Gastrin in Human Bronchogenic Carcinomas: Constant Expression but Variable Processing of Progastrin

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ABSTRACT

Using a library of radioimmunoassays against essential sequences of human progastrin and procholecystokinin, we have examined the occurrence of gastrin, cholecystokinin, and their precursors in bronchogenic adenocarcinomas, large-cell, small-cell, and squamous-cell carcinomas ($n = 17$). Progastrin and some of its bioactive (i.e., $\alpha$-carboxyamidated) products were present in all tumors, irrespective of histological classification. The concentration of progastrin varied from 0.2 to 21.9 pmol/g tissue; glycine-extended intermediates constituted <0.1 to 0.5 pmol/g; and bioactive, carboxyamidated gastrin ranged from <0.1 to 6.1 pmol/g. Chromatography showed that the bioactive gastrins were exclusively gastrin-17 peptides, half of which were tyrosine-$\alpha$-sulfated. Neither procholecystokinin nor its processing products were found in the tumor extracts. Six samples of nonneoplastic human lung tissue contained traces of progastrin (range, <0.1 - 0.8 pmol/g), but neither bioactive gastrins nor any cholecystokinin. The results show that the gastrin gene is expressed in all classes of bronchogenic carcinomas. Due to incomplete posttranslational processing measurement of progastrin may be necessary to detect such expression.

INTRODUCTION

The respiratory tract contains peptidergic neurons and endocrine cells (for review, see refs. 1 and 2). Accordingly bronchogenic carcinomas frequently synthesize neuroendocrine peptides (3, 4), of which adrenocorticotropic hormone, calcitonin, gastrin-releasing peptide (bombesin-like peptide) and vasopressin have attracted particular interest (5–14). In contrast, neither gastrin nor related hormone, CCK,7 have so far been found in lung tumors (6, 15) apart from a single carcinoma with very low gastrin content (16).

Cellular synthesis of neuroendocrine peptides involves multiple covalent modifications of the prohormones. In peptide-producing carcinomas the prohormone processing is often incomplete so that prohormones may constitute a large proportion of the hormonal peptides (6, 7, 12, 17, 18). Although prohormones generally do not induce symptoms, they may nevertheless have considerable clinical value as tumor markers (7, 12, 19, 20).

The recent deduction of the structure of human preprogastrin and preproCCK from cloned cDNA (21, 22) has made it possible to develop assays that measure progastrin and proCCK at different levels of processing (20, 23–25). Using such assays (20, 24, 26, 27) we have now examined the occurrence of gastrin, CCK and their precursors in bronchogenic carcinomas.

MATERIALS AND METHODS

Tissue. Histologically verified carcinoma tissue was obtained from 17 patients undergoing surgery for lung tumors. In addition, control samples of histologically verified nonneoplastic lung tissue were obtained from six other patients subjected to thoracotomy for nonneoplastic diseases. Within a few minutes after resection the tissue was placed on dry ice or immersed in liquid nitrogen. It was stored at -80°C until extraction.

Extraction. The frozen tissue was minced and immersed directly in boiling water (pH 6.6), 5 ml per g tissue. It was boiled for 20 min, homogenized, and centrifuged. After decantation of the supernatants, the pellets were reextracted in 0.5 M acetic acid for 20 min, homogenized, and centrifuged. Dilutions from 1:3 to 1:0000 of both neutral and acid supernatants were radioimmunoassayed.

Radioimmunoassays. In order to measure all biologically active forms of CCK and gastrin as well as their precursors, a library of sequence-specific antisera were used (Fig. 1): Antiserum 2609 is directed against the common $\alpha$-carboxyamidated C-terminus of gastrin and CCK, -Trp-Met-Asp-Phe-NH$_2$, which is the active site in both hormones. Consequently antiserum 2609 measures all biologically active forms of CCK and gastrin (26, 27). Antiserum G-160 is specific for bioactive CCK peptides. It does not bind gastrin. It is directed against sequence 25–30 of CCK-33 and requires $O$-sulfation of tyrosyl in position 27. Consequently, it measures amidated and sulfated CCK-33, -32, -22, and -8 (28). The gastrin specific antiserum were antibodies 2604 and 2605, which are directed against the C-terminal part of the bioactive gastrins and measure component I, gastrin-34, and gastrin-17 with almost equimolar potency. Antiserum 2605 measures only the nonsulfated forms, whereas 2604 binds sulfated and nonsulfated forms with equal potency. The cross-reactivity with CCK is negligible (26, 29). Antiserum 8017 is directed against the N-terminal sequence of human gastrin-17 (Fig. I). It measures gastrin-17 as well as C-terminally extended precursors of gastrin-17 (22, 30). In order to measure N-terminal extended gastrin precursors as well, the extracts were incubated with equal volumes of trypsin (2 mg/ml 0.05 mol/liter sodium phosphate, pH 7.5) at 20°C for 20 min. The enzymatic cleavage was terminated by boiling for 10 min. By the tryptic cleavage the N-terminal sequence of gastrin-17 is exposed for binding to antiserum 8017. Consequently progastrin and all its products, bioactive or not, can be measured (20). Another way of measuring both the immediate glycine-extended precursor as well as further C-terminally extended precursors is by using antiseria, which are directed against the glycine-extended C-terminal sequence of CCK and gastrin (Fig 1). After sequential cleavage with trypsin (see above) and carboxy-peptidase B (100 n/ml sample) both CCK and gastrin precursors are bound to antiserum 3208, but gastrin precursors only to antiserum 5284 (24).

