Autoantibody against Diiodinated Tyrosine-Gastrin in a Patient with Graves' Disease
Masato Noguchi, Hideki Adachi, Etsuo Aoki, Yasuhiro Iida, Kanji Kasegi, Keigo Endo, Junji Konishi, and Kanji Tortzuka

We describe autoantibodies against iodinated gastrin in a patient with Graves' disease. Values for serum gastrin differed in this case, depending on which of two different radioimmunoassay (RIA) kits was used. RIA with the dextran-coated charcoal method for separation of free tracer gastrin gave a value <9.5 pmol/L, whereas the value by a RIA kit by the double-antibody method was 318 pmol/L. The patient's serum contained a binding protein for [125I]-labeled gastrin, as detected by Sephadex G-200 column chromatography. The IgG fraction was responsible for the ability of serum to bind [125I]-labeled gastrin. Interestingly, of the two possible forms of iodinated gastrins, moniodinated (MIT) and diiodinated (DIT) tyrosine-[125I]-labeled gastrin, only the latter bound to patient's IgG. Furthermore, DIT-gastrin, but not gastrin or MIT-gastrin, inhibited the binding of DIT-[125I]-labeled gastrin. The patient's serum evidently contains autoantibodies against DIT-gastrin that interfere with RIA of gastrin.

Additional Keyphrases: radioimmunoassay • iodinated peptides

The presence of autoantibodies against peptide hormones such as thyroid hormone and insulin has been well documented in patients with autoimmune thyroid disorders and diabetes mellitus (1, 2). Because such autoantibodies will give a spuriously low or high value for hormone concentration as measured by the radioimmunoassay (RIA), depending on whether dextran-coated charcoal or the double-antibody method is used to separate bound and free tracer hormones, the discordance between clinical features and measured values for hormone concentration in blood suggests the existence of autoantibodies in these patients. The presence of hypergastrinemia has been reported in cases of atrophic gastritis, pernicious anemia, antral G-cell hyperplasia, and gastrin-producing pancreatic tumors (Zollinger-Ellison syndrome) (3). In some patients, pancreatic tumors have been sought, but not found, although Zollinger-Ellison syndrome was suspected because of hypergastrinemia (4, 5). However, there is no report of autoantibodies against gastrin and related substances, which also may cause RIA values for serum gastrin to be high.

In the present study, we describe a patient who had autoantibodies against diiodinated tyrosine-gastrin (DIT-gastrin), and whose serum gastrin value was significantly above-normal when measured by RIA with the double-antibody method. This is the first case in which only the iodinated peptide was recognized by autoantibodies.

Materials and Methods

Human gastrin I (gastrin) was purchased from Calbiochem, La Jolla, CA; Na[125I] from New England Nuclear, Boston, MA; and Protein A-conjugated Sepharose CL-4B and Sephadex G-200 from Pharmacia Fine Chemicals, Uppsala, Sweden.

The patient, a 20-year-old man with Graves' disease, had no past history of illness. His grandfather had gastric cancer. The values for serum thyroxin, triiodothyronine, reverse triiodothyronine, and thyrotropin, as measured by RIA, were >24 mg/L, 800 mg/L, 200 g/L, and <0.5 milli- units/L, respectively. Titers for anti-microsomal antibody and anti-thyroglobulin antibody, as measured by the hemagglutination method (Fuji Zoki, Tokyo, Japan), were 1:640 and 1:320, respectively (normal range, <1:80). Serum triglyceride and free cholesterol concentrations were within normal limits.

We measured serum gastrin with two RIA kits from a commercial source—one based on the single-antibody method (Dinabot, Tokyo, Japan), in which dextran-coated charcoal is used to separate bound and free fractions of [125I]-labeled gastrin, and the other based on the double-antibody method (Travenol-Genentech Diag., Cambridge, MA), in which goat anti-rabbit IgG antiserum is used to separate [125I]-labeled gastrin bound to anti-gastrin antiserum from the free fraction.

