Physiological control of cholecystokinin release and pancreatic enzyme secretion by intraduodenal bile acids

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Abstract

***Background*—The physiological relevance of duodenal bile acids in the control of cholecystokinin release and pancreatic enzyme secretion is still unknown.**

***Aim*—To provide a near physiological situation by perfusing a bile acid mixture mimicking the individual endogenous bile acid composition of the person under investigation. For maximal reduction of endogenous bile output the CCK-A receptor antagonist loxiglumide was infused intravenously.**

***Subjects and Methods*—Seven healthy volunteers were studied on four different days by a duodenal marker perfusion technique. The individual bile acid composition in duodenal juice and test meal stimulated bile acid output was assessed on day 1. Bile acids were perfused at an amount of 30 or 100% as determined on day 1 in combination with the test meal in the presence or absence of loxiglumide. Pancreatic enzymes, bilirubin, and bile acid output were determined in duodenal juice. Plasma cholecystokinin (CCK) and plasma pancreatic polypeptide (PP) were measured radioimmunologically.**

***Results*—Bile acid perfusion did not significantly alter stimulated pancreatic enzyme, bilirubin or bile acid output or plasma CCK. Loxiglumide did not alter basal CCK release but increased test meal stimulated CCK output fourfold ($p<0.05$). The addition of bile acids to the test meal at a dose resembling 30% of bile acid output as determined on day 1 prevented this increase. Plasma PP concentration remained unchanged by bile acids and were mostly undetectable during loxiglumide infusion.**

***Conclusion*—The CCK producing cell is under constant suppression by intraduodenal bile acids which cannot be further enhanced by a physiological bile acid mixture. However, removal of duodenal bile acids by inhibition of gall bladder contraction unmasks this suppression leading to a dramatic increase in plasma CCK levels. As little as one third of postprandially released bile acids completely reverse this effect. Bile acids are the most important luminal regulator of CCK release in humans.**

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Keywords: bile acids, bile, pancreatic enzyme secretion, CCK-A receptor antagonist, cholecystokinin, pancreatic polypeptide.

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The control mechanisms of the intestinal phase of exocrine pancreatic secretion are complex. Food components interact with digesting factors, such as gastric acid, bile, and pancreatic juice, and eventually come into contact with intestinal epithelial cells and probably sensory nerve endings causing release of hormones and activation of neurones, which in turn regulate exocrine pancreatic secretion.

In humans, one of the most potent local luminal regulators of exocrine pancreatic secretion was found to be bile. However, results differ with respect to the effect of bile and bile acids on basal stimulated exocrine pancreatic secretion. Basal secretion was reported to be enhanced by bile[1–4] whereas stimulated pancreatic secretion was either unaltered[4] or inhibited.[5 6] The most important hormone involved in the regulation of exocrine pancreatic secretion is cholecystokinin (CCK), which accounts for about 50% of postprandially released pancreatic enzymes.[7 8] In addition, CCK is the predominant mediator of gall bladder contraction. Postprandial duodenal bile content is reduced to about 5% of infusion of the highly specific CCK-A receptor antagonist loxiglumide.[7 8] Bile acids in turn have been found to inhibit stimulated CCK release.[4 9–11] One crucial point about all the studies investigating the effect of bile acids on either exocrine pancreatic secretion or plasma CCK release is the use of supraphysiological concentrations and amounts of bile acids. Furthermore, in most studies sodium taurocholate and chenodeoxycholic acid were used as single bile acids each accounting for only 15%–30% of all bile acids secreted.[12 13] Thus, the physiological relevance of luminal bile acids in the regulation of exocrine pancreatic secretion and CCK release is still largely unknown.

The purpose of our study was to provide a near physiological situation by duodenal perfusion of a bile acid mixture mimicking the individual endogenous bile acid composition and concentration of the person under investigation. Endogenous bile acid output was reduced by inhibiting gall bladder contraction by infusion of the highly specific CCK-A receptor antagonist loxiglumide.[14 15] We were thereby able to show that duodenal bile acids

are of minor importance in the regulation of postprandial pancreatic enzyme secretion but are a key regulator of stimulated CCK release. Bile acids appear to exert a constant inhibitory tone on the intestinal CCK producing cell.

