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Identification of gastrin molecular variants in gastrinoma syndrome

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Summary

The molecular species of gastrin in the circulation and in tumor extracts were studied in two groups of patients: (1) with benign gastrinoma and (2) with gastrinoma with liver metastases. Radioimmunoassays (RIAs) and immunoaffinity chromatography for the amino (NH_2)- and amidated COOH-terminus of gastrin-17 (antiserum G17) and the NH_2-terminus of gastrin-34 (antiserum G34) were employed. In both benign and metastatic tumors the molecular forms of gastrin in boiling water extracts measured by the gastrin-17 NH_2- and COOH-terminal assays were similar. In addition to a molecular component resembling the amidated gastrin-17, there were also significant amounts of larger molecular weight (mol. wt.) forms. The larger mol. wt. forms absorbed by the NH_2-terminus of G17 antiserum corresponded to the COOH-terminus-extended forms of gastrin-17. Furthermore, larger mol. wt. gastrins immunopurified by antiserum to the NH_2-terminus of gastrin-34 corresponded to gastrin-34 extended molecules. Sera of patients with liver metastases had higher concentrations of the NH_2-terminal of gastrin-17 whereas sera of patients with benign gastrinoma contained predominantly gastrins detected by the COOH-terminal assay. These results suggest that: (a) there are differences in the molecular pattern of gastrin in the circulation of patients with benign and metastatic gastrinomas; (b) gastrins which are fully processed with carboxy-terminal amidation predominate in the circulation of patients with benign gastrinoma; and (c) gastrins containing the gastrin-17 and COOH-terminally extended gastrin-17 and gastrin-34 precursor molecules occur

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in high concentration in the circulation of gastrinoma patients with metastases to the liver.

Gastrin; Gastrinoma; Radioimmunoassay; Liver metastase

Introduction

Gastrin in gastrinoma syndrome is heterogenous. It is found as peptides of variable amino acid chain length. The known gastrin peptides are gastrin-34, gastrin-17, gastrin-14, Component I, the NH_2-terminus tridecapeptide 1–13 gastrin-17 and a nonapeptide extension at the COOH-terminus of gastrin [1–6]. Gastrin-34, gastrin-17 and gastrin-14 have a common amidated carboxy-terminus. The nonapeptide-extended forms of gastrin contain a Phe-Gly linkage instead of Phe-NH_2 at the COOH-terminus. Since previous studies used carboxy-terminus antisera [2], and carboxy-terminus immunoaffinity absorption [3], this extension of the gastrin molecule may have precluded recognition by antisera to the amidated gastrins and dictates a need to utilize radioimmunoassays (RIAs) capable of recognition of these variants if they are to be quantitated in tissues and biologic fluids.

We have previously described the development of region-specific RIAs for gastrin [7] and their utilization in distinction between benign and metastatic gastrinoma [8]. In the present studies we have characterized the molecular forms of gastrin in extracts of tumor and in the circulation of patients with gastrinoma. The results suggest that even though tumor extracts contain multiple molecular forms of gastrin, amidated gastrin-34 is the major form in the circulation of patients with benign tumors, whereas gastrin-17 and the COOH-terminally extended gastrins predominate in the serum of patients with gastrinoma and liver metastases.

Materials and Methods

Peptides. Pure natural gastrin-34, and 1–13 gastrin-17 were gifts from Prof. R.A. Gregory and Hilda J. Tracy (Liverpool). Synthetic gastrin-17, 1–12 gastrin-17 and 1–16 [Tyr^{17}]gastrin-34 were gifts from ImmunoNuclear Corp, Stillwater, MN. Carboxy-terminal-extended fragments (EG10) were a gift from K.L. Agarwal, Chicago, IL. Gastrin-34 and 1–20 gastrin-34 (Kenner-Harris sequence) were purchased from Fluka Chemical, Hauppauge, NY. Gastrin-16 and gastrin-10 were purchased from GRB, Atlantic Beach, NY.

Patients. The clinical details of the 22 patients studied have been described previously [9–11]. All patients had surgically proven gastrinomas and the blood samples were taken prior to surgery. Of the 22 patients studied, 14 had benign gastrinomas and 8 had metastases to the liver.

Tumor extraction. The tumors obtained at operation were frozen immediately. The frozen tumors were weighed, cut into small pieces and then boiled in 10 ml of water

[1–5] (pH 7.0) for 10 min, homogenized in a Pyrex tissue grinder and centrifuged at 2000 rpm for 10 min. The supernatant was decanted and frozen at −20°C until ready for analysis.

