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IMMUNOLOGICAL RECOGNITION OF NPS-GASTRIN DERIVATIVES BY GASTRIN ANTIBODIES

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Summary: NPS-gastrin (o-nitrophenylsulfenylgastrin) derivatives are potent inhibitors of stimulated gastric acid secretion in the rat. The aim of this work is to study the recognition by a gastrin antibody of several gastrin derivatives either with or without the addition of NPS to the C-terminal tryptophan residue. We studied the following peptides and their NPS-derivatives: human gastrin I (2-17) (G), BOC-βAla-Trp-Met-Asp-PheNH$_2$ (pentagastrin) (Pg), its analog BOC-Gly-Trp-NLeu-Asp-PheNH$_2$ (NLeu-Pg), t-AOC-Trp-Met-Asp-PheNH$_2$ (tetragastrin). In addition, (Dehydro(αβ)Trp)-pentagastrin (dehydro-Pg) was also tested. In a standard immunoassay system containing constant amounts of ^{125}I-gastrin and gastrin antibody we measured the B/F ratio with varying amounts of gastrin and the above peptides. In the case of all penta and tetrapeptide derivatives the presence of the NPS radical promoted better recognition of the molecules by gastrin antibody. On the other hand the rigidity of the side chain of the tryptophan in the dehydro-Pg analog of pentagastrin drastically decreased the affinity of gastrin antibody for the molecule (D 50 = 1/1000 that of pentagastrin).

It is well known that the minimal size of the fragment of the gastrin molecule able to stimulate gastric acid secretion is the C-terminal tetrapeptide (1). On the other hand, it has been shown that the substitution of an orthonitrophenylsulfenyl group for the H of the indole ring of the C-terminal tryptophan residue changes gastrin and C-terminal tetrapeptide into competitive antagonists of the hormone (2).

The first aim of this work is to study the effect of this structural modification on the affinity of the molecule to an antibody to human gastrin (2-17) using a RIA system. The second aim is to compare these changes in immunologic acitvity with those observed at the receptor level, involving biological activity.

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Table I

Definition of the Various Reagents Used

I - GASTRIN, FRAGMENTS AND NPS DERIVATIVES	
GASTRIN I (17)	(PYRO)GLU-GLY-PRO-TRP-LEU-GLU-GLU-GLU-GLU-GLU-ALA-TYR-GLY-TRP-MET-ASP-PHE-NH_2
GASTRIN I (17) Mono NPS	(PYRO)GLU-GLY-PRO-TRP-LEU-GLU-GLU-GLU-GLU-GLU-ALA-TYR-GLY-TRP(NPS)-MET-ASP-PHE-NH_2
PENTAGASTRIN	t-BOC-βALA-TRP-MET-ASP-PHE-NH_2
PENTAGASTRIN NPS	t-BOC-βALA-TRP(NPS)-MET-ASP-PHE-NH_2
TETRAGASTRIN	t-AOC-TRP-MET-ASP-PHE-NH_2
TETRAGASTRIN NPS	t-AOC-TRP(NPS)-MET-ASP-PHE-NH_2
2 - PG : SUBSTITUTION OF MET BY NOR-LEU AND ITS DERIVATIVE	
NOR-LEU-PG	t-BOC-βALA-TRP-NOR-LEU-ASP-PHE-NH_2
NOR-LEU-PG-NPS	t-BOC-βALA-TRP(NPS)-NOR-LEU-ASP-PHE-NH_2
3 - DEHYDRO αβ-TRP-PG	
DEHYDRO PG	t-BOC-βALA-(αβ)TRP-MET-ASP-PHE-NH_2

MATERIAL and METHODS

A. Reagents used.

Synthetic human gastrin (G I), BOC-Gly-Trp-Nle-Asp-PheNH$_2$ or NLeu-Pg and dehydro(αβ)-Trp-Pg were generous gifts from Dr. Morley (ICI, Macclesfield, G.B.), Dr. De Castiglione (Farmitalia, Italy), and Dr. Maelicke (Max Planck Institute, Dortmund, RFA). t-AOC-Trp-Met-Asp-PheNH$_2$ (TG) (Farmitalia, Italy) and t-BOC-βAla-Trp-Met-Asp-PheNH$_2$ were purchased respectively from Protein Research Foundation (Japan), and Vega Chemical Co. (USA) (Table I).

The preparation of o-nitrophenylsulfenylated derivatives and tetra- and pentagastrin has already been reported (3). N-Ac (NPS) Trp-NH$_2$ (Vega Chem. Co.) and NPS-Cl (Fluka) were placed in glacial acetic acid for 15 min. The product was twice crystallized from ethyl acetate-petroleum ether (mp. 155-156°C). Purity was checked by thin layer chromatography in various solvent systems (4).

B. Radioimmunoassay system.

We used the method described by R.S. Yalow and S.A. Berson (5). The components are the following:

1. Antiserum: our 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 (6).

2. Tracer: synthetic (2-17) human gastrin was iodinated using the Hunter and Greenwood method (7). Purification was performed through either starch gel electrophoresis or ion exchange chromatography (amino-ethylcellulose AE 41 Whatman).

3. B/F ratio: the separation of the bound ^{125}I gastrin (B) from the free one (F) was achieved by adsorption of F on Amberlite IRP 58 M (Rohm and Haas, USA) (5).

C. Bioassay

1. Operative technique: male Wistar rats weighing 300 ± 25 g 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 (8) and modified by Lai (9). The rats were anesthetized by intramuscular injection of urethane (0.6 to 0.7 ml of a 25 % solution per 100 g) into both thighs.

