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Cross-Reaction of Two Different Somatostatin Antisera with Calcitonin Gene-Related Peptide[1] (42771)

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Abstract. Two different anti-somatostatin antisera, R-101 and OAL-273, cross-react with rat calcitonin gene-related peptide (1-37) (CGRP). CGRP amounts, in excess of 6.25×10^{-9} *M*, cross-react with R-101 in the somatostatin radioimmunoassay. CGRP amounts, in excess of 1.6×10^{-9} *M*, cross-react with OAL-273. Both CGRP displacement curves are parallel to that of synthetic somatostatin (1-14). Comparison of ID_{50}'s shows that the cross-reactivity of CGRP with R-101 and OAL-273 are 0.02 and 0.1% of somatostatin, respectively.

Calcitonin gene-related peptide (CGRP) is a 37-amino acid peptide that is produced by alternative processing of the RNA transcribed from the calcitonin gene (1, 2). CGRP can inhibit the release of insulin (3, 4), but the mechanism of inhibition is unknown. The finding that CGRP can increase the release of somatostatin from the perfusated stomach (5) suggests that the insulin-inhibitory effect of CGRP is mediated by release of somatostatin.

We have recently been involved in studies on the effect of CGRP on the release of insulin and somatostatin from cultured rat islet cells (6, 7). We found that CGRP, at concentrations less than 10^{-9} *M*, did not affect the release of somatostatin, while CGRP, at concentrations greater than 10^{-8} *M*, apparently increased the release of somatostatin in a dose-dependent fashion. This finding prompted us to examine the possibility of cross-reaction of our anti-somatostatin antiserum with CGRP.

Materials and Methods. *Iodination of somatostatin.* Iodination of 1-Tyr[1]-somatostatin (Peninsula Laboratories, Belmont, CA) was performed by a modification of a method of Patel and Reichlin (8). In a reaction volume of 30 μl, 5 μg 1-Tyr-somatostatin was iodinated with 1 mCi ^{125}I by the addition of 5 μg chloramine-T. After a 30-sec incubation, the reaction was terminated by the addition of 100 μl of 10% bovine serum albumin (BSA) and 1 ml of normal human plasma. Radioiodinated somatostatin was separated from free ^{125}I and from damaged somatostatin by Sephadex G-50 superfine column. The specific activity was 47 μCi/μg.

Radioimmunoassay conditions. The assay buffer consisted of 0.05 *M* phosphate-buffered saline (pH 7.5) containing 0.02% sodium azide, 0.25% BSA, and 0.1 *M* EDTA. Incubations were carried out in glass tubes in duplicate for 48 hr after adding somatostatin antiserum, and for 72 hr after adding ^{125}I-Tyr-somatostatin. The total volume of 1 ml consisted of 100 μl first antiserum, 100 μl synthetic cyclic somatostatin (1-14) standard (0.001–0.1 ng/tube) or unknown, 100 μl ^{125}I-Tyr[1]-somatostatin (~10,000 cpm/tube), and 700 μl assay buffer. Separation of bound and free somatostatin was achieved by the double-antibody technique.

Examination of cross-reactivity. We used doses of synthetic somatostatin (1-14) from 6×10^{-13} to 6×10^{-10} *M*. For examination of cross-reactivity, rat CGRP (1-37), human calcitonin (1-32), and salmon calcitonin (1-32) were used at doses from 8×10^{-10} to 10^{-7} *M*. Two different anti-somatostatin antisera were used. One was R-101 which was purchased from A. Arimura (Tulane Uni-

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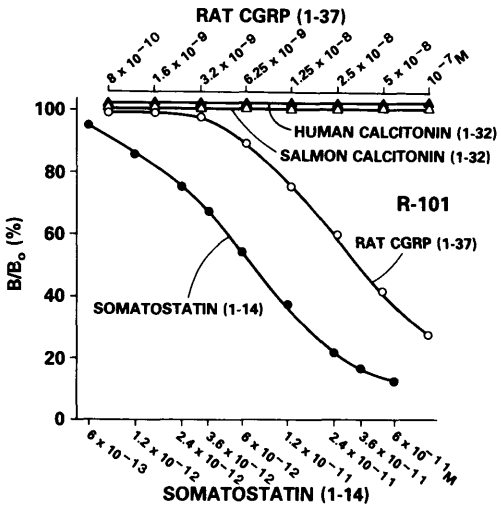

FIG. 1. Cross-reactivity of rat CGRP with anti-somatostatin antiserum, R-101. R-101 was used at a final dilution of 1:40,000.

versity, School of Medicine, New Orleans, LA). The other was OAL-273 from N. Yanaihara. Final dilutions of the two antisera were 1:40,000 and 1:100,000 in this assay system, respectively.

Results. The somatostatin antiserum R-101, in the somatostatin radioimmunoassay (RIA), cross-reacted with CGRP amounts in excess of 6.25×10^{-9} *M* and the displacement curve of CGRP was parallel to that of synthetic somatostatin (1-14) (Fig. 1). Similarly, another somatostatin antisera, OAL-273, cross-reacted with CGRP amounts in excess of 1.6×10^{-9} *M* and the displacement curve of CGRP was also parallel to somatostatin (Fig. 2). Comparison of the ID_{50}'s showed that the cross-reactivity of CGRP with R-101 was 0.02%, while with OAL-273 was 0.1% when compared to that of synthetic cyclic somatostatin (1-14). Both R-101 and OAL-273 failed to detect either human or salmon calcitonin (Figs. 1 and 2).

