Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 298–302, January 1990
Biochemistry

Progastrin expression in mammalian pancreas

(biosynthesis/gastrinoma/peptide hormone/radioimmunoassay)

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Communicated by Diter von Wettstein, August 31, 1989 (received for review May 11, 1989)

ABSTRACT **Expression and processing of progastrin were examined in fetal, neonatal, and adult pancreatic tissue from five mammalian species (cat, dog, man, pig, and rat). A library of sensitive, sequence-specific immunoassays for progastrin and its products was used to monitor extractions and chromatography before and after cleavage with processing-like enzymes. The results showed that progastrin and its products are expressed in the pancreas of all species in total concentrations varying from 0.3 to 58.9 pmol/g of tissue (medians). The degree of processing was age- and species-dependent. In comparison with adult pancreatic tissue the fetal or neonatal pancreas processed a higher fraction to bioactive, C-terminally amidated gastrin. Nevertheless, the pancreatic processing was always less complete than that of the adult antral mucosa. The moderate level of expression and the attenuated processing in the adult pancreas contribute to explain previous failures to detect gastrin in normal pancreatic tissue. Our results indicate that gastrin-producing tumors in the pancreas are not ectopic, but arise from cells that normally express the gastrin gene.**

Gastrin stimulates the secretion of gastric acid and growth of the fundic mucosa (1). Accordingly, gastrin-producing tumors (gastrinomas) induce hypersecretion of acid with ensuing duodenal ulcer disease and fundic mucosal hypertrophy, i.e., the Zollinger–Ellison syndrome. By far most gastrinomas originate in the pancreas, although gastrin normally is synthesized in the antroduodenal mucosa. Gastrinomas are nevertheless a frequent type of endocrine tumor in the human pancreas (2, 3) and have been found also in dogs (4, 5).

The pancreatic origin of gastrinomas has been an enigma. Insulinomas, glucagonomas, somatostatinomas, pancreatic-polypeptide tumors (PPomas), and vasoactive intestinal polypeptide tumors (VIPomas) all have cellular counterparts that express the corresponding hormone in the normal pancreas. In contrast, attempts to detect gastrin in the normal pancreas have so far either failed (6–10) or resulted in the misidentification of somatostatin cells as gastrin cells (11–13). The only reproducible exception has been the apparently transient gastrin synthesis in the fetal and neonatal rat pancreas (14–18).

Deduction of the sequence of mammalian preprogastrins (19–21) has paved the way for development of radioimmunoassays for progastrin and its processing intermediates (22–25). Such radioimmunoassays have proved useful for studies of gastrin biosynthesis in tissues known to express the gastrin gene (24–29). The observation of expression but attenuated processing of progastrin in the normal pituitary (29) suggested that progastrin might be expressed also in the normal pancreas, and hence explain the gastrinoma enigma (30). This hypothesis has now been examined.

NOMENCLATURE

Mammalian preprogastrins comprise 101–104 amino acid residues (19–21). They have an N-terminal signal sequence, a spacer sequence, the sequence containing the major bioactive forms of gastrin (i.e., gastrin-34 and gastrin-17), and finally a C-terminal flanking peptide. The decisive amino acid sequence during the biosynthetic maturation is shown in Fig. 1. In the present study we have distinguished three major categories of preprogastrin products: (*i*) *progastrins*, which are defined as products extended beyond glycine at the C terminus; (*ii*) *glycine-extended intermediates*, which are further processed progastrins that constitute the immediate precursors of the mature, bioactive gastrins; (*iii*) *amidated gastrins* (the bioactive gastrins), in which the C-terminal phenylalanine residue is amidated using glycine as the amide donor. Each category contains a number of peptides of different chain length. For instance, the amidated gastrins comprise component I, gastrin-34, and gastrin-17 [in both tyrosine-sulfated and nonsulfated forms (Fig. 1)].

