Isolation of two minigastrins from Zollinger-Ellison tumour tissue

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SUMMARY A pair of gastrin tridecapeptides ('minigastrins') have been isolated from Zollinger-Ellison tumour tissue; they correspond to the fragment 5-17 of the heptadecapeptides isolated from the same source. In one (type II) the tyrosine residue present is sulphated, in the other (type I) it is not. The proportion of types I and II is approximately 2:1 similar to that for the 'big' gastrins and the heptadecapeptides isolated from the same source and from human antral mucosa. Both minigastrins are potent stimulants of gastric acid secretion. Immunological evidence exists to indicate that both are present as circulating forms of the hormone gastrin in patients with the Zollinger-Ellison syndrome and in fed normal subjects.

There have previously been isolated from Zollinger-Ellison tumour tissue: (1) a pair of heptadecapeptide amides ('little gastrins') identical in amino-acid sequence and proportions with the peptides identified as the major form of the hormone gastrin in human antral mucosa (Gregory, Tracy, and Grossman, 1966; Gregory, Tracy, Agarwal, and Grossman, 1969), and (2) a pair of larger and less acidic peptides corresponding to the 'big gastrin' identified immunologically by Yalow and Berson (1970, 1971) in the serum of patients showing the Zollinger-Ellison syndrome (Gregory and Tracy, 1972, 1973).

More recently, through the kind cooperation of Dr Edward Passaro and Dr Morton Grossman of Los Angeles, we have been afforded the opportunity of studying the forms of gastrin present in a very large hepatic metastasis obtained at necropsy from a case of the Zollinger-Ellison syndrome. Preparative analysis of this material revealed the presence in it of a pair of tridecapeptides which are potent stimulants of gastric acid secretion. On the basis of their amino-acid composition, and for convenience in description it is proposed to refer to them in this report as 'minigastrins'.

Experimental

The tumour tissue was obtained at postmortem examination about 18 hours after death. It was cut into small cubes which were immediately boiled in water to arrest further enzyme action.

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The cooked cubes and liquor were frozen in dry ice and transported in this state by air from Los Angeles to Liverpool. In the routine extraction of the material, which was carried out on portions weighing approximately 100 g by a method similar to that to be described in detail elsewhere for the extraction of 'big gastrin' from hog antral mucosa, a partially purified aqueous extract (50 ml) was filtered through a column of Sephadex G-50 superfine grade 5 cm × 50 cm run at 4°C in 0.04 M ammonium bicarbonate solution saturated with toluene. Fractions of 5 ml were collected every 10 min and the UV absorption at 280 nm read in 2 mm cells. A typical result is shown in fig 1 where the shaded area is the salt zone. Region A (void volume) contained immunoreactive material corresponding

Fig 1 Filtration of a Zollinger-Ellison tumour extract on a column of Sephadex G-50. Regions A-E contained different gastrin peptides of decreasing molecular size. shaded area = salt zone.

in size to the 'big big' gastrin identified immunologically in serum and jejunal mucosal extracts (Yalow and Berson, 1972; Yalow and Wu, 1973). Region B contained material similar to 'component I' identified immunologically in serum by Rehfeld (1972). Region C contained the 'big gastrin' peptides isolated from similar material by Gregory and Tracy (1972). Region D contained very large amounts of the heptadecapeptide amides. Region E contained some heptadecapeptide amide type I (unsulphated) and also the tridecapeptides with which this report is concerned.

