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Specific stimulation of gastric acid secretion by a pentapeptide derivative of gastrin

A. M. BARRETT

The effects of gastrin, a synthetic pentapeptide (*N*-t-butyloxycarbonyl-β-Ala. Try. Met. Asp. Phe. NH_2; I.C.I. 50,123) and histamine on the secretion of gastric acid have been compared in a perfused stomach preparation using anaesthetised rats. The pentapeptide was shown to possess similar secretory activity to that of gastrin but to be 11 times less potent on a molar basis. It produced acid secretions similar to those produced by histamine when compared in several different ways. Unlike histamine, the pentapeptide was without significant effect on blood pressure or haematocrit in maximally stimulating doses.

IN a variety of clinical conditions it is necessary to assess the ability of the stomach to secrete acid. Histamine is a valuable diagnostic tool in this respect but suffers from the disadvantage that the doses required to stimulate maximal acid secretion also produce very unpleasant side-effects. In 1953, Kay proposed the concomitant use of antihistamines with histamine in his "augmented histamine test" since the action of histamine on gastric secretion is unopposed by antihistamine drugs. This technique allowed the dose of histamine to be raised to that necessary for the stimulation of maximal secretion without serious consequences for the patient.

In 1964, Gregory & Tracy announced the isolation of gastrin in pure form and described its specific effects on the gastrointestinal system. Immediate interest was aroused in the possible replacement of histamine by gastrin in the assessment of gastric secretion. A comparison of the stimulant actions of gastrin and histamine has shown potential usefulness of gastrin in this context (Mahklouf, McManus & Card, 1965). Gastrin is a polypeptide containing 17 amino-acids and although total synthesis has been achieved (Anderson, Barton, Gregory, Hardy, Kenner, MacLeod, Preston, Sheppard & Morley, 1964) it is not practicable on a commercial scale. However, in a study of the biological activity of fragments of the gastrin molecule, Tracy & Gregory (1964) found that all the physiological effects of gastrin were shared by the terminal tetrapeptide sequence Try. Met. Asp. Phe. NH_2, even though it was quantitatively less active. In a series of analogues of the active tetrapeptide, one pentapeptide (*N*-t-butyloxycarbonyl-β-Ala. Try. Met. Asp. Phe. NH_2; I.C.I. 50,123) was found to be particularly active in stimulating gastric secretion (Morley, Tracy & Gregory, 1965). This paper compares the action of this pentapeptide with that of gastrin and histamine in the stimulation of gastric acid secretion in the rat.

Experimental

METHODS

The experiments were made using male rats, 240–260 g, from the colony of specific pathogen-free albinos maintained at Alderley Park. The

From the Department of Pharmacology, Pharmaceuticals Division, I.C.I. Ltd., Alderley Park, Macclesfield.

animals were fasted for 24 hr before use but allowed free access to water. The rats were anaesthetised with urethane (8·0 ml/kg of 17·5% solution) given by the intramuscular route. They were then prepared for perfusion of the stomach by the technique of Ghosh & Schild (1958) using saline in place of buffer solution. Each rat stomach was perfused for up to 8 hr and the perfusate titrated for acidity at 10 min intervals. The rate of perfusion was 1 ml/min. Intravenous injections of stimulants were made in volumes of 0·2 ml/100 g via a cannulated jugular vein and given at intervals of not less than 90 min. Each rat received up to 5 injections and the response to each dose of stimulant was determined in 4 separate rats. Systemic arterial blood pressure was monitored in some animals by means of a Condon mercury manometer connected to a carotid artery and in others haematocrit was determined by a standard technique.

Drugs. The drugs used were gastrin II (kindly supplied by Prof. R. A. Gregory, F.R.S.), pentapeptide (prepared by Dr. J. S. Morley of the Chemistry Department at Alderley Park) and histamine acid phosphate. Doses of histamine were calculated in terms of the base.

Results and discussion

The perfused stomach preparation secreted a small but definite amount of acid throughout the whole period of perfusion. The amount secreted may vary from 2 to 6 μ-equiv. hydrochloric acid per 10 min from rat to rat but for any individual animal this background secretion is relatively constant. When gastrin, the pentapeptide or histamine is injected there is an immediate increase in acid secretion. The responses to various doses of these 3 agents are summarised in Table 1.