MATERIALS AND METHODS

Tissue Sampling. Pancreatic tissue was collected from five mammalian species. First, an extensive collection was carried out in rats at ages that allowed us to follow the ontogenetic development. We also collected antral, duodenal, jejunal, and ileal rat tissues in order to compare pancreatic gastrin expression with that of the gastrointestinal tract. In addition, muscle tissue was sampled for control. Wistar rats were reared with their mothers in temperature- and light-controlled cages. They were allowed to suckle ad libitum and had free access to food and water. When 21 days old, the young rats were separated from their mothers to separate cages with free access to food and water. Rats aged 14, 21, 30, and 100 days were fasted overnight with free access to water. Pups 7 days old, pups 4 days old, and newborn rats were separated from their mothers on the morning of the dissection day. Fetuses at 19 days of gestation were obtained from anaesthetized animals by hysterotomy. Before dissection, the newborn rats were decapitated whereas the older rats were anaesthetized with pentobarbital (60 mg/kg, intraperitoneally). A midline incision was made and the pancreas, duodenum (whole duodenal wall), and gastric antrum (corresponding to the distal third of the stomach) were excised. The antrum and duodenum were then opened, and, after removal of the luminal content, the tissue was immediately frozen in liquid N_2. For each age, 20 rats were dissected, and the tissue was divided into five pools of four pieces each.

After the results of the rat study were obtained, we continued the collection of pancreatic tissue from newborn (i.e., <3 hr postpartum) or fetal and adult cats, mongrel dogs, and pigs in numbers shown in Table 1. The pancreas was removed from appropriately anaesthetized animals after midline incision and the tissues were immediately frozen in liquid N_2. Human pancreatic tissue was obtained from human fetuses in connection with abortion induced on sociomedical indications. The ages of the fetuses ranged from 15 to 23 weeks. Moreover, adult pancreatic tissue was obtained from human organ do-

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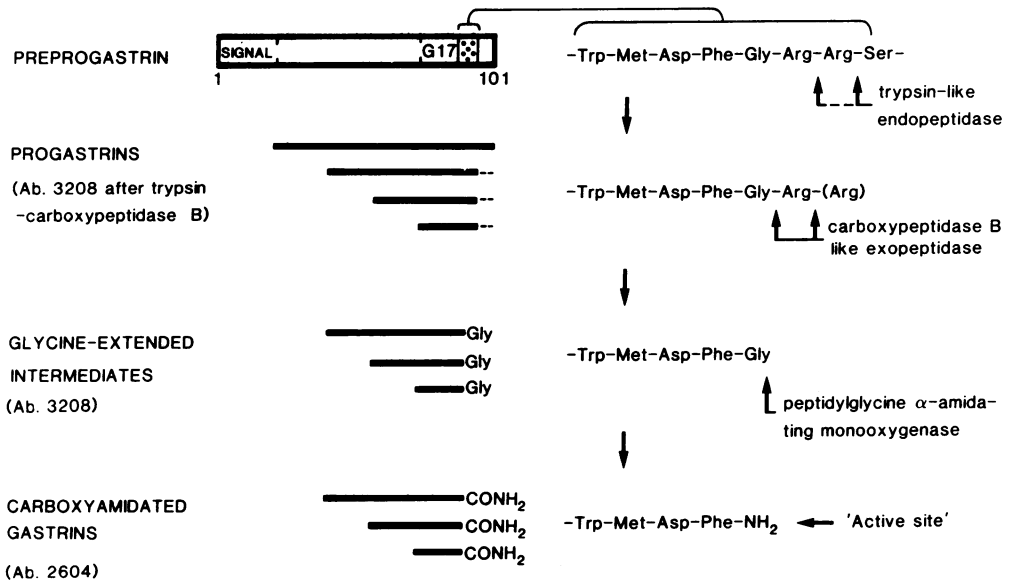

FIG. 1. Mammalian preprogastrin and core steps of its maturation. Bioactive gastrins require a C-terminal Trp-Met-Asp-Phe-NH_2 sequence, which can be obtained by cleavage with a trypsin-like endopeptidase, a carboxypeptidase B-like exopeptidase, and finally peptidylglycine α-amidating monooxygenase. The maturation process can be monitored, as indicated, by antibodies (Ab.) 2604 (which bind only C-terminally amidated gastrins), Ab. 3208 (which bind only glycine-extended gastrins), and by Ab. 3208 after trypsin and carboxypeptidase B cleavage (further C-terminally extended progastrins). G17, gastrin-17.

nors, from whom corpus and cauda pancreatis was used as transplant by the segmental duct-occlusion method, leaving parts of the caput pancreatis for our examination. The use of human tissue was approved by the local ethics committee.

