Identification of Xenin, a Xenopsin-related Peptide, in the Human Gastric Mucosa and Its Effect on Exocrine Pancreatic Secretion*

Gerhard E. Feurle, Gerd Hamscher, Reinhard Kusiek, Helmut E. Meyer, and Jörg W. Metzger

From the Stadtkrankenhaus Neuwied, 5450 Neuwied and the Medizinische Klinik, University of Bonn, 5300 Bonn, the Ruhr-Universität Bochum, Institut für physiologische Chemie, 4630 Bochum, and the Institut für organische Chemie, University of Tübingen, 7400 Tübingen, Federal Republic of Germany

The octapeptide xenopsin was first discovered in the skin of amphibians (1). Xenopsin-related peptides have also been found in avian gastric extracts (2) and in the stomach, liver, and brain of the rat (3). Recently, an extended xenopsin-related peptide has been described in acid extracts of canine stomach (4). The biological function of the different forms of xenopsin-related peptides in amphibians, birds, and mammals is unknown.

We have studied gastric mucosa of men and have previously provided chromatographic and immunohistochemical evidence for the presence of xenopsin-like material in the human stomach (5, 6). In the present investigation, we describe the isolation and identification of a 25-amino acid peptide, xenin 25, related to amphibian xenopsin in the human gastric mucosa. We further found evidence that this peptide circulates in the blood and that it exerts biological effects.

EXPERIMENTAL PROCEDURES

Purification from Tissue Extracts—Human stomachs obtained at surgery for peptic ulcer disease were immediately separated into the mucosal and muscular layers and frozen on dry ice. Frozen mucosa from the gastric antrum was homogenized in 450 ml of 2% ice-cold trifluoroacetic acid with an Ultra-Turrax homogenizer. The homogenate was centrifuged for 10 min at 3500 × g, and the pellet was reextracted with 100 ml of trifluoroacetic acid. The combined supernatants were pumped through a glass column (2.5 × 30 cm) filled with 10 g of Sep-Pak C18 material (37-55 μm) at a flow rate of 2 ml/min. Homogenization, acid extraction, and Sep-Pak C18 chromatography of tissue and plasma were performed in ice water to minimize generation of xenopsin-related peptides (7). Retained material was eluted with 50% acetonitrile in water containing 0.1% trifluoroacetic acid, frozen on dry ice, and lyophilized. We obtained 1.24 g of white amorphous powder from 70 g of fresh antral mucosa. The material was dissolved in 0.1% trifluoroacetic acid, filtered through a 0.45-μm sterile filter disc (Millipore), and then chromatographed in 20 batches on a µBondapak C18 column (7.8 mm × 30 cm) eluted with 0.1% trifluoroacetic acid and 0.01% β-mercaptoethanol in water with a gradient from 0 to 100% acetonitrile. The fractions eluting at 54–55 min were pooled and rechromatographed on an ion exchange glass column (8 × 75 mm, TSK/SP–5PW, Pharmacia LKB) equilibrated in a solution of 0.34% sodium acetate at 30°C, 0.17% acetic acid, and 9.0% acetonitrile in water. The column was eluted with a gradient from 0 to 1.0 M sodium chloride. Further purification was performed on an analytic 3.9 mm × 30-cm µBondapak C18 column, followed by a µBondapak phenyl column (3.9 mm × 30 cm). The last two steps were performed twice. Final purification was obtained by a POLY LC-polysulfonylethyl (PSE)¹ aspartamide column (4.6 × 100 mm) with a gradient from 0 to 0.5 M KCl (flow rate 1.0 ml/min) in 5 mMol of KH₂PO₄ buffer, pH 3.0, with 25% acetonitrile. Recovery during the different purification steps was always greater than 80% as determined by radioimmunoassay before and after the column passage.

Radioimmunoassay—We prepared three radioimmunoassays: the first for amphibian xenopsin with a radiolabeled analogue of amphibian xenopsin and antiserum against amphibian xenopsin, the second with an antibody against a 9-amino acid C-terminal fragment of xenin 25 using the amphibian tracer, and the third with a modified xenin 25 tracer and antibodies against xenin 25.

For preparation of tracers suitable for labeling by the chloramine-T method, amphibian xenopsin (purchased from Peninsula, St. Helens, United Kingdom) was coupled to Wood’s reagent (8) and

¹ The abbreviation used is: PSE, polysulfonylethyl.