TABLE 1. THE EFFECT OF THE INTRAVENOUS INJECTION OF DIFFERENT DOSES OF GASTRIN, THE PENTAPEPTIDE AND HISTAMINE ON THE ACID SECRETION OF PERFUSED RAT STOMACHS. Each line shows the dose injected and the mean 10 min acid output for 10 consecutive collection periods for 4 rats

Secretory stimulant	Dose (μg/kg)	Acid secretion/10 min (μ-equiv. HCl)									
		Pre-injection			Post-injection						
		1	2	3	4	5	6	7	8	9	10
Gastrin	2	4·3	4·3	4·1	8·6	12·7	9·4	8·1	5·5	4·4	3·4
	8	5·6	4·2	4·2	8·0	14·9	13·5	10·5	8·0	5·3	4·7
	32	5·0	4·6	4·6	8·5	14·7	16·0	14·5	10·3	7·1	4·6
	128	4·5	4·4	4·4	10·2	17·2	19·1	17·3	12·5	8·1	5·8
	512	4·2	4·3	4·2	9·6	17·7	20·1	19·3	17·1	13·1	10·3
Pentapeptide ..	2	3·7	4·0	4·0	9·3	10·5	9·1	7·7	4·5	3·7	3·4
	8	3·7	3·4	3·3	9·0	11·7	10·4	7·3	4·5	3·4	3·2
	32	4·4	4·0	3·9	9·8	13·0	13·1	8·5	5·2	4·4	3·9
	128	4·1	4·2	4·2	11·4	17·0	14·8	8·3	5·8	5·0	4·9
	512	4·0	3·6	3·6	11·1	17·0	16·2	11·6	9·2	6·2	4·1
	2,048	5·0	4·4	4·5	10·1	16·6	17·7	16·2	13·5	10·2	7·5
Histamine ..	80	3·7	3·5	3·3	3·5	5·3	4·8	4·1	3·4	3·2	3·2
	320	3·2	3·1	2·5	4·0	7·7	6·4	4·9	4·2	3·4	3·1
	1,280	3·7	3·4	3·0	4·9	7·5	9·4	7·6	7·0	5·6	4·5
	5,120	3·3	3·1	2·9	4·8	11·3	14·7	14·9	14·1	12·0	10·6
	20,480	3·4	3·2	3·3	11·6	12·9	14·6	14·4	13·3	13·5	15·1

The pattern of the responses to increasing doses of gastrin and the pentapeptide is illustrated in Fig. 1. At each dose level it appears that

the increase in the first 10 min period is greater with the pentapeptide than the natural hormone although this difference is not statistically significant. However, the peak value, reached in the second 10 min period after

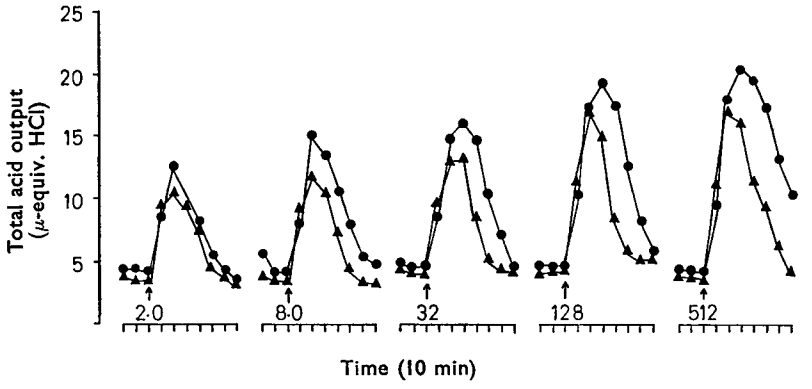

FIG. 1. The effect of gastrin and the pentapeptide on the total acid output per 10 min from perfused rat stomachs. The doses (μg/kg) were given in random order at the arrows and each point represents the mean value for 4 animals (●—● gastrin; ▲—▲ I.C.I. 50,123).

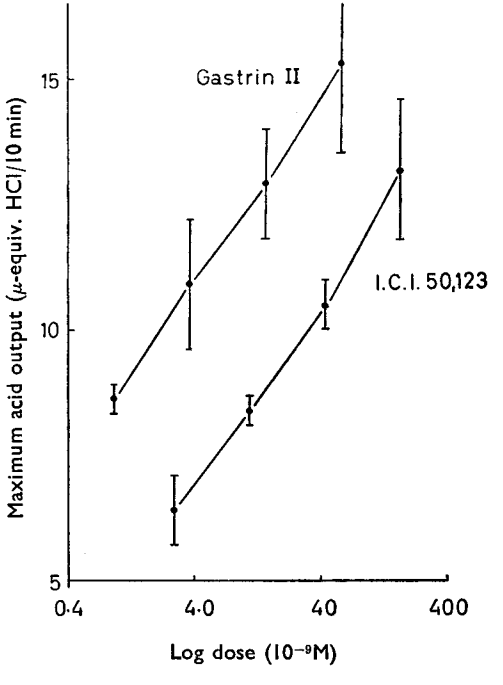

FIG. 2. Dose response curves for gastrin and the pentapeptide comparing the maximum response at different dose levels.

injection was always greater with gastrin. Further, the recovery to base-line acid secretory levels was quicker with the pentapeptide. It may be concluded therefore that gastrin has a slightly slower onset of action than equivalent doses of the pentapeptide but that it produces a larger and more sustained acid secretion. Considering that the pentapeptide represents an active fragment of gastrin, it is reasonable to conclude that the rate of access to, and removal from, the appropriate receptor sites is greater for the smaller molecule.

