TRITIUM AND IODINE LABELLING OF A GASTRIN LIKE HEXAPEPTIDE

Marie-Hélène Charon*, Paul Pham Van Chuong+, Botond Penke**,
Jean-Louis Morgat, Marcelle Dubrasquet++ and Pierre Fromageot.

Service de Biochimie, Bât. 142, C.E.N. de Saclay
91191 Gif-sur-Yvette Cédex, France.

SUMMARY

Tyrosine derivatives were introduced onto the N-terminal deprotected Pentagastrin to yield precursors of labelling. The tritiated derivative Boc-3,5-$^{3}H_2$-Tyr-Pentagastrin of high specific radioactivity (45 Ci/mmol) was obtained by catalytic dehalogenation of the corresponding di-iodo-derivative in the presence of tritium gas. Iodination of Boc-Tyr-Pentagastrin by NaI and chloramine T led to the formation of several compounds separated by HPLC. Conditions to obtain the mono-iodinated non-oxidized peptide, the only biologically active derivative, were investigated. Analytical chromatographic behaviour as well as biological activity of the tritiated and of the various iodinated peptides were reported.

Keywords : Gastrin like peptides ; Tritium and iodine labelling ; Methionine oxidation ; Iodination ; Reverse-phase HPLC.

---

* D.G.R.S.T. doctoral fellow.
\+ To whom correspondence should be addressed.
** Permanent address : Medical University, Dom Tér. 8, SZEGED, Hungaria.
++ Unité de Recherches de Gastroentérologie, INSERM U.10
Hôpital Bichat, 75877 Paris Cédex 18, France.

ABBREVIATIONS : PG = Pentagastrin or Boc-pG or Boc-βAlanyl-TG ; Boc = tert-butyloxycarbonyl ; HG = Hexagastrin or Boc-Tyr-PG ; BAW = mixture of n-butanol : Acetic acid : Water (75:10:22) (v:v:v) ; EtOAc = ethyl acetate ; ACN = acetonitrile : $AcNH_4$ = ammonium acetate ; TEA or Et_3N = triethylamine ; TEAP = buffer triethylamine-phosphate 0.1 M pH 3.5 ; TMD = 4,4'-tetramethyl-diamino-diphenylmethane.

0362-4803/84/010015-22$02.20.

Received April 25, 1983
Revised September 6, 1983

INTRODUCTION

The full range of biological activities of gastrins is confined to the C-terminal tetrapeptide Trp-Met-Asp-Phe-NH_2 or TG (1). Hundreds of derivatives of this peptide have been synthesized and tested for their biological properties (2, 3, 4, 5). One of the most active synthetic derivative of the peptide has been found to be Boc-βAla-TG or Pentagastrin (3) which is now being currently used in most studies instead of the original heptadecapeptide. Whereas the labelling of this longer peptide has been largely reported for the tritiated (6) and particularly the iodinated forms (7, 8, 9), that of the C-terminal shorter peptide has been much less investigated.

Tritiated Pentagastrin (of around 7 Ci/mmol) has already been obtained (10) by catalytic cleavage, in the presence of Tritium of the thioether group from 2-(2-nitrophenylthio) Trp^2-Pentagastrin or PG-NPS. The discovery of Gastrin antagonistic properties of PG-NPS (11, 12) prompted us to search for another site of tritiation which would allow to obtain a tritiated derivative of PG-NPS. We first synthesized (4-Cl-Phe^5)-Pentagastrin, but its tritiation using normal conditions of dehalogenation (6) was incomplete and led to low specific radioactivity. By contrast, the introduction of a di-iodo-tyrosyl residue to the N-terminal end of Pentagastrin allowed the obtention, reported in this paper, of a highly radioactive (45 Ci/mmol) tritiated derivative : Boc-$^3H_2$-Tyr-PG. This labelled peptide has the same biological activity as Pentagastrin, as expected, since the Tyr residue is at the same position as in the natural Gastrin sequence. Therefore, the addition of a Tyr residue to Pentagastrin was also used for iodination since the pentapeptide cannot be iodinated directly.

Iodination of peptides led to several compounds : mono- or di-iodinated derivatives, and when methionine is present, as in pentagastrin, oxidized products. All these iodinated peptide derivatives might not have the same biological activity as the native peptide.

To identify the various products obtained by iodination of Boc-Tyr-PG and to check their biological and immunological properties, reference peptides were synthesized : Boc-Tyr-PG (or HG), Boc-Tyr (I)-PG, Boc-Tyr (I_2)-PG and their methionyl sulfoxide derivatives. Biological tests showed that the only active iodinated derivative was the non-oxidized mono-iodinated peptide Boc-Tyr (I)-PG.

Iodination of Boc-Tyr-PG by the chloramine T method, under the same conditions as already used for iodination of Gastrin G-17 (7, 8, 9), led to oxidized hexapeptide derivatives. Therefore, various parameters for the iodination of Boc-Tyr-PG were investigated, and conditions were selected to obtain within one hour, a pure mono-iodinated radio-labelled non-oxidized derivative. These conditions might be extended to iodination by Chloramine T of other methionyl containing short peptides.

EXPERIMENTAL

I - Material

Pentagastrin Boc-βAla-Trp-Met-Asp-Phe-NH_2 was supplied by Imperial Chemical Industries (GB), amino acids and derivatives were from Sigma (USA) or Bachem (CH), current chemicals from Fluka (CH) or Sigma, solvents from Merck (D) or Prolabo (F), Tritium gas from the Commissariat à l'Energie Atomique (CEA, F), $Na^{125}I$ from Amersham International Ltd (GB).

Purity control and reaction progress were checked by analytical TLC and HPLC. Silica gel ready coated analytical TLC glass plates (Whatman, LK6DF, 0.25 mm, 20 x 20 cm) were used. Spots were revealed by

U.V. examination and by Ehrlich or Cl_2-KI-TMD system's reagent (12). Radioactivity on thin layer chromatogram was detected by autoradiography on Kodirex films or scanning with a LB 2733 scanner (Berthold).