To examine binding of [125I]-labeled gastrin to the patient's serum, we incubated [125I]-labeled gastrin obtained from a RIA kit with or without 0.5 mL of the patient's serum in barbital buffer (20 mmol/L, pH 8.0) in a total volume of 1.1 mL at 4 °C for 24 h without anti-gastrin antiserum. The incubation mixture was then applied to a 1.6 × 85 cm column of Sephadex G-200 that had been equilibrated with the barbital buffer. The column was eluted with the same buffer at a flow rate of 9.4 mL/h. We collected 1.5-mL fractions and measured their absorbances at 280 nm and their radioactivities.

IgG and non-IgG fractions were obtained from sera by affinity chromatography on a column of Protein A-conjugated Sepharose CL-4B (6, 7). Unlabeled and [125I]-labeled monoiodo-tyrosine (MIT) and diiodo-tyrosine (DIT) gastrins were prepared with use of columns of Sephadex G-25 (0.8 × 18 cm) and DEAE-Sephadex A-25 (1 × 8 cm), as reported previously (8, 9). The concentrations of MIT- and DIT-gastrin obtained were determined by RIA for gastrin with the single-antibody method, and expressed in terms of gastrin.

We examined the binding of iodinated gastrin to serum, IgG, and non-IgG fractions. The iodinated gastrin was in 0.5 mL of barbital buffer containing 1 g of bovine serum albumin per liter, unless otherwise stated. After incubation at 4 °C for 24 h, the free fraction of the tracer gastrin was separated from the bound fraction by adding 1 mL of 10 g/L dextran-coated charcoal suspension, then centrifuging (2800 × g, 15 min, 4 °C). The radioactivity of the pellet was measured in a gamma counter. Binding of gastrin was expressed as the percent of total iodinated gastrin (T-NSB), as follows: T-NSB = F/(T-NSB) (T: total iodinated gastrin added, NSB: nonspecific binding without antibodies, F: free fraction).

Protein concentrations in samples were measured by the method of Lowry et al. (10), with BSA as the standard.
Results

Initially, gastrin in the serum of the patient was measured by use of an RIA kit, because patients with untreated hyperthyroidism reportedly show increased concentrations (11). However, the value for serum gastrin in this patient was below the minimal detectable value (<9.5 pmol/L), when measured with an RIA kit based on the single-antibody method. In contrast, when an RIA kit based on the double-antibody method was used, the value for serum gastrin of the patient was remarkably high, 318 pmol/L. This marked disagreement suggested the existence of a binding protein for the tracer in his serum.

To examine this possibility, we first incubated the serum with 125I-labeled gastrin from RIA kits at 4 °C for 24 h, and then subjected the incubation mixture to Sephadex G-200 column chromatography. As shown in Figure 1, 24% of total 125I-labeled gastrin added was eluted in the fraction with a $K_{av}$ value of 0.18. When 125I-labeled gastrin alone was applied on the same column, no radioactivity was detected in the corresponding fractions (Figure 1). To further identify the binding protein in the patient's serum, we investigated binding of 125I-labeled gastrin to IgG and non-IgG fractions we had prepared. All of the binding ability in the patient's serum was associated with the IgG fraction (Table 1).

After iodination, 125I-labeled gastrin usually contains both MIT- and DIT-125I-labeled gastrin unless these are separated by further purification. We studied bindings of these two different forms of the iodinated gastrin to the patient's serum as described in Materials and Methods. Binding of MIT-125I-labeled gastrin to the patient's serum (5.2% of total radioactivity added) was similar to that of the control sera (1.4 ± 1.0%, mean ± SD, n = 10). However, binding of DIT-125I-labeled gastrin to the patient's serum (77.9%) was significantly greater than that of control sera (4.1 ± 0.5%).

