme-1 gene is located on chromosome 16q22.1 in a tight cluster with four unrelated genes. The gene has seven exons with the signal and activation peptide and the three main catalytic residues forming the active site encoded by separate exons. Northern blots of pancreatic mRNA showed a major transcript of 1.9 kilobases and a minor transcript of 1.3 kilobases due to alternative polyadenylation. No transcript was found in other tissues. Its presence in pancreatic tissue, duodenal juice, and urine was demonstrated with antisera raised against synthetic peptides from the derived amino acid sequence of the gene. The peptide sequences were chosen for being most dissimilar to chymotrypsin, and the antisera obtained did not react with purified human chymotrypsin. The proteolytically active CTRL-1 has been identified in pancreatic homogenate, duodenal juice, and urine, and a recombinant CTRL-1 has been characterized. Increased pancreatic secretion of CTRL-1 was induced by protease inhibitors indicating that the enzyme is secreted from pancreas upon feedback stimulation. Both native and recombinant CTRL-1 displayed chymotrypsin- and elastase-2-like activities and hydrolyzed the amide bonds of substrates having tyrosine, phenylalanine, or leucine residues at the P1 and hydrolyzed the amide bonds of substrates having enhanced by duodenal instillation of protease inhibitors, and it may consequently be part of a pancreatic protease backup system.

EXPERIMENTAL PROCEDURES

Materials—Kunitz trypsin inhibitor, Bowman-Birk inhibitor, and diaminobenzidine were obtained from Sigma; protease K was from Boehringer Mannheim; camostate (foy-305) was a gift from Dr. H. Babst, Sand-Schwarz, Monheim, Germany; Dynabeads oligo(dT) and anti-rabbit IgG were from Dynal, Inc.; Genescreen membranes were from DuPont NEN; restriction enzymes were from New England Biolabs Inc.; HL1163b agt11 library and 5′-RACE kit were from Clontech; Magic Miniprep was from Promega; Bluescript vectors were from Stratagene; T7 polymerase, precast 8–18 and 10–15% SDS-polyacrylamide gels, Sephadex G100 HiLoad 16/60, Superdex prep grade 75, CNBr-activated Sepharose, and the Sephasil C18 SuperPac Cartridge Pep-S column were from Pharmacia Biotech Inc.; Microse 10 K was from Filter Scandinavia; horseradish peroxidase-labeled goat anti-rabbit sera were from Southern Biotechnology; human chymotrypsin, human trypsin, anti-human chymotrypsin, and anti-human trypsin were from Calbiochem; low molecular weight SDS-PAGE standards were from Bio-Rad.

Serine proteases are enzymes utilizing an activated serine residue in their substrate binding site to catalyze the hydrolysis of certain peptide bonds. These proteases are encoded by related genes that evolved from a common ancestral protease through gene duplication (1, 2). The pancreatic subfamily of proteases comprises trypsins, chymotrypsins, kallikrein, and elastases. Their structural genes encode related enzymes of similar size, structure, and function but with different peptide bond specificities. The catalytic activity of chymotrypsin (EC 3.4.21.1) is directed at peptide bonds involving aromatic, and to a lesser degree, aliphatic amino acids (3, 4). Chymotrypsin is synthesized by pancreatic acinar cells as an inactive precursor (chymotrypsinogen) secreted to the duodenum and activated by tryptic cleavage. Two or three forms of chymotrypsin (5–7) as well as one major and one minor form of chymotrypsinogen have been reported in humans (8). Chymotrypsins are the most abundant among the pancreatic proteases and may represent 10–20% of the total protein synthesized by the exocrine pancreas (3, 4).

We describe here a novel human pancreatic serine protease (chromosome 16q22.1) with a 54% sequence identity to human chymotrypsin B (chromosome 16q22.3). A comparison with human elastases gave 45% identical residues between the new enzyme and elastase 2A. The enzyme was provisionally named chymotrypsin-like enzyme-1 (CTRL-1).1 Its expression was enhanced by duodenal instillation of protease inhibitors, and it may consequently be part of a pancreatic protease backup system.
Bio-Rad; chromogenic substrates -OMe-succinyl-Arg-Pro-Tyr-pNA (S-2586), benzoyl-Arg-Val-Trp-pNA (S-2532), benzoyl-Lys-Val-Tyr-pNA (S-2504), D-Ser-Leu-Met-pNA (S-2327), and D-Lys-Val-Phe-pNA (S-2538) were from Chromogenix, and glutaryl-Ala-Ala-Pro-Leu-pNA was from Peptide Institute; minimal essential medium (MEM) and Glasgow MEM were from Life Technologies, Inc. Patient material was obtained from the Transplantation Unit, Surgical Department, Rikshospitalet, Oslo, Norway. Two lines of BHK cells and the Semliki Forest virus (SFV) expression system were kind gifts from Dr. Henrik Garoff (Center of Biotechnology, Huddinge, Sweden). The pXT vector was kindly donated by Dr. S. J. Clark (CSIRO Division of Biomolecular Engineering, Sydney, Australia).

