Pancreatic hypertrophy with acinar cell nodules after longterm fundectomy in the rat

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Abstract

The effect of gastric fundectomy with hypergastrinaemia on the pancreas in rats was studied for 14 months. Rats with hypercholecystokinaemia that had had a pancreaticobiliary diversion (PBD) operation and sham operated rats served as controls. Fundectomised rats showed a significant increase in pancreatic weight and total DNA and protein content compared with sham operated rats. DNA flow cytometry showed a significantly higher ratio of tetraploid to diploid nuclei in pancreatic tissue after fundectomy than after sham operation. Mean values of all these variables were significantly lower after fundectomy than after PBD. Acidophilic atypical acinar cell foci of the pancreas were diagnosed in both fundectomised and PBD operated rats, but not in sham operated controls. The volume density and ³H-thymidine labelling index of the acidophilic atypical acinar cell foci were significantly lower after fundectomy than after PBD. Changes consistent with pancreatic adenoma were diagnosed in the PBD group only. In conclusion, fundectomy lasting about half of the life span in rats causes pancreatic hyperplasia and hypertrophy, as well as development of acidophilic atypical acinar cell foci. Although hypergastrinaemia is a prominent feature, it may not be the only factor responsible for this pancreaticotrophical effect of fundectomy.

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Gastrin-17 and pentagastrin given exogenously, as well as surgical procedures leading to endogenous hypergastrinaemia, have been reported to stimulate growth of the exocrine pancreas in mice and rats.¹⁻⁶ Gastrin receptors have been found in guinea pig pancreatic acini⁷ and in an azaserine induced rat carcinoma cell line.⁸ It has been shown in man that gastrin-17 augments pancreatic enzyme secretion at doses that are below the maximum for gastric acid secretion.⁹ These findings show that gastrin, under certain circumstances, may stimulate growth and function of the exocrine pancreas. There are other studies, however, showing that neither potent acid secretion inhibition with hypergastrinaemia in different rodents,^{10,11} nor gastrin-17 infusion in rats,¹² causes pancreatic growth.

In rats, resection of the oxyntic gland area of the stomach (fundectomy) induces endogenous hypergastrinaemia.^{13,14} This model has been used to evaluate the effects of chronic hypergastrinaemia on digestive organs, including the pancreas. In one study, fundectomy for 10 weeks did not induce pancreatic hypertrophy.¹⁴ The effect of fundectomy for longer than 10 weeks on the

pancreas is not known. The purpose of this study was to investigate this aspect using simultaneous measurements of morphology, DNA ploidy, and autoradiography.

Pancreaticobiliary diversion (PBD) in the rat and hamster induces persistent hypercholecystokinaemia with pancreatic hypertrophy.¹⁵⁻¹⁷ This trophic effect can be prevented by the simultaneous administration of a cholecystokinin (CCK) receptor antagonist namely L-364,718.^{18,19} In rats, longterm PBD leads to the development of hyperplastic acinar cell nodules and adenoma in the pancreas.^{20,21} We therefore decided to include this model as a positive control in this study.

Methods

ANIMALS AND STUDY DESIGN

The study was approved by the local animal welfare committee. Forty two male eight week old Wistar rats (Bantin and Kingman, United Kingdom) with a mean body weight (SD) of 268 (21) g were used. The animals were kept at 20°C, 50% humidity and a light/dark cycle of 12/12 hours. They had free access to standard rat food pellets (EWOS, Sweden) and tap water. Rats were divided into three groups. One group (n=14) was operated on with resection of the oxyntic gland area of the stomach (fundectomy). The vagal trunks were preserved, and gastric function was restored by anastomosis between the antrum and the non-oxyntic rumen of the proximal stomach.¹⁴ Another group (n=16) had PBD¹⁶ by transposing, at most, 7 mm of duodenum, including the pancreaticobiliary ducts and papilla, to the middle of the small intestine. The third group (n=12) was sham operated with small intestinal transection and gastrotomy in the oxyntic gland area. Ketamine hydrochloride (Ketalar, Parke-Davis, United Kingdom) and xylazine chloride (Rompun, Bayer, Germany) given intraperitoneally were used for general anaesthesia. Animals were fasted for 15 hours before the operation. Postoperative fasting lasted 24 hours during which the animals received two subcutaneous injections of 8 ml 0.9% saline. There was no early postoperative death, but one fundectomised rat died 10 months after the operation.

