

Pancreatic exocrine function in severe human chronic renal failure*

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SUMMARY Patients with chronic renal failure have an abnormal immunoreactive gastrointestinal hormone profile, which is characterised by raised fasting serum concentrations of hormones that have antagonistic effects on exocrine pancreatic function. In addition, in this present study we have found that in renal insufficiency cholecystokinin disappears slowly from the plasma after a constant intravenous infusion of the hormone ($p=0.05$ compared with healthy subjects). To evaluate whether the stimulatory or inhibitory hormones have a predominant effect, pancreatic exocrine function under conditions of mannitol perfusion of the duodenum and continuous intravenous cholecystokinin stimulation was studied in eight patients who had severe chronic renal failure and eight age-matched and sex-matched control subjects. Compared with healthy subjects, patients with renal insufficiency had hypersecretion of trypsin in response both to mannitol perfusion of the duodenum and to cholecystokinin stimulation ($p<0.05$). No significant differences in lipase secretion were noted between the patients with renal insufficiency and control subjects. These findings are consistent with the hypothesis that, of the abnormally raised fasting serum concentrations of gastrointestinal hormones found in renal insufficiency, hormones that stimulate rather than inhibit pancreatic exocrine function predominate. Secondly, the dissociation between trypsin and lipase outputs in chronic renal failure may suggest a differential trophic influence of stimulatory hormones – that is, hypercholecystokinaemia – on pancreatic exocrine enzyme secretion.

Patients with chronic renal failure have an abnormal gastrointestinal hormone profile, which is characterised by raised serum concentrations of cholecystokinin (CCK),¹ a powerful stimulator of pancreatic exocrine function, as well as inhibitors of pancreatic secretion such as glucagon^{1 2} and pancreatic polypeptide.³ The effect of this abnormal hormone profile¹ on pancreatic function, however, is not known. Also, several of these hormones may have trophic effects on the pancreas.⁴

To evaluate whether the stimulators or inhibitors of pancreatic secretion have a predominant effect and whether a trophic effect of these hormones can be shown, we studied pancreatic enzyme secretion in response to mannitol perfusion of the duodenum (control) and maximal pancreatic enzyme secretory

capacity (CCK-stimulated) in a group of patients with severe chronic renal failure and in age-matched and sex-matched normal subjects and then measured the disappearance of CCK after terminating a continuous CCK intravenous infusion.

Methods

SUBJECTS

Eight patients (men, aged 25 to 62 years) with severe chronic renal failure (serum creatinine concentrations 12.0 to 16.9 mg/dl, mean 14.3) and eight age-matched and sex-matched controls were studied after giving written informed consent. The patients with renal failure were on chronic haemodialysis programmes for at least 18 months before the study and had their care in medicinal programme supervised by one of us (JCM). The patients were studied on non-dialysis days, when serum ionised calcium concentrations were normal and non-fluctuating. All patients were dialysed against a 0.75–0.88 mmol/l (3.0–3.5 mEq/l) calcium bath, which usually causes a transient (three to 12 hours) increase of serum

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calcium concentrations of no more than 0.5–0.75 mmol/l (1–1.5 mg/100 ml). All subjects were within 10% of their ideal weight, instructed to eat a normal protein diet, and had no previous gastrointestinal or biliary surgery or other significant co-existent endocrinopathy. All patients had normal serum concentrations of glucose and calcium and were taking no medication known to interfere with pancreatic function.

PROCEDURE

Pancreatic lipase and trypsin secretions were studied using our standard gastroduodenal intubation perfusion technique.⁵ To summarise this technique, after an overnight fast a double-lumen duodenal tube and a separate gastric sump tube were positioned fluoroscopically so that the duodenal perfusion site was in the second part of the duodenum, the duodenal aspiration site was 20 cm distal to this at the ligament of Treitz, and the gastric sump tube was in the gastric antrum. The duodenal perfusion solution used was an isotonic solution of mannitol containing a non-absorbable marker polyethylene glycol (PEG 4000) (5 g/l) warmed to 37°C and adjusted to pH 7.0. Mannitol was used in this series of studies instead of our usual saline perfusate because of the necessity to restrict sodium in renal failure. Duodenal samples were collected at the level of the ligament of Treitz by siphonage and were pooled every 20 minutes over ice. Gastric contents were aspirated from the antrum by continuous mechanical suction. After steady-state conditions were established, samples were collected for two hours. Then collections were continued for one hour while pancreatic enzyme secretion was stimulated by the continuous intravenous infusion of 20% porcine CCK (GIH Laboratory, Karolinska Institute, Stockholm) given at a rate of 0.25 CHR U/kg/min.