Received for publication, May 12, 1992.

Copyright © 1992 by The American Society for Biochemistry and Molecular Biology, Inc.

Vol. 267, No. 31, Issue of November 5, pp. 22300–22309, 1992
Printed in U.S.A.
purified with a C18 pBondapak column. For preparation of the modified xenin 25 a custom-synthesized analogue (Bissendorf, Hanover, Federal Republic of Germany (FRG)) was used in which Met at position 1 was replaced by Leu and Phe at position 17 was replaced by Tyr.

For radioiodination, 5 nmol of modified xenin 25 was reacted with 8.9 nmol of chloramine T and 0.3 nmol of 125I (0.5 mCi) for 20 s. The reaction was stopped with 26.3 nmol of sodium metabisulfite and 50 μl of β-mercaptoacetic acid. The product was purified by HPLC on a C18 μBondapak reverse phase column (3.9 mm x 30 cm) with a gradient from 0 to 50% acetonitrile in 0.1% trifluoroacetic acid.

The antibodies were raised in rabbits after footpad injection of the peptides coupled to bovine serum albumin with glutaraldehyde as described previously (9). Antibody 2815/3 directed toward the 9-amino acid C-terminal fragment of xenin 25 was used in a final dilution of 1:30,000 and antibody 18/1 directed against xenin 25 was used in a final concentration of 1:60,000. Reaction time with radioiodinated xenin 25 was 5 days at 4°C.

Sequence Analysis—Sequence analysis of the most purified synthetic xenin 25 and the purified extract of gastric mucosa. Ion spray mass spectrometry was performed on a Sciex API III triple-quadrupole mass spectrometer with 2400-Da mass range equipped with an ion spray ion source (Sciex, Toronto, Canada). The mass spectrometer was operated under unit mass resolution conditions for all determinations, and profile spectra were obtained by acquiring data points every 0.1 Da. Ion spray voltage was +5 kV. The samples were dissolved in 25% acetonitrile in 0.1% trifluoroacetic acid and introduced into the ion spray source at a constant flow rate of 5 μl/min with a microliter syringe using a medical infusion pump (Harvard Apparatus).

Xenin in Human Plasma—Blood was taken from the antecubital vein in 1.6 mg/ml potassium EDTA and 200 units/ml aprotinin of volunteers in the fasted and postprandial state. The meals consisted of our hospital lunch (composed approximately of 50% carbohydrate, 25% protein, and 25% fat) enriched with 20 g of butter and bread. In 10 volunteers, 5 ml of plasma taken at short intervals before and after the meal were examined with the radioimmunoassay for xenin 25. In 8 additional volunteers, 30 ml of plasma obtained before and 15–30 min after a meal was acidified with an equal volume of ice-cold 2% trifluoroacetic acid, chromatographed on Sep-Pak C18 cartridges, eluted with 50% acetonitrile in 0.1% trifluoroacetic acid, and rechromatographed on a C18 μBondapak column and eluted with a gradient from 0% to 50% acetonitrile. Non-acidified human plasma was filtered through hydrazido affinity cartridges (DRG Instruments, Marburg, FRG) loaded with purified immunoglobulin G of antibody 2815/4. The eluate was chromatographed on a C18 column as described.

250 ml of postprandial plasma of one individual was chromatographed after Sep-Pak passage on C18 reverse phase HPLC followed by a PSE column as described under “Purification from Tissue Extracts.”

Exocrine Pancreatic Secretion—Six beagles (mean weight 16 kg) anesthetized with 15 mg/kg Tiletamin and Zoletil (Parke Davis, Berlin, FRG) were laparotomized after completion of electrophysiologic experiments. The duodenum was incised, the main pancreatic duct was cannulated, and pancreatic secretion was collected at 15-min intervals. Synthetic xenin 25 dissolved in normal saline solution containing 0.2% human serum albumin was infused after a basal period of 60 min. The dosages were 0.25, 1.0, 4.0, 16.0, 64.0, and 256.0 pmol/kg/min xenin 25 followed by a 30-min interval and infusion of 4 pmol/kg/min synthetic porcine secretin (Serva, Heidelberg, FRG). Each dose was infused for 30 min. Normal saline solution containing 0.2% human serum albumin was infused during the basal period and during the interval. Pancreatic secretion of more than 2 ml was returned into the duodenum after the volume had been determined. The secretion was examined with the Phadebas amylase test (Pharmacia, Freiburg, FRG) and the Monotest 10 lipase test (Boehringer, Mannheim, FRG). Blood was taken at the end of each dosage for the determination of xenin plasma concentrations.