HPLC system used included Water's M720, M730 and WISP units, Pye Unicam LC multi wavelenth detector, and a μ-Bondapak C18 or a Whatman Partisil 10-ODS 2 analytical column (4 x 300 mm). Characteristics of elution systems used are listed in Table I. Detection was monitored at 280 nm. ^{3}H radioactivity of the HPLC elution was quantified with a SL 30 Intertechnique liquid scintillation counter and ^{125}I radioactivity was determined with a Intertechnique CG 4000 counter.

U.V. and visible absorption spectra were recorded on a Cary 210 or a Zeiss DMR 10 spectrophotometer.Melting points were determined on a Mettler FP 5 apparatus connected to a FP 51 oven.

TABLE I - Characteristics of the analytical HPLC systems used. Elution rate was 1.2 ml/mn except for system n° IV (1·5 ml/mn)

N°	Support	Elution solvents
I	Partisil 10.ODS 2	ACN/TEAP 0.1M pH 3.5 (45/55)
II	Partisil 10.ODS 2	ACN/TEAP 0.1 M pH 3.5 (40/60)
III	Partisil 10.ODS 2	ACN/$AcNH_4$ 0.05 M pH 4.75 (35.6/64.4)
IV	Partisil 10.ODS 2	ACN/$AcNH_4$ 0.05 M pH 4.75 (35.6/64.4)
V	μBondapak C18	ACN/$AcNH_4$ 0.05 M pH 4.75 (38/62)
VI	μBondapak C18	ACN/$AcNH_4$ 0.05 M pH 4.75 (gradient in 15 mn from (34/66) to (42/58))
VII	μBondapak C18	ACN/$AcNH_4$ 0.05 M pH 4.75 (gradient in 20 mn from (35/65) to (65/35))

II - Bioassays and radioimmunoassays

Bioassays were performed (11) on male Wistar rats weighing 300 ± 25 g which were fasted for 18 h before experiment but were allowed access to water. The technical aspects of the operation have been described by Ghosh and Schild (14) and modified by Lai (15). The rats were anesthetized by intramuscular injection of urethane (1.25 g/kg). A polyethylene catheter, introduced into the oesophagus down to the cardia, was connected to a peristaltic pump (Desaga) set to deliver a solution of 0.9 % NaCl at a constant rate of 1 ml/mn. The infused liquid was collected by another catheter at the level of pylorus. The temperature of the rats was maintained at 34°C. The test were begun after stabilisation of the gastric infusion, usually within 60 mn after surgical operation. The studied compound was then administered by intravenous injection (vein of penis). The gastric secretion was collected every 20 mn and the acidity was measured by titrating the sample with 0.01 N NaOH to the phenolphtalein end-point. The interval between two injections was at least of 90 mn and the secretory responses were evaluated by the difference between acid output 40 mn after injection and 40 mn before injection.

Radioimmunoassays were performed according to the method described by R.S. Yalow and S.A. Berson (16).

The antiserum was obtained by subcutaneous injection to New-Zealand white rabbits of (2-17) human Gastrin coupled to bovine serum albumin using the carbodiimide technique.

Synthetic (2-17) human Gastrin was iodinated, as tracer, using the Hunter and Greenwood method (17).

The separation of the bound ^{125}I Gastrin (B) from the free one (F) was achieved by adsorption of F on Amberlite PRP 5851 (Rohm and Haas, USA).

III - Synthesis of precursors and standard peptides

N_α-deprotected Pentagastrin (H-pG) :

100 mg (0.13 mmole) of Boc-pG (Pentagastrin) were dissolved at 0°C in 20 ml of mixture of trifluoroacetic acid/dichloromethane/thioglycolic acid (49/49/2, v/v/v). The solution was stirred during 1 hr and then concentrated to 2 ml. The N-deprotected, trifluoroacetate salt (TFA, pG) was filtered, washed with ethyl acetate and then dissolved again in 2 ml of DMF. Precipitation from 100 ml of ethyl acetate filtration and dessication under vaccum allowed to obtain pure product. TLC and HPLC checks of purity are given in Tables II and III. The yield of the reaction was 95 %.

Boc-Tyr(I)-OPCP and Boc-Tyr(I_2)-OPCP :

1 mmol of t-Butyloxycarbonyl-3 iodo-tyrosine or t-Butyloxycarbonyl-3,5 di-iodo-tyrosine was dissolved in 5 ml of ethyl acetate and cooled to 0°C before addition of 1.1 mmol of pentachlorophenol and 1 mmol of dicyclohexylcarbodiimide. The solution was stirred during 1 hr at 0°C and 8 hrs at 20°C. Then the dicyclohexylurea was removed by filtration and the solvent by rotatory evaporation. The product was dissolved again in 30 ml of ethyl acetate, treated with activated charcoal and crystallized from hexane. The crystals were then filtered and dried. Purity of the pentachlorophenyl esters was controlled by TLC (Table II). The measured melting points of these two compounds were 135.7°C and 166.5°C for the mono and di-iodo derivatives respectively.

Hexagastrin (Boc-Tyr-PG or HG) :

In a 3 ml Pierce reacti-vial, 7.9 µmol of Et_3N and 8.3 µmol of commercial N-Boc-L-tyrosine-N-hydroxysuccinimide ester (Boc-Tyr-OSu) were added to a solution of 6.5 µmoles of TFA, PG in 200 µl of dimethylformamide at room temperature and under magnetic stirring. The reaction was monitored by TLC. After 3 hrs, the reaction was completed and the mixture was purified on a 75 x 1.5 cm column of Sephadex LH 20 (25-100 µm) using dimethylformamide as eluant. The main product was then purified by partition chromatography on a 30 x 2 cm column of Silica Gel Si 60 (230 Mesh) using a mixture of EtOAc/BAW (80/20, v/v) as eluant. 4.3 mg (4.6 µmoles) of pure Hexagastrin (yield : 71.2 %) was obtained and controlled by TLC and HPLC (Tables II and III).