Gel chromatography. The samples were fractionated at 4°C on columns of Sephadex G50 (superfine, 100 × 1 cm), equilibrated and eluted with 0.02 M Tris-HCl buffer, pH 8.0, containing 0.2 g/liter sodium azide and 0.001% bovine serum albumin. The columns were calibrated with gastrin-34, gastrin-17, gastrin-14, gastrin-13, 1–16 [Tyr^{17}]gastrin-34, 1–13 gastrin-17 and 1–12 gastrin-17. With each chromatographic analysis, very small amounts of purified albumin were added to mark the void volume and $Na^{125}I$ for the salt peak. The protein peak in the column eluates was detected by light absorption at 280 nm. The elution profiles are described in terms of percent of the elution volume between the protein peak (0%) and the salt peak (100%). Values are given as a % or as a fraction of the total volume (K_{av}). The columns were eluted at a flow rate of 4.0 ml/min and fractions of 0.5–1 ml were collected for the assays unless otherwise specified. Recoveries (in percent) of gastrins were: gastrin-34, 70 ± 3; gastrin-17, 84 ± 3; gastrin-14, 68 ± 2; 1–12 gastrin-17, 89 ± 1; 1–13 gastrin-17, 81 ± 1; and 1–16 [Tyr^{17}]gastrin-34, 87 ± 3, means ± S.E.M.

Antisera. Four antisera were used. They recognized (i) the amidated COOH-terminus of gastrin-17 and gastrin-34 (G); (ii) NH_2-terminus of gastrin-17 (MG2); (iii) NH_2-terminus of gastrin-34 (R34); and (iv) COOH-terminally extended gastrins (EG10). The antiserum to the COOH-terminal-extended gastrin (EG10) reacted with amidated and non-amidated gastrins equally well. Further, since the gastrin-30–39 sequence is identical in rat preprocholecystokinin, the antiserum generated using the COOH-terminally extended gastrin (EG10) was used only for confirmation of the presence of COOH-terminal-extended fragments. The antisera raised against the amidated COOH-terminus of gastrin-16 (G), NH_2-terminus of 1–13 gastrin-17 (MG2) and NH_2-terminus of gastrin-34 (R34) proved to be useful in the present studies.

RIA. The methods for the RIAs using antisera G, MG2 and R34 have been reported in detail [7,8]. Fig. 1 gives the sequence of gastrin and the recognition sites of the 3 antisera, G, MG2 and R34. Antiserum raised against the COOH-terminal-extended gastrin EG10 reacted equally with EG10, gastrin-14, gastrin-17 and gastrin-34. However, it did not react with 1–13 gastrin-17. Labeled gastrin-17 was used as a tracer. The use of labeled EG10 peptide as tracer did not alter the specificity. This suggests that EG10 antiserum needs the 31–34 aminoacid residues of the gastrin-34 sequence for the reaction. The ID_{50} was 68 pM. The intra-assay coefficient of variation was 13% at 25 pM and 6% at 250 pM of G17.

Immunoabsorption. In order to purify and establish the identity of the individual peaks measured by the 3 RIAs in gel chromatography eluates, immunoaffinity chromatography using antisera G, MG2 and R34 was used to isolate various gastrin species. The immunoglobulins from 5 ml of these antisera were precipitated by the addition of 5 ml of saturated ammonium and coupled to 300 μg of CNBr activated Sepharose 4B according to the manufacturer's instruction (Pharmacia, Uppsala, Sweden). Three separate immunoabsorbent columns (10 × 1 cm) were prepared. The immunoabsorption studies were carried out by the method described previously [7].

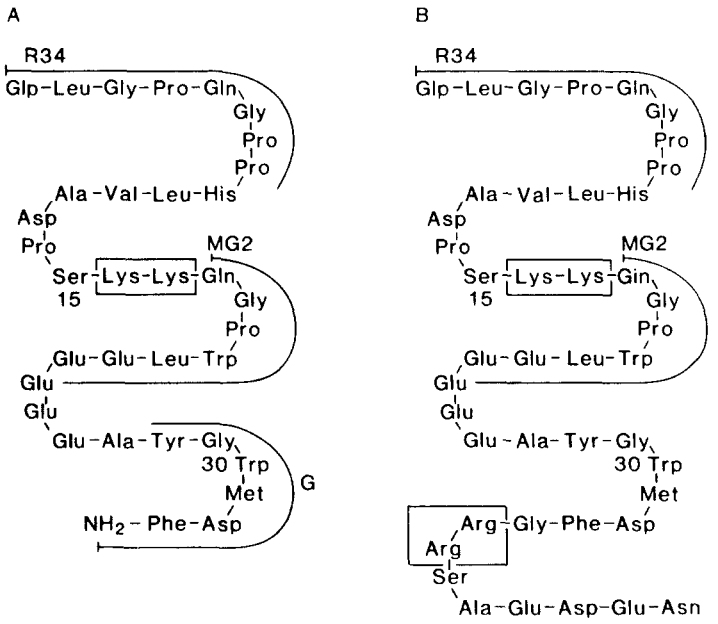

Fig. 1. The aminoacid sequence of human gastrin-34 is shown in (A) and that of COOH-terminally extended gastrin-34 as deduced from gene studies is shown in (B). Braces indicate the sequences of gastrin which the antisera recognize. Boxes indicate the bonds cleaved by trypsin.