Radioimmunoassay—Sensitivity and specificity of the antibody Xen 6 against amphibian xenopsin has been described (5). This radioimmunoassay was used for monitoring the eluates from the various HPLC columns. The second radioimmunoassay utilizing modified 125I-xenin 25 and antibody 125I/3 and antibody 2815/3 raised against the 9-amino acid C-terminal fraction of xenin 25 detected 5 fmol/ml xenin 25. Cross-reaction at 50% binding was 0.5% with neurotensin. There was no cross-reaction with other gastrointestinal peptides (data not shown). Our third radioimmunoassay using 125I-xenin 25 analogue and antibody 18/1 directed against xenin 25 had a sensitivity of 5 fmol/ml. There was <0.4% cross-reaction with neurotensin 1–13 and <0.1% with neurotensin 8–13. Cross-reactions with the following peptides were less than 0.01%: neuromedin N, glucagon, somatostatin, Met- enkephalin, Leu-enkephalin, neurotensin 1–8, kinetensin, PHI, NPY, FFY, pancreatic polypeptide, bombesin, CCKs, lant 6, secretin, and gastrin. The calibration curve is shown in Fig. 1.

Purification and Sequence—The chromatographic sequence and profiles are given in Fig. 2. The ratio of the peptide concentration determined from the optical density related to the radioimmunochemical peptide concentration was 1.3/1 in

**RESULTS**

**FIG. 1.** Standard curve of the radioimmunoassay for xenin 25 using 125I-labeled xenin 25 analogue and antibody 18/1 (incubation time 5 days at 4°C, final antibody dilution 1:60,000). F/T (%) indicates the ratio of free to total 125I-radioactivity in percent.

**FIG. 2.** Chromatography of human gastric antrum mucosa extracts. A, preparative reverse phase C18 HPLC; B, fast protein liquid ion exchange chromatography (FPLC); C, analytic reverse phase C18 HPLC; D, reverse phase phenyl HPLC; E, cationic exchange chromatography on a PSE column. Fractions reacting in the radioimmunoassay for xenopsin are given in black. Amino acid sequencing of xenin 25 was performed from the peak of trace E.
the final peak from the cationic PSE column. The sequence was determined with the material of this peak. The procedure revealed a 25-amino acid peptide (xentin 25), the sequence of which was confirmed twice in extracts of the antrum and of the corpus mucosa of man (Fig. 3). The calculated molecular mass of xentin 25 is 2971 daltons. In some stomach extracts, in addition, a C-terminal fraction thereof with 16 amino acids was found. In none of the multiple extractions was an extension of the C terminus beyond Leu detected. Xentin immunoreactivity eluting at the retention time of xentin 25 was also found in the duodenum and the jejunum. The concentration of xentin 25 in the jejunum was approximately 10% of that in the antrum and duodenum. No xentin was found in extracts of ileum, colon, and pancreas.

**Mass Spectrometry**—Ion spray mass spectra of the extracted and synthetic peptide showed both an intense ion at m/z 991 and an ion at m/z 744 (in the case of the natural peptide with low signal/noise ratio) (Fig. 4). These are triply and quadruply protonated ions ([M+H]^{3+} and [M+4H]^{4+}) from which a molecular mass of 2970 ± 1 was calculated. As xentin 25 contains a rather high content of basic amino acids (4 Lys, 2 Arg, and 1 His), which are preferential sites of protonation and (among other factors) determine the charge, triply and quadruply charged, but no doubly charged, ions were observed. Obviously, at pH 4.8 (the pH of the sample solutions) only gas phase ions, in which 3 or more residues were protonated, were formed during the ionization process.