Preparation of mRNA and Northern Blots—Human pancreas, spleen, liver, and adrenals removed surgically were immediately frozen and stored at -70 °C until use. Total cellular RNA was isolated (9), and poly(A) mRNA was purified with Dynabeads oligo(dT)25. Northern blots were prepared (9) using GeneScreen membranes and then hybridized and washed in 1× SSC (0.15 M NaCl, 0.015 M sodium citrate, pH 7.0) at 68–70 °C (10). DNA probes were labeled with32P by random priming (11). The blots used for Fig. 2B were from Clontech.

Screening for cDNA Clones and Sequencing—Agt11 cDNA library (HL1163b) was screened with a 32P-labeled 780-base pair genomic fragment ( EcoRI and NsiI) covering three putative polyadenylation signals are shown in bold. Three putative polyadenylation signals are shown in bold.

**Peptide Synthesis and Production of Antisera—**Two peptides were synthesized from the derived amino acid sequence (peptide A, EYDRSSNAEPLQVLSVSR, 18-mer, residues 89–106 in the prepro-sequence and peptide B, NVRAPAVYTRVSKFSTWINQ, 20-mer, residues 240–259), having been selected for being the most different from the corresponding sequence of chymotrypsin B and for potential antigenicity by the Jameson and Wolf algorithm (15). They were created using the Fmoc/t-butyI protecting group strategy in continuous flow solid phase peptide synthesis (16) performed by an automated Biolynx 4170 synthesizer (Pharmacia). 4-Methoxy-2,3,6-trimethylbenzenesulfonyl was used as a protector of arginine side chains (17). The Fmoc amino acids were activated for coupling as pentafluorophenyl esters (18) except for serine and threonine, which were activated as 3,4-dihydro-4-oxo-1,2,3-
benzotriazin-3-yl esters (17). Four equivalents of activated amino acid and 1-hydroxybenzotriazole were used in each coupling step, which was performed in dimethylformamide (DMF). Novasyn PA 500 (Novabiochem USA) was used as solid support, and Fmoc cleavage was done in 20% piperidine in dimethylformamide, 5% thioanisole in trifluoroacetic acid (TFA). Fmoc was added to cleave the resin and to remove side chain protecting groups. The peptides were purified and analyzed by reverse phase (C18) HPLC (Shimadzu LC5A).

Antisera were raised in rabbits against these two peptides by immunizing every 3rd week with 100 μg of peptide coupled to keyhole limpet hemocyanin and Freund’s complete or incomplete adjuvant. Their antibody responses were monitored in an ELISA using the respective peptides as antigens and horseradish peroxidase-labeled goat anti-rabbit Ig (1:5000) as the second antibody. The 3,3′-diaminobenzidine (tetrahydrochloride)-based horseradish peroxidase reaction product was visualized using 0.03% H₂O₂, 0.3 g/liter diaminobenzidine, and 0.5 g/liter NiCl₂ in 50 mM (NH₄)₂HCO₃ (19). Anti-peptide A (APA) sera from two rabbits were pooled, as were two anti-peptide B sera (APB). In an ELISA assay, APA and APB were found not to react with immunoprecipitated chymotrypsin or commercially available chymotrypsin. Anti-chymotrypsin, however, reacted with CTRL-1 immunoprecipitated by APA or APB.

