INTERACTION OF ^{3}H-LABELLED SYNTHETIC HUMAN GASTRIN I WITH RAT GASTRIC PLASMA MEMBRANES. EVIDENCE FOR THE EXISTENCE OF BIOLOGICALLY REACTIVE GASTRIN RECEPTOR SITES

M. LEWIN*, A. SOUMARMON, J. P. BALI and S. BONFILS
Unité de Recherches de Gastroentérologie, INSERM U.10, Hopital Bichat, 75877 Paris, Cedex 18, France

and

J. P. GIRMA, J. L. MORGAT and P. FROMAGEOT
Service de Biochimie, Département de Biologie, Centre d'Etudes Nucléaires de Saclay, 91190 Gif sur Yvette, France

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1. Introduction

A considerable amount of evidence supports the view that gastrin plays a crucial role in the hormonal control of HCl secretion by stomach [1]. In addition, the possible mediation of this control by 3′,5′-AMP has been suggested [2]. However, no direct evidence has been yet provided for the existence of gastrin receptors in gastric mucosa.

In this paper, we report preliminary experiments dealing with the interaction of immunologically and biologically active tritiated synthetic human gastrin I ([^{3}H]GI) [3] with gastric plasma membranes (GPM) purified from rat fundic mucosa. It will be shown that [^{3}H]GI reversibly binds to GPM with an accompanying increase in adenyl cyclasic activity which strongly suggests the existence of gastrin receptor sites in these membranes.

2. Material and methods

2.1. *Preparation of gastric plasma membranes*

Rats, Wistar males, weighing 250 g, were killed by a blow on the neck. Fundic area of the stomach was scraped and homogenized (Potter Elvehjem, 2000 rev/min, 4–5 strokes) in 250 mM sucrose medium buffered with 3 mM Tris-HCl, pH 7.4. Homogenate was centrifugated 3 min at 12 500 rev/min (Spinco L Beckman, rotor 50 Ti). Pellet was discarded and supernatant was centrifugated 30 min at 39 000 rev/min (90 000 *g*). The 90 000 *g* pellet was allowed to equilibrate 20 h in a linear sucrose gradient (Spinco L Beckman, zonal rotor Ti 14). The 1.12–1.14 g/cm^3 density band was collected (GPM).

2.2. *Preparation of tritiated synthetic human gastrin I*

Synthetic human gastrin I (Imperial Chemical Industries, England) was tritiated by a procedure including the preparation of intermediary iodinated gastrin as previously described [3]. Specific radioactivity of [^{3}H]GI was 60 Ci/mmole.

2.3. *Filter assay for gastrin binding*

In the standard assay procedure 0.3 ml GPM (0.5 to 1.3 mg protein ml^{-1}) was incubated at 20°C, pH 7.4, with [^{3}H]GI (10^{-9} to 10^{-7} M). 50 µl successive samples were taken, rapidly diluted in 500 µl NaCl 150 mM at 0°C (stop solution) and applied to 2 cm diameter Oxoid filters (Oxoid, Hampshire, UK). Filtration was vacuum operated to dry the filters within 30 sec. Filters were then placed

*To whom correspondence should be addressed.

in 15 ml of PCS solution (Amersham Searle Co) and counted (Intertechnique SL 30). Controls for [^{3}H]GI absorption to the filters were performed by omitting [^{3}H]GI in the incubation medium and adding it after dilution of the samples in the stop solution. This absorption was found to be proportional to the amount of [^{3}H]GI filtrated, and to account for 2.5 to 3% of this amount. The corresponding cpm were systematically substracted from the data.

Biochemical assays for protein, cytochrome *c* oxidase, Mg^{2+}-ATPase (pH 8) and RNA were performed as previously described [4]. Monoamine oxidase was assayed according to Weissbach et al. [5], and adenyl cyclasic activity was measured according to Rosselin and Freychet [6].

Radioimmunoassay for gastrin was performed according to Yalow and Berson [7]. Biological assays for the estimation of gastrin-stimulated HCl secretion in the Wistar rat were performed according to the method of Ghosh and Schild [8] modified [3]. Treatment for electron microscopic observation was carried out as previously described [9].

3. Results and discussion

3.1. *Gastric plasma membranes*

Enzymatic profile of the 1.12–1.14 g/cm^3 density band is shown in table 1.