Methods

SUBJECTS

Seven healthy male volunteers aged 22–27 had given informed written consent to participate in this study. The protocol had been approved by the local ethical committee of the Medical Faculty of the Philipps-University of Marburg.

Experimental protocol

On the morning after an overnight fast a triple-lumen tube was positioned with its tip in the distal part of the duodenum under fluoroscopic guidance. The most proximal lumen ended in the gastric antrum and was used for continuous aspiration of gastric acid. The second lumen terminated in the middle part of the duodenum close to the Papilla of Vateri and served as perfusion site for the recovery marker polyethylene glycol (PEG 4000, dissolved in 0·9% saline) and for the liquid test meal plus PEG with or without bile acids. The perfusion rate was 2 ml/min. Duodenal contents were continuously collected via the third lumen, which was located 10 cm distally to the perfusion site. Samples were obtained at 15 minute intervals, stored on ice, and immediately processed for determination of pancreatic enzymes. Aliquots of duodenal juice were stored at −30°C for future measurements of bilirubin and bile acid concentration. Via an indwelling venous catheter in the forearm, blood was drawn into EDTA-containing tubes at 15 minute intervals and stored on ice. Immediately after the end of the experiment the blood was centrifuged and the plasma stored at −30°C until hormone determination.

During the whole experiment the volunteers lay in a recumbent position. Perfusion of PEG was started as soon as the tube was positioned. Equilibration lasted between 60 and 90 minutes until the duodenal juice was clear, enzyme output had declined, and stable conditions had been achieved.

The duodenal stimulation period lasted for 120 minutes and perfusion of stimuli was performed according to the protocol outlined below. Each volunteer was studied on three different ways. Four of them participated in a fourth study.

Day 1

Aliquots of the first two fractions of unstimulated duodenal juice were processed for determination of individual bile acid composition. Stimulation was performed by infusion of a liquid test meal (18 g soybean oil, 15 g protein, 40 g glucose, 300 ml water) over 120 minutes in order to obtain separate control values for the first and second hour. The amount of bile acids released during the first hour of the stimulation period was used to calculate bile acid perfusates on the following days.

Day 2

During the first hour of the stimulation period the liquid test meal was mixed with 30% of bile acid output as determined on day 1. The bile acid mixture comprised all the bile acids identified in the duodenal function from day 1 mixed according to their individual proportions. During the second hour of the stimulation period the test meal was perfused without bile acids. Via an indwelling venous catheter in the forearm, a continuous infusion of saline was given over the whole study time.

Day 3

During the first hour of the stimulation period the liquid test meal was mixed with 100% of the bile acid output determined on day 1. The bile acid mixture comprised all the bile acids identified in the duodenal fraction from day 1 mixed according to their individual proportions. Over the second hour of the stimulation period the test meal was perfused without bile acids. Saline was infused intravenously over the whole experimental period.

Day 4

Duodenal perfusion during the basal and stimulation period was performed according to the conditions on day 2. Instead of saline, however, the CCK-A receptor antagonist loxiglumide was infused intravenously at a rate of 10 mg/h per kg body weight. Infusion of loxiglumide was started at the beginning of the basal period. According to previous measurements maximum steady state plasma concentrations of loxiglumide are achieved after 45–60 minutes of intravenous infusion.[7]

Days 2 and 4 were performed in random order.

BILE ACID COMPOSITION

The individual bile acid compositions were determined in duodenal aspirates using high pressure liquid chromatography.[16] In three volunteers, the day to day variation in bile acid composition was investigated. Over two weeks no significant variation in bile acid composition was observed. Furthermore, it has been shown by others that day to day variation in bile acid composition is negligible.[13]

ENZYME, BILE ACID, BILIRUBIN, AND PEG MEASUREMENTS

The volume of gastric and duodenal samples was determined to the closest millilitre and corrected for loss by reference to PEG recovery (see under Calculations and statistics below). The pH was monitored repeatedly during the test and in each 15 ml portion. It did not decrease below 6.