Mono or di-iodo-hexagastrin (HGI or HGI_2) :

75 µmoles of Boc-Tyr(I)-OPCP or Boc-Tyr(I_2)-OPCP and 50 µmoles of N-methylmorpholine were added to a solution of 50 µmoles (35 mg) of N_α-deprotected Pentagastrin in 1 ml of dimethyl formamide. The solution was stirred for 20 hours at 20°C. The hexapeptide was precipitated by addition of 50 ml of HCl 0.01 N and 15 ml of diethylether. The precipitate was then filtered, washed with ether and dried. The yield of the coupling was 60 % for the mono-iodo-hexagastrin and 71 % for the di-iodo compound. TLC and HPLC showed that the derivatives obtained were pure (Tables II and III).

Oxidized peptides :

To a solution of 10 µmoles of each peptide HG, HGI or HGI_2 in 400 µl of acetic acid were added 12 µmoles (13.4 µl) of hydrogen peroxide. After 1 hr of reaction the oxidized peptide was precipitated with diethylether, washed with hexane and then lyophilized. TLC and HPLC controls showed that the reaction was complete.

RESULTS AND DISCUSSION

I - Chromatographic behaviour, and biological properties of Hexagastrin and its iododerivatives

TLC characteristic Rf values, in five solvant mixtures of the seven peptides and three tyrosine derivatives are reported in Table II.

HPLC retention times, in the various elution systems already described in Table I, of the six hexapeptides (Hexagastrin, mono- or di-iodo-hexagastrin, and their three oxidized (Met sulfoxide) analogues) are

TABLE II - Rf values obtained on Silica Gel ready coated analytical glass plates (Whatman LK6 DF, 0.25 mm, 20 x 20 cm)

	BAW / EtOAc			Chloroform /Hexane/ Acetic acid 8/1/1	EtoAc/ Pyridine/ Acetic acid/Water 60/20/6/11
	15/85	30/70	100/0		
HG	0.13	0.65	0.87	0	0.86
HGI	0.14	0.68	0.88	0	0.90
HGI_2	0.18	0.72	0.91	0	0.93
HGox	0	0.13	0.77	0	0.41
HGIox	0	0.14	0.77	0	0.44
HGI_2ox	0	0.16	0.77	0	0.50
TFA, H-PG	0	0	0.32	0	0.16
Boc-Tyr-OSu	0.93	0.95	0.97	0.36	0.97
Boc-Tyr(I)-OPCP	0.97	0.98	0.99	0.86	0.98
Boc-Tyr(I_2)-OPCP	0.98	0.99	0.99	0.94	0.98

TABLE III - HPLC retention time (mn) of HG and derivatives in various elution systems described in Table I

Elution system	HGox	HGIox	HG	HGI_2ox	HGI	HGI_2
I	3.0	3.9	4.5	6.6	6.6	11.6
II	4.2	5.5	6.4	9.7	11.4	22.2
III	4.4	8.2	8.5	14.8	18.0	34.0
IV	2.1	3.5	4.0	6.6	8.3	16.7
V	4.3	6.1	6.7	9.2	10.7	17.7
VI	3.7	7.9	8.4	12.1	13.8	17.6
VII	2.5	6.0	7.0	9.4	10.4	12.9

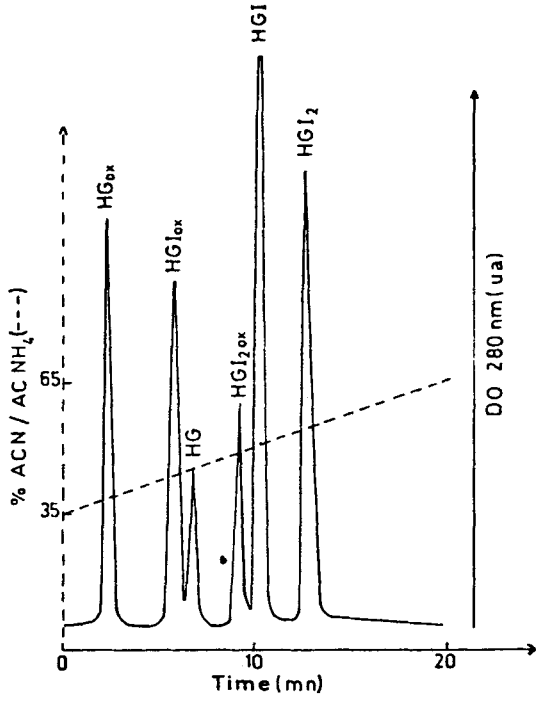

Figure 1 - HPLC separation on a µBondapak C18 column (system VII) of Gastrin like hexapeptide and its iodinated or/and oxidized derivatives.

listed in Table III. It can be noticed that these peptides could be easily separated, particularly when using elution systems n° I, II, VI or VII. As an example elution profile of separation of the six peptides by the HPLC system n° VII is reported on figure 1.

The biological activity of the six peptides investigated was measured by secretory responses of gastric HCl in rats. First of all, the results reported in Table IV showed that Boc-Tyr-pG presents similar potency as PG toward acid stimulation, as expected, since a Tyr residue is at this same position in the native Gastrin G-17. Figure 2 showed that the di-iodinated derivative was much less active than the mono-iodo compound which exhibited activity similar to HG. Oxidation of the methionyl residue decreased more drastically biological activity of all the three peptides HG, HGI and HGI_2 as shown in figure 3. The observed decrease of biological activity after di-iodination or oxidation of HG might explain the reduction of activity of gastrin G-17 after such modifications (6, 18). Thus mono-iodinated non-oxidized hexagastrin (HGI) was the only derivative retaining full biological activity.