Identification of the CTRL-1 Protein—SDS-PAGE with and without reducing agents was performed on precast gels (8–18%). The SDS-PAGE-separated proteins were electroblotted onto nitrocellulose membranes (20). Proteins from different sources of pancreatic enzymes were utilized: clam frozen slices of pancreatic tissue homogenized (25 mg/ml) and diluted in 200 mM Tris-HCl, pH 8.0, at 4°C for 24 h and activated by the addition of 1 mM bovine trypsin, duodenal juice from healthy volunteers collected every 10 min during stimulation with secretin (30 min) and 1-hydroxybenzotriazole was blocked by incubation in 1% casein for 1 h at room temperature before the antiserum solution was added. Anti-chymotrypsin was used in a 1:3000 dilution in PBS, while APA and APB were diluted 1:1000. After incubation overnight at 4°C, the membranes were washed 5 times in PBS before a further 2-h incubation in a 1:3000 dilution of horseradish peroxidase-labeled goat anti-rabbit immunoglobulin and visualized as described above (19).

Substrate specificity of immunoprecipitated enzymes was tested using Nunc-Immuno Plate Maxi Sorb (Nunc, Roskilde, Denmark) coated with APA, APB, or antisera against human chymotrypsin (10 μg/ml overnight at 4°C). Excess protein binding capacity was blocked by 1% casein in PBS for 1 h at room temperature. The source of pancreatic enzyme (diluted duodenal juice, urine from transplanted patients, or homogenate of pancreatic tissue) was added in 10-fold dilution, and the plates were incubated at 4°C for 4 h to avoid autodigestion. After washing, the substrates were added at a final concentration of 2–5 mM in 200 mM Tris-HCl, pH 8.3, and 20 mM CaCl₂. The substrate reaction was monitored in an ELISA reader (Titertek Multiskan MKII) at 405 nm after incubation for 24 h at room temperature. Optical density was recorded against the appropriate blanks. The substrates used were S-2586, 1.3-kb probe did not hybridize with the 1.5-kb transcript. The autoradiogram was overexposed to show the 1.3-kb transcript. B. Northern blot of mRNA (lanes 1-6) isolated from spleen (1), thymus (2), prostate (3), testes (4), ovary (5), small intestine (6), colon (7), peripheral blood leukocytes (8), heart (9), brain (10), placenta (11), lung (12), liver (13), skeletal muscle (14), kidney (15), and pancreas (16). The blots were hybridized with the EcoRI-NSI probe as well as with β-actin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probes, with stripping between each hybridization.

and EcoRV and blunting of the BamHI end, an 865-base pair fragment was cloned into the Smal site of pSFV to create pSFV-CTRL. RNA made in vitro from pSFV-CTRL and helper vector 1 was introduced into BHK cells by electroporation (22). Virus particles from the supernatant were stored in aliquots at −80°C.

CTRL-1 SFV particles diluted 1:10 in MEM with 0.2% bovine serum albumin were added to subconfluent BHK cells for 1 h. The cells were washed with PBS before incubation for 24 h in Glasgow MEM containing 20 mM Hepes (pH 7.4) and 2 mM glutamine. Expression of CTRL-1 was monitored by fluorescence microscopy of methanol-fixed cells stained with a mixture of APA and APB (diluted 1:500 in PBS) and goat anti-rabbit fluorescein isothiocyanate (dilute 1:1000 in PBS). Infected cells were lysed in 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, and 2% Nonidet P-40 and were left on ice for 30 min. The lysed cells were centrifuged at 10,000 rpm for 15 min, and the supernatant was stored at −80°C prior to purification of CTRL-1.

Purification of Recombinant CTRL-1—Extracts of infected cells were separated on a Sephadex G-100 column. The catalytic activity of the fractions was monitored using S-2586 and glutaryl-Ala-Ala-Pro-Leu-
NA as substrates. Fractions constituting two partially overlapping peaks of activity were pooled and concentrated by centrifugation at 7500 rpm and 4 °C for 1 h using Microsep 10-kDa filters. The pooled fractions were further separated on a Superdex 75 prep grade column, giving one distinct peak of proteolytic activity. The fractions constituting this peak were pooled and concentrated using the 10-kDa cutoff filter tubes. Enzyme specificity and Mr of the protein in the pooled and concentrated fractions were analyzed. The yield from one 180-cm² flask was determined to be about 6 mg of purified protein. The enzyme from one flask was used for 20 activity measurements with the various substrates.

