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Gastrin derivatives investigated for secretory potency and for changes in gastric mucosal histamine formation

ELSA ROSENGREN AND S. E. SVENSSON

Institute of Physiology, University of Lund, Sweden

Summary

1. Acid secretion and rate of histamine formation in the gastric mucosa were determined in rats after injection of hog gastrin II, gastrin pentapeptide and some substituted analogues of the C-terminal tetrapeptide of gastrin.

2. Accelerated mucosal histamine formation was induced by hog gastrin II, gastrin pentapeptide and those analogues which elicited acid secretion, whereas secretorily inactive derivatives did not alter histamine formation.

Introduction

In order to exert the wide range of physiological effects displayed by gastrin I and II (Gregory & Tracy, 1964), only the C-terminal tetrapeptide sequence Try.Met.Asp.Phe. NH_2 is necessary (Tracy & Gregory, 1964; Morley, Tracy & Gregory, 1965). The relationship between the structure of differently substituted gastrin analogues and their function has been extensively studied in dogs (Morley *et al.*, 1965) and in rats (Morley, 1968). These authors found that alterations in the parent tetrapeptide proper did not necessarily render the analogue inactive.

It has been reported from this laboratory that feeding as well as the individual components operating on feeding—vagus stimulation, gastrin release and distension of the stomach—all evoke an elevation of histamine formation in the gastric mucosa, a step which is believed to be essential in the process of stimulation of the parietal cells (see Rosengren & Svensson, 1969, for references). The object of the present study was to investigate possible changes in mucosal histamine formation produced by gastrin tetrapeptide analogues in which one or more substitutions of the amino-acid residue had diminished or abolished the stimulatory effect on gastric secretion. Although gastric secretion was determined, it was not considered essential to attempt a formal comparison between the ability of the compounds studied to stimulate gastric secretion and to accelerate histamine formation.

Methods

Animals. Female rats, bred at the Institute of Physiology, Lund, were used, and their body weight was 160–220 g. They were fed on a standard pellet diet (Type 142, Teknosan, Malmö, Sweden).

Drugs. The constitution of the peptide derivatives used is stated in Table 1. The structures are numbered according to Morley *et al.* (1965). The peptide

analogues 11, 12, 16, 17, 24 and 25 were dissolved in a small amount of dimethylsulphoxide and ammonia solution to which was added 0·9% NaCl to give a final concentration of 250 μg/ml. Hog gastrin II was dissolved in 0·9% NaCl to which dimethylsulphoxide and ammonia was added. Gastrin pentapeptide (Peptavlon, ICI 50,123, (29)) had been dissolved by the manufacturer in NaCl solution and ammonia, and was used without further dilution. Control animals were injected with the solution used for dissolving the analogues. All injections were made subcutaneously.

Stomach preparation. Heidenhain pouches were prepared as described by Alphin & Lin (1959). Collection of gastric juice and determination of HCl and pepsin secretion were performed as described in detail elsewhere (Svensson, 1970).

Determination of histamine forming capacity (HFC). The rate of histamine formation, that is the histidine decarboxylase activity, was determined by procedures, originally devised by Schayer, and adapted for use in this laboratory (Kahlson, Rosengren & Thunberg, 1963). The rats were fasted for 20 h before removal of the stomach. The animals were killed by a blow on the head and bled. The stomach was opened along the minor curvature, washed with 0·9% NaCl solution, blotted with gauze, and the mucosa of the parietal cell containing region was collected by scraping with a scalpel. The minced tissue was incubated for 3 h at 37° C under nitrogen in beakers containing about 150 mg of tissue, 40 μg of 2-ring [^{14}C] L-histidine (base), 10^{-4}M aminoguanidine sulphate, 10^{-1}M sodium phosphate buffer, pH 7·4 and 0·2% (w/v) glucose, and the total was finally made up to a volume of 3·2 ml. At the end of the incubation, carrier histamine and perchloric acid were added. After filtration, radioactive histidine was separated from radioactive histamine on an ion exchange resin (Dowex 50 W-X4, 100–200 mesh) and after conversion of the histamine to pipsyl histamine the radioactivity of the histamine formed was determined at infinite thickness in a flow counter. The pipsyl samples were recrystallized repeatedly from acetone until they displayed constant radioactivity. With the [^{14}C] histidine and the measuring equipment used 1 μg [^{14}C] histamine formed corresponded to about 5,000 c.p.m. Activity is expressed in μg [^{14}C] histamine formed per g tissue in 3 h.

Results

The stimulatory effect of the investigated gastrin analogues on acid secretion, as recorded by Gregory and his colleagues, was confirmed in the unanaesthetized rat provided with a Heidenhain pouch. Hog gastrin II (10 μg/kg) was the most effective in eliciting acid secretion. Gastrin pentapeptide (compound No. 29) was less active, although a high rate of secretion could be evoked by injecting 250 μg/kg. Compounds 11 and 12, in the same high dose employed as with compound 29, were still less active, the secretory responses amounting to about half of those obtained with gastrin pentapeptide. Compound 16 elicited only a small but definite secretory response. With compounds 17, 24 and 25 no stimulation of secretion could be demonstrated.

