COMPARATIVE REACTIVITIES OF ^{125}I-SECRETIN AND ^{125}I-6-TYROSYL SECRETIN WITH GUINEA PIG AND RABBIT ANTI-SECRETIN SERA

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Received April 16,1975

SUMMARY: Since secretin contains only an N-terminal histidyl and no tyrosyl residue, a synthetic secretin has been commercially prepared containing tyrosine in place of phenylalanine to facilitate the preparation of a radioiodine labeled tracer. We have found that although the rate of iodination of 6-Tyr-secretin is more rapid than that of secretin, the efficiency of iodination is not greatly increased and the shelf-life of the labeled product is not prolonged. The striking disadvantage of the use of ^{125}I-6-Tyr-secretin as a tracer in radioimmunoassay is its diminished immunoreactivity with several guinea pig and rabbit antisera compared to ^{125}I-secretin.

Because of the low concentration of peptide hormones in plasma and the fact that it is inadvisable in radioimmunoassay (RIA) to employ an amount of labeled tracer hormone whose immunochemical concentration is large compared to the concentration of the unlabeled hormone, radioiodine labeled hormones of high specific activity are generally used in these procedures. The iodinating species substitutes easily onto the ortho positions of tyrosine in peptidal linkage, and although histidyl residues can also be iodinated they are generally considered less suitable (1). Problems in the development of a RIA for secretin, a 27 amino acid peptide, have been attributed in part to the difficulty in preparing a satisfactory labeled preparation because secretin contains an N-terminal histidyl and no tyrosyl residue. Recently a secretin in which tyrosine replaces phenylalanine in the sixth position (6-Tyr-secretin) has been synthesized to facilitate the preparation of a radioiodine labeled tracer. In this report we describe methods for iodination of unmodified and of tyrosyl-modified synthetic secretins and compare the labeled preparations in terms of their suitability for radioimmunoassay.

METHODS

Synthetic human secretin (Schwarz-Mann) and synthetic 6-Tyr-secretin

(Schwarz-Mann) were made up in stock solutions of 250 µg per ml of 0.01 N HCl. Iodination with ^{125}I (Union Carbide) was carried out by a slightly modified chloramine T technique (2). A series of iodinations were performed in 40 µl phosphate buffer (pH 7.5) in which approximately 500 µCi of ^{125}I and 1 µg of secretin were exposed to 52.5 µg of chloramine T for 2, 30, 60, 120 and 180 seconds respectively prior to the addition of 48 µg of sodium metabisulfite. Efficiency of iodination was assessed by paper chromato-electrophoresis (3) on Toyo 525 filter paper. In this system ^{125}I-secretin binds at the site of application, while damaged hormone and unreacted iodide migrate anodally. Purification was effected by adsorption to and elution from Quso G-32 (4).

Standard curves were constructed to study the binding to specific anti-secretin antisera of ^{125}I-secretin and ^{125}I 6-Tyr-secretin having specific activities of approximately 250 µCi per µg. Binding of labeled secretin to antisera was determined with tracer concentrations of about 10, 20 and 40 pg/ml. Antisera were prepared in 3 guinea pigs and 1 rabbit by repeated subcutaneous injection of synthetic human secretin (Schwarz-Mann) coupled to guinea pig albumin by the carbodiimide reaction of Goodfriend (5). The same two labeled secretin preparations were evaluated with each antiserum. The studies were repeated with two lots of radioiodine and four separate iodinations of each secretin. Pure natural secretin (GIH Research Laboratory, Karolinska Institute, Stockholm, Sweden) was used for generation of the standard curves. After a 72 hour incubation at 4°C separation of antibody-bound and free labeled secretin was accomplished by adsorption of unbound ^{125}I-secretin to charcoal (6).

Shelf-lives of the labeled secretin preparations were evaluated daily over a ten day period by chromatoelectrophoretic analysis and by measuring the extent of binding to antibody.

RESULTS

In aqueous solution at pH 7.5 maximal transfer of ^{125}I onto 6-Tyr-

secretin occurred within two seconds. Efficiency of iodination generally ranged from 30 to 50%. Under the same conditions, transfer of ^{125}I onto synthetic human secretin occurred more slowly and there was a steady increase in the percent of iodination until a maximum ranging from 20 to 40% was achieved in 120 seconds.

