BIOLOGICAL ACTIVITY OF IODINATED GASTRINS

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Summary

Mono- and di-iodo heptadecapeptide gastrins were prepared by a modified chloramine-T technique. The iodinated gastrins did not differ significantly from unmodified gastrins in immunoreactivity and in stimulating acid secretion in gastric fistula dogs. The preparations should prove useful in studies of hormone binding to receptors.

Introduction

For direct studies of hormone binding to biological receptors it is desirable to have available radio-labelled preparations of hormone. Such studies with the antral hormone gastrin have been frustrated by difficulties in preparation of labelled hormone with proven biological activity.[1,2] Gastrin biological activity is inactivated by oxidation of methionine under ordinary labelling conditions. We report now that mono- and di-iodo gastrin having full biological activity on acid secretion in gastric fistula dogs can be prepared by a minor modification of the chloramine-T reaction[3] and purified by ion-exchange chromatography.[4]

Methods

Pure natural human heptadecapeptide gastrin in the unsulphated form (G-17-I) was a gift of Professor R. A. Gregory and Dr. H. J. Tracy. Synthetic [Leu^{15}] G-17-I was a gift of Professor E. Wunsch.

Two types of iodination were performed: 1) high specific activity ^{125}I G-17-I was prepared by adding 2.8 n moles (6 µg) G-17-I dissolved in 6 µl NH_4HCO_3 (0.05M) to 25 µl of phosphate buffer (pH7.4, 0.25M), followed by 10 µl Na ^{125}I (100 mCi/ml) and 9 n moles (2.4 µg) chloramine-T dissolved in 5 µl of phosphate buffer. The reaction was stopped after 10 sec by addition of 52 n moles (10 µg) sodium metabisulphite dissolved in 20 µl phosphate buffer; 2) larger quantities of low specific activity iodinated gastrins were prepared by adding 480 n moles G-17-I (1.0 mg) in 200 µl of NH_4HCO_3(0.05M) to 100 µl phosphate buffer (0.25M, pH 7.4), followed by 480 n moles KI in 10 µl phosphate buffer, 10 µl Na ^{125}I

(100 mCi/ml), 1.42 μ moles chloramine-T in 20 μl phosphate buffer, and after 30 secs 6.5 μ moles sodium metabisulphite in 25 μl phosphate buffer.

Both reaction mixtures were applied to columns (1 x 10 cm) of Sephadex G10 equilibrated and eluted with 0.05M ammonium bicarbonate. Fractions of 1.0 ml were collected and tubes containing iodinated gastrin (fractions 3-5) were pooled for further purification by ion-exchange chromatography. Amino-ethyl cellulose (Whatman, AE 41) was equilibrated with 0.05M ammonium bicarbonate, and packed into columns (1 x 10 cm); the mixture of iodinated gastrins was applied to the column and eluted with a gradient to 0.5M ammonium bicarbonate. Column eluates were analyzed by absorbance at 280nm (Zeiss M4 QII U V Spectrophotometer), radioimmunoassay (RIA) and bioassay.

Gastrin concentration was estimated from UV absorbance by application of molar extinction coefficient (12,261) for G-17-I.[5]

Radioimmunoassay of gastrin was performed by methods already described,[6,7] using an antibody with specificity for the C-terminal portion of G-17-I (Ab 1296, 1:300,000).[7] Specific activity of ^{125}I G-17 was estimated by comparison of binding curves for standard G-17-I and labelled preparations (autoinhibition curves).

The action of iodinated gastrins on acid secretion was studied in two dogs prepared with gastric fistulas several months previously. After an overnight fast each dog received an infusion of saline into a leg vein for a basal period of 40 min and thereafter graded doses of stimulant which were doubled every 40 min. On separate days the dogs received pentagastrin, standard G-17-I and fractions corresponding to peaks I, II or III of iodinated G-17-I or [Leu^{15}] G-17-I preparations. Gastric acid secretion was collected every 10 min and acid determined by titration to pH 7.0 with 0.2 M NaOH.

Results

Figure 1 shows the separation on AE cellulose of the products obtained when 1 mg G-17-I was labelled with a mixture of ^{125}I and ^{127}I. Three peaks of immunoreactive gastrin (I - III) could be identified in the eluates and for each there was a matching peak of material with absorbance at 280 nm. Two of the peaks (II and III) also corresponded to peaks of radioactivity. Essentially similar results were obtained when [Leu^{15}]G-17-I was iodinated, and when μg quantities of G-17-I were labelled to high specific activity with ^{125}I only.