After an initial wash with 40 ml of 0.02 M Tris HCl (pH 8.0), gastrins bound to the column were desorbed by elution with 1 M acetic acid. The eluates were lyophilized and reconsituted in the assay buffer. Table I shows the binding capacity of the immunoaffinity columns for gastrin-34, gastrin-17, gastrin-14, 1–13 gastrin-17, and 1–16 [Tyr^{17}]gastrin-34. As shown, the immunoaffinity column using antiserum G bound only peptides containing the amidated carboxy terminus of gastrin; antiserum MG2 bound only the peptides containing the NH_2-terminus of gastrin-17; and antiserum R34 bound only the peptides containing the NH_2-terminus of gastrin-34.

TABLE I

Absorption (%) of 10 nM of various gastrin molecular forms of gastrin by 3 immunoaffinity columns

	Immunoaffinity columns		
	G	MG2	R34
Peptide			
1–16[Tyr^{17}]gastrin-34	0%	0%	95%
Gastrin-34	100%	1%	100%
Gastrin-17	100%	97%	0%
Gastrin-14	100%	0%	0%
1–13 Gastrin-17	2%	95%	0%

The gastrin content of washings and desorbed material was measured by RIA's using appropriate antisera.

Results

Gastrins in tumor extracts

Chromatography on Sephadex G50 (superfine) of the tumor extracts resolved the immunoreactive fractions measured by antiserum G (which recognizes the amidated COOH-terminus of gastrin) into 5 peaks (Fig. 2). These peaks eluted at K_{av} values of 0.0, 0.25, 0.40, 0.55 and 0.75, that correspond to the known gastrin molecules big big gastrin (BBG), Component I, gastrin-34, gastrin-17, and gastrin-14 [3]. An additional peak at a K_{av} of 0.85 was also identified in 3 tumors [12]. The Sephadex G50 profile of G-immunoaffinity-purified material was similar to that of the extracted tumor as measured by antiserum G (Fig. 2). The differences between the profiles shown in Fig. 2 are due to marked differences in the gastrin-14 content in the tumors of patients 1–3. Antiserum MG2 identified only a peak corresponding to gastrin-17, whereas R34 identified a peak resembling gastrin-34 in the G-immunoaffinity-purified eluates (data not shown). These data indicated that all 5 identified species contain the COOH-terminus of gastrin. Furthermore, the position of individual peaks remained unchanged on Sephadex G50 column chromatography in 8 M urea, indicating that the large molecular weight species were not polymers held together by non-covalent forces. The incubation of BBG, Component I and gastrin-34 with trypsin displaced the immunoreactivity to the elution position of synthetic G17 as measured by antiserum G, confirming that all the molecular species contained the COOH-terminus of gastrin-17.

Studies of the Sephadex G50 elution profile of the tumor extracts using antiserum MG2 resolved the immunoreactive fractions into 7 peaks (Fig. 3). The peaks eluted at K_{av} values of 0.05, 0.18, 0.25, 0.42 (all 4 peaks (I–IV) resembled those identified by Rehfeld [5]), 0.49 (resembling pure 1–13 gastrin-17), 0.53 (peak V) and 0.55 (resembling synthetic gastrin-17. The Sephadex G50 profile of MG2-immunoaffinity-desorbed material was similar to that of the tumor extracts as measured by antiserum MG2 (Fig. 3). Antiserum G detected only a peak resembling gastrin-17 at a K_{av} of 0.55 whereas antiserum R34 did not recognize any of the peaks detected by MG2 in these MG2-immunoaffinity-purified eluates. This confirmed that all the peaks measured contained the NH_2-terminus of gastrin-17 and only the peak resembling gastrin-17 at K_{av} of 0.55 had the amidated COOH-terminus of gastrin-17.

Further studies of MG2-immunoaffinity-purified eluates using an antiserum that reacts with carboxy-terminally extended gastrin and amidated COOH-terminus of gastrin measured peaks I–V, and one resembling gastrin-17 but not the one resembling 1–13 gastrin-17. This suggested that peaks I–V, which are not measured by antiserum G, contain the NH_2-terminus of gastrin-17 and are extended at the carboxy-terminus of gastrin-17. Furthermore, in 8 M urea, the position of individual peaks on the Sephadex G50 column chromatography remained unchanged as measured by antiserum MG2. Separate incubation of peaks I–IV with trypsin displaced the immunoreactivity to the position corresponding to peak V as measured by antiserum MG2. None of the displaced peaks were measurable by antisera G or R34. Peak V and peaks resembling 1–13 gastrin-17 and gastrin-17 were not displaced by treatment with trypsin. This further confirmed that peaks I–V contain the NH_2-ter-