A polyethylene catheter, introduced into the oesophagus and passed to the level of the cardia was connected to a peristaltic pump (Desaga) set to deliver a solution of 0.9 % NaCl at a constant rate of 0.8 to 1.0 ml/min. This perfusate was collected by another catheter placed through the pylorus and secured with a ligature. If necessary, the temperature of the rats was maintained at 34°C with the aid of electric lamps.

2. Experimental protocol: the tests were begun after stabilization of the gastric perfusion, usually within 30 to 60 min after completion of the surgical preparation. The gastric secretion, diluted with perfusate of 0.9 % NaCl, was collected every 10 min and the acidity was measured by titrating the entire sample with 0.01 N NaOH to the phenolphtalein end point. Each had an average of 3 to 4 intravenous bolus injections (dorsal vein of penis) with an interval of 2 h between injections: the secretory output was allowed to stabilize at the basal level before injection of the subsequent agent. Each experimental group consisted in 6 rats.

A single agonist was given during each experiment; NPS derivatives in various doses, were administered simultaneously. The pharmacological model of the perfused stomach in the anesthetized rat allows the injections given to each rat to be considered independently. The order in which the combinations are used in each experimental group was randomized.

3. Evaluation of secretory responses: secretory responses were evaluated by comparison between acid output 40 min before injection and 40 min after injection.

RESULTS

Our results are reported in table II.

DISCUSSION

All the native derivatives which we tested (gastrin, pentagastrin, tetragastrin, N-Leu pentagastrin) give the same maximal response in our bioassay (15 µEq/40 min acid output); their ED 50 ranged from 280 pmoles/-kg for gastrin to 1,960 pmoles for tetragastrin. The NPS derivatives do

Table II

Biological Activity (acid secretion) and Immunoreactivity of some Peptide Analogues of Pentagastrin and their NPS-derivatives

	NATIVE PEPTIDES		NPS DERIVATIVES		
	Biological activity ED 50 *pmoles*	Immuno-reactivity ID 50 *pmoles*	% inhib. biological activity antag./ag. 1/1	antag./ag. 4/1	Immuno-reactivity ID 50 *pmoles*
G	280	0.008	54	100	0.017
PG	650	0.75	61	100	0.003
t-AOC-TG	1960	0.80	77	100	0.022
NOR-LEU-PG	1330	0.25	69	92	0.045

Acid secretion is expressed in terms of pmoles of native peptides able to show 50 % of the maximum secretory activity (ED 50). The inhibiting effect is given in % of the maximum secretion elicited by the native peptide + NPS derivative.
Immunoreactivity in the RIA system is given by the ID 50 (dose of peptides able to provoke a fall of 50 % of the B/F ratio).

not present any agonist effect and are competitive inhibitors of their natural homologues and of pentagastrin.

The NPS derivative of G-17 on the C-terminal tryptophan has been shown to be competitive with ^{3}H-G-17 in the fixation to isolated gastric cells and fundic plasma membranes; apparently NPS G-I-17 and ^{3}H-G-I-17 have similar affinity (10).

Substitution of nitrophenylsulfenyl tryptophan (NPS-Trp) for tryptophan in gastrin G-17 produces a gastrin analogue that competitively blocks gastrin binding on a 1:1 basis, showing that the binding affinity is not modified by NPS. The stoichiometry of inhibition is 1:1 on acid secretion. Thus the receptor activation property has been lost. This implies that tryptophan is involved not only in binding specificity, but also in activation of the receptor. The above data conflict somewhat with previous conclusions (11, 12). Since more modifications in the aspartyl residue were shown to abolish potency for H^{+} secretion, aspartyl residue was considered critical for activity, whereas the three other amino acids were thought to be involved only in binding.

Our antiserum showed good specificity for G-17 (ID 50 = 0.008 pmoles and 0.75 pmole for pentagastrin). The ID 50 for N-Leu pentagastrin is less than that of native Pg (ID 50 = 0.25 pmole). This phenomenon could be due to the fact that the structure of N-Leu Pg is more easily recognizable by the antibody.

For the NPS series, the addition of this group results in an ID 50 for each derivative which is lower than that of the natural product. The most marked difference exists in the case of ID 50 of Pg (0.75 pmole) in regard to its NPS derivative (ID 50 = 0.003 pmole).

This could be accounted for on the basis of a change in the hydrophobia of the molecule carried by the NPS group. In fact, without this group, the tetragastrin molecule is folded so that it contains an hydrophobic area which is composed by the side chains of Phe-Asp-Met-Trp. Hydrophylia is related to the COOH groups, CO and NH from Asp-Trp and terminal $CONH_2$ group. The addition of an NPS group to the C_2 of indole, provokes extension of the hydrophobic area (NPS being an hydrophobic group).

Thus, change in hydrophobia may explain why our antibody has better recognition and greater affinity for NPS derivatives than for the parent molecules. This suggests that the antibody recognizes more a conformation than a group and that it contains an hydrophobic pouch.

The data concerning dehydro-Pg showed that its agonistic activity is very poor (estimated ED 50 = 42,000 pmol) in regard to Pg (ED 50 = 650 pmoles). In our RIA system, ID 50 for this Pg-derivative is 100 pmoles (0.75 pmole for Pg). This suggests that the rotation of Trp around the connection (α-β) (which is hidden by the presence of the double bond) is indispensable to the activation of the biological sites and to the binding with the antibody.

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