Complete tyrosine *O*-sulfation of gastrin in adult and neonatal cat pancreas

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We have found gastrin in both the adult and neonatal cat pancreas. In contrast with the main production sites, antrum and duodenum, gastrin in the pancreas occurs in a single molecular form, tyrosine *O*-sulfated gastrin-17. Since tyrosine sulfation increases the pancreozymic effect of gastrin, the complete sulfation seems functionally expedient.

Cholecystokinin *Gastrin* *Ontogeny* *Tyrosine* *Sulfation*

1. INTRODUCTION

Tyrosine *O*-sulfation has recently attracted interest as a widespread post-translational modification of secreted proteins [1–6]. Also the hormones cholecystokinin (CCK) and gastrin are tyrosine *O*-sulfated [7,8]. While this sulfation does not affect targets like gastric parietal cells and central neurons [9–12], it dramatically increases the effect on pancreatic secretion and gallbladder contraction [13–15].

In contrast to CCK, only half of the cerebral and gastrointestinal gastrin peptides are sulfated [7,11,16,17]. Recently, however, we found that the transiently occurring gastrin in fetal human jejunum and neonatal rat pancreas is completely *O*-sulfated [18,19].

We now report that the feline pancreas in both neonatal and adult state contains gastrin. In contrast with other tissues and species, however, the feline pancreatic gastrin occurs in a single molecular form, completely tyrosine *O*-sulfated gastrin-17.

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2. MATERIALS AND METHODS

Pancreatic, antral, and duodenal mucosal tissue from adult ($n = 6$) and neonatal ($n = 6$) cats as well as from 3 adult dogs were sampled immediately postmortem and frozen in liquid nitrogen. Tissue specimens were collected from the head, body and tail of the adult pancreas, while whole pancreas was extracted from the neonatals. The frozen tissue was cut in pieces weighing a few milligrams and immersed into boiling water (0.1 g/ml, pH 6.6). After boiling for 20 min, the tissue was homogenized, centrifuged at 4°C and the supernatants, which contain all the gastrins, were stored at −20°C until assay.

The occurrence and degree of tyrosine *O*-sulfation of gastrin peptides were determined in the following way: First, gastrins in the extracts were measured by radioimmunoassay using antiserum no.2604, that binds tyrosine *O*-sulfated and non-sulfated gastrins equally [20]. Monoiodinated gastrin-17 [22] was used as tracer. Next, sulfated and non-sulfated gastrins were separated by ion-exchange chromatography using AE-41 cellulose columns (1 × 15 cm) eluted at 20°C with a linear 0.05–0.2 M NH_4HCO_3 gradient as

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described [16,22]. The fractions were assayed using both antiserum nos 2604 and 2605. Finally, tyrosine *O*-sulfation of the gastrins was examined by shift of the chromatographic elution and antibody binding after treatment with arylsulfatase. Abalone entrail arylsulfatase (Sigma, lot 64 F-9560, 0.8 mg in 400 μl of 0.2 M sodium acetate, pH 5.0) was added to a 200 μl sample (extract or chromatographic fraction) and incubated at 37°C for 3 h. After inactivation of the sulfatase by boiling for 2 min, the mixture was applied to AE-41 cellulose chromatography.

3. RESULTS AND DISCUSSION

Pancreatic tissue from both adult and neonatal cats contained significant amounts of gastrin, while the canine pancreas was devoid of gastrin. The concentrations in the pancreatic head, body and tail of the adult cat were 4.4 ± 2.2, 1.9 ± 1.2 and 0 pmol/g (mean ± SE), respectively, while the neonatal pancreas contained 6.5 ± 1.6 pmol/g. As shown by the reactivity with antiserum nos 2604 and 2605, and its elution position by ion-exchange chromatography before (fig.1A) and after incubation with arylsulfatase (fig.1B), all the pancreatic gastrin was tyrosine *O*-sulfated gastrin-17. In contrast, antro-duodenal gastrin was highly heterogeneous, consisting of gastrin-34, -17, and -14 in both sulfated and non-sulfated forms (fig.1C and D).

