Gastrin-releasing Peptide-related Peptides in a Human Malignant Lung Carcinoid Tumor

Kevin A. Roth, Christopher J. Evans, Eckard Weber, Jack D. Barchas, David G. Bostwick, and Klaus G. Bensch

Nancy Pritzker Laboratory of Behavioral Neurochemistry, Department of Psychiatry and Behavioral Sciences [K. A. R., C. J. E., E. W., J. D. B.], and Department of Pathology [D. G. B., K. G. B.], Stanford University School of Medicine, Stanford, California 94305

ABSTRACT
Recent immunohistochemical findings have indicated the presence of gastrin-releasing peptide in normal and pathological human lungs. Gastrin-releasing peptide is a 27-amino acid peptide isolated from porcine gut which bears considerable carboxy-terminal homology with bombesin. We have characterized the gastrin-releasing peptide-like peptides present in a human malignant lung carcinoid tumor by gel chromatography and reverse-phase high-performance liquid chromatography. Our results show that this tumor did not contain bombesin; however, this tumor expressed a gastrin-releasing peptide-like compound, several amino-terminal fragments, and a carboxy-terminal fragment of gastrin-releasing peptide.

INTRODUCTION
Tsutsumi et al. (10) have recently reported the presence of GRP in normal lungs and in pulmonary tumors, using immunohistochemical methods. GRP is a 27-amino acid peptide isolated from porcine gut which bears a heptapeptide carboxy-terminal sequence corresponding to the region of bombesin (Chart 1). We have examined the GRP-like peptides in a human malignant carcinoid tumor of the lung using antisera directed at several different regions of the porcine GRP sequence.

MATERIALS AND METHODS

Clinical History. The patient, a 61-year-old female, presented with a 2-month history of coryza and intermittent chest pain. Radiographic examination disclosed a localized 3.0-cm-diameter right infralobar mass. Sputum cytologies, bronchoscopy, mediastinoscopy, and scalse lymph node biopsies were nondiagnostic. Ectopic hormone production was not observed clinically. The patient, who underwent a right lower lobectomy and resection of hilar lymph nodes, was found to have a submucosal tumor mass in the bronchus. Massive bleeding from the main pulmonary artery, which occurred postoperatively, could not be controlled.

Pathological Studies. The pathological specimen consisted of the right lower lobe and attached hilar lymph nodes. The bulk of the specimen was prepared for light microscopy by fixation in 10% buffered formalin, and representative sections were embedded in paraffin. For electron microscopy, small blocks of tissue were immediately fixed in 4% buffered glutaraldehyde, postfixed in osmium tetroxide, and embedded in Epon. Thin sections were stained with uranyl acetate and lead citrate, and were examined with a Hitachi H-300 electron microscope. For biochemical and immunohistochemical studies, representative sections of tumor were immediately frozen and stored in liquid nitrogen at −70°C. The bulk of the specimen was prepared for light microscopy by fixation in 10% buffered formalin, and representative sections were embedded in paraffin.

The tumor was originally classified as a small-cell (oat cell) carcinoma on frozen sections. However, upon review of the paraffin-embedded material and on the basis of ultrastructural findings, it was reclassified as a malignant (aggressive) carcinoid tumor. The tumor had the appearance of a carcinoid architecturally, cytologically, and by virtue of the abundant neurosecretory-like granules. The presence of hilar lymph node metastases indicated that the neoplasm was a malignant form of carcinoid tumor, and it confirmed the pulmonary origin of the lesion. Also suggestive of the aggressive nature of the tumor was the large number of mitoses and the foci of necrotic tumor cells.

RIA. Three GRP antisera were generated in rabbits against synthetic porcine GRP and GRP(1-16) (Peninsula Laboratories, San Carlos, Calif.), linked by a water-soluble carbodiimide to bovine thyroglobulin, as described previously (12). RIAs were performed, as described in Weber et al. (11). Synthetic GRP and GRP(1-16) were iodinated as described in Weber et al. (12). Three different GRP RIAs were used in this study. The specificity of these antisera in RIAs is shown in Table 1. One antiserum [Code GRP(1-27) R3-2] was directed at the carboxy-terminal region of porcine GRP; this antiserum is approximately 30% cross-reactive with bombesin and would detect endogenous bombesin if present. A second antiserum [Code GRP(1-16) R2-6A] is equally cross-reactive with porcine GRP(1-13), GRP(1-16), and GRP(1-27), and thus is directed to the amino-terminal region of GRP. The third antiserum [Code GRP(1-16) R2-6B] is...
Cross-reactivity of GRP RIAs