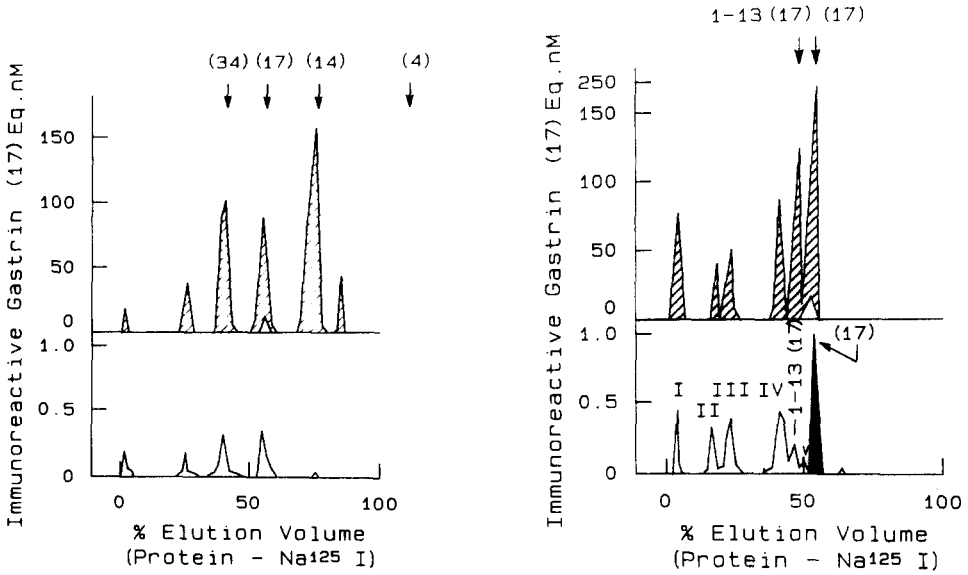

Fig. 2 (left). Chromatography of Sephadex G50 (superfine) of tumor extracts. Gastrin immunoreactivity as measured by antiserum G using synthetic gastrin-17 (17) as standard is shown on the ordinate. The hatched bars indicate the ranges of concentration observed in the tumors examined. On the abscissa, percentage of elution volume between the protein peak (void volume 0%) and $Na^{125}I$ peak (100% volume) is given. Elution volume of natural gastrin-34 (34) and synthetic gastrin-17 (17), gastrin-14 (14) and gastrin-4 (4) are given. Elution profile of boiling tumor extract (top) and G-immunoaffinity-desorbed tumor (No. 3) extract (bottom).

Fig. 3 (right). Chromatography of tumor extracts on Sephadex G50. For details see Fig. 2. Elution volume of pure 1–13 gastrin-17 (1–13 (17)) and synthetic gastrin-17 (17) measured by antiserum MG2 are given. Elution profile of boiling-water tumor extract measured by antiserum MG2 (upper panel) and MG2-immunoaffinity-desorbed tumor (No. 2) extracts measured by antiserum MG2 (–) and G (dark area; bottom panel) are given. The hatched bar in the upper panel indicates range of concentrations between patients in tumors examined. Note: the peak resembling gastrin-17 was recognized by antisera G and MG2.

minus of gastrin-17 and are extended at the COOH-terminus of gastrin-17.

Table II gives the concentration of immunoreactive gastrin peaks measured by antisera G and MG2 in the gastrinomas. Individual peak concentrations varied considerably in benign gastrinoma. However, the total COOH-terminus immunoreactivity was lower than total NH_2-terminus immunoreactivity in cases of gastrinoma with liver metastases. Furthermore, one of the tumors examined (No. 15) had peaks measured by antiserum MG2, i.e., containing the NH_2-terminus of gastrin-17, and amidated gastrin-17, but had no gastrin-34 or Component I as measured by antiserum G. No attempts were made to quantify peak V due to the low concentration in benign gastrinoma and extremely high concentrations in patients with liver metastases.

Gastrins in the circulation

Chromatography on Sephadex G50 columns of serum from patients with benign gastrinoma and gastrinoma with liver metastases resolved the immunoreactivity into 4 peaks resembling Component I, gastrin-34, gastrin-17 and gastrin-14 as measured

TABLE II

Immunoreactive gastrin concentrations using antisera G and MG2 (nmol/g wet wt.) of immunoreactive peaks in tumor extracts from 8 patients

Patient	Gastrins			Antiserum G			Antiserum MG2						
	BBG or Compo-nent I	34	17	14	Other	Total	I	II	III	IV	a	1–13 gastrin-17	Total
Benign													
1		4.4	86.1	154.4	41.7	286.6	11.1		33.3		91.7		136.1
2	18.7	34.0	33.3	15.7		101.7	11.2	8.4	17.0	12.2	36.4	5.0	90.2
3	7.1	9.5	11.6	0.1		28.3	1.1		1.4	6.4	17.3	5.0	31.2
4	1.6	2.5	34.5			38.6	9.5				36.7	3.6	49.8
5	37.5	103.9	58.9		31.4	231.7	13.6	5.7	36.8	11.8	63.2	49.6	180.7
Liver metastases													
15			1.5			1.5	78.5	29.2	51.7	78.5	170.7	123.1	531.7
16		15.0	57.5		11.2	83.7	23.8	41.9	40.0	40.0	78.7	106.3	330.7
17		1.0	250.0			251.0	10.0	13.0	10.0	40.0	250.0		323.0

RIA's were performed using antiserum G for the COOH-terminus and MG2 for the amino-terminus of gastrin-17 measurement. The results are expressed relative to the gastrin-17 standard. Concentrations in all eluates were estimated corresponding to the peaks from chromatography on Sephadex G50 (Figs. 2,3). In column a, peak V and gastrin-17 were not quantified individually by RIA using antiserum MG2 due to their close elution on chromatography.