II - Production of tritiated hexagastrin

Tritiation

Tritiated hexagastrin was produced by catalytic substitution in di-iodo-hexagastrin, of the two iodine atoms on tyrosyl residue by two tritium atoms.

TABLE IV - Secretory responses (in µmol of HCl/40 mn, corrected from basal) to injected doses of 1.3 nmol/kg and 5.2 nmol/kg of PG and HG (values averaged on 8 rats)

	1.3 nmol/kg	5.2 nmol/kg
PG	5.8 ± 1.0	9.0 ± 1.5
HG	5.6 ± 1.6	8.9 ± 0.8

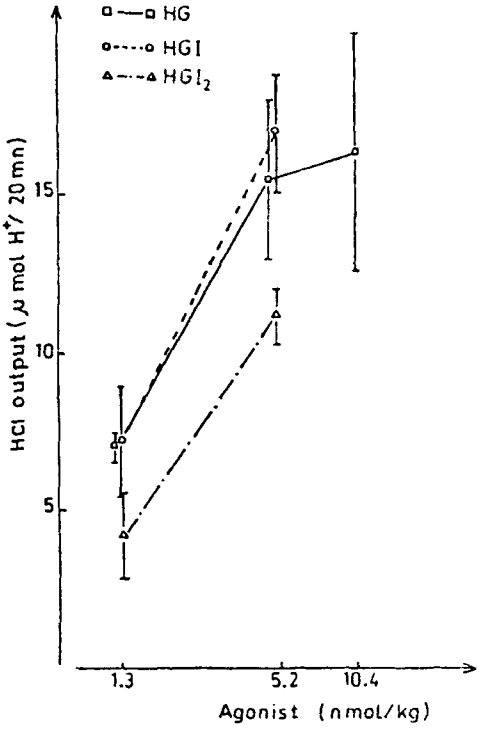

Figure 2 - Dose response curves of Hexagastrin (HG), mono-iodinated (HGI) and di-iodinated (HGI_2) derivatives.

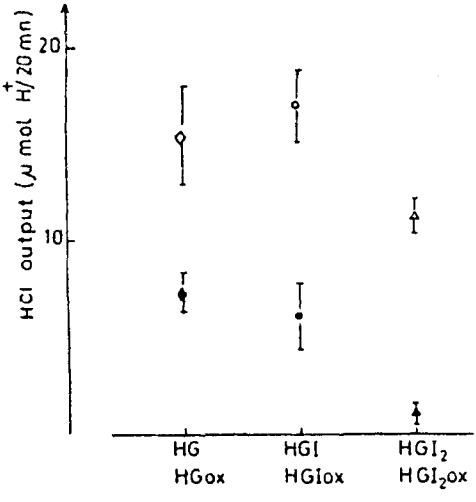

Figure 3 - Secretory responses obtained by infusion of HG, HGI, HGI_2 (clear symbols) and their methionyl sulfoxide derivatives (dark symbols).

Di-iodo-hexagastrin (2 mg, 1.7 μmol) was put into a 2.5 ml round bottom flask (tritiation reactor) with a magnetic stirrer and dissolved in 100 μl of ammonia 0.1 M and 300 μl of ethanol. The solution was frozen and 20 mg of PdO were added. The flask was then fixed on a tritiation line (19) kept frozen and the whole circuit was placed under vacuum. Tritium was then introduced to obtain a pressure of 0.95 bar. The sample was then thawed slowly and stirred for 1.5 hours. The reaction was stopped by freezing and excess tritium was removed under vacuum. After a second thawing the catalyst was filtered on Millipore LS filter. The solution was concentrated by rotatory evaporation, then redissolved into