Standard curves employing ^{125}I-synthetic secretin and ^{125}I-6-Tyr-secretin as tracers are shown in Fig. 1. The antisera obtained from the

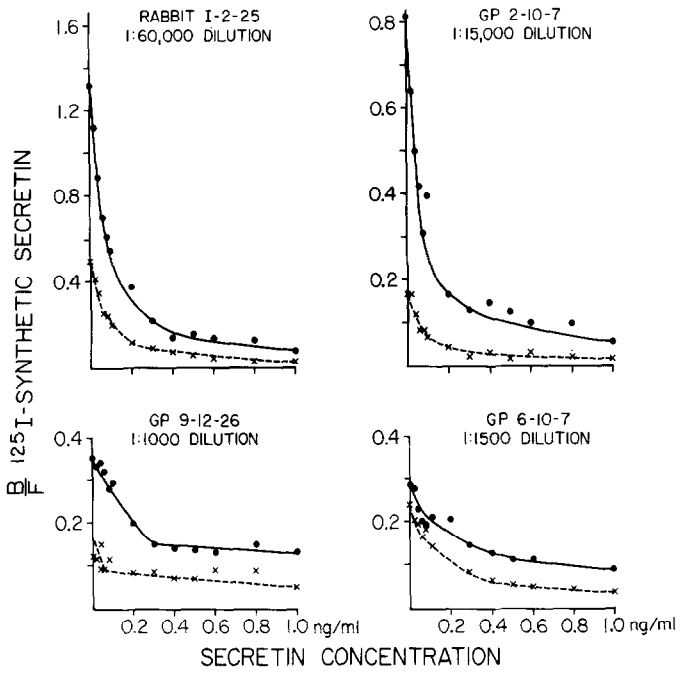

Fig. 1 - Standard curves for secretin assay generated using ^{125}I-synthetic secretin and ^{125}I-6-Tyr-secretin as tracers and pure natural secretin for standard. Note that in the three guinea pigs and one rabbit antisera studied there was diminished binding of the labeled preparation to antibody and the standard curve was less sensitive when the ^{125}I-6-Tyr-secretin was employed.

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three guinea pigs and rabbit differ markedly both in sensitivity and in the titer of antibody-combining sites. However for a given dilution of each antiserum the binding of ^{125}I-secretin was greater than that of the ^{125}I-6-Tyr-secretin. The observed differences between the labeled preparations were not due to differences in the specific activity of the two preparations since increases in the concentration of tracer from 10 to 40 pg per milliliter

produced no reduction in B/F ratios. The observed differences in immunoreactivity varied among the antisera. There was no appreciable deiodination or damage or loss of immunoreactivity with either iodinated preparation during ten days of storage at -20°C with daily thawing and refreezing.

DISCUSSION

Preparation of high specific activity labeled hormones for RIA is generally effected by substitution of radioiodine onto tyrosyl residues. The absence of tyrosine in a peptide has been considered a hindrance in the development of a RIA for the peptide (7) and in the case of secretin, has prompted the synthesis of a peptide in which a tyrosyl residue is substituted for phenylalanine in the sixth position (8).

The tyrosyl substitution appears to confer an advantage in the iodination procedure in that the reaction time is reduced considerably and there is a slight improvement in overall iodination yield. Both preparations have satisfactory shelf-lives since they appear to remain essentially unchanged even after ten days storage at -20°C with daily thawing and refreezing.

The striking disadvantage in the use of ^{125}I-6-Tyr-secretin is its diminished immunoreactivity compared to ^{125}I-secretin. It is not surprising that the reduction in immunoreactivity is variable and dependent on the individual antiserum since antibodies developed against a single molecular species may be directed against different antigenic sites or, even if directed against the same site, may manifest differences in the conformational fit. Thus these studies do not rule out the possibility that with some anti-secretin sera, the 6-Tyr-secretin may be as fully immunoreactive as secretin itself. Nonetheless our results suggest that the substitution of tyrosine for the phenylalanine in the sixth position and the iodination of this tyrosyl residue results in a conformational alteration in the secretin molecule which renders it less immunoreactive with antibodies developed in response to secretin immunization and diminishes the sensitivity of the secretin RIA.

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