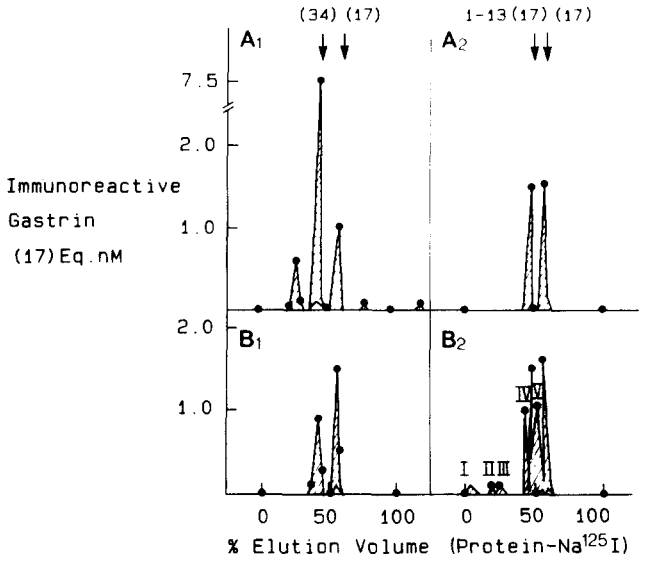

Fig. 4. Chromatography of sera on Sephadex G50. For details see Figs. 2 and 3. Elution profile of sera from patients with sporadic gastrinoma (upper panels) and liver metastases (bottom panels) are given. The left panels (A_1, B_1) were measured by antiserum G and right panels (A_2, B_2) by antiserum MG2. The hatched bar indicates range of concentrations between patients in sera examined.

by antiserum G (Fig. $4A_1$, B_1). However, antiserum MG2 measured peaks resembling gastrin-17 and 1–13 gastrin-17 in gastrinoma (Fig. $4A_2$) and various amounts of all 7 peaks in gastrinoma with liver metastases (Fig. $4B_2$).

The total immunoreactivity containing the COOH-terminus of gastrin as measured by antiserum G was higher than NH_2-terminus of gastrin-17 immunoreactivity as measured by antiserum MG2 in the serum of patients with benign gastrinoma (Table III). In addition, the relative concentration of gastrin-17 was less than 20% of total COOH-terminus immunoreactivity in all patients with benign gastrinoma as measured by antiserum G (not shown).

In contrast, total NH_2-terminus of gastrin-17 immunoreactivity measured by antiserum MG2 was higher than COOH-terminus gastrin as measured by antiserum G in 7 out of 8 patients with liver metastases (Table III). Furthermore, the relative concentration of gastrin-17 was higher than 20% of total COOH-terminus immunoreactivity in patients with liver metastases as measured by antiserum G (data not shown).

COOH-terminally extended big gastrin

Chromatography on Sephadex G50 of the 3 tumor extracts resolved the immunoreactivity into 8 fractions eluting at K_{av} of 0.02, 0.08, 0.2, 0.35 (peaks A–D), 0.4 (resembling pure G34), 0.5 (resembling NH_2-terminus tryptic peptide of G34 (peak E), 0.68 (peak F) and 1.00 (peak G) as measured by antiserum R34 (Fig. 5). The 3 later peaks have been identified in human plasma [13]. The Sephadex G50 profile of R34-immunoaffinity-desorbed material was similar to that of the tumor extracts as

TABLE III

Immunoreactive gastrin concentration (pmol) of individual peaks in serum of 22 patients

Patient	Antiserum G					Antiserum MG2							
	Component I	Gastrin-34	Gastrin-17	Gastrin-14	Total	I	II	III	IV	V	Gastrin-17	1–13 Gastrin-17	Total
Sporadic													
1	6	46	6	40	98						3		3
2		196	16		212						10		10
3	600	7500	1227		9327						1550	1500	3050
4		210	42		252						5		5
5		392	36		428						12		12
6		96	6		102						3		3
7		256			256						5		5
8	9	43	6	4	62						5		5
9		312	8		320						10		10
10	10	100	6	8	124						11		11
11	140	300			440						11		11
12	120	275	61		456						45	10	55
13		78	14		92						16		16
14		38	16	40*	94						10		10
Liver metastases													
15			1148		1148				80	668	1048	160	1956
16		36	384		420				140	148	362		650
17		105	209		314	20	8		8	16	124	20	196
18		132	772		904	5	17	17	264	320	896	148	1667
19		344	408		752				220	260	576		1056
20		224	512		736	15	33	19	820	1000	1848	120	3855
21		864	1472		2336	24	43	50	1000	1100	1550	1521	5288
22		20	17		37	6	8	20	22	52	44		152

RIAs were performed using antiserum G for carboxy-terminus and MG2 for the NH_2-terminus of gastrin-17 measurements. The results are expressed relative to gastrin-17 standard. Concentrations were estimated by integration of all eluates corresponding to the peaks from chromatography on Sephadex G50 (Fig. 4). Peak V was quantified by rechromatography in 0.5 ml eluate volume of pooled lyophilized eluates in gastrin-17 region (K_{av} of 0.5–0.6).