Changes in mucosal HFC produced by the tetra- and pentapeptide analogues were first investigated after a single injection of 250 μg/kg. With this administration the resulting change in mucosal HFC was inconsistent and difficult to evaluate, and therefore the same dose of the analogues was successively injected eight times at 30 min intervals and the rats were killed 30 min after the last injection. Hog

TABLE 1. *HFC (μg/g) in the gastric mucosa of rats after eight injections at 30 min intervals of control solution, hog gastrin II (10 μg/kg), gastrin pentapeptide (250 μg/kg) and analogues of gastrin tetrapeptide (250 μg/kg)*

Treatment		HFC μg/g	Mean±S.D.
Control		2·5, 3·2, 3·2, 3·9, 6·4, 6·4,	4·3±1·7
Hog gastrin II	$\lceil$Glu. Gly. Pro. Try. Met. $(Glu)_5$. Ala. Tyr(SO_3H). Gly. Try. Met. Asp. Phe. NH_2	21, 25, 31, 36, 48,	32·2±10·5
Peptavlon, ICI 50,123 (29)	BOC.β–Ala. Try. Met. Asp. Phe. NH_2	29, 31, 45, 48, 49, 51,	42·2±9·64
(11)	Z. Try. Met(O, O). Asp. Phe. NH_2	29, 33, 41, 48, 67,	43·6±15·0
(12)	Z. Try. Met(O). Asp. Phe. NH_2	25, 31, 35, 37, 39, 48,	35·8±7·75
(16)	Z. Try. Met. Asp(Phe. NH_2).	8·8, 11, 12, 14, 19, 19,	14·0±4·54
(17)	Z. Try. Met. Glu. Phe. NH_2	3·9, 5·3, 6·0, 7·3, 8·2, 11,	7·0±2·49
(24)	BOC. Try. Met. Asp. Phe. OH	3·5, 3·8, 4·9, 6·6, 7·4, 7·7,	5·7±1·84
(25)	BOC.D.Try.D.Met.D.Asp.D.Phe.NH_2	5·3, 5·9, 6·4, 6·6, 6·8, 7·8,	6·5±0·85

BOC=Me_3 C. O. CO; Z=$PhCh_2$. O. CO.

gastrin II, 10 μg/kg, and the control solution were similarly administered. The results are summarized in Table 1. Hog gastrin II, gastrin pentapeptide and compounds 11 and 12, each generated strikingly high HFC in the gastric mucosa. In compounds 11 and 12, the sulphur atom has been oxidized to the sulphoxide or to sulphone ; these changes do not diminish the power to elevate HFC.

Compounds which are structurally altered at the aspartic acid position are less effective in elevating the HFC. Compound 16, which excites the secretion of acid and pepsin only slightly, produces an elevation of HFC about three times the controls. Compound 17, in which aspartic acid has been replaced by glutamic acid, is incapable of elevating HFC significantly.

The acid analogue of the parent tetrapeptide amide, compound 24, and the D-analogue of gastrin tetrapeptide, compound 25, are likewise incapable of producing definite increase of mucosal histamine formation.

Discussion

The physiological effects of gastrin and its analogues have been amply recorded. On the other hand, little is known about the mechanism by which gastrin exerts its action on the parietal cells. Observations in this laboratory have led to the view that accelerated formation of histamine in the gastric mucosa, brought about by feeding, vagal excitation and gastrin, constitutes an important step in the sequence of events leading to HCl-secretion. The process of acid secretion *per se* is, however, not associated with an alteration of mucosal HFC, since injection of histamine (Kahlson, Rosengren, Svahn & Thunberg, 1964) or of stable choline esters (Rosengren & Svensson, 1969) produce HCl secretion without involving accelerated histamine formation.

In extensive studies it has been found that the entire range of physiological effects of gastrin is produced by the parent tetrapeptide amide (Tracy & Gregory, 1964 ; Morley *et al.*, 1965). These authors suggested that a definite kind of configuration in the tetrapeptide is concerned with the binding of the molecule to a receptor site and another grouping with the activation of the receptor.

The present results show that among the compounds investigated a correspondence exists between their power to excite acid secretion and to induce elevation of mucosal HFC. Pure hog gastrin II is the most active in exciting gastric secretion and accelerating mucosal histamine formation. Compounds 11 and 12, although secretorily less active than gastrin pentapeptide, did elevate mucosal HFC distinctly on repeated injections of these compounds. On single injection compound 16 elicited a small acid response and a significant acceleration of mucosal HFC. The compounds 17, 24 and 25 produced a very small elevation of HFC, but did not evoke appreciable secretory response. This occasional lack in correspondence between secretion and HFC should, however, not be taken to indicate the absence of a fundamental coupling beween the two changes. In the first place, as already said, the pertinent elevation of HFC was trivial, and further, a corresponding minor secretory response may have escaped detection.

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