Discussion. Although somatostatin and CGRP are not structurally related peptides, the cross-reaction of somatostatin antisera with CGRP indicates that they have a common epitope within their primary amino acid structures. As shown in Fig. 3, both peptides have the cys–cys bridge, serine–cystine sequence, and asparagine–phenylalanine sequence. Both human and salmon calcitonin also have a cys–cys bridge, and human calcitonin has the phenylalanine–asparagine sequence. Salmon calcitonin has the cystine–serine sequence, although the two different antisomatostatin antisera do not cross-react with either human or salmon calcitonin. Taken together, the binding of CGRP to somatostatin antisera may be attributable to the tertiary structure of CGRP.

The present findings raise the following possibilities. (i) A portion of the pancreatic islet cells identified originally as D cells by immunohistochemistry techniques are actually CGRP-containing cells. Hence, another cell type that secretes CGRP may exist in the pancreas. Therefore, earlier reports (3, 9) that suggest CGRP-containing cells are also somatostatin-containing cells may not be altogether correct. (ii) We speculate that some RIA measurement of high levels of somatostatin in plasma of patients with putative somatostatinomas may actually represent CGRPomas, at least in part.

In immunohistochemical studies of D cells, somatostatin antisera, which are usually used at low dilutions, are preabsorbed with normal sera or well-known pep-

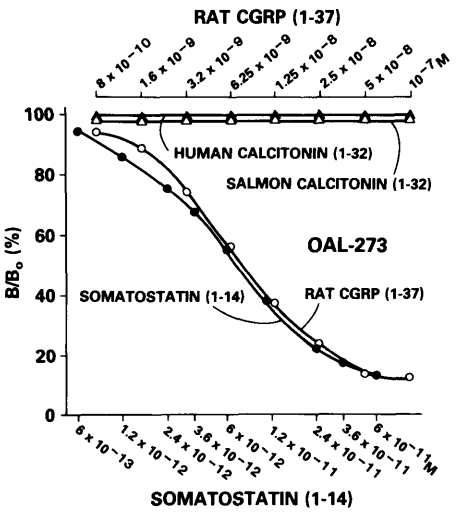

FIG. 2. Cross-reactivity of rat CGRP with anti-somatostatin antiserum, OAL-273. OAL-273 was used at a final dilution of 1:100,000.

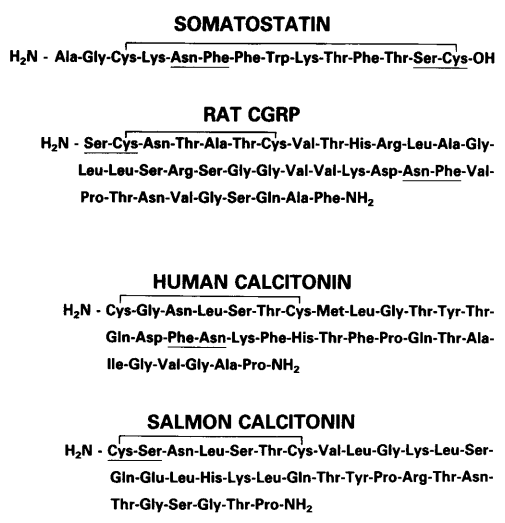

FIG. 3. Amino acid structure of cyclic somatostatin (1-14), rat CGRP (1-37), human calcitonin (1-32), and salmon calcitonin (1-32). Underscores show common amino acid sequences, although those of human and salmon calcitonin are reversal sequence.

tides, such as vasoactive intestinal polypeptide, insulin, glucagon, or gastrin. The CGRP content of these normal sera may be negligible; hence, somatostatin antisera may not be effectively preabsorbed. To our knowledge, only one immunohistochemical study (3) in which somatostatin sera preabsorbed with excessive amounts of CGRP has been reported. Therefore, we would like to suggest that preabsorption of somatostatin antisera with an excessive amount of CGRP should be done in localization studies of somatostatin-secreting cells by immunohistochemical methods.

Biological effects of CGRP may explain some of the symptoms of somatostatinoma syndrome (10). CGRP can inhibit the release of insulin, and it can inhibit the motility of gallbladder smooth muscle (unreported finding). Again, we would like to point out that elevated circulating levels of immunoreactive somatostatin, as measured by RIA, should be verified by high pressure liquid chromatography, or at least by gel chromatography.

Two different anti-somatostatin antisera, R-101 (11) and OAL-273, used in this examination may be unique: another somatostatin antiserum may not cross-react with CGRP. Despite this possibility, the present findings indicate that the cross-reactivity of newly discovered peptides with antisera which are used for RIA or immunohistochemical studies should be examined since polyclonal antisera may detect structurally dissimilar peptides. Parenthetically, these data argue for the use of monoclonal antisera and not polyclonal antisera when addressing some research questions. Furthermore, data derived from studies that utilize polyclonal antisera should be supported by other techniques that characterize the peptide in question chemically (i.e., primary structure) or according to a specific biological action.

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