Gastrin in pituitary tumours

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Abstract. Twelve of 87 pituitary adenomas from patients with acromegaly, Cushing’s syndrome, Nelson’s syndrome, hyperprolactinaemia and without symptoms of hormone hypersecretion contained gastrin in concentrations from 0.5 to 166 pmol/l. Only ACTH-producing tumours contained gastrin, which occurred in forms smaller than those present in the normal adenohypophysis. The results indicate that corticotropic tumours may synthesize gastrin in moderate amounts.

Like other gut hormones, gastrin is synthesized also in extra-gastrointestinal tissues. Thus, the neonatal pancreas (Larsson et al. 1976), vagal neurons (Uvnäs-Wallensten et al. 1977), and the pituitary (Rehfeld 1978) produce gastrin. The neurohypophysis always contain gastrin, whereas there are considerable species variations in the adenohypophysal occurrence (Rehfeld et al. 1984). Normally, the human adenohypophysis contains only traces of gastrin.

In order to determine whether the sporadic adenohypophysal synthesis of gastrin might be of oncogenic significance, we have examined the occurrence and molecular nature of gastrin in 87 human pituitary tumours.

Material and Methods

The tumours were obtained by transsphenoidal microsurgery. Thirty-one of the adenomas were from patients with clinically typical acromegaly and elevated plasma levels of growth hormone non-suppressible by hyperglycaemia. Cushing’s syndrome was present in 15 patients with characteristic clinical presentation and elevated urinary excretion of cortisol. Five patients, in whom bilateral adrenalectomy had been performed 2–24 years prior to pituitary surgery, suffered from Nelson’s syndrome (Nelson et al. 1958; Lindholm et al. 1969). They all had high plasma concentrations of ACTH (162–4400 ng/l). Fifteen tumours were prolactinomas (prolactin concentrations in plasma > 200 µg/l). Finally, 21 tumours were apparently non-functioning adenomas operated upon because of suprasellar tumour extension and visual disturbances.

After removal the tumour tissue was immediately frozen on dry ice or in liquid nitrogen and stored at −80°C until extraction and analysis. Normal pituitaries (N = 10) were obtained 6–16 h post-mortem from patients without pituitary or other endocrine disorders. After removal the lobes were rapidly separated, frozen in liquid nitrogen and stored at −80°C until extraction and analysis.

Extraction

The frozen tissue was minced and immersed directly in boiling water (pH 6.6, 5 ml/g tissue). It was boiled for 20 min, homogenized and centrifuged. After decantation of the supernatants, the pellets were reextracted in 0.5 mol/l acetic acid for 20 min, homogenized and centrifuged. Both neutral and acid extracts were examined for gastrin by radioimmunoassays.

Radioimmunoassays

Two sequence-specific antisera were used. Antiserum No. 2604 is directed against the C-terminus of gastrin-17 and measures all bioactive forms of gastrin such as component 1, gastrin-34, and gastrin-17 (sulphated or non-sulphated) with equimolar potency (Rehfeld et al. 1972). The cross-reaction with cholecystokinin is negligible, i.e. below 0.1%. Antiserum No. 1295 (a kind gift...
from J. Walsh, UCLA, USA) is entirely specific for gastrin. It is directed against the N-terminus of gastrin-17, and measures gastrin-17, N-terminal fragments of gastrin-17, and the inactive forms extended beyond the C-terminus of gastrin-17 (Rehfeld 1980).

**Chromatography**

One ml of tissue extract was applied to Sephadex G-50 superfine columns (10 x 1000 mm). The columns were eluted at 4°C with 0.02 mol/l barbital buffer (pH 8.4), at a flow rate of 4 ml/h in fractions of 900 µl. The columns were calibrated with highly purified gastrin-34, gastrin-17, and gastrin-14 (kind gifts from R. Gregory, Liverpool, UK). The void and total volumes of the columns were determined with 125I-labelled albumin and 32NaCl, respectively. All fractions were assayed with the above-mentioned antisera. In order to expose the N-terminal sequence of gastrin-17 before measurement with antisem No. 1295, the fractions were incubated with equal volumes of 2 g trypsin/l in 0.05 mol/l sodium phosphate, pH 7.5, at 20°C for 30 min. The enzymatic reaction was terminated by boiling for 10 min.

**Results**

Only four of the normal pituitaries contained gastrin and only in concentrations below 2 pmol/g. Twelve adenomas contained gastrin. In two the concentrations were below the upper limit of that of normal adenohypophysal tissue. The highest concentration (166 pmol/g) was found in an adenoma from a patient with Nelson's syndrome. Another Nelson tumour and a Cushing tumour contained substantially increased amounts, whereas only moderate levels were found in six growth hormone-producing tumours and one Cushing adenoma (Fig. 1). Gel chromatography of the extracts (Fig. 2) showed that normal pituitary tissue (upper panel) contained almost exclusively gastrin-34, whereas the tumours contained both

**Fig. 1.**

Concentrations of gastrin in 4 of 10 normal human anterior pituitaries and 12 of 87 pituitary tumours. In the remaining normal pituitaries and tumours the concentrations of gastrin were below the detection limit of the assays.