Gel Chromatography. Tissue extract (0.5–1 ml) was applied to Sephadex G-50 superfine columns (10 x 1000 mm). The columns were eluted at 4°C with 0.02 mol/liter barbital buffer containing 15 mmol/liter bovine serum albumen per liter, pH 8.4, at a flow rate of 4 ml/h in fractions of 1.0 ml. The columns were calibrated with sulfated CCK-33, sulfated and nonsulfated CCK-8, CCK-4, gastrin-34, and sulfated and nonsulfated gastrin-17. The fractions were assayed with the antisera described and with antiserum 8017 after treatment with trypsin as described.

RESULTS

As shown in the table all the carcinomas contained $\alpha$-amidated gastrin and/or its precursors. The concentrations of amidated gastrins varied more than 60-fold, i.e., from <0.1 to 6.1 pmol/g tumor tissue. The immediate precursors for the bioactive amidated gastrins, the glycine-extended gastrins were present only in low concentrations (<0.5 pmol/g). In contrast, the further C-terminally extended gastrins, the progastrins,
GASTRIN IN HUMAN BRONCHOGENIC CARCINOMAS

HUMAN PREPROGASTRIN
(SEQUENCE 54-101)

HUMAN PREPROCHOLECYSTOKININ
(SEQUENCE 65-115)

Fig. 1. Sequence-specificity of radioimmunoassays toward the COOH-terminal half of human preprocholecystokinin (preproCCK) and preprogastrin (left). The filled circles constitute the homologous sequence. Arrows indicate tryptic cleavage sites. Shadowed brackets indicate the sequence for which the antisera are specific. The active site of CCK and gastrin peptides is within the homologous sequence, i.e. 100-103 of preproCCK and 89-92 of preprogastrin. Activation of this site requires carboxyamidation of the COOH-terminal phenylalanine. Accordingly, the subsequent Gly-Arg-Arg sequence constitutes the signal for amidation with glycine as amide donor. Antibody (Ab) 2609 is specific for the amidated, i.e., active tetrapeptide sequence. Hence, it binds only bioactive fragments of preproCCK (and gastrin). Antibodies 2604 and 2605 are also directed against the carboxyamidated phenylalanine. But they also require tyrosine in position six as counted from this C-terminus. Therefore, they are specific for bioactive gastrins. In contrast, antibodies 3208 and 5284 require COOH-terminal glycine. Therefore, they do not measure active peptides.

DISCUSSION

This study has given three novel features of bronchial carcinomas. First, gastrin is synthesized in all the carcinomas irrespective of histological class, whereas the related CCK was not detectable. Second, although the concentrations of bioactive gastrin in the tumors varied, they were low in comparison with for instance gastrin-producing pancreatic carcinomas (the Zollinger-Ellison tumors). Third, the concentrations of the biosynthetic precursor, progastrin, were higher than those of mature gastrins suggesting that the posttranslational processing in lung tissue is incomplete.

That gastrin and/or progastrin occurred in all bronchial carcinomas and, moreover, in concentrations of the same magnitude in the different tumor types is surprising, because it has been stated that peptide hormones occur especially in small-cell carcinomas and bronchial carcinoids (31-33). A possible explanation of our finding is that bronchial carcinomas rarely consist of only one cell type, but are mixed. Thus, the cells expressing the gastrin gene probably constitute a minor population in all bronchial carcinomas. We do not know the nature of the bronchial gastrin cells, because the gastrin concentrations are too low to identify the cells with techniques presently available.

It has been reported that bronchial mucosa synthesizes small amounts of CCK (1, 34), whereas no gastrin has been found so far. The study on bronchial CCK, however, employed a C-terminal directed CCK-8 antiserum that also binds gastrins (see Fig. 1). Moreover, only cat, guinea pig and rat tissues were studied (1, 34). The occurrence of progastrin rather than CCK in human nonneoplastic lungs (Table 1) suggests that the normal human lung indeed expresses the gastrin gene. Our results

occurred in concentrations varying from 0.2 to 21.9 pmol/g. Neither α-amidated CCK, glycine-extended CCK, nor proCCK were present in detectable amounts. The control samples from nonneoplastic lung tissue contained traces of progastrin (Table 1).