Table 1

<table>
<thead>
<tr>
<th>Synthetic peptide</th>
<th>GRP(1-27)</th>
<th>GRP(1-16)</th>
<th>GRP(1-13)</th>
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<tbody>
<tr>
<td>Code R3-2</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Code R2-6A</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Code R2-6B</td>
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<td>0</td>
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</tr>
<tr>
<td>GRP</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>GRP(18-27)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>o-N-Acetyl-GRP(20-27)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bombesin</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

An antisera dilution of 1:20,000 for the GRP(1-27) R3-2 antisera, and a dilution of 1:15,000 for the GRP(1-16) R2-6A were used for RIA. For these RIAs, GRP(1-27) was used as a reference tracer and standard. An antisera dilution of 1:20,000 for GRP(1-16) R2-6B and GRP(1-16) trace and standard were used in the GRP(1-16) carboxy-terminal assay. Cross-reactivity was based on the amount of unlabeled peptide needed to obtain a 50% displacement of the 125I tracer from the antisera. The highest concentration of unlabeled peptide tested was 1 µM. Synthetic peptides were synthesized by standard solid-phase methods and purified by gel filtration and partition chromatography. Purity of the peptides was shown by RP-HPLC and partition chromatography. Purity of the peptides was shown by RP-HPLC and partition chromatography. Purity of the peptides was shown by RP-HPLC and partition chromatography.

RESULTS

RIAs and Gel Chromatography. RIA of the acid:acetone extracts of the tumor detected large amounts of GRP-like immunoreactive peptide. The carboxy-terminal GRP RIA measured 270 pmol/g tissue, the carboxy-terminal GRP(1-16) RIA measured 170 pmol/g tissue, and the GRP(1-27) amino-terminal assay measured 110 pmol/g tissue. The molecular size of the GRP-immunoreactive peptides in brain was determined by gel-filtration chromatography on a Sephadex G-50 column eluted with a dissociating solvent (11, 14). The GRP carboxy-terminal RIA identified 2 major peaks of immunoreactivity; the first peak had a molecular weight of approximately 2800 and chromatographed with 125I-GRP, and the second peak had a molecular weight of approximately 1200 (Chart 2A). The GRP amino-terminal RIA gave a complex pattern of immunoreactivity; a minor peak of immunoreactivity of ~2800 was observed (~15% of the GRP carboxy-terminal RIA amounts were measured at this weight), as well as a major peak of M~ ~1900. This second peak had a shoulder with a peak at M~ ~1700 (Chart 2B). The GRP(1-16) carboxy-terminal RIA identified a major peak of immunoreactivity at M~ ~1700.

RP-HPLC. The 2 peaks of GRP carboxy-terminal immunoreactivity were further analyzed by RP-HPLC. The M~ 1200 peak of GRP carboxy-terminal immunoreactivity was found to cochromatograph with the GRP fragment (18-27) (Chart 3C). The M~ 2800 peak of GRP carboxy-terminal immunoreactivity eluted slightly later than porcine GRP, indicating that it is more hydrophobic than the porcine GRP (Chart 34). This peak of immunoreactivity produced upon trypsin digestion an equimolar amount of a peptide which cochromatographed on RP-HPLC with the M~ 1200 peak of GRP carboxy-terminal immunoreactivity and GRP(18-27) (Chart 3B). These results indicate that the large-molecular-weight peptide contains within it a peptide with the same HPLC characteristics and immunoreactivity as GRP(18-27). No peaks of immunoreactivity were found at the bombesin-retention time.

The major peak of GRP(1-16) carboxy-terminal immunoreactivity was also analyzed by RP-HPLC. This material eluted slightly later than porcine GRP(1-16), indicating that it is more hydrophobic at pH 2.7 than is porcine GRP(1-16) (Chart 3D). This peak of GRP(1-16) carboxy-terminal immunoreactivity and the M~ 2800 RP-HPLC peak of GRP carboxy-terminal immunoreactivity were recognized ~15% by the GRP amino-terminal RIA.

