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Antral content, secretion and peripheral metabolism of N-terminal progastrin fragments

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Abstract

Objectives: In addition to the acid-stimulatory gastrins, progastrin also release N-terminal fragments. In order to examine the cellular content, secretion and peripheral metabolism of these fragments, we developed an immunoassay specific for the N-terminal sequence of human progastrin.
Results: The concentration of N-terminal progastrin fragments in human antral tissue was 6.7 nmol/g tissue ($n=5$), which was only half of that of acid-stimulatory gastrins (12 nmol/g tissue). Gel chromatography of antral extracts showed that the progastrin fragment 1–35 and 1–19 constitute the major part of the N-terminal progastrin fragments. The basal concentration of N-terminal fragments in normal human plasma was almost 30-fold higher than that of the amidated, acid-stimulatory gastrins (286 pmol/l versus 9.8 pmol/l, $n=26$, $P<0.001$). In contrast, the concentration of N-terminal fragments in hypergastrinemic plasma was only 2.7-fold higher than the concentration of amidated gastrins (540 pmol vs. 198 pmol/l, $P=0.02$). During meal stimulation, the plasma concentrations of N-terminal progastrin fragments and amidated gastrins increased in a correlated manner ($r=0.97$, $P=0.005$). The half life for progastrin 1–35 in circulation was 30 min, and a pig model revealed the kidneys and the vasculature to the head as the primary sites of degradation.
Conclusion: The cellular and circulatory concentration profiles of N-terminal progastrin fragments differ markedly from those of the acid-stimulatory gastrins. The high basal plasma concentrations of N-terminal progastrin fragments cannot be explained by differences in elimination.

Keywords: Elimination; Gastrin; Hypergastrinemia; Progastrin; Radioimmunoassay; Secretion

1. Introduction

Gastrin is the major regulator of gastric acid secretion and gastric mucosal growth [1] Gastrin is synthesized in antroduodenal G-cells by elaborate posttranslational processing into acid-stimulatory peptides of which gastrin-17 and gastrin-34 are the most abundant ([2–6], for review see Ref. [7]). The bioactive site of the gastrins is the evolutionary conserved C-terminal tetrapeptide amide, Trp–Met–Asp–Phe–NH_2. In addition to the acid stimulatory gastrins, progastrin also release other fragments. The N-terminal fragments have for instance been identified in extracts from gastrinoma tissue [8–10], and in normal human antral tissue [11]. Hence, in addition to the large amidated gastrin-71, fragments 1–35, 6–35, and 20–35 from the N-terminal part of progastrin were also present in G-cells with fragment 1–35 being the most abundant after purification [11].

So far, the biology of N-terminal progastrin fragments has not been examined. But recently, we reported that the N-terminal fragment 1–35 is not required for cellular sorting to the secretory pathway and subsequent regulated secretion of gastrins [12]. Other reports have suggested that the intact progastrin molecule in itself can stimulate colonic cell growth [13–17]. However, the active site within progastrin has not always been defined, which hampers a molecular interpretation of the observed effects. Furthermore, the normal and pathophysiological occurrence of the N-terminal progastrin derivatives is virtually unknown. In order to examine the secretion and metabolism of N-terminal progastrin fragments, we therefore developed a sensitive and specific radioimmunoassay for the N-terminus of human progastrin. Our results reveal a surprisingly different cellular and circulatory profile compared to gastrin.

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2. Methods and materials

2.1. Peptides

Human progastrin fragments 1–35, 1–19 and 6–35 were custom synthesized by Cambridge Research Biochemicals Ltd (Cheshire, UK). Also, progastrin 1–10 extended C-terminally with a tyrosyl residue and fragment 1–10 extended C-terminally with cysteine were synthesized for tracer and standard, and for immunization, respectively. The structure and identities of all synthetic peptides were verified by reversed-phase HPLC, amino acid analysis and mass spectrometry before use.