Tubes corresponding to peaks I - III in Figure 1 were separately pooled for analysis of biological and immunochemical activity. All three fractions were found to stimulate gastric acid secretion in dogs (Figure 2), and over the range 20 - 60% of maximal response to pentagastrin dose-response lines were parallel to that for standard G-17-I. The concentration of biologically active gastrin in the three samples (determined by visual comparison of doses of sample and standard required for 50% maximal response) were similar to those estimated

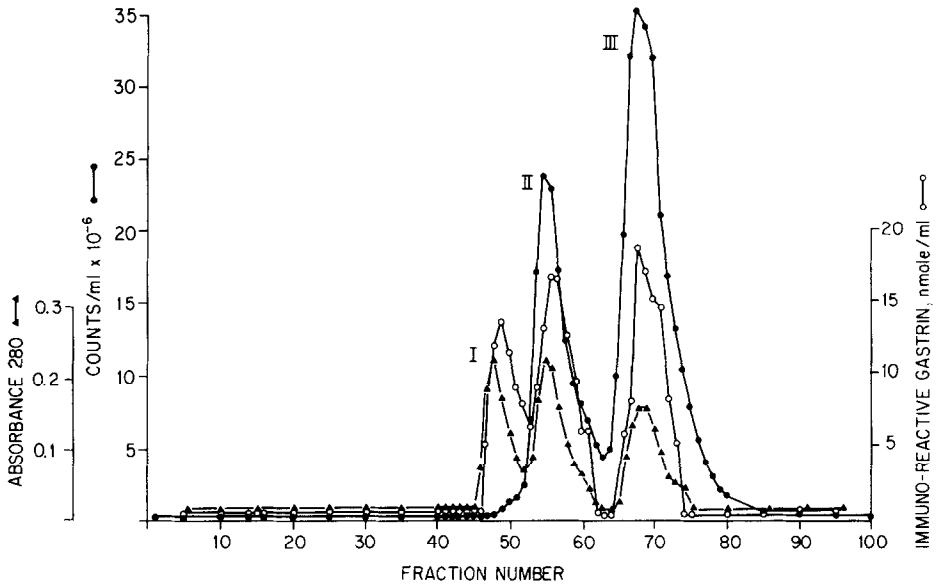

Figure 1. Separation of iodinated gastrins on AE cellulose. 1 mg HG-17-I iodinated with KI and tracer amounts of Na ^{125}I (see Methods). Reaction mixture separated first on Sephadex G10 (1 X 10 cm) and tubes corresponding to first peak pooled and applied to an AE cellulose column (1 X 10 cm) equilibrated and eluted with 0.05M ammonium bicarbonate. A gradient to 0.5M ammonium bicarbonate was started from fraction 10. Mixing vessel 125 ml; flow 6 ml/hr; fractions 1.1 ml. ●---● counts per ml; o---o gastrin estimated by radioimmunoassay; Δ---Δ optical density at 280 nm. Peaks identified by Roman numerals. For further analysis tubes pooled as follows: 40-51, peak I; 53-59, peak II; 66-71, peak III.

by RIA and by absorbance at 280 nm (Table 1).

The UV absorbance spectra of peaks I and II were similar and were comparable to the spectrum of unlabelled G-17-I, but peak III was characterized by a slightly lower maximum at 280 nm. We attribute the altered spectrum to changes in conformation or organization of the tryptophan and tyrosine residues induced either by the substitution of two iodine atoms onto tyrosine or by oxidation by chloramine-T. In spite of this, the concentration of gastrin in peak III material estimated by UV absorbance using the molar extinction coefficient for G-17-I was essentially similar to the concentration determined by bioassay and RIA.

RIA inhibition curves for peaks II and III material (prepared to high specific activity) were parallel to that for standard G-17-I with our C-terminal

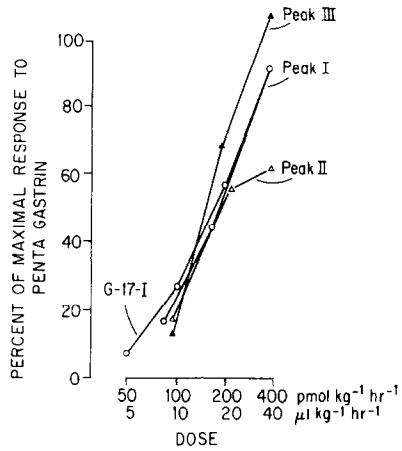

Figure 2. Dose response relationships for action of standard G-17-I and pooled samples of peaks I, II, and III (AE column, Fig. 1), or acid secretion in gastric fistula dogs. Abscissa: dose of G-17-I expressed in pmol kg^{-1} hr^{-1} and of AE peaks in µl kg^{-1} hr^{-1}. Ordinate: responses normalised to percent of the peak acid output in response to pentagastrin (2.8 mEq 10 min). ● G-17-I; o peak I; Δ peak II; ▲ peak III. Each point mean of 6 experiments in 2 dogs.

Table 1

Concentration of gastrin in AE peaks estimated by 3 methods.

All values, n mole/ml.