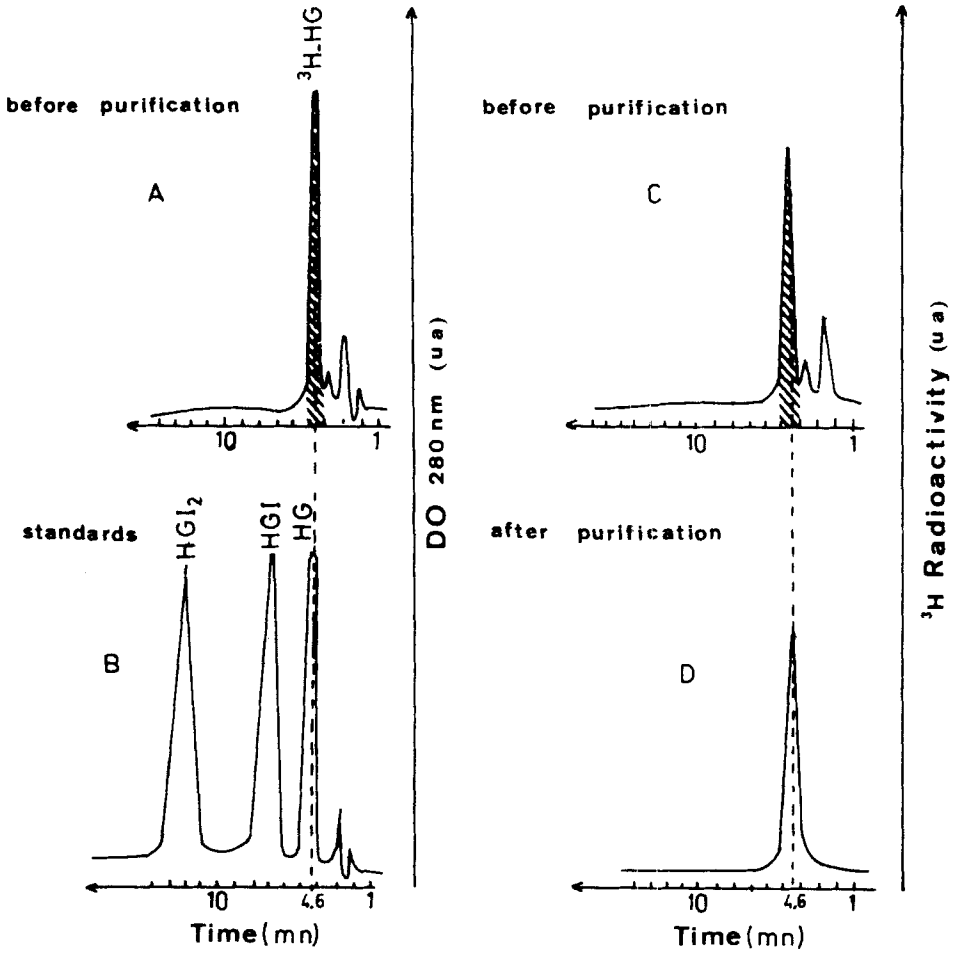

Figure 4 - HPLC purification of tritiated Hexagastrin on a analytical column of Partisil 10.ODS 2 using the eluant mixture Acetonitrile/TEAP 0.1 M, pH 3.5 (45/55) at an elution rate of 1.2 ml/mn.

100 ml of acetic acid 1 % in water and flash evaporated to remove labile tritium. TLC control showed that one main spot was observed, as detected by radioactivity scanning and autoradiography, at the same position as reference Hexagastrin.

Purification of the tritiated Hexagastrin was performed by HPLC, using system n° I (Tables I and III) which gives a good separation of the possible reaction products. Both optical density at 280 nm (Waters U.V. Model 450 variable wavelength detector) and ^{3}H radioactivity (Berthold HPLC radioactivity monitor LB 503) were recorded during the elution. The reaction was complete as seen in figure 4 since neither di-iodo hexagastrin nor mono-iodohexagastrin was observed. The main peak was collected and an aliquot was reinjected on the column to verify the purity of the labelled compound thus obtained.

Measurement of absorption at 280 nm and radioactivity after tritiation and purification, led to a 72 % yield and a specific activity 45.5 Ci/mmol.

This result represents the highest specific radioactivity obtained so far by tritium labelling of Pentagastrin analogues.

Biological activity of the tritiated Hexagastrin was controlled in rats by i.v. injections of different doses of this compound. The dose-response curve is represented on figure 5, where responses of Pentagastrin and Hexagastrin are also given for comparison.

Radioimmunoassay results, reported in figure 6 showed that both HG and tritiated HG behave similarly, against Gastrin antibodies.

III - ^{125}I labelling of Hexagastrin

In a preliminary study, iodination of Boc-Tyr-PG by various methods using ICl (20), Iodogen (21) or NaI-chloramine T (17) according to the quoted conditions, led in every case to oxidation of the methionyl residue of the peptide.

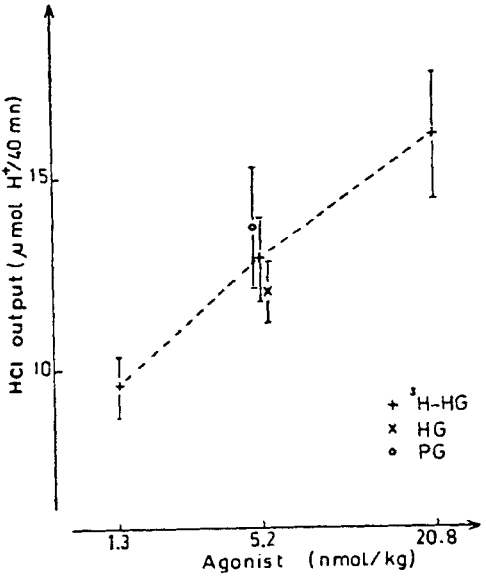

Figure 5 - Dose-response curve of tritiated Hexagastrin (+), secretory responses of PG (o) and HG (x) at the doses of 5.2 nmol/Kg were also reported.

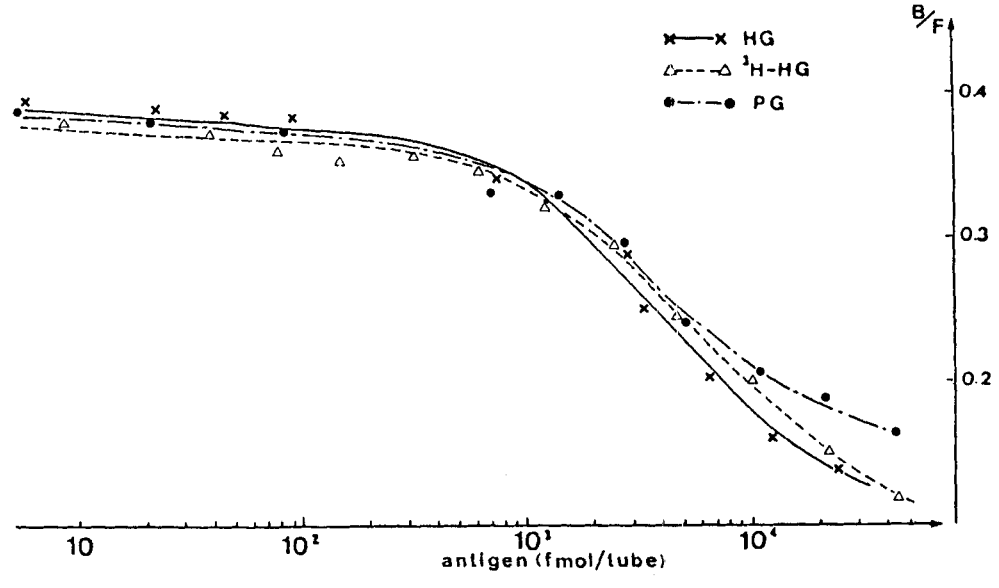

Figure 6 - Radio-immunoassay of tritiated Hexagastrin (Δ---Δ) and standard HG (x — x) or PG (● - ●).