**Xentin 25 in Human Plasma**—Plasma purified by Sep-Pak C18 cartridges followed by C18 chromatography and by cation exchange chromatography revealed xentin immunoreactivity exactly at the retention time of xentin 25 (Fig. 5). The same retention time of xentin immunoreactivity was obtained when human postprandial plasma first was affinity-filtrated using our antibody 2815/4 and then chromatographed on the C18 column. Xentin immunoreactivity in fractions 48–54 min of the C18 reverse phase chromatography of 30 ml of plasma of 10 volunteers before and after a meal rose significantly from 6.7 ± 2.2 to 14.7 ± 6.0 fmol (p < 0.05) paired Wilcoxon test (mean ± S.E.). Xentin immunoreactivity in the plasma of 8 volunteers rose significantly 15–30 min after a meal (p < 0.05) (Fig. 6). The postprandial response was very variable. In some persons the immunoreactive xentin plasma concentrations rose only by a few femtomoles, in others up to 20000 fmol.

**Exocrine Pancreatic Secretion**—Xentin 25 infusion in the dog induced a dose-dependent stimulation of exocrine pancreatic secretion by volume, amylase, and lipase beginning at a dosage of 4 pmol/kg/min. The highest dose of 256 pmol/kg/min induced a submaximal response (Fig. 7). 64 pmol/kg/min xentin 25 were more efficacious than 4 pmol/kg/min human secretin in enzyme secretion and equipotent in volume secretion. Plasma concentrations of xentin 25 during infusion in 6 dogs are also shown in Fig. 7.

**DISCUSSION**

The present work has revealed a pentacosapeptide in gastric, duodenal, and jejunal mucosa of man and evidence for the presence of the same peptide in circulating plasma. We propose the term xentin 25 as the peptide is related to xenopsin and consists of 25 amino acids. In addition, we found in some extracts a peptide with the 16 C-terminal amino acids of xentin 25. As this fragment was not found in all extracts, we are not sure whether it represents an extraction artifact or a naturally occurring peptide. The 6 C-terminal amino acids of xentin 25 are identical to those of the amphibian octapeptide xenopsin. This identity has facilitated the recognition of xentin by an antibody raised against frog xenopsin. Other peptides showing C-terminal amino acid similarity with xenopsin are the neurotensins and neuropeptide N. Neurotensin is a tridecapeptide originally discovered in bovine hypothalamus (11) and later also found in the mucosa of the ileum of various mammals (9, 12). Neuropeptide N, a 6-amino acid peptide, has been extracted from the spinal cord of pigs (13).

Xentin 25 in the jejunum was approximately 10% of that in the antrum and duodenum. No xentin was found in extracts of ileum, colon, and pancreas.

**Human Xenin**

Human xenin 25, canine xenopsin-related peptide (4), amphibian octapeptide xenopsin, neurotensin, and neuropeptide N sharing common C-terminal amino acids can be classified, therefore, to belong to a neurotensin/xentin family of peptides (Fig. 3). Unlike many other gastrointestinal peptides, this peptide family lacks a C-terminal amide. The conservation of the C-terminal 6-amino acid sequence of these xenopsin-related and neurotensin peptides from *Xenopus laevis* to *Homo sapiens* suggests that this sequence harbors a biologically important part of the molecule, which has been an advantage during selection and evolution from amphibia to mammals. Recently, canine xenopsin with 9 amino acids and two segments of its precursor with 25 and 27 amino acids have been described in the gastric mucosa of the dog (4). The amino acid sequence of this peptide is otherwise identical to the sequence of human xentin 25 we have found in gastric mucosa of man. The presence of the C-terminal extension of the peptide described in the dog was deduced from the results of amino acid analysis and enzymatic digestion of canine gastric mucosal extracts with carboxypeptidase (4), but was not verified by sequence analysis or mass spectrometry. Our investigation using sequence analysis and ion spray mass spectrometry did not reveal a C-terminal extension in extracts from human stomachs. It remains to be seen whether the 9-amino acid peptide is characteristic for canine xenopsin.

At any rate, the xentin 25 found in human gastric mucosa without this C-terminal extension has biological activity in the dog.

The cellular origin of xentin in the gastric antrum seems to be a specific endocrine cell, as immunohistochemistry clearly locates xentin immunoreactivity to the gastric antral G-cells (5, 6). However, the presence of xentin 25 in the mucosa of the body of the stomach indicates that xentin is also contained in other tissue compartments. These immunohistochemical studies, however, were performed with antibodies against frog xenopsin.