Extraction of Tissue. While frozen the tissue was cut into pieces weighing a few milligrams, immersed in boiling water (5 ml/g of tissue, pH 6.5) for 20 min, homogenized, and centrifuged at 10,000 × *g* for 30 min. The supernatant was decanted (the neutral extract) and the pellet was reextracted by addition of 0.5 M CH_3COOH (5 ml/g of tissue), rehomogenized, left for 20 min at room temperature, and then centrifuged as above.

Radioimmunoassays. The extracts were assayed for progastrins, glycine-extended intermediates, and amidated gastrins (Fig. 1) by two types of radioimmunoassay. For measurement of glycine-extended gastrins we used antiserum 3208, which was raised in rabbit 3208 against a tetradecapeptide corresponding to sequence 5–17 of human gastrin-17 extended with glycine (22). Gastrin-(5–17)-Gly was monoiodinated by mild chloramine-T treatment and purified on AE-cellulose (21). Gastrin-(5–17)-Gly (custom-synthesized by Cambridge Research Biochemicals, Harston, U.K.) was also used as standard. Antiserum 3208 crossreacts <0.2% with amidated gastrin-17 (22). The antiserum recognizes gastrin-34-Gly with 80% the potency with which it recognizes gastrin-17-Gly (27). For measurement of amidated gastrins we used antiserum 2604, raised in rabbit 2604 against human gastrin-(2–17) (31). Human gastrin-17 was monoiodinated by a mild chloramine-T technique (32). For standard we used nonsulfated human gastrin-17 (Imperial Chemical Industries). Antiserum 2604 crossreacts <0.2% with gastrin-(5–17)-Gly compared to gastrin-17 (22). It binds sulfated and nonsulfated gastrin with equimolar potency. It also binds gastrin-34 and gastrin-17 with almost equimolar potency (33).

For human pancreatic tissue we also used antiserum 8017 in control measurements. Antiserum 8017 was raised against the N-terminal tridecapeptide sequence of human gastrin-17 and is specific for human gastrin (34). Used in combination with tryptic cleavage, it recognizes unprocessed or partly processed human progastrin (23).

The detection limits for measurement of progastrins, glycine-extended gastrins, and amidated gastrins in solution have been detailed elsewhere (22, 23, 31, 34). For measurement in tissue extracts, the detection limit is 0.1 pmol/g (wet weight) with our methods of extraction.

Enzymatic Treatment. Determination of gastrins extended beyond the C-terminal glycine residue [gastrin-Gly-Arg-Arg (-R)] was made by measurement of glycine-extended gastrins before and after sequential treatment of the extracts with trypsin (LS 0003741, Worthington) and carboxypeptidase B (batch 103 233, Boehringer Mannheim), as described (refs. 22 and 27 and Fig. 1). To evaluate the size of these precursors, fractions from gel filtration chromatography of undigested extract were assayed before and after treatment with trypsin and carboxypeptidase B (27).