At 14 months, all rats were killed by exsanguination under general anaesthesia and after fasting for 15 hours. One hour before being killed, each rat received ³H-thymidine through the internal jugular vein (specific activity 20.0 Ci/mmol, Du Pont de Nemours GmbH, Germany) in a dose of 1 µCi/g body weight. To rule out the possible influence of intraperitoneal sequelae of the operation, only animals with no or slight

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adhesions in the area of the operation were included. This resulted in 10 fundectomised, 13 PBD operated, and 10 sham operated rats for further studies. The entire pancreas was removed, trimmed of fat, and weighed. The presence of macroscopic nodules was noted and their diameter recorded. The pancreatic tail was removed and fixed in formalin for histological studies. A piece of tissue adjacent to the pancreatic tail was excised and kept in citrate buffer at -70°C for flow cytometry.²² The rest of the gland was quick frozen and stored at -70°C for DNA and protein analysis. Blood samples of the fasted state of the rats were taken at the time when the animals were killed for measurement of gastrin and CCK in plasma and were collected in EDTA tubes from the internal jugular vein. Plasma was stored at -20°C until analysed.

DNA AND PROTEIN ANALYSIS

Pancreatic tissue DNA and protein contents were analysed according to the methods described by Labarca and Paigen²³ and Lowry *et al.*,²⁴ respectively.

Flow cytometry was performed blindly. The tissue was thawed and dissected with a pair of scissors at room temperature. For preparation of nuclei suspension, a detergent trypsin procedure specified by Vindeløv *et al.*²² was used. The trypsinised nuclei, which were stabilised by spermine tetrahydrochloride, were stained with propidium iodide after filtration. A FACscan flow cytometer (Becton Dickinson) and a 15 mW argon laser (488 nm) were used to estimate DNA ploidy and S phase fraction. Histograms with at least 15 000 events were recorded. Chicken and trout red blood cells were used as internal reference standards.²⁵ S phase was calculated using a rectangular distribution.²⁶ The number of channels between the G₀/G₁ and G₂/M peak were multiplied by the mean number of cells in the channels interactively selected in the S phase region of the histogram.

AUTORADIOGRAPHY AND HISTOLOGICAL ANALYSIS

For autoradiographical and morphological studies, tissue specimens fixed in 4% buffered formalin were embedded in plastic (JB4 Kit, Polaron Equipment, Watford, United Kingdom) and cut in 2 μm thick sections. These were coated with Kodak NTB₂ emulsion (Eastman Kodak, NY, USA), developed after four weeks of incubation in darkness at 4°C , and counterstained with haematoxylin and eosin. Atypical acinar cell foci were identified histologically and

classified as acidophilic or basophilic according to established criteria.²⁷ Identification of pancreatic adenoma or carcinoma was also made according to previously described criteria.^{28, 29} Sections from the pancreatic tail were screened for such lesions.

The volume density (%) of atypical acinar cell foci was determined blindly with a modified point counting method,³⁰ using a magnification of $\times 400$ and 25 μm between the points. In each animal, a total of 30 000 points were counted over consecutive visual fields across the tissue sections. The ³H-thymidine labelling index of the atypical acinar cell foci was determined blindly on autoradiographed sections with a magnification of $\times 1000$.²⁷ Five or more grains overlying a nucleus were considered a significant labelling. In each animal, a total of 200 labelled and non-labelled acinar cell nuclei within the foci were counted in consecutive visual fields. Labelling index was expressed as the number of labelled cells per 1000 cells. The diameter of the foci was determined microscopically, taking the mean of at least two measurements.

PLASMA GASTRIN AND CHOLECYSTOKININ ASSAY

The concentrations of gastrin in plasma were measured by a specific radioimmunoassay, as previously described.^{31, 32} The concentrations of CCK in plasma were measured by a radioimmunoassay using C-terminal directed antiserum samples without cross reactivity towards gastrin.³³ The assays have been described in detail elsewhere.^{34, 35}

STATISTICAL ANALYSIS

Results are expressed as mean (SEM). Two tailed Student's *t* test as well as the Mann-Whitney U test were used. Differences were considered significant when $p < 0.05$ in both tests. *p* Values are those derived from the *t* test unless otherwise stated.

Results

BODY WEIGHT AND PLASMA GASTRIN AND CHOLECYSTOKININ

The animals seemed healthy during the experiment. At the time of death, the mean body weight of the fundectomised rats was 80% of that in the sham operated rats, while the corresponding figure in PBD operated rats was 96% (Table I). Figure 1 shows the fasting plasma concentrations of gastrin and CCK at the time of death.

TABLE I Mean (SEM) of the body weight (BW), pancreatic weight (PW), ratio of pancreatic weight to body weight (PW/BW), total pancreatic protein, total pancreatic DNA, and the ratio of tetraploid to diploid nuclei (T/D) in pancreatic tissue 14 months after fundectomy, pancreatobiliary diversion (PBD), and sham operation (control) in the rat.