In terms of marker enzymes this band is seen to be relatively free from mitochondria and rough reticulum as illustrated by cytochrome *c* oxidase, monoamine oxidase and RNA respectively. By contrast it is seen to be highly enriched in 5'AMPase (X 8.9 over the homogenate) and Mg^{2+}-ATPase (X 5.7). The former enzyme is usually considered as mainly associated with plasma membranes in various tissues [10], the latter has been suggested to be a relevant marker enzyme for plasma membranes in rat gastric mucosa [4].

Electron microscopic observation of the 1.12–1.14 g/cm^3 density band reveals smooth surfaced vesicles of a mean diameter of 1 μm and additional unclosed membrane fragments.

These findings and also the fact detailed later on (Table III) that the 1.12–1.14 g/cm^3 density band exhibits adenyl cyclase activity, allow the assume that this band accounts for plasma membranes. Precise cellular origin of GPM is a point of interest since gastrin is presumed to be specific for HCl secretion. Admittedly, it can be assumed that, for a part, GPM are derived from the acid secreting cells (parietal cells). However, because of the cellular heterogeneity of the gastric mucosa, GPM presumably also account for membranes originating from peptic and mucous cells. In order to further specify GPM origin, studies are going on involving gastric cells separation [9] prior to subcellular fractionation.

2. *Gastrin binding to gastric plasma membranes*

Time course of [^{3}H]GI binding to GPM is typified in fig.1. For all hormonal and membrane protein concentrations tested (10^{-9} to 10^{-7} M and 0.5 to 1.2 mg ml^{-1} respectively), binding increased with time

Table 1
Recovery of biochemical markers in subcellular fractions from rat fundic mucosa

	5'AMPase	ATPase	Cytox	MAO	RNA
Homogenate	6.4	199	74	520	53
90 000 *g* pellet	16.7	417	60	760	80
1.12–1.14 g/cm^3 density band (GPM)	57.1	1135	0	34	13

ATPase (Mg^{2+}-ATPase, pH 8) and 5'AMPase (plasma membrane) nmoles min^{-1} per mg protein. MAO (monoamine oxidase: mitochondria outer membrane) and cytox (cytochrome *c* oxidase: mitochondria inner membrane): nmoles min^{-1} per mg protein and units (3.1 $\Delta \log_{10}$ DO min^{-1}) per mg protein, respectively. RNA (ribonucleic acid: rough reticulum): μg per mg protein.

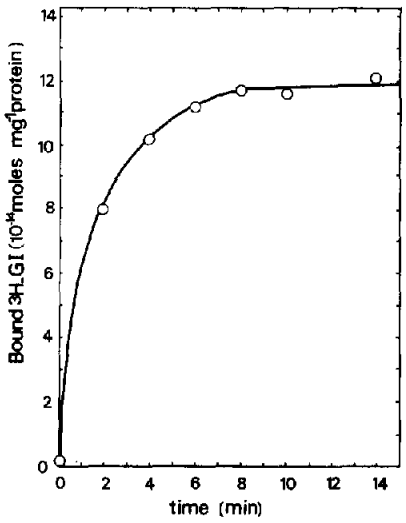

Fig.1. Time course of [^{3}H]GI binding to GPM at 20°C. [^{3}H]GI: 1.15 10^{-8} M, GPM: 0.7 mg protein ml^{-1}. [^{3}H]GI bound was estimated from cpm retained after filtration (see Material and methods) on the basis of the specific activity of the hormone (60 Ci/mmoles) and a counter yield of 33%. Data are corrected for aspecific absorption to the filters.

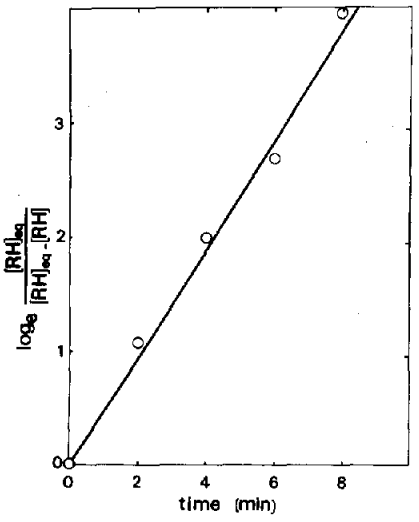

Fig.2. Semi-logarithmic plot of [^{3}H]GI association to GPM (initial points from fig.1). According to the equation,

$$R + H \xrightarrow{k_1} RH \text{ (see results)} \quad \log_e \frac{[RH]_{eq}}{[RH]_{eq} - [RH]} = k_1 \cdot [H] \cdot t$$

