Isolation from chicken antrum, and primary amino acid sequence of a novel 36-residue peptide of the gastrin/CCK family

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A peptide that cross-reacted with C-terminal gastrin/CCK antisera was isolated from chicken antral extracts by a combination of gel filtration and reversed-phase HPLC. The sequence was: Phe-Leu-Pro-His-Val-Phe-Ala-Glu-Leu-Ser-Asp-Arg-Lys-Gly-Phe-Val-Gln-Gly-Asn-Gly-Ala-Val-Glu-Ala-Leu-His-Asp-His-Phe-Tyr-Pro-Asp-Trp-Met-Asp-Phe(NH_2). Aside from the C-terminal tetrapeptide and the Tyr residue, the molecule does not resemble other known forms of gastrin or CCK. The peptide was a potent stimulus of avian gastric acid but not pancreatic secretion. The results have important implications for the structure-activity and evolutionary relationships of the gastrin/CCK family.

Amino acid sequence *Antral peptide* *Tryptic digestion* *HPLC* *Gastrin* *Cholecystokinin*

1. INTRODUCTION

The mammalian peptides gastrin and cholecystokinin (CCK) share a C-terminal pentapeptide sequence that contains the minimal fragment (Trp-Met-Asp-Phe-NH_2) with biological activity. It seems possible that these peptides might share a common evolutionary history, and it has been speculated that they may have arisen by divergence following duplication of a common ancestral gene [1–3]. Many immunochemical and chromatographic, and limited bioassay studies have identified gastrin/CCK-like peptides in a wide range of submammalian vertebrates, and invertebrates [2–9]. To date, however, no structural information has been obtained on such peptides in submammalian gut or brain. We report here the isolation from chicken antrum, and primary amino acid sequence of a novel peptide containing the C-terminal tetrapeptide of gastrin/CCK.

2. MATERIALS AND METHODS

2.1. *Extraction and purification*

Chicken antrums, collected from a local abattoir, were boiled in water for 10 min (50 *g* in 500 ml), homogenised, the pH adjusted to 8.0 with ammonia, and centrifuged. The supernatant was added to 2 vols isopropanol and stirred overnight at 4°C. The aqueous phase was partitioned by addition of dichloromethane, aspirated, reduced to 40 ml by rotary evaporation and applied to a column of Sephadex G-50 fine (5 × 100 cm) equilibrated with 0.05 M ammonium bicarbonate. Some extracts were concentrated on Sep Pak C_{18} cartridges before gel filtration. Radioimmunoassay was performed on the G-50 eluates according to published methods using antisera (L112, L48) specific for the C-terminus of gastrin/CCK [10,11]. Immunoreactive G-50 eluates were applied to a Waters radial pak C_{18} cartridge equilibrated with 0.1% trifluoroacetic acid (TFA) and eluted with a gradient to 40%

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acetonitrile. Immunoreactive peaks in the C_{18} cartridge eluates were purified to homogeneity on a Techsil 5 μm C_{18} column and a Vydac 5 μm C_{18} wide-pore column, equilibrated with 0.1% TFA, and eluted with a gradient to 30 or 35% acetonitrile.

2.2. *Trypsinization*

Pure peptide (2–3 nmol) was incubated with 20 μg TPCK trypsin (Sigma, 12.5 BAAE units/μg) in 0.05 ammonium bicarbonate at 37°C. After 1 h the reaction was halted by the addition of 10 μl TFA, and the digest microfuged and applied to a 5 μm C_{18} Vydac wide pore column equilibrated with 0.1% TFA. The sample was eluted with a gradient to 35% acetonitrile and the column eluate assayed with a C-terminal gastrin/CCK antiserum.

2.3. *Amino acid sequence determination*

Approx. 1 nmol pure peptide and its C-terminal tryptic fragments were subjected to sequence analysis by standard methods using an Applied Biosystems gas-phase sequenator. The phenylthiohydantoin-derivatives amino acids were identified by reversed-phase HPLC.