Selecting the chloramine T method we carried out a series of experiments to produce non oxidized mono- ^{125}I hexagastrin.

A first study was performed with non radioactive iodide, ^{127}I, using various ratios of Hexagastrin, sodium iodide, and chloramine T, at a fairly high concentration of peptide (40 nmoles, 5.10^{-4} M/L) in order to be able to detect by optical absorption the different compounds obtained after reaction.

For each assay the protocol was :

to 40 nmoles of Hexagastrin (PM = 931) dissolved in 40 µl dimethylformamide-water (50-50), various solutions of NaI and chloramine T in phosphate buffer 0.5 M pH 7.6 were added.
the final volume was adjusted to 80 µl with the same phosphate buffer.
after magnetic stirring for 1 mn, 467 nmol sodium metabisulfite was added to stop the reaction.

The reaction mixture was then analyzed directly on HPLC (system VI). The percentage of each derivative was determined by comparison of integrated areas of corresponding absorption peaks at 280 nm (all of these derivatives have a same molar extinction coefficient at 280 nm).

The results obtained with four different fixed ratios NaI/HG (0.25 ; 0.5 ; 1 ; 2) and increasing amount of chloramine T are reported on figure 7.

Among all these cases, the best yields of HGI were obtained with the molar ratios HG-NaI-Chl T = 1-1-1 (36 % HGI) and 1-2-1 (39.5 % HGI).

To reduce the quantity of oxidized and/or di-iodinated analogues in these conditions we have studied the protection of Hexagastrin against oxidation and di-iodination by the addition of dimethylsulfoxide (DMSO),

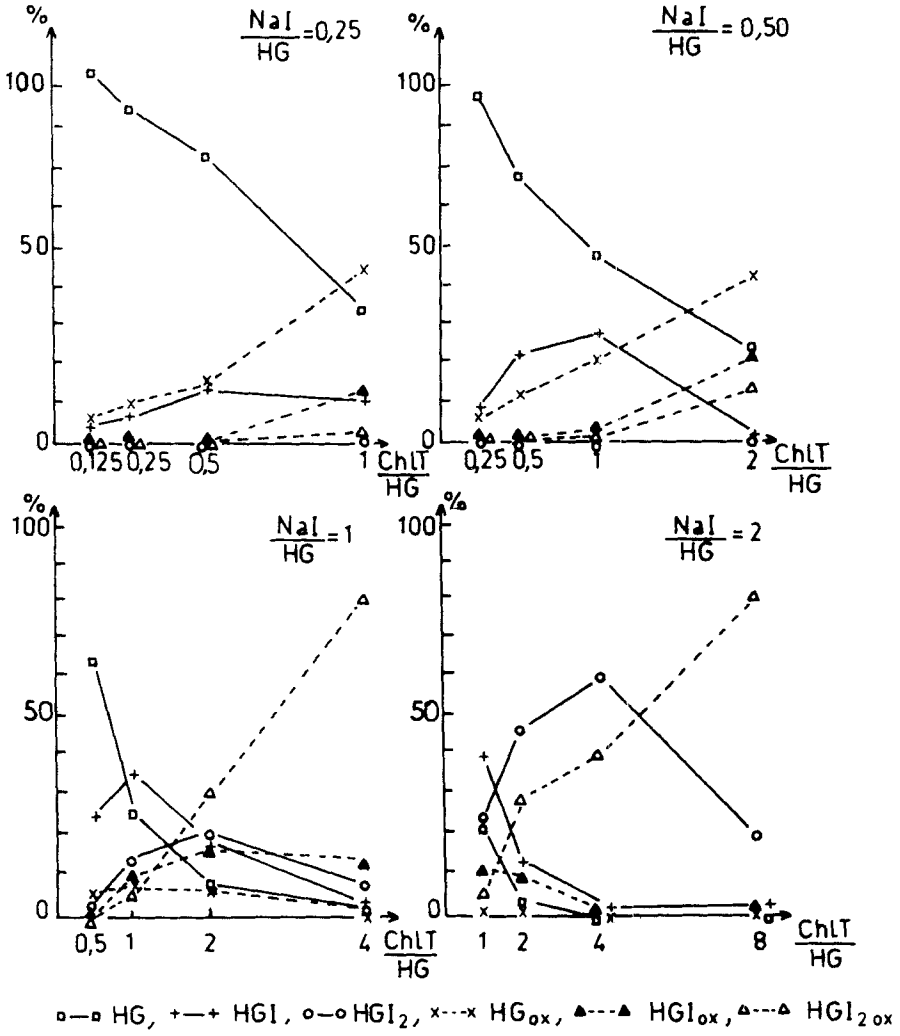

Figure 7 - Formation of derivatives from HG as a function of Chloramine T. Sodium iodide was kept constant with NaI/HG = 0.25, 0.50, 1,0, 2.0.

which has been reported (18) to be oxidized by chloramine T competitively with the methionine residue, and can thus reduce oxidation of the latter. The influence of DMSO as a scavenger is shown in figure 8 in the case of the molar ratios HG-NaI-Chl T = 1-2-1 (A) and 1-1-1 (B) where best yields of HGI were obtained and the ratios 1-2-2 (C) and 1-1-2