Four normal subjects and five of the patients with renal insufficiency donated blood at two, four, seven, nine, 12, 15, and 20 minutes after the end of the one-hour continuous intravenous infusion of 20% porcine CCK. Of the 7 ml of peripheral venous blood collected, 3 ml was collected in aprotinin (Trasylol)-ethylenediaminetetra-acetate tubes, and 4 ml was allowed to clot. These samples were centrifuged at 44°C, and plasma and serum were divided into aliquots and frozen at –20°C until assays could be performed.

Cholecystokinin was measured by a radioimmunoassay recently developed in our laboratory.⁶ Radioiodination of 99% pure porcine CCK (kindly supplied by Dr V Mutt, Karolinska Institute, Stockholm, Sweden) was accomplished by having the hormone react with succinimide ester of ¹²⁵I-

labelled 3-(4-hydroxyphenyl) propionic acid according to the new Bolton-Hunter method.⁷ The specific activity was 50 μ Ci/ μ g. Antiserum (No 3482) used in the assay was raised in New Zealand rabbits by repeated intradermal injections of 20% pure porcine CCK. It was used at a final dilution of 1:25 000. This antiserum had no significant cross-reactivity with human heptadecapeptide gastrin (G-17). The assay detected serum CCK at a concentration of 50 pg/ml. The intra-assay and interassay coefficient of variation for duplicate systems was less than 13%. Results of the serum CCK were expressed in picograms per millilitre, with CCK-33 used as the standard.

Concentrations of polyethylene glycol,⁸ trypsin,⁹ and lipase⁹ were measured in all duodenal samples. Trypsin and lipase concentrations were determined by means of an automatic titration method using TAME (*p*-toluene sulfonyl-2-arginine methyl ester) and fat emulsion (Lipomul) as substrates for the respective enzyme activities.⁹ The enzyme outputs were calculated, based on recovery of polyethylene glycol⁵ and expressed in 10³ U/(kU) per hour.

Differences between enzyme secretion during mannitol perfusion of the duodenum and CCK-stimulated pancreatic enzyme secretion obtained from patients with chronic renal failure and those obtained from the normal controls were tested using Student's *t* test, with *p*<0.05 being significant.

Results

TRYPSIN SECRETION

In response to duodenal mannitol perfusion the mean pancreatic trypsin output in chronic renal failure of 51±10 kU/h (mean±SD) was significantly higher than the mean trypsin output of 23±8 observed in healthy controls (*p*<0.01) (Fig. 1). There was no overlap in trypsin outputs between these groups.

Despite the patient's raised trypsin outputs in response to duodenal mannitol perfusion and their raised fasting serum CCK concentrations,¹ the intravenous infusion of exogenous porcine CCK further increased the trypsin output in every patient with chronic renal failure (*p*<0.01) (Fig. 1). Trypsin output increased fourfold after intravenous CCK stimulation (51±10 kU/h to 213±108). In the control group the trypsin output increased from 23±8 kU/h to 37±11 with intravenous CCK stimulation (Fig. 1). Thus the pancreatic trypsin secretion, determined in response to the maximal CCK stimulus, was greater in patients with chronic renal failure than in healthy controls (*p*<0.01). Furthermore, the increase in trypsin secretion, expressed as a percentage of secretion in response to mannitol