DISCUSSION

Our finding indicates that a human malignant lung carcinoid tumor produced large amounts of GRP-related products. Tsur-
sumi et al. (10) have reported the presence of GRP in bronchial endocrine cells, pulmonary tumorlets, and in several endocrine tumors, using immunohistochemical techniques. Our results suggest that the immunohistochemically reactive GRP material may represent human GRP and several amino- and carboxy-terminal GRP fragments.

Since the GRP carboxy-terminal antiserum cross-reacts with bombesin, we would have been able to detect endogenous bombesin if this material was present in the human lung carcinoid examined. We observed a peak of immunoreactivity of similar size as bombesin; however, this material did not coelute with bombesin on RP-HPLC. This material had an identical retention time as GRP(18-27). Tsutsumi et al. (10) did not detect any bombesin immunoreactivity in normal or pathological human
lungs, using bombesin antisera that did not cross-react with GRP. These findings are in contrast to several reports of bombesin-like peptide in human lung small-cell carcinoma (3, 5, 9, 13). It is possible that bombesin is a specific marker for small-cell carcinoma, and the human lung carcinoids do not produce this material. Alternatively, the material characterized as bombesin in small-cell carcinoma and cell lines actually represents the GRP fragment GRP(18-27). Additional work must be performed to clarify these possibilities.

In the human lung carcinoid tumor examined, the GRP(1-16) carboxy-terminal RIA detected the presence of a peptide of similar size and immunoreactive determinants as GRP(1-16). The GRP carboxy-terminal RIA also identified a peptide of similar size and immunoreactive determinants as GRP(1-27). Both of these immunoreactive peaks eluted later on RP-HPLC than did their porcine counterparts. This result, in conjunction with the observation that the antiserum directed at the porcine GRP(1-13) sequence had only a slight recognition of these peptides, indicates that the amino acid sequence of the human GRP expressed by the malignant lung carcinoid tumor tissue is different from the porcine GRP sequence in the (1-13) region. In extracts from guinea pig brains, the GRP carboxy-terminal RIA and the GRP amino-terminal RIA measure equimolar amounts of a M, 2800 peptide which has identical RP-HPLC characteristics as porcine GRP(1-27) (8). Also, in guinea pig brain, the GRP amino-terminal RIA and the GRP(1-16) carboxy-terminal RIA measure equimolar amounts of a peptide of identical molecular weight and RP-HPLC characteristics as GRP(1-16). Thus, in comparison with guinea pig brain, the malignant human lung carcinoid tumor produced GRP-like peptides which were not identical to the porcine GRP fragment (1-16) and GRP(1-27). The sequence of GRP isolated from dog intestine has also recently been reported (7). The dog GRP sequence differs by 3 amino acids, all in the (1-16) region, from the porcine GRP sequence.

We have reported the presence of a bombesin-like peptide of similar size and RP-HPLC characteristics as GRP(18-27) in guinea pig brain (8). This appears to be identical to the peptide present in the tumor studied here. This fragment, GRP(18-27), is also present in dog intestine (7).

The GRP amino-terminal RIA, although only recognizing the GRP(1-27)- and GRP(1-16)-sized material approximately 15 to 20%, measured a large amount of immunoreactivity at M, ~1900. Furthermore, this material was not recognized by the GRP(1-16) carboxy-terminal RIA or the GRP(1-27) carboxy-terminal RIA. The molecular weight of this peak in conjunction with its antibody characteristics and the identification of GRP(18-27) as a major product in the tumor would be consistent with the presence of a GRP(1-17)-like peptide.

Our results indicate the presence of several GRP-related peptides in a malignant lung carcinoid tumor; it remains to be investigated whether the measurement of GRP or its fragments can be used as a useful marker for the identification of certain neuroendocrine-derived tumors.

ACKNOWLEDGMENTS

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Fig. 1. In A, the tumor consisted of nests of cells which were separated by a scant delicate connective tissue network. The tumors cells were large and relatively uniform in appearance, with big nuclei and abundant, finely granular, sometimes translucent cytoplasm. × 300. In B, electron micrographs showed the tumor cell cytoplasm to contain large numbers of uniform, neurosecretory-like granules with an electron-opaque core and narrow electron-translucent halo beneath a limiting membrane. × 22,400.
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