* Eluted in the position of G4.

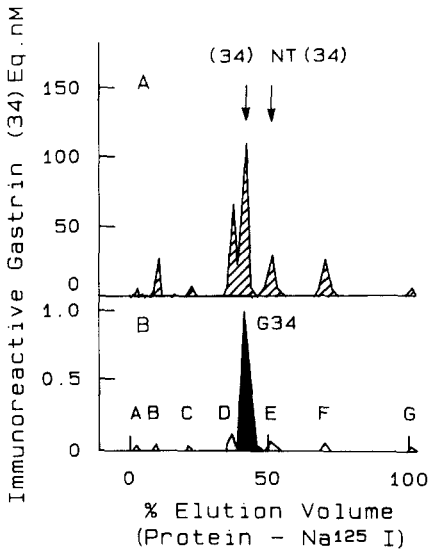

Fig. 5. Gel chromatography of tumor extracts on Sephadex G50. For column details see Fig. 2. Elution volume of pure gastrin-34 and NH_2-terminal heptadecapeptide of gastrin-34 (NT(34)) measured by antiserum R34 are given. Elution profile of boiling-water tumor extracts as measured by antiserum R34 (upper panel) and R34-immunoaffinity-desorbed tumor (No. 6) extracts measured by antisera R34 (−) and G (dark area; bottom panel) are given. The hatched bar in upper panel indicates range of concentration between patients in tumor examined.

measured by antiserum R34 (Fig. 5). Only the peak eluting at a K_{av} of 0.4 was measured by antiserum G, whereas antiserum MG2 did not recognize any peak in R34-immunoaffinity-purified eluates (data not shown). These data indicate that the peaks measured contain the NH_2-terminus of gastrin-34 and only the peak resembling gastrin-34 has the amidated COOH-terminus of gastrin.

Further studies of R34-immunoaffinity-purified eluates using an antiserum that reacts with carboxy-terminally extended gastrin and the amidated COOH-terminus of gastrin recognized peaks A–D and one resembling gastrin-34. This suggests that peaks A–D, which are not measured by antiserum G, contain the NH_2-terminus of gastrin-34 and are extended at the carboxy-terminus. Furthermore, incubation of pooled peaks (A–D) with trypsin displaced the immunoreactivity to the position of the NH_2-terminus tryptic peptide of gastrin-34 at a K_{av} of 0.5 as measured by antiserum R34. Antiserum G did not recognize any peaks in the eluates. In contrast, trypsin treatment of the peak eluting at a K_{av} of 0.4 (resembling pure gastrin-34) displaced the immunoreactivity to the position of the NH_2-terminus tryptic peptide of gastrin-34 at a K_{av} of 0.5 as measured by antiserum R34 and to the position of gastrin-17 as measured by antiserum G. Peaks eluting at a K_{av} of 0.5, 0.68 and 1.00 were not displaced by trypsin. This confirms that peaks A–D contain the NH_2-terminus of gastrin-34 and are extended at the COOH-terminus of gastrin.

Since the gastrin gene contains the sequence of both gastrin-17 and gastrin-34 and their extension, the reason for the finding of one less peak of carboxy-terminally extended gastrin-34 than gastrin-17 is not clear. However, this was attributed to poor

TABLE IV

Immunoreactive gastrin concentration (nmol/g wet wt.) of individual peaks in tumor extracts estimated using antiserum R34.

Patient	A	B	C	D	Gastrin-34	E	F	G	Total
Benign									
1	0.20	1.70	1.10	3.90	6.60	0.22	0.22		13.94
5	2.60	25.00		62.60	106.60	26.00	21.10		243.90
Liver metastases									
15									<0.01
16	2.50	7.80	4.40	4.70	12.50	12.50	12.00	1.70	58.10

RIAs were performed using antiserum R34 for the amino-terminus of gastrin-34 measurements. The results are expressed relative to gastrin-34 standard. Concentrations were estimated by integration of all eluates corresponding to the peak from chromatography on Sephadex G50 (Fig. 5).

resolution on the Sephadex G50 columns. Attempts to resolve them further were not successful. No striking differences were observed in the individual peak concentrations in two benign sporadic and one gastrinoma tumor with liver metastases (Table IV).

The chromatography of the 3 sera from patients with sporadic gastrinoma showed a major peak eluting at a K_{av} of 0.4 (resembling G34) along with the minor peaks eluting at K_{av} values of 0.5, 0.68 and 1.00 corresponding to fragments of gastrin-34 (Fig. 6, Table V). In contrast, patients with liver metastases showed a peak corre-

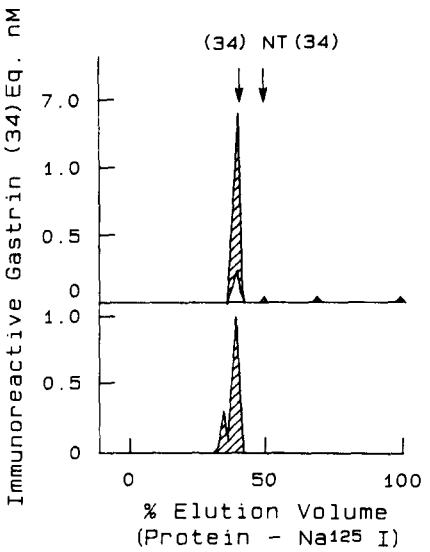

Fig. 6. Gel chromatography of sera on Sephadex G50. For details see Figs. 2, 5. Elution profile of sera from patients with sporadic gastrinoma (upper panel) and liver metastases (bottom panel), measured by antiserum R34 are given. The hatched bar indicates the range of concentrations in sera examined. Note: peak D was not observed in sporadic gastrinoma.