**RESULTS**

Characterization of the CTRL-1 Transcription Unit and the Deduced Protein Sequence—The CTRL-1 gene has seven exons with an open reading frame of 264 amino acids (Fig. 1). By screening a human cDNA library from the pancreas, 50 clones were obtained, 11 of which were sequenced. Seven of the sequenced clones were complete at the 3' end including a poly(A) tail. There was a difference in the 3' end of the cDNA caused by alternative polyadenylation. Three putative polyadenylation signals (A, B, and C) were identified in the genomic sequence (Fig. 1). The use of signal A (AUUAAA) results in a minimum transcript of 861 bases plus the poly(A) tail, in accordance with the 1.0-kb transcript seen on Northern blots (Fig. 2). On the basis of the sequenced cDNA clones (5 out of 7) and Northern blots, this is the major signal for polyadenylation. The actual polyadenylation site varied. Poly(A) tails were added 15, 21, and 26 bases downstream of the polyadenylation signal A, with the latter probably as the major site (3 out of 5). Two minor polyadenylation signals, B (AAUACA) and C (AAUAAA), were probably used in two of the sequenced cDNA clones with the poly(A) tail added 17 and 21 bases downstream of the signals, respectively (Fig. 1). The use of the latter signal creates minimum transcripts of 1129 and 1167 bases plus poly(A) tails corresponding to the 1.3-kb transcripts seen on the Northern blots (Fig. 2). A probe from the 3'-untranslated region identified the larger transcript (Fig. 2). Only three clones were close to full-length cDNA covering the translation start, and two cDNA clones had a 5' -untranslated sequence of 10 bases. This was confirmed by sequencing of 5' -RACE PCR-amplified fragments, which showed a sequence of 10 bases upstream of the putative translation start. Thus, the transcription unit starts 10 bases above the translation start codon.

No transcript was found in tissues other than pancreas (Fig. 2B). By alignment of the deduced CTRL-1 protein sequence to the sequences of five chymotrypsins (Fig. 3) the similarities were evident. Putative leader and activation peptides, active site residues, and disulfide bridges show perfect alignment in spite of only 54% identical residues (Fig. 3). Among themselves the chymotrypsins were 82–88% identical. By analogy to other serine proteases CTRL-1 is probably synthesized as a prepro-protein with a signal peptide consisting of 18 amino acids encoded by exon 1 and the first two bases of exon 2 (Fig. 1). The zymogen must be cleaved, probably between Arg33 and Ile34, to form an active protease. The 15 residues of this activation peptide as well as 19 residues of the active enzyme were encoded by exon 2. The three active site residues His75, Asp121, and Ser214 were encoded by exons 3, 5, and 7 as in chymotrypsin B. The CTRL-1 zymogen of 246 residues had a calculated Mr of 26,100 and a pI of 8.1 in the absence of glycosylation. A putative N-glycosylation site was the
asparagine at residue 114.

Identification of an Exocrine Enzyme—APA sera from two rabbits were pooled, as were APB sera. None of the antisera reacted with purified human chymotrypsin A (Calbiochem). Under non-reducing conditions both APA and APB sera reacted in Western blotting with a protein of an apparent Mr of 25,000–27,000 in pancreatic homogenates (Fig. 4). A marked band at about 55–60 kDa and a more diffuse band above 106 kDa were seen (Fig. 4, lane 2). The band above 106 kDa was caused by direct nonspecific binding of the secondary antibody (goat anti-rabbit). The 55–60-kDa band was seen both with APA and APB sera. None of the antisera reacted with purified human chymotrypsin A (Calbiochem).

Under non-reducing conditions both APA and APB sera reacted with proteins in pancreatic secretions (similar results with APB). The 55–60-kDa band was seen both with APA and APB, Coomassie-stained; lane 3, proteins after immunopurification using APA and APB; Coomassie stained; lane 4, Western blot showing APB reaction toward the proteins in lane 3 detected with horseradish peroxidase-labeled goat anti-rabbit and visualized according to Adams (19).

The substrate specificity of recombinant CTRL-1 and immunoprecipitated CTRL-1 using APA/APB antibodies revealed the presence of a specifically bound peptidolytic activity in urine from pancreas-transplanted patients (Table I). Partial purification of CTRL-1 using urine from such patients as well as APA and APB resulted in a mixture of two main proteins (Fig. 5, lane 3). The N-terminal amino acid sequence of the protein with the lower Mr was found to start with IS-(C?)-PEGTNAYRS. Search in the Swissprot data base gave a 91% identity with human lithostathine precursor (pancreatic stone protein, ISCPEGTNAYRS). The protein with Mr of 24,000–25,000 was identified by Western blotting to be CTRL-1 (Fig. 5, lane 4). Further purification of this material was not achieved. We therefore decided to express CTRL-1 in the Semliki Forest virus system using BHK cells.