Chromatography. Extracts were applied to 10 × 1000 mm columns of Sephadex G-50 superfine (Pharmacia) and eluted with 0.125 M NH_4HCO_3 at a flow rate of 4 ml/hr. Fractions of 1.0 ml were collected. The columns were calibrated with ^{125}I-labeled albumin and $^{22}NaCl$ for indication of void and total volume, respectively, with synthetic human gastrin-17, gastrin-17-Gly, and gastrin-34 (Cambridge Research Biochemicals), and with natural amidated gastrins and glycine-extended gastrins as well as progastrins from antral extracts of some of the corresponding animal species. To ensure identity of pancreatic gastrin, extracts of feline pancreatic and antral mucosal tissue were characterized by anion-exchange chromatography on calibrated 10 × 150 mm Whatman AE-41 cellulose columns, using a linear gradient from 0.05 to 0.20 M NH_4HCO_3 (pH 8.2, 300 ml). The flow rate was 33 ml/hr and fractions of 2.5 ml were collected. Finally, samples of feline pancreatic heptadecapeptide-like gastrin isolated first by gel filtration chromatography were characterized by reverse-phase HPLC on a calibrated 4 × 240 mm C_4 Vydac column, using a gradient from 0.1% CF_3COOH in water to 0.06% CF_3COOH in CH_3CN (as indicated in Fig. 5) at room temperature. The flow rate was 1 ml/min and fractions of 0.5 ml were collected.

RESULTS

In all species examined the pancreatic tissue contained progastrin, glycine-extended intermediates, and amidated (i.e., bioactive) gastrins (Table 1). Generally, the fetal and

Table 1. Progastrin, glycine-extended intermediates, and amidated gastrins in adult and fetal or neonatal pancreatic tissue

Species	Age	*n*	Median (range), pmol/g of tissue wet weight		
			Progastrin	Glycine-extended intermediates	Amidated gastrins
Cat	Neonatal	3	2.3 (2.0–2.8)	0.6 (0.6–0.8)	2.4 (1.6–3.6)
	Adult	11	1.2 (0.2–3.3)	0.7 (0.2–3.1)	0.6 (0.2–4.8)
Dog	Neonatal	8	1.5 (1.1–2.3)	0.3 (0.2–0.6)	0.8 (0.3–1.6)
	Adult	3	1.9 (1.2–4.2)	0.6 (0.3–1.2)	0.1 (<0.1–0.4)
Human	Fetal	6	<0.1 (<0.1–0.1)	<0.1 (<0.1–0.1)	0.3 (0.1–2.3)
	Adult	5	1.0 (0.2–6.2)	0.3 (0.1–0.6)	0.1 (<0.1–0.8)
Pig	Fetal	4	0.7 (0.5–0.8)	0.4 (0.1–0.8)	0.8 (0.1–1.8)
	Adult	5	2.1 (1.3–2.7)	0.5 (0.3–0.8)	0.1 (<0.1–0.1)
Rat	Fetal	20	43.9 (34–73)	2.0 (0.8–3.0)	13.0 (10–28)
	Adult	20	5.9 (2.1–7.8)	0.1 (<0.1–0.4)	<0.1 (<0.1–0.2)

neonatal rat pancreas contained the highest concentrations of the various gastrins (Table 1).

The adult rat pancreas expressed small but significant amounts of progastrin that was processed neither to glycine-extended intermediates nor to amidated gastrins (Figs. 2 and 3 and Table 1). Thus, in the rat pancreas progastrin expression and processing changes in a direction opposite to that in the antral mucosa, in which expression and processing to amidated bioactive gastrins increases drastically after birth (antral data are shown in ref. 28). Expression and processing in the rat duodenal mucosa was less characteristic, and generally the duodenal level of expression was low, whereas neither fetal, neonatal, nor adult jejunoileal tissue contained traces of preprogastrin products (data not shown).

The feline and canine pancreas contained progastrin and its amidated products in both the neonatal and the adult state. Generally, the expression of amidated gastrins was higher in the neonatal pancreas (Table 1).

The human and porcine pancreas contained small but significant amounts of progastrin and its amidated products in both the fetal and the adult state. In the fetal pancreas, progastrin was processed to bioactive, amidated gastrins to a higher extent than in the adult pancreas (Table 1). In the control tissue (muscle) neither progastrin nor gastrin peptides were detectable.

Gel filtration chromatography revealed a partial processing of the progastrin (Fig. 3). The degree of processing, first to the glycine-extended intermediates, and then to bioactive, amidated gastrins, varied considerably between species and with age (Table 1). For instance, while progastrin was poorly processed in the adult, rat, dog, and pig pancreas, progastrin was partly processed in the human and feline pancreas. There were also individual variations in the processing within the various species. In comparison with the processing in the main production site of gastrin in adults (the antral mucosa), the pancreatic processing was always incomplete (Table 2).