Trypsin activity was determined using TAME (p-toluolsulphonyl-l-arginin-methylester) as substrate by the method of Hummel.[17]

Amylase and lipase activities and bilirubin and bile acid content were determined by the use of commercially available kits. PEG was measured by turbidimetry using the method of Hyden.[18]

CCK RADIOIMMUNOASSAY

Plasma CCK concentrations were determined, with minor modifications, by the use of a highly specific and sensitive antibody (G-160, kindly provided by Professor Schafmayer, Göttingen, Germany) as described previously.[4 19] In summary, plasma was extracted with 99% ethanol before the assay procedure, lyophilised and reconstituted with assay buffer. The total assay volume was 600 µl, comprising 150 µl CCK octapeptide (CCK_8) standard or sample, 150 µl buffer or charcoal-extracted plasma, 200 µl antibody and 100 µl Bolton-Hunter ^{125}I-CCK_8.

PANCREATIC POLYPEPTIDE (PP) RADIOIMMUNOASSAY

Plasma PP determinations were performed as described by Schwartz *et al*[20] using bovine PP for iodination, human PP as standard, and antihuman PP antibody kindly provided by Dr R E Chance, Indianapolis, IN, USA.

CALCULATIONS AND STATISTICS

Results are expressed as mean (SEM). The integrated hormone output was calculated as described by Stern and Walsh.[21] It represents the area under the curve formed by the stimulated values minus the basal output over the time of the stimulation period investigated. The integrated enzyme, bilirubin, and bile acid output was calculated by subtracting the basal output over 60 minutes from the sum of all portions collected after the stimulus was given. For calculation of statistical significance between integrated responses, a Kruskal-Wallis non-parametric analysis of variance test was performed. If the p value was below 0·05 a Dunn's multiple comparison test[22] was added. Differences with a p value below 0·05 were considered significant.

PEG recovery and correction of duodenal volume loss were calculated using the following equation: $V=F\times[PEG]_{in}/[PEG]_{out}$. V is the calculated duodenal volume (ml/15 min); F is the flow rate of PEG solution perfused (150 ml/15 min); $[PEG]_{in}$ is the concentration of PEG in the perfused solution (mg/ml); $[PEG]_{out}$ is the concentration of PEG in the duodenal juice collected for 15 min (mg/ml).[23] The recovery of PEG in all experiments was 80·4 (2·9)%. Duodenal-gastric reflux was intermittent and did not exceed 7%.

MATERIALS

All bile acids were purchased from Sigma Chemicals (Deisenhofen, Germany). Activities of amylase and lipase and concentration of bilirubin and bile acids were determined using commercially available kits from Boehringer, Mannheim, Germany and Merck, Darmstadt, Germany respectively. Loxiglumide was kindly provided by Dr Rovati, Rotta Research Company, Monza/Milano, Italy. Synthetic sulphated CCK_8 was purchased from Bachem, Bubendorf, Switzerland; Bolton-Hunter ^{125}I-CCK_8 was purchased from NEN, Boston, MA, USA.

Results

Bile acid composition

Determination of bile acids in duodenal aspirates showed a wide individual variation (Fig 1).Glycine conjugates amounted to 79% and taurine conjugates 21% of duodenal bile acids. Glycoursodeoxycholic acid, glycolithocholic acid, tauroursodeoxycholic acid, and taurolithocholic acid were only inconsistently detected in duodenal aspirates.