Purification of CTRL-1—Direct immunoprecipitation of CTRL-1 using APA/APB antibodies revealed the presence of a specifically bound peptidolytic activity in urine from pancreas-transplanted patients (Table I). Partial purification of CTRL-1 using urine from such patients as well as APA and APB resulted in a mixture of two main proteins (Fig. 5, lane 3). The N-terminal amino acid sequence of the protein with the lower Mr was found to start with IS-(C?)-PEGTNAYRS. Search in the Swissprot data base gave a 91% identity with human lithostathine precursor (pancreatic stone protein, ISCPEGTNAYRS). The protein with Mr of 24,000–25,000 was identified by Western blotting to be CTRL-1 (Fig. 5, lane 4). Further purification of this material was not achieved. We therefore decided to express CTRL-1 in the Semliki Forest virus system using BHK cells.

Purification of the recombinant CTRL-1 resulted in a single protein band with Mr of 25,000 visualized by silver staining and a peptidolytic activity (Fig. 6, lane 6, and Table II). The peptidolytic activity of the purified fractions of recombinant CTRL-1 was removed by APA/APB bound to M280 Dynabeads, identifying CTRL-1 as the only protein with catalytic activity in the samples.

Substrate Specificity of CTRL-1—The specificity of immunoprecipitated CTRL-1 toward a number of elastase and chymotrypsin substrates was compared with that of chymotrypsin immunoprecipitated from the same source (duodenal juice, homogenates of pancreatic tissue, or urine from pancreas-transplanted patients). The specificity of CTRL-1 was significantly different from that of chymotrypsin (Table I), but the differences were not dramatic and indicated proteolytic activity at amino acids with aromatic side chains (Tyr, Phe, and Trp) and with aliphatic (Leu) and sulfur-containing (Met) side chains. The substrate specificity of recombinant CTRL-1 and immunoprecipitated CTRL-1 was compared with that of human chymotrypsin and BLG.

### Table I

| Substrates                      | Mr (kDa) | Peptide Bonded to | Ratio |
|---------------------------------|----------|------------------|-------|
| OMe-succinyl-Arg-Pro-Tyr-pNA    | 0.42 ± 0.14<sup>a</sup> | 0.17 ± 0.02<sup>b</sup> | 2.47  |
| d-Ser-Leu-Met-pNA               | 0.011 ± 0.004<sup>a</sup> | 0.066 ± 0.002<sup>b</sup> | 18.33 |
| Benzoyl-Lys-Val-Tyr-pNA         | 0.98 ± 0.37<sup>a</sup>  | 0.04 ± 0.02<sup>b</sup>  | 20.94 |
| d-Lys-Val-Phe-pNA               | 0.70 ± 0.04<sup>a</sup>  | 0.14 ± 0.03<sup>b</sup>  | 4.86  |
| Benzoyl-Arg-Val-Trp-pNA         | 0.36<sup>b</sup>          | 0.15<sup>b</sup>         | 2.34  |
| Glutaryl-Ala-Ala-Pro-Leu-pNA    | 0.23<sup>b</sup>          | 0.21<sup>b</sup>         | 1.13  |

<sup>a</sup> Data (nmol · h<sup>−1</sup>) from three experiments (each in triplicate) given as mean ± S.E.

<sup>b</sup> Data (nmol · h<sup>−1</sup>) from mean of one experiment in triplicate.

### FIG. 4

Western blots showing the reactions of antiserum APB with proteins in pancreatic secretions (similar results with APA). Lane 1, 0.05 mg/ml human chymotrypsin A; lane 2, human pancreas homogenate; lane 3, trypsin-activated human pancreas homogenate; lane 4, duodenal juice harvested under basal conditions about 18 cm below the pyloric region; lane 5, duodenal juice harvested at the same site during intraduodenal instillation of a mixture of protease inhibitors (20); lane 6, duodenal juice harvested at the end (60 min) of intraduodenal instillation of the protease inhibitors (20).

### FIG. 5

Purification of CTRL-1. Lane 1, extract of BHK cells infected with CTRL-1 SFV particles; lane 2, immunoprecipitated CTRL-1 from BHK cell extract in lane 1, using APA; lane 3, extract of uninfected (control) BHK cells; lane 4, immunoprecipitate from control BHK cell extract using APA; lane 5, pool of active fractions of extract of CTRL-1 SFV-infected cells after separation on Sephadex G-100; lane 6, pool of two fractions containing the peptidase activity after separation on a Superdex prep grade column.