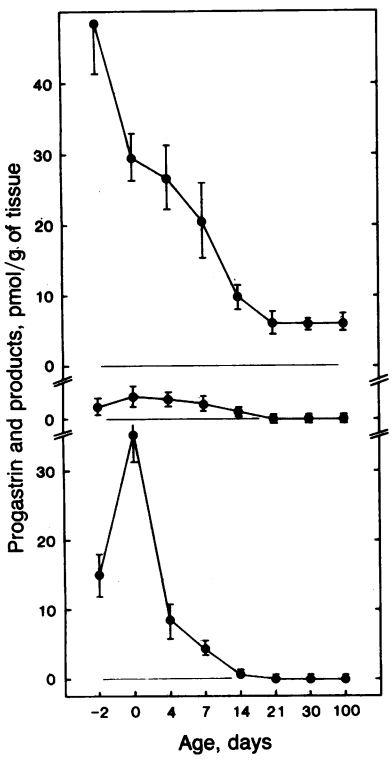

FIG. 2. Expression and processing of progastrin in the rat pancreas during ontogenesis. The preprogastrin products were measured as progastrins (*Top*), glycine-extended intermediates (*Middle*), and bioactive, amidated gastrins (*Bottom*).

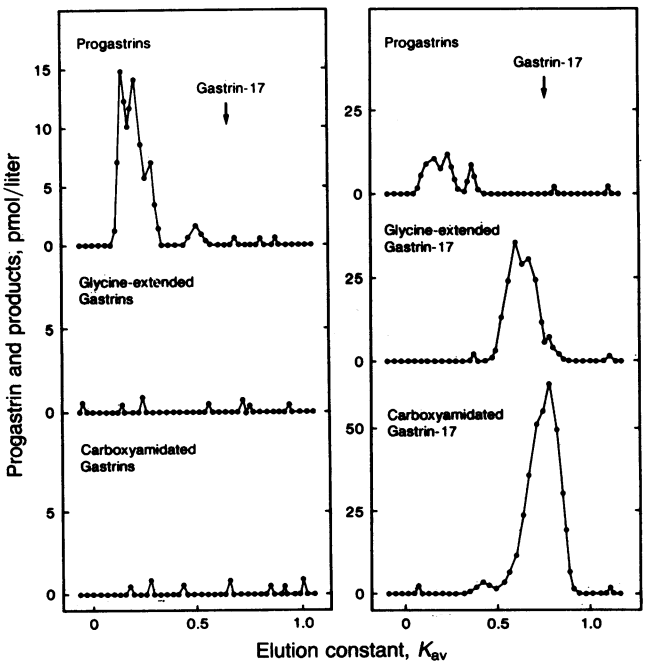

FIG. 3. Gel filtration chromatography of progastrin and its products in extracts of adult pancreatic tissue from rat (*Left*) and human (*Right*). One or two milliliters of neutral water extracts was applied to a Sephadex G-50 superfine column (10 × 1000 mm) and eluted by 0.125 M NH_4HCO_3 (pH 8.2). The fractions were dried, reconstituted in 0.02 M sodium barbital buffer (pH 8.4), and analyzed for progastrin, glycine-extended gastrins, and amidated gastrins as described in the text and in the legend to Fig. 1.

Table 2. Progastrin, glycine-extended intermediates, and amidated gastrins in adult pancreatic and antral mucosal tissue

Species	Tissue	*n*	Median (range), pmol/g of tissue wet weight			Amidated gastrins, % of total*
			Progastrin	Glycine-extended intermediates	Amidated gastrins	
Human	Pancreas	5	1.0 (0.2–6.2)	0.3 (0.1–0.6)	0.1 (<0.1–0.8)	7.3
	Antrum	11	130 (37–281)	39 (7–80)	2500 (580–5700)	93.7
Pig	Pancreas	5	2.1 (1.3–2.7)	0.5 (0.3–0.8)	0.1 (<0.1–0.1)	3.7
	Antrum	7	160 (66–297)	152 (93–446)	3400 (1300–6100)	91.6
Rat	Pancreas	20	5.9 (2.1–7.8)	0.1 (<0.1–0.4)	<0.1 (<0.1–0.2)	<1.5
	Antrum	20	53 (22–108)	112 (94–319)	1240 (1000–2200)	88.3

*Percent of total progastrin product.