2.4. *Bioassays*

Gastric acid secretion in anaesthetised chickens and exocrine pancreatic secretion in anaesthetised turkeys and rats were determined according to [12,13]. The effects of the chicken peptides, human heptadecapeptide gastrin (G17) and CCK octapeptide (CCK8) were compared in these three systems. Doses were established by amino acid analysis.

3. RESULTS AND DISCUSSION

Reversed-phase HPLC using the Waters radial compression C_{18} cartridge resolved the G-50 eluates into two well separated peaks of material that reacted with antisera specific for the C-terminus of gastrin/CCK (fig.1). The later eluting peak was purified to homogeneity on Techsil and Vydac reversed-phase C_{18} columns (fig.2), and subjected to sequence analysis. The first 18 residues were determined, which revealed that positions 12 and 13 were Arg-Lys. Further purified material was treated with trypsin and the digest fractionated by reversed-phase HPLC. Two tryptic

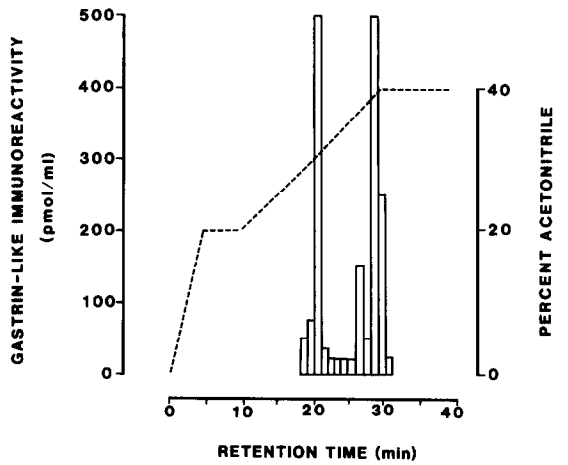

Fig.1. Elution profile of gastrin/CCK-like immunoreactivity in chicken antral extracts after reversed-phase HPLC. G-50 eluates were adjusted to pH 6.0 and loaded onto a Waters μ bondapak C_{18} radial compression cartridge equilibrated with 0.1% TFA. The column was eluted with a gradient to 40% acetonitrile (broken line), at a flow rate of 3 ml/min and eluates assayed with C-terminal gastrin/CCK antiserum, L48.

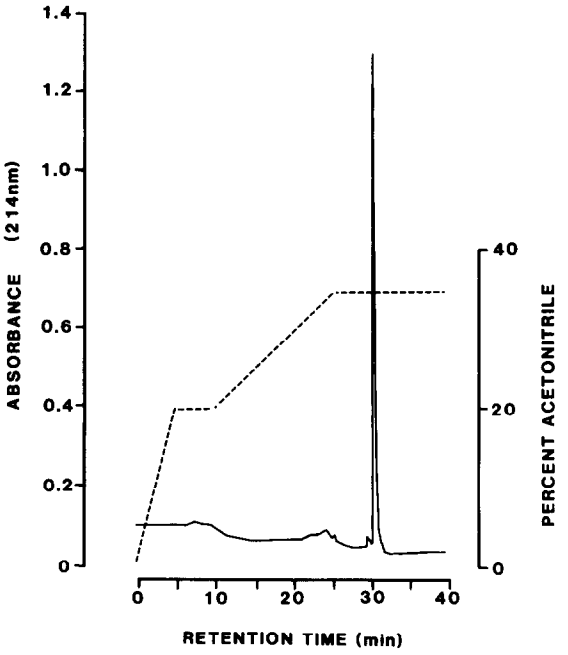

Fig.2. Final purification of the later eluting material shown in fig.1. HPLC-purified material was applied to a 5 μm Vydac C_{18} wide-pore column (0.46 × 25 cm) equilibrated with 0.1% TFA, and eluted with a gradient to 35% acetonitrile (broken line) at a flow rate of 1.25 ml/min. Absorbance was monitored at 214 nm.