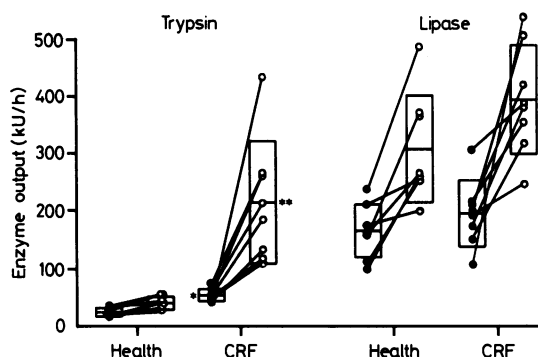


Fig. 1 Pancreatic trypsin and lipase outputs in response to intraduodenal (ID) mannitol perfusion (●) and intravenous cholecystikinin (CCK) (○) in health and severe chronic renal failure (CRF). Vertical columns represent mean \pm 1 standard deviation; * $p < 0.01$ (chronic renal failure ID mannitol compared with health ID mannitol); ** $p < 0.01$ (chronic renal failure CCK compared with health CCK); kU represents 10^3 units.

perfusion, was greater in chronic renal failure than in health ($431 \pm 263\%$ v $172 \pm 43\%$, $p < 0.01$) (Fig. 2).

LIPASE SECRETION

In contrast with the higher than normal trypsin output observed in chronic renal failure there was no significant difference in lipase output in response to mannitol perfusion between patients with chronic renal failure and healthy controls (194 ± 58 v 163 ± 46 kU/h, $p > 0.05$) (Fig. 1). The intravenous infusion of porcine CCK resulted in a similar significant increase in lipase output in every patient with chronic renal failure (194 ± 58 to 393 ± 95 kU/h, $p < 0.05$) and in health (163 ± 46 to 307 ± 94 kU/h, $p < 0.05$). Lipase output with CCK administration in

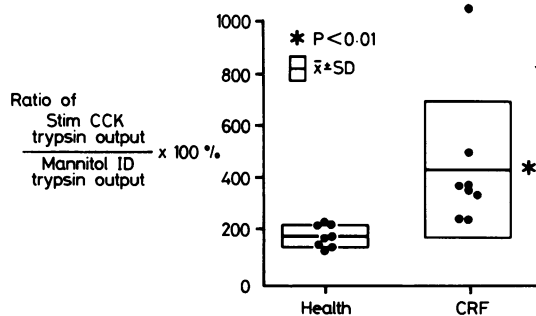


Fig. 2 Maximal stimulated pancreatic trypsin secretion in response to CCK expressed as a percentage of that elicited by duodenal mannitol perfusion is shown for healthy controls and patients with severe chronic renal failure (CRF). Individual data and group means \pm SD are shown; * $p < 0.01$.

chronic renal failure was not significantly different from that observed in healthy controls.

IMMUNOREACTIVE CCK DISAPPEARANCE STUDY

After the end of the continuous intravenous infusion of 20% porcine CCK, the disappearance of CCK from the blood during the first seven minutes (Table, Fig. 3) appeared to be the same for normal subjects and patients with chronic renal failure. After the initial seven minutes, however, CCK disappearance in the patients with renal failure was significantly slower ($p = 0.05$; Table).

Discussion

We have previously shown that the fasting gastrointestinal immunoreactive hormones are significantly higher in renal insufficiency compared with normal subjects and suggested that they could alter gastrointestinal functions in these patients.¹ We have now shown that patients with renal failure have hypersecretion of pancreatic trypsin but not lipase in response to a mannitol duodenal perfusion and to intravenous infusion of CCK. These findings might be explained by the augmentation of the raised fasting serum concentrations of gastrointestinal hormones in which stimulatory hormones such as CCK rather than inhibitory hormones have the predominant effect.¹⁰⁻¹⁶ Indeed, we have found that in patients with renal insufficiency the plasma disappearance of CCK is slow, and normal fasting levels are not achieved. These data emphasise that in renal insufficiency there is continuous CCK stimulation of the exocrine pancreas, which may cause pancreatic hypertrophy, hyperplasia, or increased sensitivity of the acinar cells to stimulating hormones such as CCK or gastrin or both.

In the rat^{4, 17, 18} chronic injection of CCK increases pancreatic weight and pancreatic content of protein, DNA, RNA, amylase, and trypsin. Furthermore, Barrowman and Mayston¹⁷ reported that the chronic injection of CCK in rats resulted in a striking increase of pancreatic content of amylase

Table Disappearance of blood during first seven minutes

Slope (min)	Normal subjects (n=4)	Patients with renal insufficiency (n=5)
0 to 7	-4400 \pm 1655	-3627 \pm 1580
9 to 20	32 \pm 12	148 \pm 70*

Mean and standard error of the first seven and last 12 minutes of the CCK disappearance curves for normal subjects and patients with renal insufficiency. Slopes calculated by linear regression. * $p = 0.05$ compared with 9-20 minute mean value in the normal subjects.