TABLE V

Immunoreactive gastrin concentration (picomolarity) of immunoreactive peaks in sera from 6 patients using antiserum R34

Patient	A	B	C	D	Gastrin-34	E	F	G	Total
Benign									
1					225	25	10	10	270
3					7000	80	16	24	7120
5					470	10	50	10	540
Liver metastases									
15									< 1
16				20	26				46
18				24	74				98
21				300	1000				1300

RIAs were performed using antiserum R34 for the amino-terminus of gastrin-34 measurements. The results are expressed relative to gastrin-34 standard. Concentrations were estimated by integration of all eluates corresponding to the peak from chromatography on Sephadex G50 (Fig. 6).

sponding to carboxy-terminally extended gastrin (described in tumor) at a K_{av} of 0.35 and resembling pure gastrin-34 at K_{av} of 0.4. Additional experiments confirmed the identity of the peaks (Fig. 6, Table V). As noted earlier, gastrin-34 was not identified in patient No. 15 (Table V).

Discussion

The immunochemical studies using antisera directed at the different regions of the gastrin molecule, in combination with chromatography, indicate that gastrin present in the tumor extracts and serum from patients with the gastrinoma syndrome is heterogenous. Furthermore, the molecular species in serum differ from that in the tumor. The predominant molecular forms in the serum of patients with sporadic gastrinoma are the COOH-terminally amidated gastrin peptides whereas the sera of patients with liver metastases contained gastrin molecular forms that are extended at its COOH-terminus and gastrin-17.

Even though efforts were made to study gastrin molecular heterogeneity in the gastrinoma syndrome using an antiserum directed at the COOH-terminus of gastrin-17 [2,3] (Fig. 2) COOH-terminally extended peptides containing the Phe-Gly linkage are not recognized by the antisera used. Using a NH_2-terminus of gastrin-17- and gastrin-34-directed antisera, molecules with a COOH-terminus extension could be identified.

Previous studies in the gastrinoma syndrome have defined the presence of 1–13 gastrin-17 [4] and 4 peaks corresponding to COOH-terminally extended gastrin-17 [5] as measured by NH_2-terminus-directed antisera. In addition to identifying peak V corresponding to COOH-terminally extended gastrin-17, the present study has also identified peaks corresponding to COOH-terminally extended gastrin-34 (Figs. 3,5,6).

Whether the larger molecular weight peaks measured by antisera MG2 and R34 reflect extension beyond the nonapeptide sequence [6] (Fig. 1) is speculative. Noyes et al. [14] have shown that porcine gastrin mRNA has 620 nucleotides of which 250–300 nucleotides were 3′ to the coding region for gastrin-17, which could embrance a greater-than-nonapeptide extension, although it is unlikely that the molecular species identified with COOH-terminal extension represent translation of the 3′ end of the gastrin gene. The large forms probably comprise unprocessed products of nonapeptide-extended forms as shown by Sugano et al. [15], who measured different immunoreactive peaks in porcine antral extract using 3 antisera specific for the COOH-terminus of gastrin-17, its extension by Gly and Gly-Arg-Arg. The apparently high molecular weight may be due to altered elution of COOH-terminal-extended molecules on the Sephadex column. The failure of COOH-terminal antiserum to recognize COOH-terminally extended gastrin and the use of NH_2-terminal antisera in the present study, and detailed classification of our patients may explain the discrepancy between this and earlier reports [2,3].

The distribution pattern of gastrin in the circulation of patients with sporadic gastrinoma differed from that in 7 patients with liver metastases. While the latter contained high concentrations of gastrin-17 NH_2-terminal immunoreactivity, the gastrin COOH-terminal immunoreactivity predominated in the circulation of patients with benign gastrinoma (Table III). These data further verify our earlier finding of a high NH_2:COOH ratio in patients with liver metastases [8,16]. The reason for this differential pattern is unknown, however, it may be due to the difference in the rate of processing of the gastrin molecule, particularly α-amidation of Phe residue in position 34 of gastrin (Fig. 1) [17]. Since COOH-terminally extended gastrin-34 has the same dibasic Arg-Arg sequence as COOH-terminally extended gastrin-17, the finding of COOH-terminally extended gastrin-34 and gastrin-17 in the serum of patients with liver metastases supports the hypothesis that metastatic tumors incompletely clear the extended precursors. The reason for finding high COOH-terminus gastrin in the serum of patient No. 17 is unknown.