fragments showed immunoreactivity with C-terminal gastrin/CCK antisera (fig.3, peaks A and B), and were sequenced. The tryptic peptides differed by an amino terminal lysine residue, and one was sequenced to completion. Overlapping sequence information on the tryptic fragment and the intact molecule enabled assignment of the complete amino acid sequence of the chicken antral peptide (fig.4). C-terminal amidation was inferred from the fact that the antisera used to monitor purification are virtually unreactive with the deamidated forms of gastrin/CCK [10,11]. Moreover, deamidated gastrin is biologically inactive [14]. The early eluting immunoreactive peak from the radial compression C_{18} eluates was also purified further and subjected to sequence analysis. However, the apparently homogeneous material gave equivocal sequence data; two peptides were identified and partially co-sequenced as Ala-Glu-Leu-Ser-Asp-Arg-Lys-Gly-Phe-( )-Gln-Gly... and Asp-Arg-Lys-Gly-Phe-Val-Gln... These sequences correspond to residues 7–18 and 11–17 of the 36-residue peptide (fig.4). Plainly then, the early and late eluting fractions are not related in the same way as mammalian G34 and G17. For convenience however, we refer to them as big and little chicken antral peptides. These data provide an explanation for previous results obtained from gel-filtration studies on antral extracts [6–8]. The concentration of gastrin/CCK-like immunoreactivity in the antrum (L112) was 6.0 ± 0.8 nmol/g (mean ± SE, n = 6), which is about 1000-fold higher than duodenal concentrations. Recovery at each purification step was approx. 70% and 50 g antrums yielded about 15 nmol pure 36-residue peptide, together with a similar quantity of the earlier eluting material (fig.1). These peptides, then, represent the major antral forms that react with C-terminal gastrin/CCK antisera.

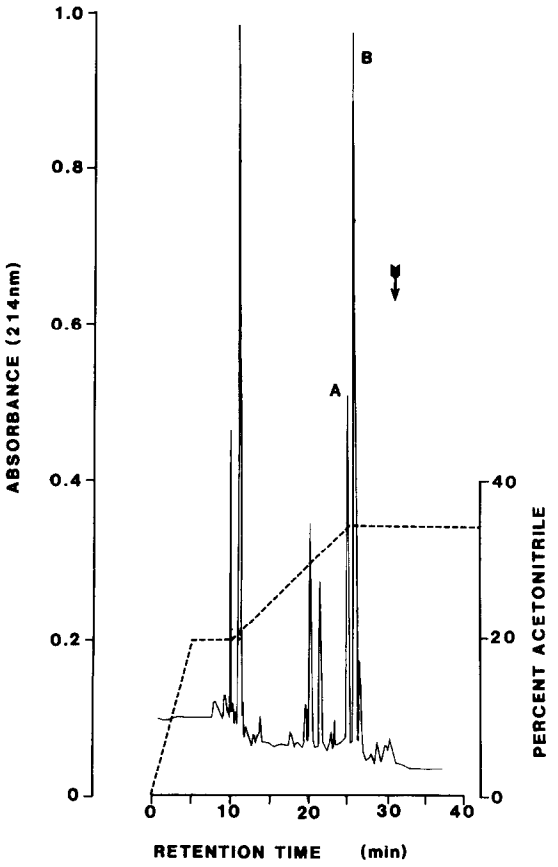

Fig.3. Elution profile of trypsinized chicken antral peptide after reversed-phase HPLC. The digest was applied to a 5 μm Vydac C_{18} wide pore column. See fig.2 for elution conditions. Absorbance was monitored at 214 nm, and column eluates assayed with C-terminal gastrin/CCK antiserum L112; only peaks A and B were immunoreactive. Arrow indicates the elution position of undigested peptide.