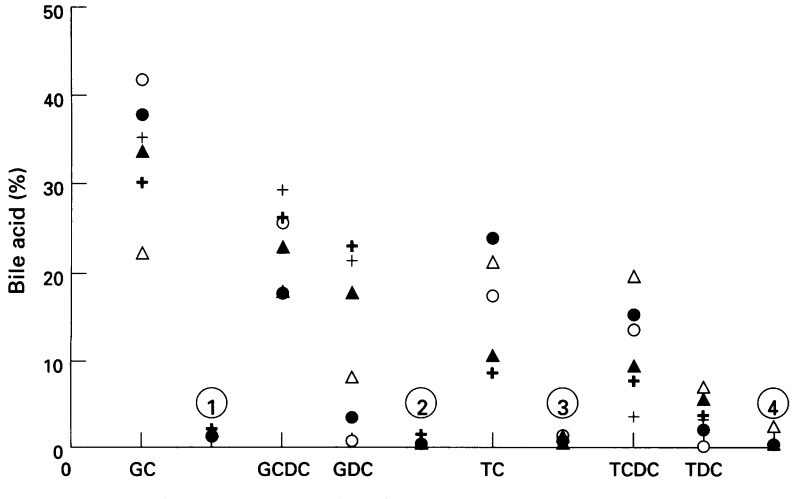

Figure 1: Bile acid composition in duodenal juice obtained from healthy volunteers. Each symbol represents one person. GC=glycocholate, GCDC=glycochenodeoxycholate, GDC=glycodeoxycholate, TC=taurocholate, TCDC=taurochenodeoxycholate, TDC=taurodeoxycholate. 1=Glycoursodeoxycholate, 2=glycolithocholate, 3=tauroursodeoxycholate, 4=taurolithocholate.

Bile acid and bilirubin output

During test meal perfusion, bile acid output was 4·5 (0·1) mmol during the first hour and this was reduced to 2·1 (0·5) mmol over the second hour (Fig 2A). Concomitant perfusion of 30 or 100% of the individual bile acid mixture during the first hour of stimulation did not change bile acid output significantly. However, loxiglumide almost completely abolished endogenous bile acid release. The slight increase in duodenal bile acid content during the second hour is probably due to left-over exogenous bile acids perfused during the first hour.

Bilirubin output remained unchanged during duodenal bile acid perfusion. Loxiglumide suppressed bilirubin output by 86% (Table I).

Pancreatic enzyme output

Duodenal test meal perfusion significantly stimulated amylase, trypsin, and lipase output. During the second hour of stimulation values

