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COOH-TERMINAL EXTENDED ENDOGENOUS GASTRINS

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SUMMARY

Extracts of antral mucosa, gastrinoma tissue and a gastrinoma serum were subjected to Sephadex G-50 high-resolution chromatography monitored by two radioimmunoassays specific for the NH_2- and the COOH-terminal sequence of heptadecapeptide gastrin. Both tissue extracts and serum contained in addition to known NH_2-terminal extended forms also gastrins corresponding to heptadecapeptide gastrin extended at the COOH-terminus. Four COOH-terminal extended gastrins with mean elution constants (K_{av}) of 0.14, 0.32, 0.44 and 0.49 were encountered. Re-chromatography in urea gradient and tryptic cleavage corroborated their existence. The COOH-terminal extended gastrins are supposed to represent early stages in the modification of the gastrin m-RNA product.

INTRODUCTION

The antral hormone gastrin was originally purified as a heptadecapeptide amide, gastrin-17 [1], in which the biological activity is confined to the COOH-terminal tetrapeptide amide [2]. Larger molecular forms, gastrin-34 [3,4] and component I [5], in which gastrin-17 is extended at the NH_2-terminal glutamic acid residue, have been discovered later on. Component I and gastrin-34 probably represent the pre-pro- and the pro-form of gastrin-17 [6,7].

It has been suggested [8,9] that the different active forms of peptide hormones are synthesized not only by proteolytic cleavage in the NH_2-terminal part of the translated polypeptide chain, but also by transamidation to form a COOH-terminal amide. If the COOH-terminal Phe-NH_2 in the

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known gastrins is produced by such transamidation, COOH-terminal extended gastrins might be present in gastrin producing tissue.

Using two radioimmunoassays, one specific for the NH_2-, the other specific for the COOH-terminal sequence of gastrin-17, the present study demonstrates that COOH-terminal extensions of gastrin-17 are present in gastrin producing tissue and in serum from a patient with excessive gastrin synthesis.

METHODS

Materials

Antral mucosa from six hogs was obtained from a local abbatoir. Pancreatic and hepatic gastrinoma tissue from three patients were obtained through the courtesy of Dr. F. Stadil, Copenhagen. Peripheral serum with a gastrin concentration of 2.3 µM was obtained from a previously described gastrinoma patient [10]. The tissue was immediately frozen on dry ice and stored at -70° C until extraction. While frozen the tissue was cut in pieces weighing a few mg and immersed in boiling water (pH 6.6, 10 ml per g tissue). After boiling for 20 min, the tissue was homogenized, centrifuged and the supernatant saved for radioimmunoassay and chromatography.

Fractionation procedure

Samples of 2 ml extract or serum was applied to calibrated Sephadex G-50 superfine columns (25 x 2000mm, bed volume 700 ml) eluted with 0.25 M NH_4HCO_3, pH 8.2, at 4°C with a flow rate of 20 ml/h. Fractions of 2.5 ml were collected. The immunoreactive components separated by gel chromatography were pooled, lyophilized, reconstituted in 2-4 ml elution buffer and divided in two equally large portions. One portion was refiltrated in a urea gradient (8-0 M) on Sephadex G-50 columns as previously described [5]. The other portion was refiltrated after incubation for 20 min at 20°C with 10 µg trypsin TPCK per ml (Worthington, Freehold, New Jersey). The action of trypsin was terminated by boiling for 5 min. The trypsin incubation was repeated for the largest component with a dose of 1 mg trypsin TPCK per ml for 20 min at 20°C.

Radioimmunoassays

The concentrations of gastrin (equiv. of synthetic human non-sulfated gastrin-17) in extracts, serum and chromatography fractions were measured radioimmunochemically using monoiodinated human gastrin-17 as tracer [11] and two different antisera raised in rabbits against human gastrin-17 coupled by carbodiimide to bovine serum albumin [12,13]. Antiserum 2609 is specific for the COOH-terminal tetrapeptide amide, and modification of this tetrapeptide amide abolishes the binding [Fig. 1]. Antiserum 1295 is specific for the NH_2-terminal pGlu-Gly-Pro-Trp sequence of gastrin-17 [13 and Fig. 1].