Gel chromatography of extracts from tumors with the highest concentrations of gastrin and progastrin showed a uniform pattern as the α-amidated gastrins in all instances eluted as gastrin-17 peptides, of which half were tyrosine O-sulfated (Fig. 2). The concentrations of glycine-extended gastrin were too low to be measured in the chromatographic fractions. In contrast, progastrin was easily detected. The progastrin immunoreactivity eluted in a position corresponding to a peptide larger than C-terminally extended component I (Fig. 3, left). Trypsin and carboxypeptidase B cleavage prior to chromatography converted progastrin to a molecule eluting in a position corresponding to glycine-extended gastrin-17 (Fig. 3, right).
Table 1 The concentrations of bioactive, α-amidated gastrin and its biosynthetic precursors in extracts of bronchogenic carcinomas and nonneoplastic lung tissue (pmol/g tissue (wet weight)).

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at operation (years)</th>
<th>α-amidated Glycine-extended gastrin</th>
<th>Progastrin</th>
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<tr>
<td>Adenocarcinoma*</td>
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<td></td>
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<td>1</td>
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<td>54</td>
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<td>4</td>
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<tr>
<td>5</td>
<td>F</td>
<td>77</td>
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<tr>
<td>7</td>
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<td>53</td>
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</tr>
<tr>
<td>8</td>
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<tr>
<td>Squamous-cell*</td>
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</tr>
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<td>9</td>
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<td>49</td>
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<tr>
<td>10</td>
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* Carcinoma class.
† Mean ± SEM (n = 6).

Fig. 2. Gel chromatography of neutral water extracts from a bronchogenic adenocarcinoma (left, patient no. 2 in Table 1) and a squamous-cell carcinoma (right, patient no. 17 in Table 1). Calibrated Sephadex G50-superfine columns (10 × 1000 mm) were eluted with 0.02 mol barbital buffer containing 15 μmol bovine serum albumin per liter, pH 8.4, at a flow rate of 4 ml/h at 4°C. Fractions of 1.0 ml were collected, incubated with trypsin (see “Materials and Methods” section) and then assayed using gastrin antiserum no. 8017, which is specific for the N-terminal sequence of gastrin-17 (see Fig. 1); right, another sample of the same neutral water extract, which was first incubated with trypsin and carboxypeptidase B. After boiling the sample was applied to a calibrated Sephadex G50-superfine column (as above) and the elution monitored using antiserum no. 5284, which is specific for the C-terminal sequence of glycine-extended gastrin-17 (see Fig. 1).

Fig. 3. Gel chromatography of neutral water extracts from a squamous-cell carcinoma (patient no. 10 in Table 1). A calibrated Sephadex G50-superfine column (10 × 1000 mm) was eluted with 0.02 mol barbital buffer containing 15 μmol bovine serum albumin per liter, pH 8.4, at a flow rate of 4 ml/h at 4°C. Left, fractions of 1.0 ml were collected, incubated with trypsin (see “Materials and Methods” section) and then assayed using gastrin antiserum no. 8017, which is specific for the N-terminal sequence of gastrin-17 (see Fig. 1); right, another sample of the same neutral water extract, which was first incubated with trypsin and carboxypeptidase B. After boiling the sample was applied to a calibrated Sephadex G50-superfine column (as above) and the elution monitored using antiserum no. 5284, which is specific for the C-terminal sequence of glycine-extended gastrin-17 (see Fig. 1).

...also suggest that restudy of lungs from other mammals using specific precursor assays may be worth while.

...In view of the easy access to gastrin assays during the last 15 years, it may seem strange that gastrin so far has been found in only one bronchial carcinoma and in a concentration (0.3 pmol/g) too low for chromatographic characterization (16). Moreover, two studies reported in 1974 mention that gastrin was undetectable in lung tumor extracts (6, 15). These meager results may have prevented further search for gastrin. The negative results are, however, easy to explain. They are partly due to insufficient sensitivity of the early gastrin radioimmunoassays. Moreover, previously used gastrin assays have measured only bioactive amidated gastrins, and not the biosynthetic precursors, which occur in higher concentrations. Thus, there is no real discrepancy between earlier negative results (6, 15) and the present findings (Table 1).

...The synthesis of progastrin and the subsequent incomplete processing to bioactive gastrins in all classes of bronchial carcinomas are of pathogenetic and biochemical interest. We believe, however, that the expression of the gastrin gene in bronchial carcinomas is without clinical implications. For such purposes the synthesis appears far too small in comparison with that of the gastroduodenal mucosa. Accordingly, neither gastric acid hypersecretion nor duodenal ulcers have so far been associated with peptide-producing bronchial carcinomas. Unfortunately, no plasma samples were available from the tumor patients in this study. Recently, however, we measured amidated gastrin as well as precursor gastrins in plasma from 67 patients with small-cell carcinomas. The concentrations were slightly increased in seven patients. The moderate hypergastrinemia in these patients was, however, due to increased antral secretion caused by hypo- or achlorhydria rather than to bronchial hypersecretion of gastrin.

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