Our investigation has yielded additional evidence that xentin 25 is also present in the circulation before and after a meal and therefore may act as a hormone. The radioimmunoassay revealed a rise of xentin-like immunoreactivity after a meal, and reverse phase C18 chromatography and cationic exchange chromatography showed that this immunoreactivity is concentrated exactly at the retention time of synthetic xentin 25. Apparently, however, xentin 25 immunoreactivity in fasting and postprandial human plasma as demonstrated by C18-HPLC represents only a fraction of the total xentin immunoreactivity.

**Fig. 3. Amino acid sequence of xentin 25 and some other member of the neurotensin/xentin family.**

**Amphibian xenopsin**:<Glu-Gly-Lys-Arg-Pro-Trp-Ile-Leu-Off>
**Xentin 25**:<Glu-Leu-Thr-Lys-PhEq-Glu-Thr-Lys-Ser-Ala-Arg-Val-Lys-Gly-Leu-Ser-Phe-His-Pro-Lys-Pro-Trp-Ile-Leu-Off>
**Neurotensin**:<Glu-Leu-Tyr-Glu-Leu-Ala-Lys-Pro-Arg-Pro-Tyr-Ile-Leu-Off>
**Neuropeptide N**:Lys-Ile-Pro-Tyr-Ile-Leu-Off
**Kinetensin**:Ala-Arg-Arg-His-Pro-Tyr-Phe-Leu-Off
Human Xenin

Fig. 4. Ion spray mass spectra of synthetic xenin 25 (A) and (B) a peptide extracted (see Fig. 2E) from human gastric mucosa. Both spectra contain the triply (m/z 744) and quadruply (m/z 991) protonated ions from which a molecular mass of 2970 Da for both peptides can be calculated.

Fig. 5. Xenin immunoreactivity (in black) in analytic reverse phase C18 chromatography (A) and in cationic ion exchange chromatography (B) on a PSE column of 250 ml of postprandial human plasma. Arrows indicate the time of elution of synthetic xenin 25.

Fig. 6. Xenin immunoreactivity plasma concentration (i-Xp) in 10 volunteers before and after a hospital meal enriched with 20 g of butter and bread (mean ± S.E.).

reactivity as measured by the radioimmunoassay. Hence, it seems possible that in addition to xenin 25 other peptides with xenin immunoreactivity are present in human plasma.

We have examined one possible biological function of this peptide: its effect on exocrine pancreatic secretion. This model was chosen as it is known from previous work that neurotensin and the amphibian xenopsin harboring a common C terminus have stimulatory effects on the exocrine pancreatic secretion (14, 15). The present study shows that xenin 25 also stimulates exocrine pancreatic secretion. Xenin 25 was approximately equipotent with secretin in releasing pancreatic amylase and less efficacious than secretin in stimulating volume secretion. The secretagogue effect of xenin 25 com-

Fig. 7. Pancreatic secretion of volume (panel A), amylase (panel B), and lipase (panel C) in 6 dogs before, during, and after intravenous infusion of rising doses of synthetic xenin 25. The various doses of the peptide are indicated on the abscissa (b = basal, S = infusion of 4 pmol/kg/min synthetic secretin). Panel D shows the immunoreactive xenin plasma concentrations (i-Xp) obtained in this experiment. (mean ± S.E.).
menced at a mean plasma concentration of 180 fmol/ml xenin 25. This concentration was somewhat higher than the mean postprandial plasma concentration of xenin in man. However, two of our volunteers had postprandial plasma concentrations of more than 200 fmol/ml. As anesthesia reduces the responsiveness of the canine pancreas (16), our findings may underestimate the potency of xenin 25 in the stimulation of the exocrine pancreatic secretion of mammals.

In conclusion, we have identified a new peptide in human gastric mucosa and provided evidence that this peptide is released into the circulation after a meal. One of its biological functions may be the stimulation of exocrine pancreatic secretion. These findings suggest that xenin 25 may be another gastrointestinal hormone.

Acknowledgments—We thank C. Iwersen, M. Herwegh, H. Korte, and K. Wilmersdorf for excellent technical help. We thank Prof. Dr. H. Goebell, Medizinische Universitatsklinik Essen, and Prof. Dr. H. J. Dengler, Medizinische Klinik, University of Bonn, who generously allowed us to perform this investigation in their laboratories.

REFERENCES
Identification of xenin, a xenopsin-related peptide, in the human gastric mucosa and its effect on exocrine pancreatic secretion.
G E Feurle, G Hamscher, R Kusiek, H E Meyer and J W Metzger