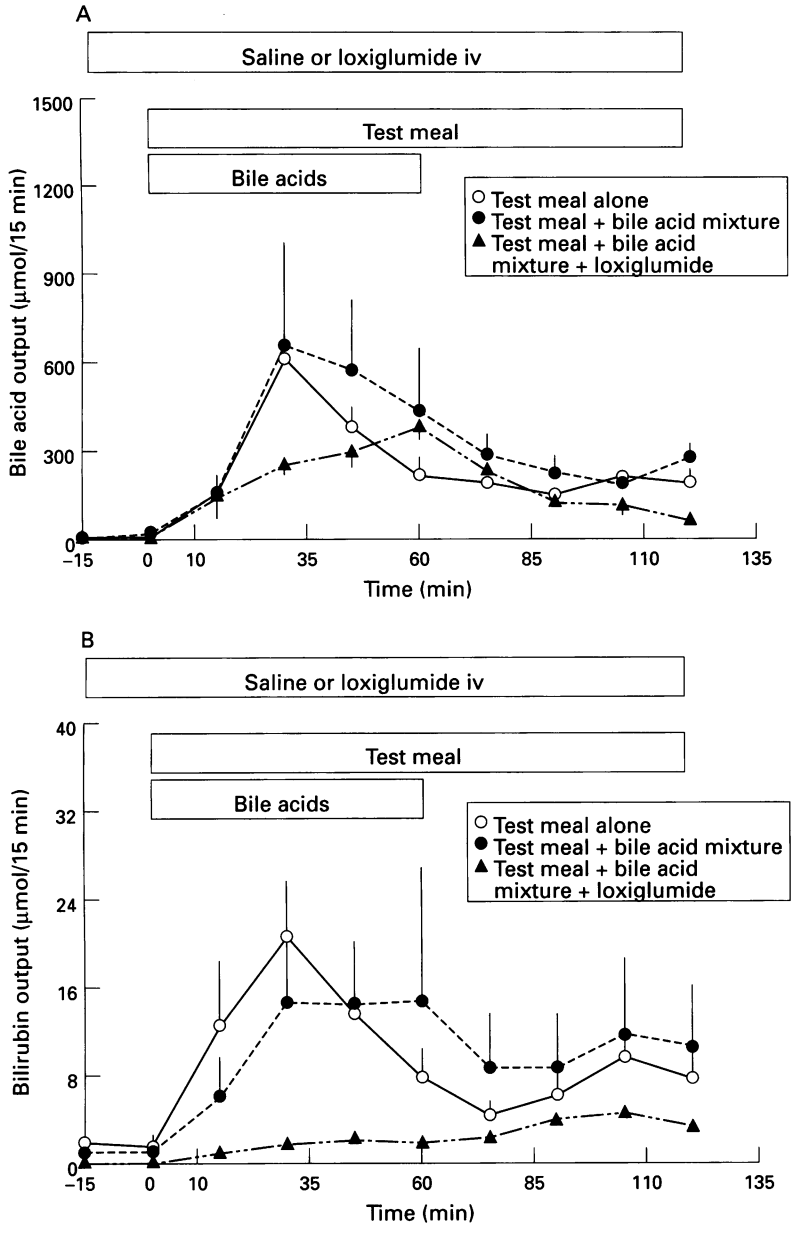

Figure 2: Bile acid (A) and bilirubin (B) output during continuous duodenal perfusion of a test meal (day 1), a test meal plus an individual bile acid mixture as determined by day 1 in a quantity that equalled 30% of the output measured on day 1 during the first hour of stimulation, with saline infused intravenously (iv) (day 2), and a test meal plus an individual bile acid mixture as determined on day 1 in a quantity that equalled 30% of the output measured on day 1 during the first hour of stimulation, with loxiglumide infused iv at a concentration of 10 mg/h per kg; this infusion was started at least 60 minutes before the test meal period and was continued throughout the study.

were significantly higher than during the first hour ($p<0.05$).

The addition of 30 or 100% of the individual bile acid mixture to the test meal diminished these differences resulting in almost identical enzyme output over both stimulation periods.

Loxiglumide infusion reduced enzyme output by 65%–75%. In parallel to the test meal perfusion alone, enzyme output was higher during the second hour than during the first hour, irrespective of concomitant perfusion of bile acids (Fig 3).

CCK release

CCK release was stimulated 2·5-fold by the test meal. The addition of bile acids at both amounts (30 and 100% of the individual bile acid mixture) did not alter plasma CCK levels. During loxiglumide infusion basal CCK concentrations remained constant and did not differ from those obtained with saline infusion. When loxiglumide infusion was continued and the test meal was perfused together with bile acids, CCK levels increased only slightly and insignificantly above the values obtained with intravenous perfusion of test meal alone and saline. However, cessation of bile acid perfusion but continuation of test meal perfusion led to a sharp rise in CCK concentrations during continuous loxiglumide infusion (Fig 4). This fourfold increase compared with the preceding hour was significant when calculated as integrated output ($p<0.05$; Table II).

PP release

Plasma PP concentrations gradually increased during test meal perfusion (Fig 4). The addition of 30% of bile acids slightly suppressed PP values. Owing to the wide interindividual variations, the differences showed no statistical significance. Loxiglumide almost completely abolished PP release (Table II).

Discussion

The role of intraduodenal bile acids in the control of basal and postprandial pancreatic enzyme and CCK release remains controversial. A major reason for the diverging results is the use of unphysiologically high concentrations of single bile acids or supraphysiological amounts of bile acids. We therefore used an experimental approach that mimicked physiological postprandial conditions to study the role of intraduodenal bile acids in the control of CCK release and pancreatic enzyme secretion. In order to achieve this, we determined the duodenal bile acid composition and