It was desirable to be able to compare the potency of these two agents and analysis of the results showed that the best way of doing this was to calculate the maximum response for each dose level. The mean basal level for each rat was calculated by averaging the values of 3 pre-injection and 3 post-recovery 10 min secretions and this was subtracted from the maximum acid output produced by the particular injection. When the results were summated and averaged, a plot of the maximum response against the logarithm of the dose gave two parallel straight lines over the dose range of 2 to 32 μg/kg. Because of the difference in molecular weight of gastrin (2154) and the pentapeptide (769), the acid output was plotted against the logarithm of the molar doses (Fig. 2). On a molar basis gastrin is 11 times the more active (95% confidence limits 4·8–27), but on a weight for weight basis gastrin is 4 times more potent (1·7–11·0).

Comparison of the patterns of response to the pentapeptide and histamine showed certain differences (Fig. 3). There was a marked difference in sensitivity, it being necessary to inject over 5 mg/kg of

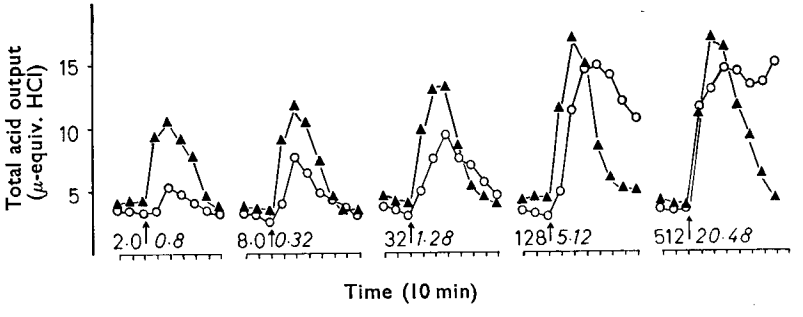

FIG. 3. The effect of histamine and the pentapeptide on the total acid output per 10 min from perfused rat stomachs. The doses, I.C.I. 50,123 in μg/kg (roman); histamine in mg/kg (italic), were given at the arrows in random order and each point represents the mean value for 4 animals (○—○ histamine; ▲—▲ I.C.I. 50,123).

histamine to produce a similar acid response to that of 128 μg/kg of the pentapeptide. Further, the response to histamine was of longer duration than that to the pentapeptide. The maximum response to both substances is similar and on a weight for weight basis histamine is 80 times less active than the synthetic peptide.

The maximum acid output per 10 min is not always the most useful index of secretory activity and many workers compare stimulant activity

in terms of the peak hour output. This value is calculated by adding the two highest 10 min acid outputs, subtracting the contribution of the basal secretion and multiplying by 3. Such a comparison is presented in Fig. 4.

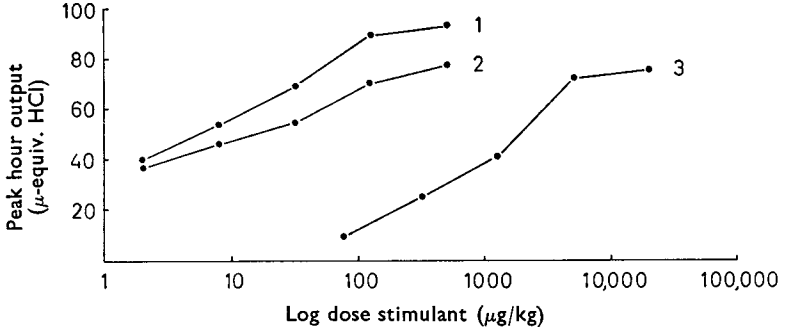

FIG. 4. Mean peak hr output of HCl (μ-equiv./hr) for gastrin (1), the pentapeptide (2) and histamine (3). (Each point represents the results from 4 rats.)

There is a clear similarity in the overall slope of the curves for the three secretagogues studied. It appears that the maximal response for histamine and the pentapeptide is only 82% of that for gastrin but these differences are not statistically significant.

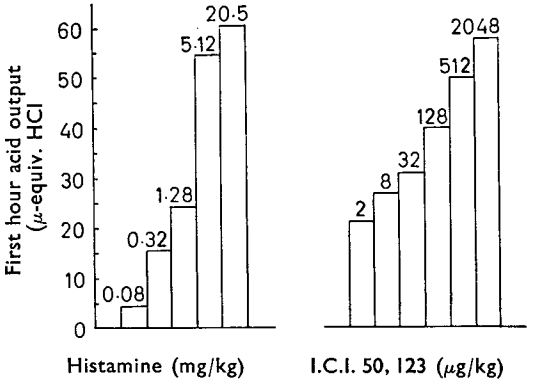

FIG. 5. Mean total acid output in the first hour after the intravenous injection of I.C.I. 50,123 and histamine (4 rats per column).