Apart from the neonatal rat [19,23,24], the neonatal and adult cat (this study), gastrin does not occur in normal pancreatic tissue, either from adult and fetal man [18,25], pig [26], dog (this study) or guinea pig [27]. However, when gastrin does occur, it is processed to completely sulfated small molecular forms ([19]; this study), a pattern that is unique for gastrin producing tissues. Thus, in the main production site, the G-cells of the gastro-duodenal mucosa, approximately half of the gastrins are unsulfated ([17,18]; fig.1). So far, the cat is the only species in which the normal adult pancreas synthesizes gastrin. Since tyrosine sulfation increases the effect of gastrin on enzyme secretion from the pancreas [13–15], it is likely that the complete sulfation has a functional significance. Thus, pancreatic gastrin may in the neonatal and adult cat contribute to the regulation of enzyme secretion in a paracrine manner. Such a paracrine

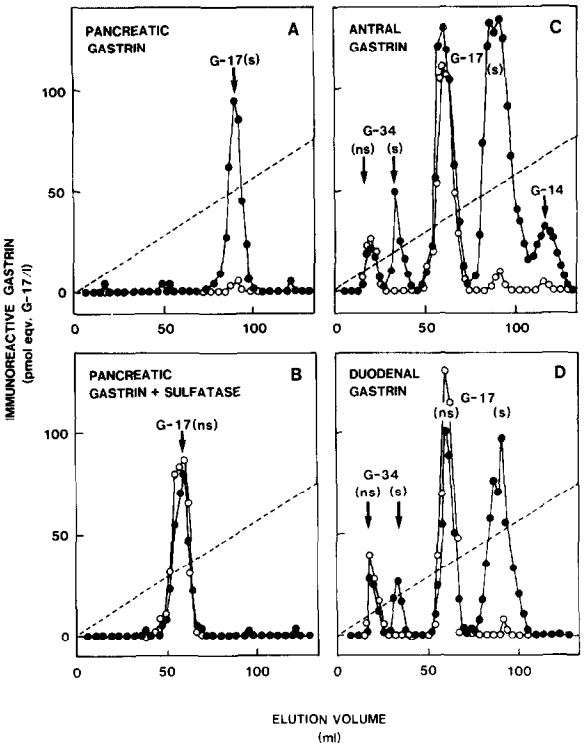

Fig.1. Ion-exchange chromatography of extracts from feline neonatal pancreas (before (A) and after (B) incubation with arylsulfatase), antral (C) and duodenal mucosa (D). Diluted samples of the extracts were applied to AE-41 cellulose columns (1 × 15 cm) eluted at 20°C in fractions of 2.5 ml with a linear 0.05–0.2 M NH_4HCO_3 gradient (– – –). The elutions were monitored with 2 gastrin radioimmunoassays using antiserum no.2604, which binds sulfated and non-sulfated gastrins with equimolar potency (●——●), and antiserum no.2605, that binds only non-sulfated gastrins (○——○). Extracts from feline adult tissue eluted in the same pattern as that of the extracts from corresponding neonatal tissue shown above.

role is supported by the observation that the gastrin cells are scattered among exocrine pancreatic cells [23,26] and not collected in the islets of Langerhans.

Expression of the gastrin gene [28] in pancreatic tissue has attracted much interest, since more than 95% of all gastrin-synthesizing tumors (gastrinomas) originate in the pancreas [29]. In this context, the complete tyrosine sulfation of gastrin from the pancreas may be of particular significance. Thus, Lin and Lipmann [5] recently found a drastic

decrease in the degree of tyrosine *O*-sulfation of proteins secreted from cells transformed with Rous or Fujinami sarcoma virus. It is consequently possible that the degree of sulfation in gastrinoma secreted gastrin may be a marker of malignancy.

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