TABLE I *Integrated output of bile acids, bilirubin, and pancreatic enzymes*

	Test meal (n=7)		*Test meal, saline iv*		*Test meal*		*Test meal, loxiglumide iv*	
	1 hour, -BA	*2 hours, -BA*	*1 hour, 30% BA*	*2 hours, -BA*	*1 hour, 100% BA*	*2 hours, -BA*	*1 hour, 30% BA*	*2 hours, -BA*
Bile acids (mmol/h) (a)*	4·5 (0·1)	2·1 (0·5)	3·7 (2·8)	2·9 (0·4)	2·2 (0·8)	3·9 (0·8)	-0·1 (0·1)†	0·6 (0·1)‡
(b)			4·8 (1·0)		6·2 (8·5)		1·1 (0·1)†	
Bilirubin (mg/h)	28 (8)	12 (40	29·5 (11·8)	23 (11)	23·2 (6·9)	18 (8)	3·9 (0·9)†	8 (2)‡
Amylase (kU/h)	20 (4)	29 (6)	14 (2)	21 (5)	24 (1)	20 (1)	4 (1)†	8 (2)‡
Trypsin (kU/h)	11 (2)	15 (2)	8 (1)	12 (2)	11 (2)	11 (3)	2·3 (0·5)†	5 (2)‡
Lipase (kU/h)	343 (132)	420 (140)	195 (38)	315 (72)	325 (116)	294 (34)	61 (20)†	109 (24)‡

*(a)=endogenous bile acids; (b)=endogenous plus exogenous bile acids. BA=bile acids; iv=intravenous.
†$p<0.05$ compared with 1 hour test meal alone.
‡$p<0.05$ compared with 2 hour test meal alone.

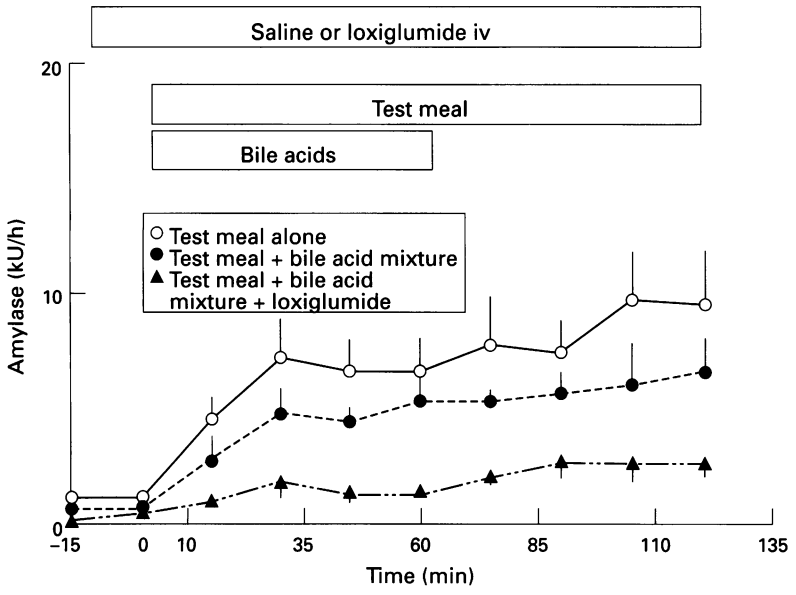

Figure 3: Amylase output during continuous duodenal perfusion of a test meal (day 1), a test meal plus an individual bile acid mixture as determined on day 1 in a quantity that equalled 30% of the output measured on day 1 during the first hour of stimulation, with saline infused intravenously (iv) (day 2), and a test meal plus an individual bile acid mixture as determined on day 1 in a quantity that equalled 30% of the output measured on day 1 during the first hour of stimulation, with loxiglumide infused iv at a concentration of 10 mg/h per kg; this infusion was started at least 60 minutes before the test meal period and was continued throughout the study.

the total amount of bile acids released during continuous duodenal perfusion of a test meal in each volunteer. We then used this individual bile acid pattern for perfusion of exogenous bile acids calculated to reproduce the postprandial situation in an identical manner.