Groups	Animals	BW (g)	PW (mg)	PW/BW (mg/g)	Protein (mg)	DNA (mg)	T/D
Control	10	591 (22)	1147 (42)	2.0 (0.1)	137 (5)	6.7 (0.2)	0.108 (0.016)†
Fundectomy	10	470 (32)**	1505 (46)***	3.3 (0.2)***	202 (10)***	12.2 (0.7)***	0.212 (0.038)*
PBD	13	583 (16) ^{NS}	2774 (126)***	4.8 (1.2)***	355 (20)***	16.9 (1.3)***	0.360 (0.049)**

NS=not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ when compared with the controls and according to two tailed Student's *t* test.
†No of rats=7.

Figure 1: Mean and SEM of the fasting plasma gastrin and cholecystikinin (CCK) concentrations in groups of sham operated (C) ($n=9$), fundectomised (F) ($n=10$), and pancreaticobiliary division operated (PBD) ($n=12$) rats 14 months after the operation. (p Values are from two-tailed Student's t test, NS=not significant).

PANCREATIC WEIGHT, PROTEIN, AND DNA CONTENT

The mean pancreatic weight was increased by 31% in the fundectomy group and by 142% in the PBD group, compared with the sham operated group (Table I). Correspondingly, the relative pancreatic weight (mg pancreas per g body weight) was increased by 65% after fundectomy and by 140% after PBD. The mean total pancreatic content of protein and DNA was increased by 47% and 82%, respectively, in the fundectomy group, and by 159% and 152%, respectively, in the PBD group (Table I). All these increases were statistically significant, as were the differences between fundectomised and PBD operated animals ($p<0.001$, $p<0.001$, $p<0.001$, $p<0.005$, respectively).

DNA PLOIDY

There was a significant increase in the ratio of tetraploid to diploid nuclei in pancreatic tissue in both fundectomised and PBD operated animals, when compared with the sham operated controls (Table I). The ratios were higher in the PBD group than in the fundectomy group ($p<0.04$). The fraction of cells in S phase did not differ significantly between the groups. Figure 2 shows representative DNA flow cytometry histograms.

MACROSCOPICAL AND MICROSCOPICAL FINDINGS

Multiple nodules with a maximum diameter of 1 mm were seen on macroscopical examination on the surface of two of 10 pancreases in the fundectomy group, and on four of 13 pancreases in the PBD group. Nodules with a maximum diameter of 3 mm were seen on three of the 13 pancreases in the PBD group only. No nodules were seen in the sham operated animals.

Acidophilic atypical acinar cell foci were diagnosed by microscopical examination in four (40%) of 10 animals in the fundectomy group and in 11 (85%) of 13 animals in the PBD group (Fig 3). No foci were seen in the sham operated

animals. The volume density of the acidophilic atypical acinar cell foci was significantly lower in the fundectomy group than in the PBD group (Table II). There was a similar difference in the mean diameter of the acidophilic atypical acinar cell foci. Furthermore, ^3H -thymidine labelling index of the acidophilic atypical acinar cell foci was significantly lower after fundectomy than after PBD (Table II). Some nodules with a diameter of 4–6 mm in three of 13 pancreases in the PBD group, but none of those in the fundectomy group, were surrounded by a fibrous capsule and maintained an acinar cell differentiation suggesting adenomatous change (Fig 4). Basophilic atypical acinar cell foci and carcinomas were not found.

Discussion

In agreement with others,^{20,21} we found that PBD induces chronic endogenous hypercholecystikinaemia with pancreatic hyperplasia and hypertrophy. These changes persisted after 14 months at which time acidophilic atypical acinar cell foci and changes consistent with adenoma were also present. Fundectomy caused a persist-

Figure 2: Flow cytometry DNA histograms showing the proportion of tetraploid to diploid nuclei (T/D) in pancreatic tissue from a sham operated (A), a fundectomised (B), and a PBD operated rat (C). The two peaks with the lowest DNA content represent chicken and trout red blood cells (CRBC and TRBC, respectively).

Figure 3: Autoradiographed pancreatic section from a fundectomized rat showing acidophilic atypical acinar cell focus (aACF). The cells are large, arranged as acini, and contain a big oval to round nucleus and an intensely eosinophilic granular cytoplasm. The focus is clearly defined from the adjacent pancreas and is not encapsulated. Normal pancreas (left) and aACF (right). (Haematoxylin and eosin, original magnification $\times 400$).

ent endogenous hypergastrinaemia with a significant, although less pronounced, increase in pancreatic weight, total DNA and protein content, and development of acidophilic atypical acinar cell foci. The finding of a more pronounced trophic response and more advanced morphological changes after PBD than after fundectomy was paralleled by a significant increase in the ratio of tetraploid to diploid nuclei in pancreatic tissue.