In anaesthetised chickens, infusion of the 36-residue peptide at a dose of 9 pmol/kg per min promptly stimulated acid secretion from a basal level of 158.1 ± 39 μmol/10 min to 287.5 ± 37.7 μmol/10 min (mean ± SE, n = 7). Similar increases over basal were elicited by the smaller chicken peptides at a dose of 5 pmol/kg per min or by human G17 at 18 pmol/kg per min. At these doses, all peptides also markedly increased the rate of pepsin secretion. In contrast, neither chicken peptide fraction, nor human G17 significantly stimulated pancreatic flow rate or enzyme secretion in rats or turkeys at doses up to 2 nmol/kg; CCK8 markedly stimulated pancreatic flow rate and enzyme secretion at a dose of 100 pmol/kg. Previous studies have shown that partially purified extracts of chicken antrum stimulate acid secretion in mammals [8,15]. Moreover, the extracts had a gastrin-like rather than CCK-like action in radioreceptor assays that clearly distinguish the two types of bioactivity [8].

However, there are several striking structural features that distinguish the chicken antral peptide from known mammalian gastrins. First, the

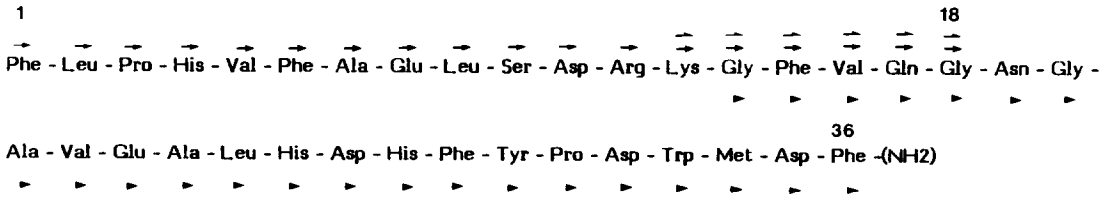

Fig.4. Amino acid sequence of chicken antral peptide. →, residues determined from sequencing of intact peptide. ⇀, residues determined from sequencing of tryptic peptide A. ⇁, residues determined from sequencing of tryptic peptide B. Trp at position 33 was equivocal, but confirmed by absorbance at 280 nm. The C-terminal amide was assigned on the basis of biological activity, and cross-reactivity with C-terminal gastrin/CCK antisera (see text).

Chicken gastrin	F L P H V F A E L S D R K G F V Q G N G A V E A L H D H F Y P D W M D F
Porcine CCK33	K A P S G R V S M I K N L Q S L D P S H R I S D R D Y M G W M D F
Porcine G34	*Q L G L Q G P P H L V A D L A K K Q G P W M E E E E E A Y G W M D F

Fig.5. Amino acid sequences of chicken antral peptide (labelled chicken gastrin), porcine big gastrin (G34) and porcine CCK33, aligned at the C-terminus. *Q, pyrrolidone carboxylic acid; C-terminal phenylalanine residues are all amidated. Common residues are boxed.

tyrosine residue occurs in the seventh position counting from the C-terminus which is characteristic of CCK, rather than gastrin where Tyr is the sixth residue from the C-terminus. Second, the amino terminus is not blocked, in contrast with the major mammalian forms of gastrin, G17 and G34. Third, the polyglutamic acid sequence of mammalian G17 is absent. When the biologically active C-terminal regions are aligned, the chicken peptide shares only 4 residues with porcine G34, and 7 with porcine CCK33 (fig.5). In mammals gastrins occur in sulphated and unsulphated forms, whereas CCK is always sulphated. It remains to be determined if the tyrosine residue in the chicken peptide may be substituted in this way; such a modification would not be expected to alter the potency for gastric acid secretion [16].

Thus, although the chicken antral peptide resembles CCK more than gastrin in its structure, it originates from a region that is considered to be the counterpart of the mammalian pyloric antrum [7], and has the biological properties of gastrin. The idea deserves consideration therefore that this peptide represents 'avian gastrin'.

There is evidence that birds have separate CCK-like molecules in brain and intestine [6,8] so the present finding has important implications for the evolutionary and structure-activity relationships of the gastrin/CCK family.

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