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. 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 x 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.](http://gut.bmj.com/)

Received for publication 29 May 1974.
in size to the 'big big' gastrin identified immuno-
logically 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 amidase. Region E contained
some heptadecapeptide amidase 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 con-
ductivity 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,
and re-run in chromatographic systems similar to
that just described. In each case, the fractions cor-
responding 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.

<table>
<thead>
<tr>
<th></th>
<th>Minigastri</th>
<th>II</th>
<th>Residues</th>
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<tr>
<td>Aspartic</td>
<td>1.00</td>
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</tr>
<tr>
<td>Glutamic</td>
<td>5.14</td>
<td>4.99</td>
<td>5</td>
</tr>
<tr>
<td>Glycine</td>
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<td>1.06</td>
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<td>Alanine</td>
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<td>Methionine</td>
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</tr>
<tr>
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<td>0.99</td>
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</tr>
<tr>
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</tr>
<tr>
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<td>1</td>
</tr>
<tr>
<td>Proline</td>
<td>Nil</td>
<td>Nil</td>
<td>0</td>
</tr>
</tbody>
</table>

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

It is clear that both peptides have the same amino
acid composition, viz, Leu1, Glu5, Ala3, 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
ninyhydrin-positive, as would be expected from the
N-terminal leucyl residue, in contrast to the hepta-
decapeptides which are ninyhdrin-negative due to
the N-terminal pyroglutamyl residue. Both pep-
tides 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 minigastri 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.

References