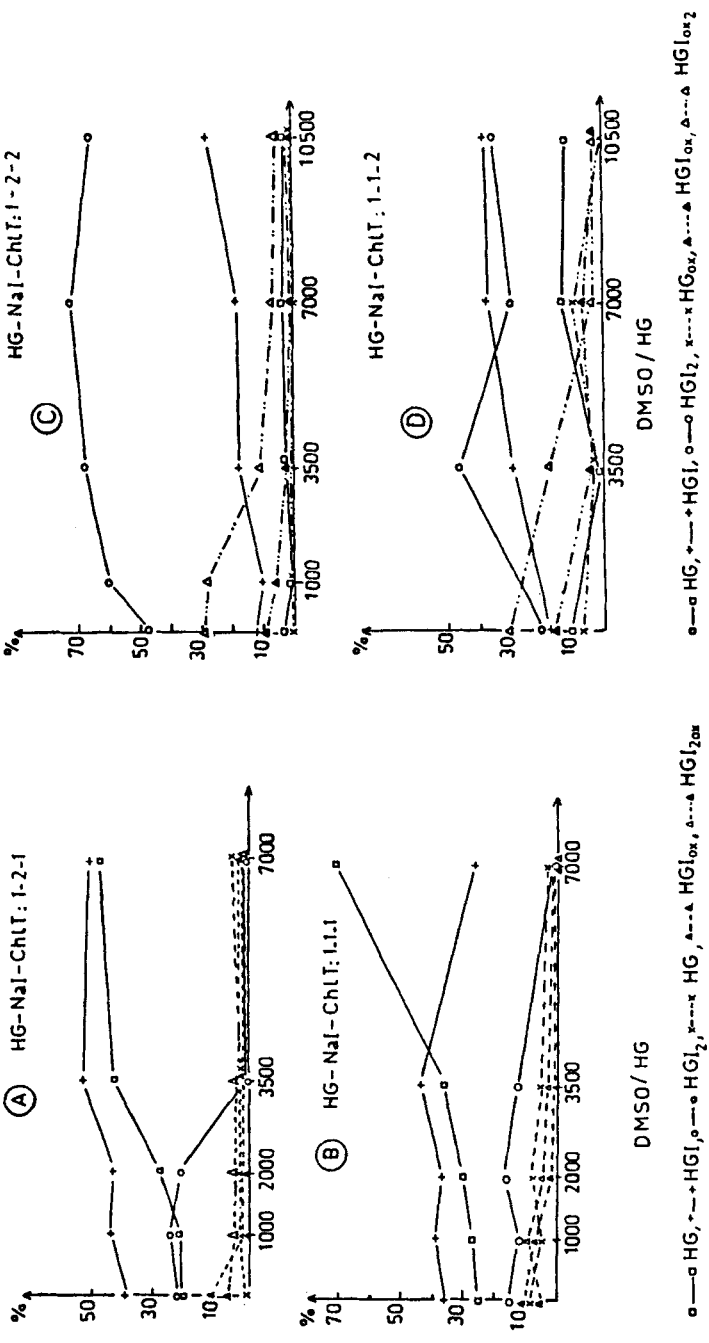

Figure 8 - Influence of DMSO added as a scavenger on the formation of various derivatives in four conditions A, B, C, D.

(D) where we expected a decrease of di-iodinated compounds oxidized or not, in favour of the formation of HGI.

The results obtained showed that in each case the addition of DMSO led to a decrease of the oxidized compounds. In cases A and B a significant decrease of the di-iodinated peptide was observed.

Thus in the optimal molar ratio (HG-NaI-ChI T = 1-2-1) (A) addition of DMSO (DMSO/HG = 3500 to 7000) allows the production of mono-iodo-hexagastrin, with a yield of about 50 %, without detectable oxidized or di-iodinated derivative. Besides HGI, only non reacted native Hexagastrin was present in the reaction mixture and could be easily removed by HPLC.

Another scavenger, free methionine, was also tried. Figure 9 shows that small quantities of methionine could help to avoid oxidation reactions, but larger quantities (ratio Met/peptide > 1) prevented iodination.

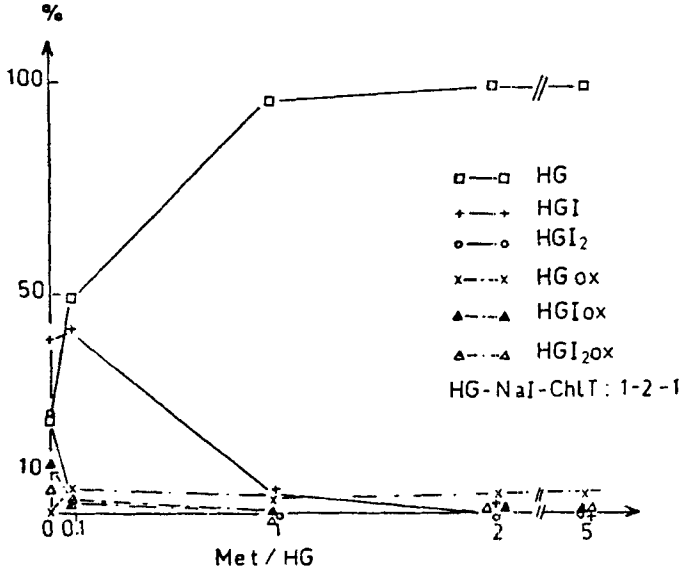

Figure 9 - Influence of L-methionine added as a scavenger (HG-NaI-ChlT : 1-2-1, HG = 40 nmol, final volume 80 µl).

When handling radioactive iodide, its absolute amount is necessarily much lower than above. The reduction in concentration of the reagents prevented iodination. To overcome the dilution problem, the concentration of Chloramine T was increased. Table V shows two noteworthy results : 1) Extrapolation of macroscale iodination conditions (with iodine 127) to microscale (with iodine 125) prevented iodination. 2) To overcome this dilution and the effect of DMSO, the concentration of Chloramine T had to be increased to around tenfold the NaI concentration ; higher concentration of Chl T oxidized HGI.