The identity of the bioactive pancreatic gastrin as a heptadecapeptide corresponding to sulfated gastrin-17 from antral tissue was ensured by identical elution in three different chromatographic systems fractionating according to size, charge, and hydrophobicity (Figs. 3–5).

DISCUSSION

This study has shown that the normal mammalian pancreas expresses the gastrin gene at peptide level. Since expression was detectable in all the five species examined, it is apparently a general phenomenon.

Except for fetal and neonatal rats (Fig. 2), the pancreatic expression is lower than that of the antrum (Fig. 2 and Table 2). It is, however, possible that a low level of expression has physiological significance. Hence, the concentration of locally released gastrin may be sufficient to affect the growth of neighboring pancreatic cells, as illustrated by the islet cell hyperplasia (35–37) and hyperinsulinemia (38) accompanying hypergastrinemia in man. In this context, the complete sulfation of pancreatic gastrin (refs. 16 and 39 and Fig. 4) seems expedient, since the pancreozymic effect of gastrin is increased by sulfation (40, 41). In fetal and neonatal pancreatic tissues of all five species, progastrin was processed to amidated bioactive gastrin to a higher degree than in adult pancreatic tissue (Table 1). This observation supports the idea of a trophic role of pancreatic gastrin in the early stages of development.

In the neonatal rat pancreas, it has been shown that the gastrin gene is expressed in cells having an ultrastructural similarity to the antral gastrin cells (14). The pancreatic gastrin cells are disseminated in both exocrine tissue and islets (14, 42, 43). Such distribution also supports the hypothesis of local, paracrine effects of pancreatic gastrin. Early suggestions about gastrin synthesis in the delta cells of the pancreatic islets (11, 13) have not been confirmed with highly specific antisera (8, 9, 14). Instead, it is now well known that the delta cells produce somatostatin (2, 37). In the adult cat, dog, and pig, a few gastrin cells of a structure similar to those of the neonatal rat pancreas have been seen scattered in the exocrine pancreas (ref. 44 and F. Sundler, personal communication). In the human pancreas, the cellular origin of progastrin and gastrin remains to be determined. On balance, however, it looks as if the gastrin gene is expressed in a few endocrine cells throughout the mammalian pancreas, perhaps including a subfraction of the beta cells (45).

Due to the preferential occurrence of gastrinomas in the pancreas, much attention has been given to the possible expression of gastrin in the normal pancreas. However, previous studies have either been unable to detect gastrin in the adult pancreas (6–10, 14–17) or (as mentioned) mistakenly suggested that somatostatin cells produce gastrin (11, 13). There are several factors that may explain the discrep-

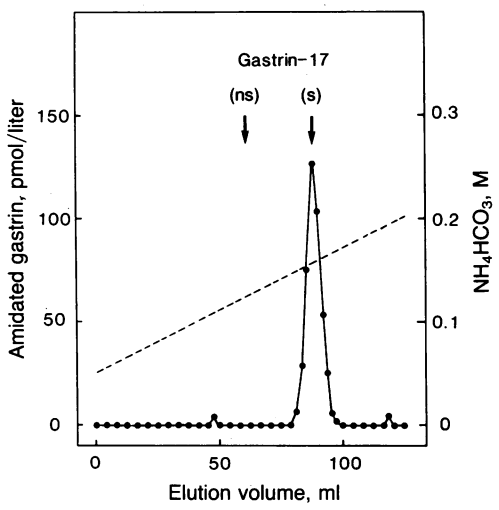

FIG. 4. Ion-exchange chromatography of amidated gastrins from feline pancreatic tissue. A pooled sample of heptadecapeptide gastrin isolated first by gel filtration chromatography was applied to a calibrated AE-41 cellulose column (10 × 150 mm) and eluted at 20°C in fractions of 2.5 ml with a gradient (---) from 0.05 to 0.2 M NH_4HCO_3. Elution was monitored by a radioimmunoassay specific for amidated gastrin (●) using antiserum 2604, which binds sulfated (s) and nonsulfated (ns) gastrins equally.