[RH] and $[RH]_{eq}$ stand for the amount of hormone bound at a given time and at the equilibrium respectively. [H] = 1.15 × 10^{-8} M.

up to an equilibrium value reached within 15 min. At the equilibrium 1 to 5% of the total [^{3}H]GI was found to be bound per mg protein. Association of [^{3}H]GI with GPM can be adequately described by the equation,

$$R + H \xrightarrow{k_1} RH$$

in which R and RH are free and bound receptors respectively, H the hormonal concentration in the medium and k_1 the association constant [11] (fig.2). Calculation of k_1 from fig.2 gives the value of $k_1 = 4.2 \times 10^7\ M^{-1}\ min^{-1}$.

Binding was reversed by a 1:10 dilution of the incubation medium. Dissociation of [^{3}H]GI from GPM agrees with the equation

$$RH \xrightarrow{k_{-1}} R + H$$

(fig.3). The dissociation constant calculated from fig.3 was found to be $k_{-1} = 0.35\ min^{-1}$, the half time for dissociation being 2 min.

[^{3}H]GI and unlabeled synthetic human gastrin I

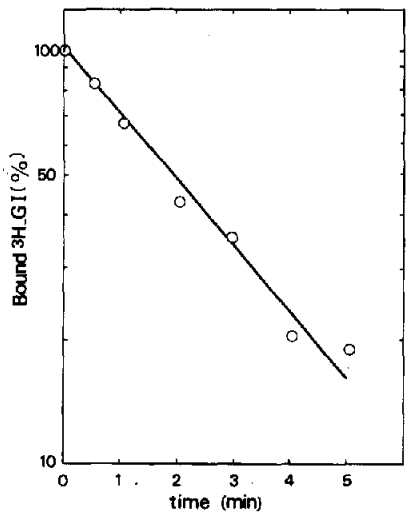

Fig.3. Semi-logarithmic plot of the time course of [^{3}H]GI dissociation from GPM after a 1:10 dilution of the incubation medium at 20°C. According to the equation,

$$RH \xrightarrow{k_{-1}} R + H \text{ (see results)},$$

$$\log_e \frac{[RH]}{[RH]_{eq}} = \log_e (\%\ \text{bound}\ [^3H]GI) = k_{-1}\ t$$

Table 2
Competition between native and tritiated gastrin for binding to GPM (equilibrium values). GPM (0.7 mg protein ml^{-1}) were incubated in the presence of native and/or tritiated hormone at 20°C.

Native (M)	[^{3}H]GI (M)	[^{3}H]GI bound (moles mg^{-1} protein)
0	0.9×10^{-8}	20.2×10^{-14}
0	1.8×10^{-8}	29.5×10^{-14}
0.9×10^{-8}	0.9×10^{-8}	12.1×10^{-14}

competed for binding. A 1:1 dilution of [^{3}H]GI in native hormone resulted in a 60% decrease in [^{3}H]GI binding value (0.295 pmol mg^{-1} compared to 0.121 pmol mg^{-1}) while the same dilution in buffer resulted in only a 30% decrease (0.295 pmol mg^{-1} compared to 0.200 pmol mg^{-1}) (table 2).

Equilibrium values for a given concentration in [^{3}H]GI were proportional to membrane protein concentration (fig.4). For a given concentration in membrane protein, Scatchard plot [11] of equilibrium values obtained at various concentrations in [^{3}H]GI gives a linear relationship (fig.5). From this plot, calculation of binding capacity and equilibrium constant gives the values of $N = 42 \times 10^{-14}$ moles per mg of membrane protein and $K = 1.7 \times 10^8$ M^{-1} respectively. It can be noticed that, in agreement with the overall equation

$$R + H \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} RH,$$

the value found for equilibrium constant is consistent with that which can be calculated as $K = k_1/k_{-1}$ (1.7×10^8 M^{-1} and 1.2×10^8 M^{-1} respectively).