The total acid output in a period of 1 hr following injection is another widely used index of gastric secretory function. As shown in Fig. 5 the pentapeptide is capable of producing values similar to those produced by histamine.

It is well known that histamine exerts marked effects on the blood pressure. As shown in Table 2 the stimulation of high acid output by histamine was accompanied by marked falls in blood pressure. Gastrin had no effect on mean arterial pressure whereas the pentapeptide exerted slight pressor effects which appeared unrelated to dose.

Previous studies have shown that both urethane and histamine bring about marked increases in the packed cell volume. Two groups of 18 rats were sub-divided into 3 further groups of 6 rats. The main group

TABLE 2. A COMPARISON OF THE EFFECTS OF GASTRIN, THE PENTAPEPTIDE AND HISTAMINE ON GASTRIC ACID SECRETION AND BLOOD PRESSURE. The parameters were recorded in different animals, there being the mean of 4 observations ± standard error for each dose level. The mean starting blood pressure was 125 ± 8 mm Hg

Drug	Dose (μg/kg)	Peak hr output (μ-equiv. HCl)	Change in blood pressure (mm Hg)
Gastrin	2	39·9 ± 5·1	+ 2 ± 1·2
	8	54·2 ± 3·1	+ 2 ± 1·2
	32	69·0 ± 5·1	+ 3 ± 1·4
	128	89·9 ± 13·3	+ 1 ± 1·6
	512	94·1 ± 14·1	+ 4 ± 1·8
Pentapeptide ..	2	37·1 ± 3·3	+ 7 ± 2·5
	8	46·1 ± 0·9	+ 8 ± 2·1
	32	55·0 + 1·8	+ 8 ± 2·1
	128	71·4 ± 4·2	+ 7 ± 2·1
	512	78·2 ± 6·9	+ 5 ± 1·0
Histamine	80	10·0 ± 2·7	− 31 ± 2·1
	320	24·8 ± 6·8	− 39 ± 3·5
	1,280	41·3 ± 10·7	− 54 ± 2·5
	5,120	73·2 ± 17·7	− 60 ± 5·1
	20,480	75·8 ± 22·4	− 65 ± 1·9

received a sham injection and the second was anaesthetised with urethane. Within the main groups, 1 hr after injection, one group received a sham injection, one histamine at 5·12 mg/kg and the third the pentapeptide at 512 μg/kg. Thirty min later the packed cell volume was determined and the results are summarised in Table 3. In conscious rats histamine

TABLE 3. THE EFFECTS OF THE PENTAPEPTIDE AND HISTAMINE ON THE PACKED CELL VOLUME (HAEMATOCRIT) OF CONSCIOUS AND ANAESTHETISED RATS. Each value denotes the mean for six rats together with the standard error of the mean

(*a*) *Conscious rats*	Treatment	Packed cell volume (%)	Increase	Change %
	Saline	43·3 ± 1·2	—	—
	I.C.I. 50,123 512 μg/kg	44·8 ± 0·5	1·5 ± 1·3	3·5 ± 3·0
	Histamine 5120 μg/kg	52·3 ± 2·3	9·0 ± 2·6	20·8 ± 6·0
(*b*) *Anaesthetised rats* (Urethane i.m.)				
	Saline	52·2 ± 1·2	8·9 ± 1·7	20·6 ± 3·9
	I.C.I. 50,123 512 μg/kg	50·2 ± 1·2	6·9 ± 1·7	16·0 ± 3·9
	Histamine 5120 μg/kg	63·5 ± 0·5	20·2 ± 1·3	46·7 ± 3·0

Estimates of significance			P value
Conscious controls	vs.	I.C.I. 50,123	N.S.
	vs.	Histamine	<0·01
	vs.	Urethane	<0·001
Anaesthetised controls	vs.	I.C.I. 50,123	N.S.
	vs.	Histamine	<0·01

increased the haematocrit by 21% whereas the pentapeptide had no effect. In rats anaesthetised with urethane there was a 21% increase in haematocrit due to the anaesthetic alone. When histamine was given to rats anaesthetised with urethane there was a further increase in packed cell volume up to 47%. The pentapeptide slightly reduced the effects of urethane on haematocrit but this effect was not significant.

From these experiments it may be concluded that I.C.I. 50,123 possesses the properties necessary to replace histamine as a diagnostic acid for the assessment of gastric secretion. The synthetic pentapeptide appears to behave similarly to the natural hormone, gastrin. It exerts a specific effect on gastric secretion and obviates the need for the concomitant administration of antihistamines which are not themselves devoid of unwanted side-effects.

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