Exocrine pancreatic nodules after longterm pancreaticobiliary diversion in rats. An effect of raised CCK plasma concentrations

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SUMMARY Surgical diversion of bile and pancreatic secretions to the mid small bowel has been shown to provoke increased CCK plasma concentration and growth of the pancreas in rats. This study was undertaken to investigate the effects of chronic pancreaticobiliary diversion on pancreatic morphology as well as the circulating concentrations of pancreatic polypeptide, secretin, gastrin, and CCK. Fifteen month diversion provoked 73 and 86 % increases in pancreatic weight and volume ($p < 0.001$). Cholecystokinin blood concentration increased by 98 %, from 20.9 ± 5.7 pg/ml in controls to 41.3 ± 5.4 after diversion ($p < 0.05$), but pancreatic polypeptide, secretin, and gastrin levels were not affected. The volume of the exocrine pancreas doubled from 1104.6 ± 78.2 mm³ in controls to 2201.2 ± 229.2 ($p < 0.001$), with a matching increase in interstitial tissue. On the contrary, the volume of the endocrine pancreas remained unchanged. Hyperplastic nodules developed in the exocrine pancreas, in 71 % of diverted rats, but not in transected controls. We conclude from these observations that chronic diversion of bile and pancreatic juice stimulated pancreatic growth, most likely through a persistent rise of CCK plasma concentrations. Furthermore, this long lasting stimulation induced the development of exocrine pancreatic nodules.

Diversion of bile and pancreatic secretions to the mid small bowel induces both intestinal and pancreatic growth. The increases in intestinal mucosal and pancreatic mass are accompanied by corresponding changes in absorptive and secretory capacity of these organs.^{1,2} The growth promoting effects of pancreaticobiliary diversion are certainly multifactorial, including hormonal trophic factors, such as enteroglucagon and cholecystokinin.³

Recent studies from our laboratory (see these proceedings) have shown that pancreatic growth occurred exclusively during the first two weeks after bile and pancreatic juice diversion, no further increase was detected thereafter. Cholecystokinin was probably responsible for this adaptation, as plasma concentrations were 81 % greater than in controls and were already significantly increased 48 hours after the operation, at a time when no change in pancreatic mass could be detected. Additional evi-

dence of the role of this peptide exists, as CCK-receptor antagonists, proglumide and benzotript, inhibited pancreatic growth.⁴

Raw soya bean flour also causes rat pancreatic hypertrophy mediated by a greater release of CCK in the circulation.^{5,6} Moreover, chronic feeding of trypsin inhibitors induces hyperplastic nodules, adenomas and carcinomas in the exocrine pancreas. A greater yield of neoplasias is obtained with additional chemical carcinogens.^{7,8}

The aim of the present study was to investigate the effects of chronic pancreaticobiliary diversion on pancreatic structure and blood concentrations of pancreatic trophic peptides, and to look for the appearance of neoplastic changes.

Methods

ANIMALS

The effects of 15 month bile and pancreatic juice diversion^{1,2} on pancreas and hormones was studied in male Wistar rats (approximately 225 g) and compared

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with transected control animals ($n = 7$ in each group).

At the time of death, blood was taken in non-fasted rats by cardiac puncture for the measurements of postprandial plasma concentrations of pancreatic polypeptide, secretin, gastrin, and CCK, by specific radioimmunoassays.^{9,10}

For morphological studies, the glands were carefully dissected from adjacent organs, mesenteries, lymph nodes and freed of fat. Weight and volume were recorded before fixation in formalin mercury for optic microscopy. Serial specimens were taken from the duodenal to the splenic part of the gland, embedded in paraffin, cut at 5μ and stained with haematoxylin-eosin or special stains (van Gieson-elastin, Alcian-blue, Gomori-silver), as appropriate. The morphometric evaluation of pancreatic structure was done according to a modified point counting method.¹¹ The following points were counted: total points, epithelium, ductules, interstitium, islets, and the number of acini were registered.

STATISTICAL ANALYSIS

The results were expressed as mean $\pm$ standard error of the mean. The significance of the differences between mean values was tested by Student's unpaired t test. p Values less than 5% were considered significant.

Results

At the end of the experiment, the animals of both control and experimental groups appeared healthy. Body weights were comparable at 485.0 ± 9.4 g in controls and 440.0 ± 7.8 after diversion (ns). Mean weight gains were also similar at 254 and 241 g respectively.