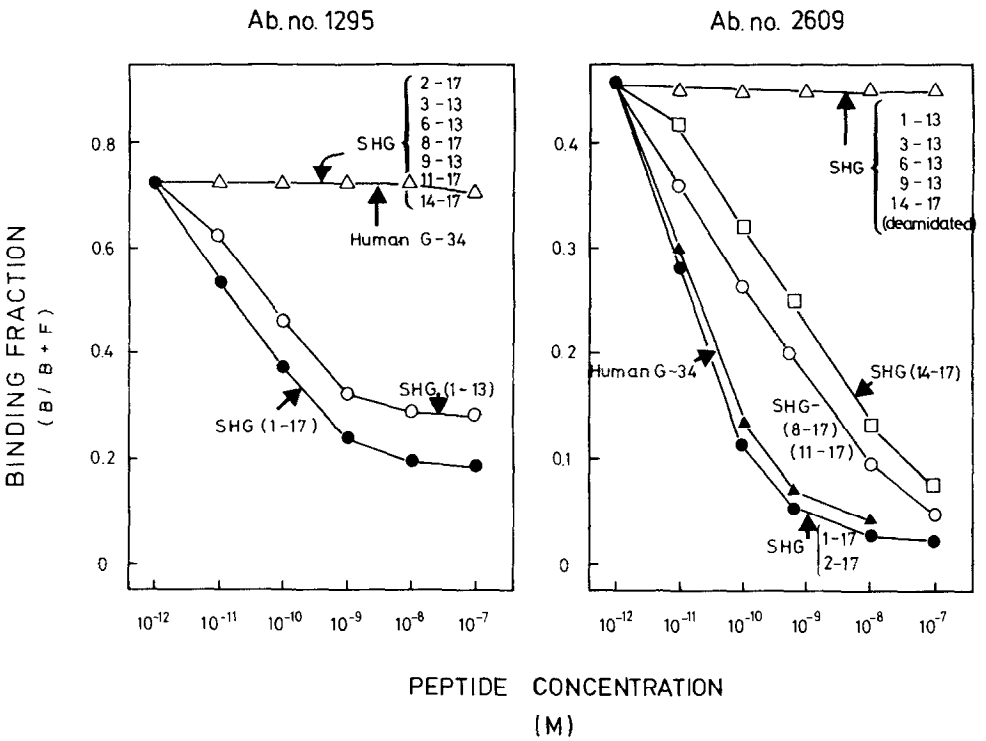

Fig. 1. Antibody displacement of monoiodinated heptadecapeptide amide gastrin by fragments of gastrin(1-17) and by its precursor, gastrin-34. Only peptides containing a NH_2-terminus identical with gastrin-17 could displace the tracer from Ab. 1295; whereas displacement of the tracer from Ab. 2609 required an intact amidated COOH-terminus.

RESULTS

The extracts of antral mucosa and gastrinoma tissue and the serum contained the well-known four main gastrin components [14, 15] all of which contain COOH-terminal Trp-Met-Asp-Phe-NH_2 necessary for binding to antiserum 2609 (data not shown). In addition the tissue and serum contained five components measurable with antiserum 1295 (Fig. 2). These components consequently contain an NH_2-terminal sequence similar to that of gastrin-17. The smallest of these corresponded to the NH_2-terminal tridecapeptide fragment of gastrin-17 [13]. Refiltration in urea gradient confirmed the