### FIG. 6

Silver staining of an SDS-polyacrylamide gel showing purification of recombinant CTRL-1. Lane 1, extract of BHK cells infected with CTRL-1 SFV particles; lane 2, immunoprecipitated CTRL-1 from BHK cell extract in lane 1, using APA; lane 3, extract of uninfected (control) BHK cells; lane 4, immunoprecipitate from control BHK cell extract using APA; lane 5, pool of active fractions of extract of CTRL-1 SFV-infected cells after separation on Sephadex G-100; lane 6, pool of two fractions containing the peptidase activity after separation on a Superdex prep grade column.

### FIG. 7

Western blot showing the reactions of antiserum APB with proteins in pancreatic secretions (similar results with APA). Lane 1, 0.05 mg/ml human chymotrypsin A; lane 2, human pancreas homogenate; lane 3, trypsin-activated human pancreas homogenate; lane 4, duodenal juice harvested under basal conditions about 18 cm below the pyloric region; lane 5, duodenal juice harvested at the same site during intraduodenal instillation of a mixture of protease inhibitors (20); lane 6, duodenal juice harvested at the end (60 min) of intraduodenal instillation of the protease inhibitors (20).
motrypsin (Tables I and II). The pH optimum for CTRL-1 was tested from pH 5 to 9 and found to have a peak at 8.0–8.5, similar to chymotrypsin in the same assay (results not shown).

**DISCUSSION**

CTRL-1 is a novel human exocrine pancreatic enzyme with substrate specificity similar to that of chymotrypsin. It contains the conserved sequences in the vicinity of the active site serine, aspartic acid, and histidine residues found in this family of serine proteases (25). The pancreatic secretion of this proteinase is feedback-regulated.

Previously, the genes for human and rat chymotrypsinogen B have been cloned and their amino acid sequences derived (26, 27). The amino acid sequences of both bovine chymotrypsin A and B (28, 29) and dog chymotrypsin II (30) have been determined. The antibody and the solid phase assays, the endogenous anti-chymotrypsin-reactive material of each patient was used, whereas the enzyme in solution was commercial chymotrypsin B. The antibody and the solid phase of the enzyme may affect CTRL-1 and the various chymotrypsins differently. It is therefore not surprising that the activity ratios show differences.

Because it is being expressed in pancreas and is present in intestinal fluid after stimulation of pancreatic secretion, it is highly likely that the CTRL-1 protein is a novel digestive enzyme. It is not the human homologue of bovine chymotrypsinogen A. The enzyme reported here may represent a new kind of pancreatic chymotrypsin-like enzymes. The Northern analysis and the number of positive clones in the cDNA library (0.2%) suggest the presence of abundant transcripts of the gene. The gene organization, similar to that of rat chymotrypsin B, as well as the fact that the protein is produced solely in pancreas and that the deduced protein sequence shows highest similarity to the chymotrypsins make it reasonable to classify this as a novel chymotrypsin-like enzyme. Accordingly, the name chymotrypsin-like enzyme-1 and the symbol CTRL-1 have been assigned to it.

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**TABLE II**

Peptidolytic activities of chymotrypsin (final concentration, 10 µg/ml) and purified recombinant CTRL-1.

| Substrates                      | Chymotrypsin | Recombinant CTRL-1 | Ratio |
|---------------------------------|-------------|---------------------|-------|
| OMe-succinyl-Arg-Pro-Tyr-pNA    | 13.54 ± 4.67* | 2.87 ± 0.66*       | 4.72  |
| t-Ser-Leu-Met-pNA               | 0.88*       | 0.07 ± 0.02*       | 12.57 |
| Benzyol-Lys-Val-Tyr-pNA         | 8.86 ± 0.95* | 0.09 ± 0.01*       | 98.44 |
| t-Lys-Val-Phe-pNA               | 10.05 ± 4.97* | 0.54 ± 0.02*       | 18.67 |
| Benzyol-Ang-Trp-pNA             | 6.12 ± 1.03* | 0.05 ± 0.02*       | 122.40|
| Glutaryl-Ala-Ala-Pro-Leu-pNA    | 1.51 ± 0.22* | 1.14 ± 0.14*       | 1.32  |

* Data (nmol h⁻¹) from three experiments (each in triplicate) given as mean ± S.E.
* Data (nmol h⁻¹) from mean of one experiment in triplicate.