**Fig. 2.**

Gel chromatography of boiling water extracts from a normal human anterior pituitary (upper panel) and from two pituitary tumours causing acromegaly (lower panel). One ml of extract was applied to Sephadex G-50 superfine columns (10 x 1000 mm). The fractions were assayed with antiserum No. 2604 directed against the C-terminus of gastrin-17. Synthetic human gastrin-17 was used as tracer and standard.
Gel antiserum trin-17 measured pituitary.

This causes the hormone mas fraction, resulting in gastrin. Fig.

ACTH-producing hormone ing products Co-synthesis D) c chromatography

Fig. 20 40

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Fig. 3.

Gastrin-17 (patient 1) or only gastrin-17 (patient 2). Tryptic cleavage of the eluted fractions, followed by radioimmunoassay with antiserum 1295 resulted in the pattern shown in Fig. 3, which corroborates the specificity of the gastrin measurements.

Discussion

This study has shown that some pituitary adenomas in addition to synthesis of a proper pituitary hormone also produce gastrin. We presume that the gastrins originate from the corticotrope, because gastrin immunoreactivity has been localized to corticotropes of normal anterior pituitaries (Larsson & Rehfeld 1981), and because only ACTH-producing tumours contained gastrin, i.e. Cushing and Nelson tumours and some growth hormone adenomas which also contained excessive amounts of ACTH. In these the ACTH concentrations were 0.05–1.45 nmol/g and in Cushing and Nelson tumours 3–1468 nmol/g.

Co-synthesis of gastrin with ACTH and other products of the proopiomelanocortin (POMC) gene has been observed in several normal tissues (Larsson 1977, 1981; Rehfeld & Larsson 1981; Rehfeld 1986). Usually the quantitative relationship has been inverse. Thus, when the concentration of POMC-gene products has been high, the production of gastrin-gene products has been low and vice versa. This relationship was also found in the present study, as the content of gastrin in corticotropic tumours was several fold below the concentrations of ACTH (3–1468 nmol/g). Considering this and the 10 000-fold higher concentration of gastrin in normal antral tissue, which is the predominant source of circulating gastrins, the pituitary production of gastrin will hardly contribute significantly to the circulating levels of gastrin in patients with acromegaly, Cushing’s and Nelson’s syndrome as demonstrated in the present study. Pre-operative serum concentrations of gastrin were not available, but there were no clinical evidence of hypergastrinaemia. In contrast, however, the co-expression of gastrin- and ACTH-peptides in pancreatic endocrine tumours often suffer from both Cushing’s and Zollinger-Ellison’s syndrome (Law et al. 1965; O’Neal et al. 1968; Larsson et al. 1975; Waldum et al. 1977; Joffé et al. 1978; Lamers et al. 1978; Maton et al. 1986). At present we know neither the factors that induce expression of both the gastrin- and the POMC-gene in the same cells, nor the factors that induce high synthesis of both gastrin and ACTH peptides in pancreatic tumours only.

Curiously, some Nelson-tumours also synthesize substantial amounts of cholecystokinin (CCK) (Rehfeld et al. 1987). CCK-peptides contain a sequence homologous to the C-terminus of the gastrins. Consequently, some gastrin radioimmunoassays measure also CCK-peptides. However, the determinations described in this study do not reflect cross-reaction with CCK-peptides for the following reasons. First, we have used specific gastrin radioimmunoassays. Second, there was no correlation between the concentrations of CCK and gastrin in the Nelson-tumours studied. Finally, the results of the chromatographic elutions before and after tryptic cleavage show that the peptides measured are gastrins (Figs. 2 and 3).

The chromatography demonstrated that the normal human anterior pituitary mainly contained gastrin-34 (Fig. 2), which also is the predominant form of gastrin in the porcine anterior pituitary (Rehfeld 1978; Rehfeld & Larsson 1981). In the corticotrophic tumours, gastrin-17 predomi-
nated (Figs. 2 and 3). Similar increased processing has also been observed for CCK, as large CCK-peptides were found in normal corticotropes, whereas Nelson-tumours synthesized only smaller forms (Rehfeld et al. 1987). Such altered processing in tumour cells emphasizes the necessity of hormone assays that measure precursor products irrespective of variations in the posttranslational processing pattern.

Acknowledgments

The measurements of ACTH were performed by Mogens Fenger, Rigshospitalet, Copenhagen. The skilful technical assistance of Mette Simons Hansen and Susanne Hummelgaard is gratefully acknowledged.

References