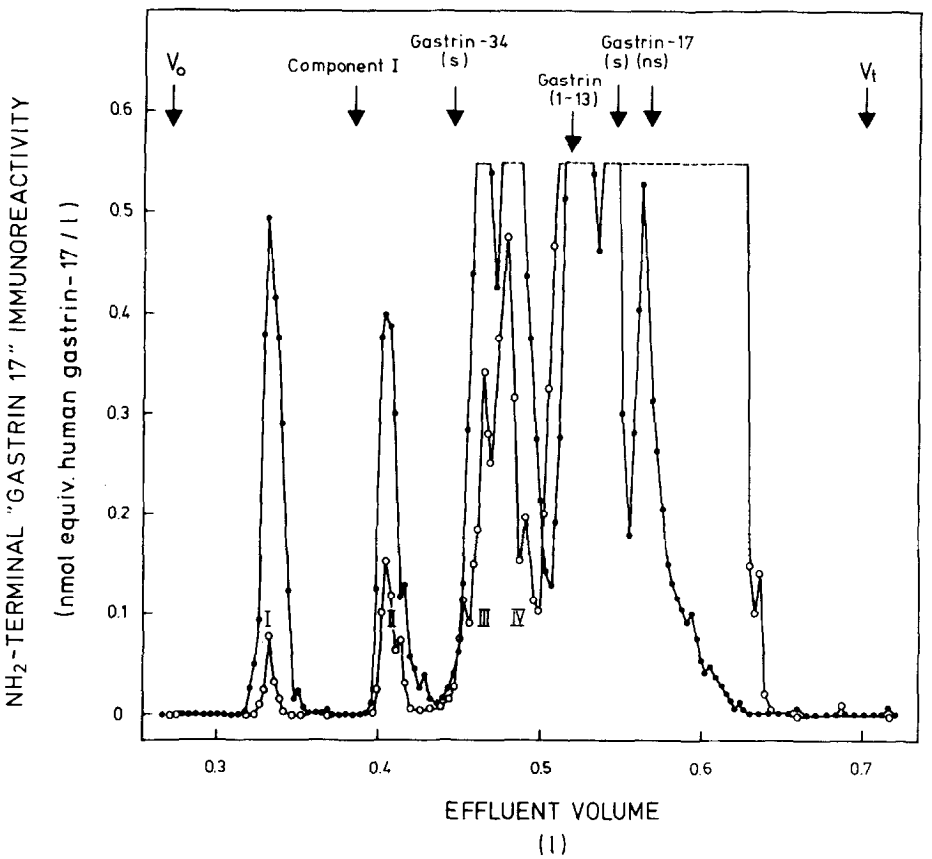

Fig. 2. Gel chromatography of COOH-terminal extended gastrins in gastrinoma serum (●——●) and an extract of porcine antral mucosa (o——o). The samples were applied to Sephadex G-50 superfine columns (25 × 2000 mm) calibrated with ^{125}I-albumin (V_o), ^{22}NaCl (V_t), highly purified porcine gastrin component I, highly purified human gastrin-34 (sulphated (s)), gastrin-17 (sulphated and non-sulphated (ns)) and synthetic human gastrin 1-13 (ns). The fractionations were monitored with a radioimmunoassay specific for the NH_2-terminal sequence of gastrin-17 (ab. 1295; and another specific for the COOH-terminal sequence of gastrin-17 (ab. 2609; data not shown here; but corresponding data are shown in ref. 16 and 20). Only one of six antra contained the COOH-terminal extended gastrins.

existence and elution positions of the new components (Fig. 3). Trypsin cleaved the two larger components, I and II (K_{av} = 0.14 and 0.32) to the intermediate components, III and IV (K_{av} = 0.44 and 0.49) and to components of heptadecapeptide size (Fig. 3, I and II). The two intermediate components,

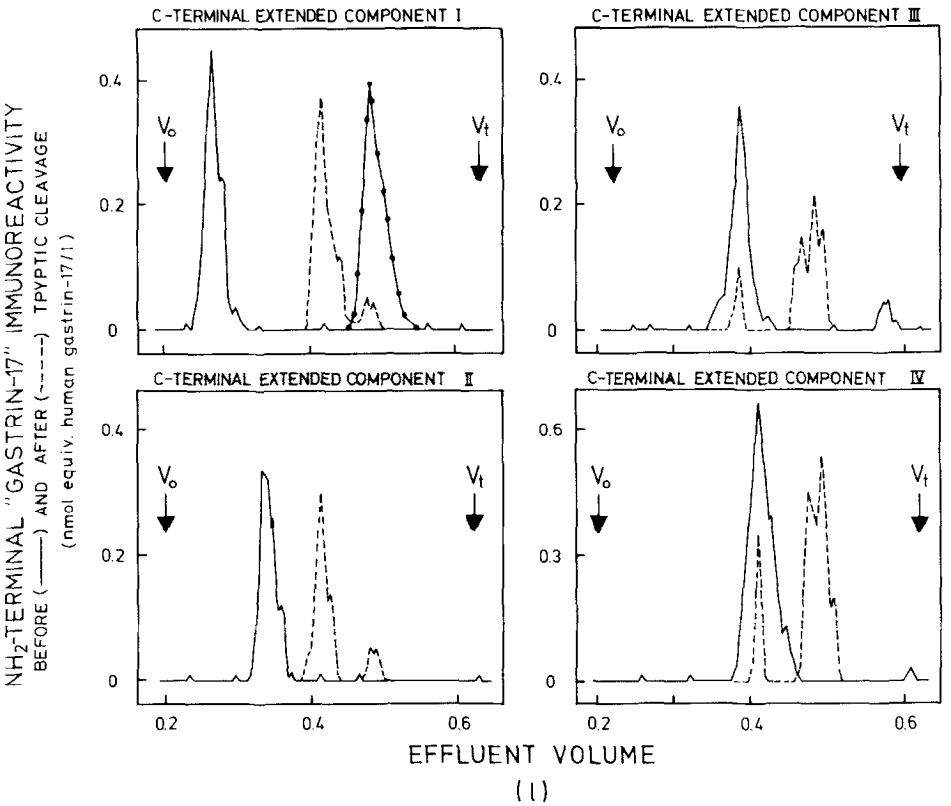

Fig. 3. Gel chromatography of COOH-terminal extended gastrins from gastrinoma serum. Fractions corresponding to each of the components I-IV (see Fig. 1) were pooled, lyophilized, reconstituted in 2-4 ml elution buffer and divided into two equally large portions. One portion was refiltrated in a 8-0 molar urea gradient (——) on Sephadex G-50 columns; the other portion was refiltrated after incubation for 20 min with 10 µg (---) or 1 mg trypsin per ml (•——•). For details about column chromatography and radioimmunoassay, see legend to Fig. 2. Only data using the NH_2-terminal specific radioimmunoassay are shown.