2.2. Radioimmunoassay

A radioimmunoassay directed against sequence 1–10 of human progastrin was developed using 10 mg of the 1–10 fragment extended C-terminally with cysteine and coupled to 20 mg bovine serum albumin using the *m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester conjugation method [18]. The coupled product was dissolved in 15 ml of distilled water (conjugate solution). The antigen solution (2 ml) was mixed with 2.5 ml isotonic saline and emulsified with an equal volume of complete Freund's adjuvant (The Danish Serum Institute, Copenhagen, Denmark) and used for the first immunization. For booster injections, 1 ml of the antigen solution was mixed with 4 ml of saline and an equal volume of incomplete Freund's adjuvant. Eight random-bred white Danish rabbits were immunized subcutaneously over the lower back at 8-week intervals. Twenty millimeter of blood was collected from an ear vein 14 days after each immunization, and the serum was stored at −20 °C for evaluation. For tracer use, the tyrosine-extended 1–10 fragment (4.5 nmol) was monoiodinated using the chloramine-T method as previously described [19] and purified on reversed-phase HPLC (Pierce C_8 column, 4.6×220 mm) eluted by a linear ethanol gradient (5–25%) in 1% trifluoroacetic acid. The gradient was selected to ensure separation of the non-labeled peptide from the iodinated tracer. The specific tracer radioactivity was determined by self-displacement [20]. For measurement of gastrins, we used a radioimmunoassay based on antiserum no. 2604, which is specific for the C-terminal octapeptide sequence of the α-amidated gastrins [21]. This assay measures all amidated gastrins with equimolar potency and the reactivity with the related hormone cholecystokinin is negligible.

2.3. Human antral tissue

Tissue biopsies of human antral mucosa ($n=5$) were obtained from patients undergoing surgery for pancreatic cancer (Whipple's operation). The use of human tissue was approved by the local ethics committee (KF01-352/96) and informed consent was obtained from all patients. The tissue biopsies were immediately frozen in liquid nitrogen and stored at −80 °C until extraction. While frozen, the tissues were sliced into small pieces and immersed in boiling water (10 ml/g tissue) for 20 min, homogenized and centrifuged at 10000 ×*g* for 30 min. The supernatant was stored and the pellet extracted in 0.5 mol/l acetic acid (10 ml/g tissue), homogenized and centrifuged as above.

2.4. Gel filtration chromatography

The molecular composition of N-terminal progastrin fragments in tissue was examined by gel chromatography (Sephadex G-50 Superfine, 10×1000 mm, Pharmacia, Sweden). Columns were calibrated with synthetic progastrin 1–35 and 1–19. A barbital buffer containing 0.1% bovine albumin was used as eluent at a flow rate of 4 ml/h (4 °C). The buffer was supplemented with 0.05 M NaCl to avoid protein binding. Void and total volumes were determined by eluting ^{125}I-albumin and ^{22}NaCl, respectively.

2.5. Plasma

Blood samples were collected from 26 healthy and fasting volunteers. Six of the volunteers were later served at protein-rich meal, and blood samples were obtained from a cubital vein at set time intervals (−35,−5, 0, 10, 20, 30, 40, 50, 70, 95 min). Plasma was stored at −20 °C until analysis. In addition, plasma from 48 patients with hypergastrinemia due to gastrinoma or achlorhydria was collected from the local gastrin analysis facility in our department.

2.6. Elimination of progastrin 1–35 in humans

Synthetic progastrin 1–35 (100 pmol/kg) was injected intravenously as a bolus into fasting, healthy subjects ($n=6$). Blood samples were collected from the opposite arm (0, 2, 4, 6, 8, 10, 15, 20, 25, 30, 40, 50 and 60 min after injection, and then every 15 min until 180 min from the time of bolus injection). After plasma measurement, the pharmacokinetics was analyzed according to a two-compartment open model: $C_t = Ae^{-\alpha t} + Be^{-\beta t}$, where A and B is the zero intercepts with the ordinate of the individual exponential terms and α and β are the slopes of the curves.

2.7. Organ extraction of progastrin 1–35 in pigs

To further elucidate the metabolic fate of progastrin 1–35, we used a previously described animal model [22]. Eight pigs (Danish landrace–Yorkshire breed, 30–40 kg) were anesthetized and intubated. Isotonic saline was infused at 10 ml/kg/h during the whole experiment, and donor blood from siblings was additionally infused in volumes equal to that removed during sampling. Cardiac output was measured regularly with a Swan–Ganz thermodilution catheter (Swan–Ganz pediatric 5F; Baxter Health Care, CA, USA). Polyethylene catheters for blood sampling were placed in the thoracic aorta via the left carotid artery and cephalically in the left internal jugular vein. Via the external jugular veins, two angiography catheters were introduced into a major hepatic vein and the left renal vein.