In a previous study, fundectomy did not result in pancreatic hypertrophy.¹⁴ Some factors should be considered to find an explanation for the difference between that study and this one. It was recently shown that the trophic response in rat pancreas is inversely related to the age of the animals at the onset of stimulation.³⁶ In this aspect there seems to be no difference between the studies. The difference between the study periods, however, was a maximum of 10 weeks in the previous study compared with 14 months in this study. This would correspond to several decades in man. Furthermore, although pancreatic weight, DNA, RNA, and protein contents were analysed in the previous study, results of simultaneous investigations of morphology, morphometry, autoradiography, and DNA flow cytometry were not included. Using a positive control group with PBD, as in this study, was also useful for comparative purposes and in the interpretation of morphological changes in the fundectomy group.

A recent study in rats, which considered effects of gastric resection, showed that pronounced duodenogastric reflux (induced by a split gastrojejunostomy) was associated with

hyperplasia and adenoma development in the pancreas after 14 months.³⁷ Whether these findings were a result of stagnation of secretions with increased CCK release in the afferent jejunal loop, a changed alkaline gastric content, or both is unclear. There is, however, no reason to believe that fundectomy is associated with increased duodenogastric reflux.

The occurrence of acidophilic atypical acinar cell foci after longstanding fundectomy has not previously been reported. Morphologically, these foci did not differ from those seen in the PBD group of from lesions previously defined as acidophilic atypical acinar cell foci, which may be induced by a chemical carcinogen in rat.²⁷ In the sham operated controls, acidophilic atypical acinar cell foci were not detected within an area of the pancreatic tail corresponding to at least 30 000 counted points. While some previous studies have shown absence of foci in old, untreated and control rats,^{20, 21} others have shown that foci do occur spontaneously with increasing age.^{38, 39} Neither is contradicted by the results of this study.

Acidophilic atypical acinar cell foci in the rat have a high proliferative capacity and have been shown to be responsive to CCK, whereas the basophilic atypical acinar cell foci seem to have a low growth potential, not appreciably affected by CCK.^{27, 38–41} This study supports this concept as no basophilic atypical acinar cell foci were seen after longterm PBD. Furthermore, fundectomy does not seem to influence the development or growth of basophilic atypical acinar cell foci.

The ³H-thymidine labelling index of the acidophilic atypical acinar cell foci in the fundectomy group was significantly lower than that in the PBD group, showing that PBD has the strongest stimulatory effect. This conclusion is further strengthened by the fact that the pancreatic weight and volume density of the acidophilic atypical acinar cell foci in the fundectomy group were also significantly lower than those in the PBD group.

The fraction of cells in S phase did not differ significantly between the groups. Although being significant, the difference in proliferative activity of the acidophilic atypical acinar cell foci between the fundectomy group and the PBD group may not have been large enough to cause measurable differences in the S phase, especially as flow cytometry was performed on total pancreatic tissue and not on isolated acidophilic atypical acinar cell foci.

We conclude that fundectomy for about half of the life span in rats stimulates pancreatic hyperplasia and hypertrophy as well as growth of acidophilic atypical acinar cell foci. These effects are less pronounced than those of PBD with hypercholecystokinaemia. Although hypergastrinaemia is a prominent feature, it may not be the only or main factor responsible for the pancreaticotrophic effect of fundectomy. Changes in the balance of other hormones or growth factors, which are as yet unknown, may result from fundectomy. Another factor which may be of relevance is the potentially carcinogenic substrate (N-nitrosamines and N-nitrosamides) formed with achlorhydria in the fairly large gastric remnant (rumen and antrum) after

TABLE II Mean (SEM) of the volume density (%), diameter, and acinar cell ³H-thymidine labelling index (LI) of acidophilic atypical acinar cell foci (aACF) of the pancreas 14 months after fundectomy and pancreaticobiliary diversion (PBD) in the rat.

Groups	Animals (n)	Animals with aACF	aACF (%)	Diameter (mm)	LI (per 1000)
Fundectomy	10	4	0.4 (0.2)	0.22 (0.05)	6 (2)
PBD	13	11	22.8 (7.5)**	2.34 (0.61)**	27 (3)**

**p < 0.01 according to two tailed Mann-Whitney U test. aACF were not observed in the sham operated controls.

Figure 4: Autoradiographed pancreatic section from a PBD operated rat showing part of a nodule with a mean diameter of 5.4 mm and surrounding fibrous capsule (A) with an acinar cell differentiation (B), suggestive of adenoma. (Haematoxylin and eosin, $\times 100$ and $\times 400$, respectively).

fundectomy. Certainly, further investigations aimed to clarify these aspects are warranted. Although these findings were on rats, they may also be relevant with regard to the possible effects of achlorhydria with or without hypergastrinaemia in man. Epidemiological studies have shown that, apart from an increased risk of developing gastric cancer, patients with pernicious anaemia, as well as patients operated on with gastric resection more than 20 years ago, have an increased risk of developing cancer in other digestive organs, including the pancreas.^{42–45} It should be emphasised, however, that this study did not show pancreatic neoplasia, including adenoma, in any of the fundectomised animals.

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