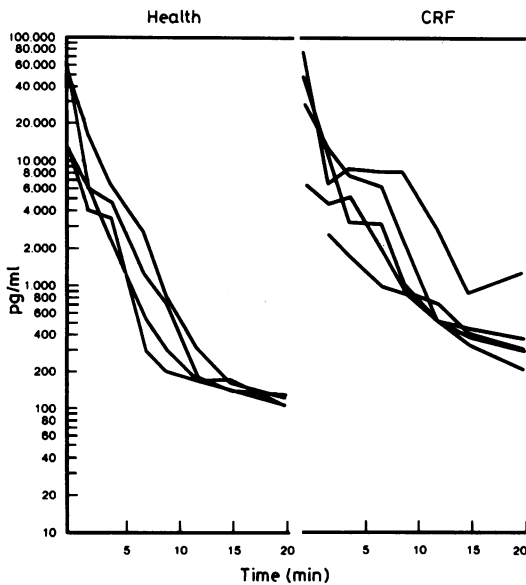


Fig. 3 Plasma CCK disappearance curves after end of a constant intravenous infusion of cholecystokinin for four normal control subjects (health) and five patients with severe chronic renal failure (CRF).

and proteolytic enzymes but not of lipase. Our data, which show a dissociation between trypsin and lipase outputs in patients with chronic renal failure in whom we have shown hypercholecystokinaemia, are consistent with this differential trophic influence on pancreatic exocrine enzymes.

Theoretically, the differences between trypsin and lipase secretion could be due to adaptation of pancreatic enzymes to dietary composition.¹⁹ Recently, Felber and his colleagues²⁰ reported that carbohydrates, lipids, and proteins induced the preferential secretion of amylase, lipase, or trypsin and chymotrypsin, respectively. Our patients, however, were receiving a normal protein diet. Therefore, it is unlikely that dietary manipulation had a major role in influencing pancreatic enzyme secretion in our patients.

In our healthy subjects trypsin output in response to duodenal mannitol perfusion was three to four times the amount secreted in response to duodenal saline perfusion^{10 11} but is submaximal and the same as that observed with duodenal perfusion of an essential amino-acid mixture.^{10 12} Thus mannitol perfusion of the intestine probably activates a neurohormonal mechanism that stimulates pancreatic secretion, which is augmented in renal failure. Similarly, intravenous infusion of porcine CCK at a dose of 0.25 CHR U/kg/min produces maximal secretion of pancreatic enzymes in healthy

subjects.^{11 12} In chronic renal failure, however, the pancreatic trypsin secretion in response to this CCK stimulus was significantly greater than in healthy controls.

In all other studies normal or decreased pancreatic secretion has been found in chronic renal insufficiency. Normal²¹ or decreased^{22 23} bicarbonate secretion in response to secretin and normal²⁴ or decreased^{21 23 25} CCK-stimulated amylase output have been reported. When trypsin^{21 23 25} or lipase²¹ outputs have been measured they have usually been normal. Lipase outputs, however, were found to be abnormally low in 15 of 25 patients.²⁵ That our results differ from these studies may be due to different techniques (we measured outputs by recovery of an unabsorbable marker), awareness of the possibility of increased secretion, and selection of patients.

The clinical importance of hypersecretion of pancreatic trypsin in chronic renal failure is uncertain at present. None of the eight subjects whom we studied had experienced a typical episode of pancreatitis. A high prevalence of pancreatic disease in chronic renal failure, however, has been reported,^{26 27} and in one necropsy report²⁷ pathological changes in the pancreas were present in 56.8% of the cases in which maintenance haemodialysis had been used before death from uraemia. Histological changes included duct ectasia, periductal fibrosis, ductular proliferation, acinar ductular metaplasia, and interstitial fibrosis.²⁷ It is tempting to speculate that pancreatic trypsin hypersecretion may initiate pancreatic disease in this group of patients. The significance of this finding, however, still awaits further clinical and pathological studies.

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