Further to analyze the property of binding of DIT-125I-labeled gastrin to the patient's serum, we examined the inhibitory effects of gastrin, MIT-gastrin, and DIT-gastrin on the binding. Only DIT-gastrin inhibited the binding, whereas neither gastrin nor MIT-gastrin affected the binding (Table 2). In addition, neither MIT alone nor DIT alone in concentrations up to 380 nmol/L inhibited the binding (data not shown).

Discussion

In this report, we describe the existence of a binding protein for 125I-labeled gastrin in the serum of a patient with Graves' disease. The binding protein was eluted in gel filtration in the fractions with a $K_{av}$ value of 0.18, suggesting that the binding protein is IgG or IgM. However, because the IgG fraction from the patient's serum, prepared by using a Protein A affinity column, could bind 125I-labeled gastrin, we conclude that the binding protein in the patient's serum is IgG. These findings suggest that the patient's serum contained autoantibodies against gastrin or related substances.

Interestingly, the autoantibodies recognized only DIT-gastrin. Because DIT-gastrin is not a naturally occurring form in humans, the reason for the production of human autoantibodies against it is unknown. No autoantibodies against thyroxin, triiodothyronine, or "reverse" triiodothyronine were detected in the patient's serum; however, anti-thyroglobulin antibody was. Because the production of autoantibodies to thyroid hormone is speculated to result from abnormal recognition of thyroglobulin or thyroid hormone-thyroglobulin complex (1), perhaps the autoantibody against DIT-gastrin is a kind of anti-thyroglobulin antibody.

Fatty acid interference in RIA measurement of gastrin in heparinized samples is known to cause discrepant results for different kits (12). However, such interference was not responsible for the discordance of values for gastrin concentration in this patient, whose fatty acid concentrations in serum were normal. The interference of this patient's autoantibody led to spurious results in RIAs for gastrin, except when no DIT-125I-labeled gastrin was present in the radiolabeled gastrin preparation.

Although hypergastrinemia determined by RIA is essential for the clinical diagnosis of ulcerogenic syndrome, including Zollinger–Ellison syndrome (13), some studies

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![Image of Sephadex G-200 column chromatograms of 125I-labeled gastrin incubated with the patient's serum (O, O) and 125I-labeled gastrin alone (△).](image)

125I-labeled gastrin was incubated with or without the patient's serum in barbital buffer (20 mmol/L, pH 8.0) in a total volume of 1.1 mL at 4 °C for 24 h, then applied to a Sephadex G-200 column (1.8 × 85 cm). The column was eluted with barbital buffer at 4 °C, and fractions of 1.5 mL were collected. Note differences in scale for the radioactivity measurements.

<table>
<thead>
<tr>
<th>Table 1. Binding of 125I-Labeled Gastrin to IgG and Non-IgG Fractions of Serum</th>
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<tbody>
<tr>
<td>Serum fraction eluted</td>
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<td>Patient</td>
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<td>IgG</td>
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IgG and non-IgG fractions, prepared by using a Protein A affinity column, were incubated with 125I-labeled gastrin (7000 counts/min).

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<th>Table 2. Inhibitory Effects of Various Agents on Binding of DIT-125I-Labeled Gastrin to the Patient's Serum</th>
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<td>Agent</td>
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<tr>
<td>None</td>
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<tr>
<td>Gastrin</td>
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<tr>
<td>MIT-gastrin</td>
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<td>DIT-gastrin</td>
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DIT-125I-labeled gastrin (0.1 mL, 7000 counts/min) and 0.1 mL of the 10-fold diluted patient's serum were incubated with the indicated agent as described in the text.
have found some patients without a gastrin-producing tumor in pancreas or duodenum, even though Zollinger-Ellison syndrome was suspected from the increased concentration of gastrin in serum (4, 5). In such cases, the existence of autoantibodies against D1T-gastrin should also be considered as a possible reason for hypergastrinemia, if an RIA based on the double-antibody method was used to measure serum gastrin.

References