III and IV were cleaved to fragments of heptadecapeptide size (Fig. 3, IV and V). Using high concentrations of trypsin (1 mg/ml) all of component I was cleaved to a fragment of heptadecapeptide size, which, however, was not detected by antiserum 2609 (Fig. 3, I).

DISCUSSION

Previously known molecular forms of gastrin have all contained the COOH-terminus, Trp-Met-Asp-Phe-NH_2 (for review,

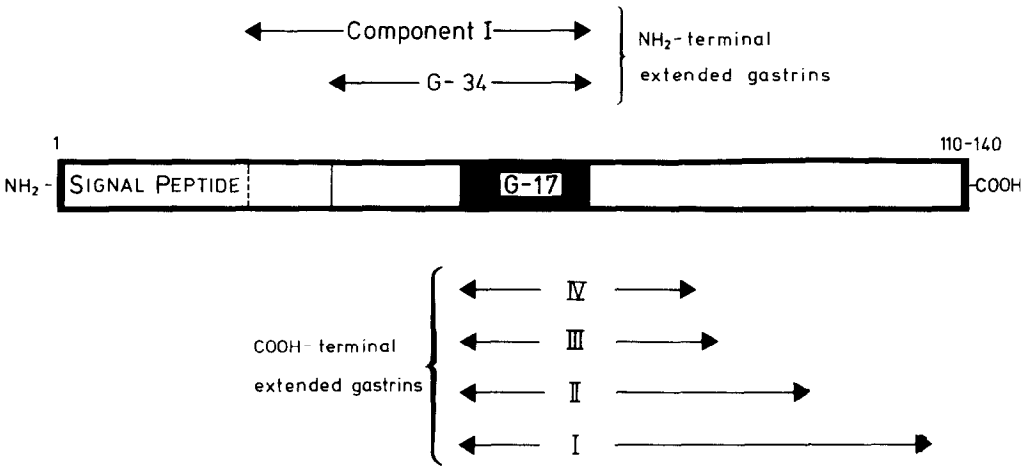

Fig. 4. Suggested biosynthetic interrelationship between the known NH_2-terminal extensions of heptadecapeptide gastrin (G-17) (component I [5] and gastrin-34 [4]) and the present COOH-terminal extensions of G-17. The primary translated polypeptide product of the m-RNA coding for gastrin has been estimated to contain 110-140 amino acid residues [19]. Moreover 250-300 nucleotides of the gastrin m-RNA are 3' to the region coding for the COOH-terminus of gastrin-17 [19].

see 15). The presence of this tetrapeptide amide at the COOH-terminus is crucial for biological activity [2], and even a minor modification like deamidation renders gastrin-17 inactive [2,16]. This study indicates, however, that gastrin cells synthesize also peptides, which correspond to gastrin-17 extended at the COOH-terminus.

The tryptic cleavages indicate that the COOH-extensions contain arginine and/or lysine. Some of the arginyl or lysyl bonds were easily cleaved at a distance from the COOH-terminus of gastrin-17, which may correspond to a size of a 10-20 amino acid peptide (Fig. 3). Other less easily cleaved bonds were closer to the COOH-terminal phenylalanyl in gastrin-17 at a distance corresponding to a few amino acid residues.

The COOH-extended gastrins probably reflect early steps in the modification of the polypeptide chain translated by the m-RNA coding for gastrin. This m-RNA was recently detected in mucosa from hog antra [17]. It contained 620 nucleotides, and could hence code for a gastrin precursor containing 110-140 amino acids. Interestingly 250-300 of the nucleotides were 3' to the region coding for the COOH-terminus of gastrin-17 [17]. Thus, the gastrin m-RNA has a size that easily allows synthesis of COOH-terminal extended gastrins of the size found in the present study (Fig. 4).

It is not surprising that COOH-extended gastrins were abundant and hence detected first in gastrinoma tissue and serum. The tumours examined were large, and the synthesis and release of gastrin excessive [10]. Hence, whereas the putative transamidation in the synthesis of biological active gastrins may be an efficient process in the normal gastrin cells, the rate of translation in malignant gastrin cells may be too high for the capacity for transamidation.

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