The fractions corresponding to region E were pooled and lyophilized. The residue was dissolved in 50 ml H₂O and brought to pH 2.5 with 2N H₂SO₄. Ammonium sulphate was then added to a concentration of 10% w/v. After standing at 4°C overnight, the pale precipitate was collected by suction filtration on a small filter paper impregnated with acid-washed Hyflo Supercel. The precipitate was dissolved in 0.05M triethylamine, filtered to remove Hyflo, and the solution gassed with carbon dioxide to pH 6.0-6.4. It was then applied to a column (1 cm × 10 cm) of aminoethylcellulose (Whatman AE 41) prepared in 0.05 M triethylamine carbonate buffer pH 6.4; the column was run at 4°C. Before application to the column, the conductivity of the sample was adjusted by dilution with water to be lower than that of the column buffer. After a preliminary wash with starting buffer, a gradient was established to 0.5 M buffer pH 7.4 through a closed magnetically stirred mixing flask volume 150 ml. Fractions of 1 ml were collected every 10 min and the UV absorption read in 1 cm microcells at 280 nm. A typical result is shown in figure 2.

The fractions corresponding to the three well defined peaks were separately pooled, lyophilized,

Fig 2 Chromatography on aminoethyl cellulose of fraction E shown in figure 1.

I heptadecapeptide type I; II minigastrin type II (sulphated); III minigastrin type I (unsulphated)

and re-run in chromatographic systems similar to that just described. In each case, the fractions corresponding to the single major peak observed were pooled, lyophilized, and aliquots of the residue taken for analysis. Quantitative amino-acid determinations were performed after: (a) acid hydrolysis using 6N HCl or by the method of Liu and Chang (1971) which preserves tryptophan, and (b) 30 hours of hydrolysis at 110°C in 5N NaOH, which preserves tyrosine sulphate. The results are shown in the table.

	Minigastrin		Residues
	I	II	
Aspartic	1.00	1.05	1
Glutamic	5.14	4.99	5
Glycine	1.09	1.06	1
Alanine	0.91	1.00	1
Methionine	0.83 ¹	1.03	1
Leucine	1.00	0.92	1
Tyrosine	0.97	0.99 ²	1
Phenylalanine	0.86	0.86	1
Tryptophan	1.00	0.99	1
Proline	Nil	Nil	0

Table Amino-acid composition of two minigastrins isolated from Zollinger-Ellison tumour tissue

¹Sulphoxide also present

²tyrosine sulphate

It is clear that both peptides have the same amino-acid composition, viz, Leu₁, Glu₅, Ala₁, Tyr(SO₃H)₁, Gly₁, Trp₁, Met₁, Asp₁, Phe₁. They obviously correspond to the N-terminal tridecapeptide of the heptadecapeptide, lacking the N-terminal sequence pyroglutamyl-Gly-Pro-Trp-. Both peptides were ninhydrin-positive, as would be expected from the N-terminal leucyl residue, in contrast to the heptadecapeptides which are ninhydrin-negative due to the N-terminal pyroglutamyl residue. Both peptides were potent stimulants of gastric acid when injected intravenously into conscious dogs provided with denervated pouches of the gastric fundus. Samples of the pure peptides were supplied to Dr Morton Grossman for study of their acid-stimulatory properties and disappearance rates from the circulation (Debas, Walsh, and Grossman, 1974).

Discussion

The tumour tissue from which the minigastrins were isolated as described above became available for preservation 18 hours after death. For this reason, and also the fact that they were found in the same proportion of sulphated and unsulphated forms as are the 'big' gastrins and the heptadecapeptides isolated from the same tissue, it was at first suspected

that they might be artefacts produced from larger forms of the hormone by postmortem digestion. However, since their isolation immunological evidence has been obtained by others which indicates that they are present in the circulation. Rehfeld and Stadil (1973) reported that in patients with the Zollinger-Ellison syndrome, and in fed normal subjects, immunoreactive gastrin present in the serum was separable by gel filtration on Sephadex G-50 into four components, the last and smallest of which (component IV) was eluted just before the salt zone. We have supplied preparations of the minigastrins to Dr Rehfeld; and in further studies (Rehfeld, Stadil, and Vikelsøe, 1974) it has been shown that component IV can be resolved into two peaks, the elution volumes of which correspond to those of minigastrins I and II on the same column.

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