Johnson and Fabri [18] have reported that G17 accounts for more than 20% of the total gastrin in all patients with metastatic gastrinoma and less than 20% in patients with benign sporadic gastrinomas. Our studies using a COOH-terminus-directed antiserum support this finding.

Carboxy-terminally extended gastrins (residues 18–43 and 1–43) and the gastrin-34 sequence contain gastrin-17. This raises the possibility that they are precursors of gastrin-17. However, the finding of amidated G17 in the circulation in the absence of gastrin-34 in tumor extracts and the circulation in patient No. 15 suggests that the COOH-terminally extended gastrin-17 may be the precursor of gastrin-17. Furthermore, the finding of both carboxy-terminally extended gastrin-34 and gastrin-17 in the same tumor (Figs. 3,5) suggests that these are the independent precursors of gastrin-17 and gastrin-34. This will have to be demonstrated more directly.

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References

1 Yalow, R.S. and Berson, S.A., Radioimmunoassay of gastrin, Gastroenterology, 58 (1970) 609–615.
2 Dockray, G.J., Walsh, J.H. and Passaro, Jr., E., Relative abundance of big and little gastrins in tumors and blood of patients with the Zollinger–Ellison syndrome, Gut, 16 (1975) 353–358.
3 Rehfeld, J.F., Schwartz, T.W. and Stadil, F., Immunochemical studies on macromolecular gastrins, Gastroenterology, 73 (1977) 469–478.
4 Dockray, G.I. and Walsh, J.H., Amino terminal gastrin fragment in serum of Zollinger–Ellison syndrome patients, Gastroenterology, 68 (1975) 222–230.
5 Rehfeld, J.F., COOH-terminal extended endogenous gastrins, Biochem. Biophys. Res. Commun., 92 (1980) 811–818.
6 Boel, E., Vuust, J., Norris, K., Wind, A., Rehfeld, J. F. and Marcker, K.A., Molecular cloning of human gastrin DNA: evidence for evolution of gastrin by gene duplication, Proc. Natl. Acad. Sci. U.S.A., 80 (1983) 2866–2869.
7 Kothary, P.C., Vinik, A.I., Owyang, C. and Fiddian-Green, R.G., Immunochemical studies of molecular heterogeneity of cholecystokinin in duodenal perfusates and plasma in humans, J. Biol. Chem. 258 (1983) 2856–2863.
8 Kothary, P.C., Fabri, P.J., Gower, W., O'Dorisio, T.M., Ellis J. and Vinik, A.I., Evaluation of NH_2-terminus gastrins in gastrinoma syndrome. J. Clin. Endocrin. Metab., 62 (1986) 970–974.
9 Glowniak, J.V., Shapiro, B., Vinik, A.I., Glaser, B., Thompson, N.W. and Cho, K.J., Percutaneous transhepatic venous sampling of gastrin: value in sporadic and familial islet-cell tumors and G-cell hyperfunction, N. Engl. J. Med., 3077 (1982) 293–297.
10 Vinik, A.I., Strodel, W.E., Cho, K.J., Eckhauser, F.E. and Thompson, N.W., Localization of hormonally active gastrointestinal tumors. In N.W. Thompson and A.I. Vinik (Eds.), Endocrine Surgery Update, Grune and Stratton, New York, 1983, p. 195–218.
11 Vinik, A.I. and Glaser, B., Pancreatic endocrine tumors. In T.L. Dent, F.E. Eckhauser, A.I. Vinik and J.G. Turcotte (Eds.), Pancreatic Disease. Diagnosis and Therapy, Grune and Stratton, New York, 1981, p. 427.
12 Fiddian-Green, R.G., Pittenger, G., Kothary, P. and Vinik, A.I., Role of calcium in stimulus-secretion coupling of antral gastrin release, Endocrinology, 112 (1983) 753–760.
13 Pauwels, S. and Dockray, G.J., Identification of NH_2-terminal fragment of big gastrin in plasma, Gastroenterology, 82 (1982) 56–61.
14 Noyes, B.E., Merarch, M., Stein, R. and Agarwal, K.L., Detection and partial sequence analysis of gastrin mRNA by using an oligodeoxy-nucleotide probe, Proc. Natl. Acad. Sci. U.S.A., 76 (1979) 1770–1774.
15 Sugano, K., Aponte, G. and Yamada, T., Sequential processing of progastrin: Identification of a glycine-extended intermediate prior to alpha-amidation, Clin. Res., 32 (1984) 287A.
16 Kothary, P.C., Fiddian-Green, R.G. and Vinik, A.I., NH_2-terminus gastrin: a marker of metastatic gastrinoma, Clin. Res., 30 (1982) 397A.
17 Kothary, P.C. and Vinik, A.I., Biosynthesis of gastrin-17, Gastroenterology, 86 (1984) 1143.
18 Johnson, J.A., Fabri, P.J. and Lott, J.A., Serum gastrin in Zollinger–Ellison syndrome: identification of localized disease, Clin. Chem., 26 (1980) 867–870.