TABLE V - Iodination of Hexagastrin by Chloramine T (final volume = 40 µl)

	REAGENTS nmoles (molar ratio to HG)				PRODUCTS nmoles					
	HG	DMSO	NaI	ChlT	HG	HGox	HGI	HGIox	HGI_2	HGI_2ox
^{127}I	40 (1)	280.000 (7000)	80 (2)	40 (1)	18.8	1.2	19.8	0.2	0	0
	2 (1)	14.000 (7000)	4 (2)	2 (1)	2		0	0	0	0
^{125}I	40 (1)	280.000 (7000)	1 (0.025)	40 (1)	39.3		0.1	0.7	0	0
	40 (1)	280.000 (7000)	1 (0.025)	10 (0.25)	39.2		0.5	0.3	0	0

A typical iodination was carried out with 1.8 nmol of $Na^{125}I$ (3 mCi), 71 nmol of Boc-Tyr-PG dissolved in 30 µl DMF and 60 µl phosphate

buffer 0.2 M, pH 7.4, 3.6 µl DMSO and 17.8 nmol Chloramine T, the latter added under stirring during 1 minute. The reaction was then stopped by addition of 835 nmol sodium metabisulfite. The whole mixture was passed through a Sep-Pak C-18 cartridge and eluted with water (3 x 0.5 ml) to remove mineral constituents, particularly unreacted ^{125}I, and solvents DMF and DMSO. All the peptides were recovered by methanol elution (3 x 0.5 ml). The peptide solution was concentrated to c.a. 0.3 ml under a nitrogen stream and chromatographed on HPLC (system III). 0.83 nmoles of pure mono-iodo-non oxidized ^{125}I Hexagastrine was recovered and controlled to be fully biologically active.

CONCLUSION

The radioactive labelling of Pentagastrin , a biologically active analogue of the C-terminal part of Gastrin has met with difficulties in the past since the molecule does not contain a suitable reacting side chain like tyrosine or histidine. Furthermore, the presence of a methionine residue, playing an important role in the biological activity of the peptide, renders the latter quite susceptible to oxidation. These remarks and the need for labelled Pentagastrin prompted the preparation of a derivative, Boc-Tyr-Pentagastrin, that we called Hexagastrin and which has the same biological activity as Pentagastrin. The presence of a tyrosine ring did not solve all the difficulties since the preparation, by direct iodination of Hexagastrin, of a di-iododerivative as a precursor for tritiation led to extensive oxidation of the methionine residue. This side reaction was avoided by synthesis of the iodohexapeptide using a suitably protected and activated di-iodo-tyrosine. The catalytic substitution of the iodine atoms by tritium was then

carried out, and a 45 Ci/mmol (^{3}H)-Hexagastrin obtained, presenting full biological activity. For ^{125}I-labelling this chemical synthesis is less appropriate, especially when small amounts are required. Direct iodination with Na^{125}I and Chloramine T could be obtained with incorporation of about half the ^{125}I input into the peptide to yield a non-oxidized mono-iodinated derivative, provided strict experimental conditions were selected. The present work emphasized the need for an adaptation to individual cases of efficient general labelling principles. Comparison of the behaviour of the heptadecapeptide Gastrin and of its C-terminal analogue Hexagastrin towards iodination, provides a good example of this point.

ACKNOWLEDGEMENT

The authors wish to express their greatful thanks to Dr. J.P. Accary and Mr. J.P. Laigneau for their assistance and advice in radioimmunology.

REFERENCES

1. Tracy H.J. and Gregory R.A. - Nature 204 : 931 (1964)
2. Davey J.M., Lair A.H. and Morley J.S. - J. Chem. Soc. (c) : 555 (1966)
3. Morley J.S. - Proc. Roy. Soc. B 170 : 97 (1968)
4. Higaki K., Danno T. and Miyoshi M. - Pharmacometrics 8(2) : 147 (1974)
5. Kisfaludy L., Schon I., Nafradi J., Varga L. and Varro V. - Hoppe Seyler's Z. Physiol. Chem. 359 : 887 (1978)
6. Girma J.P., Morgat J.L., Fromageot P., Dubrasquet M., Accary J.P., Vatier J. and Bonfils S. - Biochimie 56 (5) : 763 (1974)

7. Mac Guigan M.D. - Gastroenterology 54 (6) : 1005 (1968)
8. Stadil F. and Rehfeld J.F. - Scan J. Clin. Lab. Inves. 30 : 361 (1972)
9. Dockray G.J., Walsh J.H. and Grossman M.I. - Biochem. Biophys. Res. Comm. 69 (2) : 339 (1976)
10. Marche P., Girma J.P., Morgat J.L., Fromageot P., Ghelis C., Dubrasquet M. and Bonfils S. - Eur. J. Biochem. 50 : 375 (1975)
11. Dubrasquet M., Ory-Lavollée L., Pham Van Chuong P., Morgat J.L., Fromageot P. and Bonfils S. - Gastroentérol. Clin. Biol. 4 : 786 (1980)
12. Pham Van Chuong P., Morgat J.L., Fromageot P., Dubrasquet M., Ory-Lavollée L. and Bonfils S. - In : Hormone receptors in digestion and nutrition. G. Rosselin, P. Fromageot and S. Bonfils, eds. Elsevier/North Holland Biomedical Press, Amsterdam : 157 (1979)
13. Accary J.P., Laigneau J.P., Pham Van Chuong P., Morgat J.L. and Dubrasquet M. - Biochem. Biophys. Res. Comm. 99 : 511 (1981)
14. Ghosh M.H. and Schild H.D. - Brit.J. Pharmac. 13 : 54 (1958)
15. Lai K.S. - Gut 5 : 327 (1964)
16. Yalow R.S. and Berson S.A. - Gastroenterology 58 : 1 (1970)
17. Hunter W.M. and Greenwood F.C. - Nature 19 : 495 (1962)
18. Stagg B.H., Temperley J.M., Rochman H. and Morley J.S. - Nature 228 : 58 (1970)
19. Morgat J.L., Desmares J. and Cornu M. - J. Label. Comp. XI : 257 (1975)
20. Mac Farlane A.S. - Nature 182 : 53 (1958)
21. Fraker P.J. and Speck J.C. - Biochem. Biophys. Res. Comm. 80 : 849 (1978).