In contrast, pancreatic weights and volumes were increased by 73 and 86% respectively in comparison with control animals ($p < 0.001$), as shown in Figure 1. The morphometric analyses revealed that most of these changes were caused by an increased volume of the exocrine pancreas and also, but to a lesser degree, by interstitial tissue ($p < 0.001$). No significant differences were detected for the volume occupied by the ductules, however, nor by the endocrine part of the gland. Figure 2a shows the well defined acinar structure with its polarised cells and their basally located nuclei in control animals. In contrast, several features emerge from a pancreatic nodule after 15 months pancreaticobiliary diversion. As shown in Figure 2b, cells had undergone hypertrophy, containing bigger nuclei with prominent nucleoli. The number of acini was reduced from 531.7 ± 23.0 to 302.6 ± 15.3 after diversion ($p < 0.001$), caused by increased volume (Fig. 3).

Fig. 1 Morphological analysis of the pancreas after 15 month bile and pancreatic juice diversion. PBD = pancreatico-biliary diversion ($n = 7$ in each group).

The effects of pancreaticobiliary diversion on postprandial concentrations of peptides are shown in Figure 4. Cholecystokinin blood concentrations increased by 98% ($p < 0.05$) in comparison with controls, whereas diversion of pancreaticobiliary juice did not significantly affect pancreatic polypeptide, secretin nor gastrin.

Discussion

The present study was part of a series of experiments on pancreatic adaptation after bile and pancreatic juice diversion. Having first shown how pancreatic growth developed (see Miazza *et al*, these proceedings), we investigated the effect of long term diversion on pancreatic structure and on circulating concentrations of peptides, which might be trophic to the organ.

The results have shown that 15 months diversion of bile and pancreatic secretions to the mid small bowel provoked, (1) a persistent increase in the mass of the

Fig. 2 Details of Fig 2a: control pancreas; b: hyperplastic acini. H-E.

Fig. 3 Gomori-silver staining. a: normal acini surrounded by sharp limits. b: grossly enlarged acini with indistinct borders after diversion.

Fig. 4 Postprandial plasma concentrations of gastrointestinal peptides after diversion. PP = pancreatic polypeptide, CCK = cholecystokinin, PBD = pancreatico-biliary diversion. NS = nonsignificant.

gland, (2) a persistent increase in plasma CCK and, (3) the development of hyperplastic-adenomatous nodules in the exocrine pancreas.

The morphometric analysis revealed findings that are fundamental for our knowledge of pancreatic adaptation. Diversion had, indeed, a trophic effect exclusively on the exocrine pancreas, the endocrine part being not involved. Thus, the overall increase in pancreatic volume was due to only two components of the gland, the acinar tissue and, albeit much less, the interstitial tissue. By contrast, the volumes of ductules and islets were not modified. The morphometric observation concurred with our previous results showing that bicarbonate secretion was similar in controls and diverted rats (unpublished data), and that there were no changes in postprandial plasma concentrations of insulin, pancreatic glucagon³ nor pancreatic polypeptide, as markers of pancreatic endocrine function.

The mechanism of pancreatic growth after diversion has not been fully elucidated. There is, however, evidence for a priming role of CCK, the main trophic factor for the exocrine pancreas. First, CCK increased rapidly after diversion, preceding the change in DNA synthesis and pancreatic growth. Second, additional indirect evidence came from a previous work using CCK-receptor antagonists. High doses of proglumide and benzotript, given subcutaneously, inhibited the pancreatic adaptation of diversion.⁴

The hormonal results obtained in these experiments have confirmed that diversion had provoked a permanent release of CCK in the blood-stream. This finding is at variance with the results recently reported

by Fölsch and Creutzfeldt in rats fed raw soya flour.¹² This diet induced only a transient rise in CCK plasma concentrations, as these returned 'towards' control values after 20 days, despite continuing treatment. A nearly six fold increase, however, in CCK was measured in controls over the same period, which remains unexplained. In contrast with these results, Adrian *et al*⁶ have reported that CCK concentrations were three times higher after 21 days of soya flour diet. Controversy exists therefore as to the effect of trypsin inhibitor feeding on the release of CCK. Nevertheless, both diversion and soya bean diet increase endogenous CCK and stimulate pancreatic growth. These results suggest again that CCK plays a central role in pancreatic adaptation, but they cannot rule out the participation of other factors.

Pancreaticobiliary diversion also shares another similarity with trypsin inhibitor containing diet. In both models, stimulation of pancreatic growth results in the development of acinar cell neoplasias. These developed after long term diversion or soya flour treatment and have been shown to undergo malignant changes in soya bean fed rats. After diversion, numerous foci of nodular hyperplasia were found scattered throughout the gland in 71 % of animals. The architecture of acini became distorted and tubular, their normal limits became illdefined, but in some places nodules were surrounded by a thick fibrous capsule, suggestive of adenomatous change. The exact sequence of events leading to the development of nodules and adenomas is still unknown, however it would appear that the continuing CCK-stimulus of proliferation induces phenotypic changes in particularly sensitive cells.

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