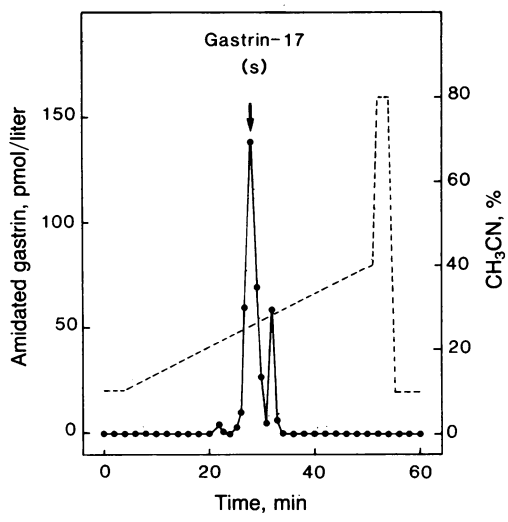

FIG. 5. Reverse-phase HPLC of amidated gastrins from feline pancreatic tissue. A pooled sample of heptadecapeptide gastrin isolated first by gel filtration chromatography was applied to a calibrated C_4 Vydac column (4 × 240 mm) and eluted at 20°C in fractions of 0.5 ml (per 0.5 min) with a gradient (---) from 0.1% CF_3COOH in water to 0.06% CF_3COOH in CH_3CN. After the fractions were dried and reconstituted in the initial volume of assay buffer, elution was monitored by radioimmunoassay (●) as in Fig. 4.

ancy between earlier results (6–10) and the present data. First, the state of the tissue and the methods of extraction are crucial. Autopsy material cannot be used, because postmortem degradation is extensive in pancreas. Since the pancreas is so rich in proteolytic enzymes, complete denaturation of the enzymes during extraction is also necessary. On the other hand, acid/ethanol, used to extract insulin, glucagon, and somatostatin, cannot be used for pancreatic gastrins, which dissolve poorly in ethanol. Instead, careful boiling in water and homogenization are essential.

Second, the sensitivity and specificity of the assays used to detect gastrin expression are crucial. Gastrin bioassays (6), mRNA detection methods such as Northern and dot blots (17, 45, 46), and some radioimmunoassays are too insensitive to detect the low levels of expression in all but the neonatal rat pancreas (17). Moreover, polyclonal gastrin antisera raised against antral extracts contain somatostatin antibodies that may give misleading immunocytochemical results (11, 13). Finally, the fact that much of the expressed polypeptide is progastrin, especially in the adult pancreas, explains why previously used bioassays and radioimmunoassays are less useful, since they detect only the mature, amidated gastrins, which are present in only minute amounts. Thus, only sensitive progastrin assays (22, 23) can detect the expression in adult rat, pig, dog, and human pancreas (Table 1).

Incomplete, attenuated processing of progastrin has also been found in normal pituitary corticotrophs (29). We do not know the biological significance of such expression (47). Silent expression may, however, reach significance by transformation of normal cells to tumor cells in which prohormones are processed to mature, bioactive peptides (48). Hence, the present results suggest that the expression of gastrin in pancreatic gastrinomas does not develop by ectopic dedifferentiation of transcription mechanisms, but rather by acceleration of already existing translational and posttranslational processing mechanisms.

The skillful technical assistance of Susanne Hummelgaard, Alice Lieth, and Bente Rotbøll is gratefully acknowledged. We thank the head of faculty animal house, Dr. H. J. Skovgaard, and his staff for expert assistance. The study was supported by grants from the Danish Medical Research Council, the Danish Cancer Union, and the Einar Willumsen Foundation.

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