AE peak	I	II	III
Gastrin by UV absorbance	11.4	11.8	10.0
Gastrin by RIA	8.7	12.1	13.4
Gastrin by bioassay	9.4	8.9	11.3

antibody (Figure 3) indicating that the iodinated gastrins were immunochemically indistinguishable for standard G-17-I. By comparing the amounts of standard and labelled gastrins required for 50% inhibition of binding, the specific activity of peak II was estimated to be 2,200 cpm/fmol compared to 4,700 cpm/fmol for peak III.

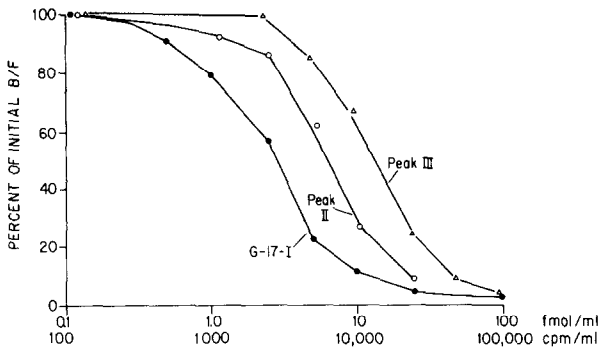

Figure 3. Inhibition curves of standard G-17-I (o), peak II (0) and peak III (Δ) samples. In this experiment peaks II and III were obtained from iodination of G-17-I with ^{125}I alone. All tubes contained Ab 1296 (1:300,000) and 2000 cpm of mono-iodo [^{125}I] G-17-I as tracer. Abscissa: graded amounts of standard G-17-I expressed in fmol/ml; and graded amounts of ^{125}I substituted G-17's expressed in cpm/ml. Ordinate: binding expressed as percentage of maximal ratio bound/free [^{125}I] G-17-I seen with antibody and trace alone.

The results obtained with [Leu^{15}]G-17-I were similar to those for G-17-I in that the biological and immunochemical properties of the iodinated and unreacted peptides were similar. However, it is noteworthy that the biological activity of [Leu^{15}]G-17-I was about 50% higher than G-17-I whereas immunoreactivity was only about 40% of that of the native hormones (Table 2).

Discussion

Three main components have been identified in the reaction mixture following iodination of gastrin by the chloramine-T method. One of these (designated peak I) corresponds to the original unreacted gastrin, the other two are iodo-derivatives. When gastrin was iodinated with ^{125}I the two iodinated products had specific activities of 2200 and 4700 cpm/fmol respectively; allowing for counting efficiency of 60% the observed specific activities agree well with the calculated specific activities of mono- and di-iodo G-17-I respectively. Results presented in Figure 3 indicate that the iodinated peptides are immunochemically indistinguishable from unlabelled G-17-I. The two derivatives were also fully active in stimulating gastric acid secretion (Figure 2) and the ratio of biological to immunochemical potencies were broadly comparable to those of unlabelled

Table 2

Comparison of biological and immunochemical potencies of iodinated G-17-I and Leu^{15} G-17-I. Concentrations calculated from UV absorbance at 280 nm.

	Dose required for 50% maximal H^+secretion	Dose required for 50% inhibition of antibody binding
G-17-I	172 pmol kg^{-1} hr^{-1}	2.4 pmol l^{-1}
Mono-iodo G-17-I	180	2.3
Leu^{15} G-17-I	115	6.0
Mono-iodo Leu^{15} G-17-I	100	7.1

peptides. Morley[8] showed that oxidation of the methionine residue at position 15 in G-17-I abolished biological activity. During iodination of G-17-I it is widely believed that the chloramine-T oxidizes methionine and so inactivates the gastrin molecule. However, Stagg et al[1] found loss of activity only when G-17-I was exposed to a four-fold molar excess (or greater) of chloramine-T. Lower ratios of G-17-I to chloramine-T did not cause loss of activity. In the present study G-17-I was successfully iodinated using a 3-fold molar excess of oxidizing agent and under these conditions both the mono- and di-iodo products have been shown to have full biological and immunochemical activity.

The results suggest that by using appropriate conditions iodinated gastrins may be prepared which are similar to those currently used in RIA and which could also be used for binding studies with biological receptors. The advantages of using monoiodo G-17-I of high specific activity and uncontaminated with unlabelled peptide in these binding systems are obvious.

An alternative solution of the problem of preparing biologically active radio-labelled gastrins is provided by the [Leu^{15}]G-17-I analogue, since this molecule lacks the methionine residue (position 15) it is less susceptible to inactivation by oxidation. [Leu^{15}]G-17-I was up to 50% more active than G-17-I

in stimulating acid secretion although its immunoreactivity was about 40% less than that of G-17-I with the antibody employed for RIA. The present study has shown that monoiodo [Leu^{15}]G-17-I has a similar pattern of biological and immunochemical properties to the altered peptide.

Recently a method for the preparation of high specific activity ^{3}H G-17-I has been described.[2] It is likely that the biological and immunochemical properties of the ^{3}H G-17-I and our ^{125}I G-17-I are similar. However, the method of preparing radio-labelled gastrins which was used here offers advantages in terms of speed and simplicity.

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