There is no previous reference dealing with gastrin binding to gastric membranes. However the values reported here appear to be in agreement with those previously reported for a number of other hormone–receptor models [11,12].

3.3. *Biological tests*

The immunoreactivity and the biological activity of [^{3}H]GI were tested prior to binding studies and found to be identical to that of unlabelled native human synthetic gastrin I.

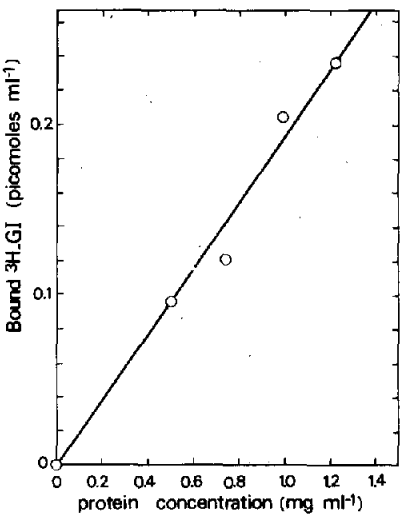

Fig.4. Amount of hormone bound at the equilibrium according to membrane protein concentration in the incubation medium at 20°C. [^{3}H]GI concentration was 0.9×10^{-8} M.

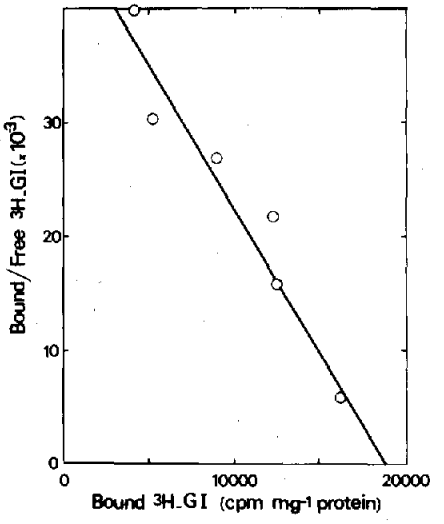

Fig.5. Scatchard analysis of [^{3}H]GI binding to GPM plotting bound: free hormone versus bound hormone. Each point was obtained from equilibrium values at various concentrations in [^{3}H]GI. GPM: 0.7 mg protein ml^{-1}; 20°C.

Table 3
Stimulation of adenyl cyclase activity in subcellular fractions from rat gastric mucosa by [^{3}H]GI with reference to stimulation by native gastrin

	Basal (pmoles cAMP min^{-1} mg^{-1} protein)	% Stimulation	
		Native gastrin	[^{3}H]GI
Homogenate	3.2	53	–
90 000 *g* pellet	5.4	94	83
1.12–1.14 g/cm^3 density band (GPM)	9	277	178

Incubation medium contained 100 to 200 μg protein ml^{-1}, ATP 0.8 mM, $MgCl_2$ 5 mM, theophylline 10 mM, EDTA 1 mM, creatine phosphate 20 mM, creatine phosphokinase 0.5 mg/ml, Tris 20 mM, pH 7.4, and 10^{-8} M [^{3}H]GI or native gastrin. Reaction was run 10 min at 37°C.

In table 3 are reported the results of adenyl cyclasic activity determination. Basal activity in GPM is enriched 2 fold over the homogenate. It is comparable to that previously reported in guinea-pig [13] and rabbit [14] gastric mucosa preparations (22.5 and 12 pmol min^{-1} mg^{-1} protein respectively), but higher than that reported in crude membrane fractions prepared from *Necturus* gastric mucosa [2].

Adenyl cyclasic activity in GPM is seen to be stimulated by 10^{-8} M [^{3}H]GI. This finding is of interest as far as the role of 3′,5′-AMP in the regulation of acid secretion is controversial [13]. However, as it will be discussed elsewhere (in preparation), our data are in agreement with the 60% stimulation reported in *Necturus* gastric mucosa by pentagastrin [2].

From these data, it can be therefore assumed that membrane bound radioactivity evidenced in this work accounts for gastrin binding to target sites in relation to the stimulatory effect of the hormone on HCl secretion. Furthermore they support the concept of 3′,5′-AMP involvement in the regulation of gastric acid secretion in the rat.

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