Our findings suggest that postprandial (that is, test meal stimulated) duodenal bile acids are of minor importance in the direct control of pancreatic enzyme release but are pivotal for the control of CCK release. In previous studies, single bile acids were used to study the effect of bile acids on stimulated pancreatic enzyme release. Taurine-conjugated bile acids at concentrations of 5–10 mmol/l or 6 mmol/h inhibited amino acid- or oleic acid-stimulated enzyme output.[5 6] The most pronounced effect was obtained with taurocholate. However, under physiological conditions taurine-conjugated bile acids amount to only 15–31%.[11 12] In a previous study we failed to observe any effect of 5 mmol chenodeoxycholic acid on test meal stimulated pancreatic enzyme secretion.[4]

We therefore conclude from these studies that exogenous bile acids comprising the individual bile acid mixture have no significant influence on test meal stimulated pancreatic enzyme secretion.

During loxiglumide infusion pancreatic enzyme output was reduced to 25%–35% which is in accordance with previously reported data.[7 8] Perfusion of bile acids did not alter enzyme response although during the second hour enzyme output was increased by twofold. However, enzyme output was higher during the second hour of stimulation in all groups and for all enzymes (except during 100% bile acid perfusion). Thus, the discrepancy between the first and the second hour under loxiglumide infusion was not due to bile acid perfusion.

Bile acid and bilirubin output during test meal perfusion were significantly higher during the first hour of stimulation and this was probably due to maximal gall bladder contraction during this time. Exogenous bile acids at both dosages used had no significant influence on bile output. Loxiglumide infusion reduced bile acid and bilirubin output by 85%–100%. The increase in bile acid output during the second hour when exogenous bile acid perfusion had been stopped is probably due to duodenal bile acids left over from the first 15 minutes of the second hour. Bilirubin output was not altered by intraduodenal bile acid perfusion. The results are at variance with previously reported data showing complete suppression of amino acid stimulated bilirubin output by 10 mM taurocholate and reduction of amino acid stimulated gall bladder contraction by 6 mM sodium taurocholate.[5 9]

Thus, when given as a single bile acid in supraphysiological amounts, taurocholate appears to inhibit gall bladder contraction. The more physiological approach we have chosen excluded any influence of endogenous bile acids on bile release.

As observed in previous studies, plasma PP concentrations were not significantly altered by intraduodenal bile acids.[4] The complete suppression of PP release by loxiglumide has been described previously[8 24] and underlines the importance of functional CCK-A receptors in the control of PP release. It has not been clarified whether circulating CCK acts directly on the pancreatic PP producing cell or indirectly on neurones, which in turn stimulate the PP producing cell. The latter is more likely since postprandial and caerulein induced PP release is abolished by both loxiglumide and atropine.[24]

In contrast with previous studies in which supraphysiological amounts of single bile acids were used,[4 9 11] the imitation of physiological conditions by perfusion of an individual bile acid mixture at physiological concentrations failed to show any effect on plasma CCK concentrations. Differences in study conditions (bolus application versus continuous perfusion of test meal) and the only weak stimulation of plasma CCK by the test meal (twofold over basal) may account for this discrepancy.

TABLE II *Integrated output of plasma CCK and plasma PP. Results are mean (SEM)*

	Test meal (n=7)		*Test meal, saline iv*		*Test meal*		*Test meal, loxiglumide iv*	
	1 hour, -BA	*2 hours, -BA*	*1 hour, 30% BA*	*2 hours, -BA*	*1 hour, 100% BA*	*2 hours, -BA*	*1 hour, 30% BA*	*2 hours, -BA*
CCK (pM×60 min)	131 (63)	159 (56)	82 (44)	98 (46)	104 (21)	137 (17)	229 (48)	586 (80)*
PP (pM×60 min)	4·6 (3·0)	7·6 (3·3)	1·7 (0·5)	5·7 (2·4)	3·7 (1·4)	1·9 (1·5)	0·3 (0·2)	0·4 (0·6)

*p<0·05 compared with 1 hour test meal plus loxiglumide and compared with 2 hour during test meal alone.

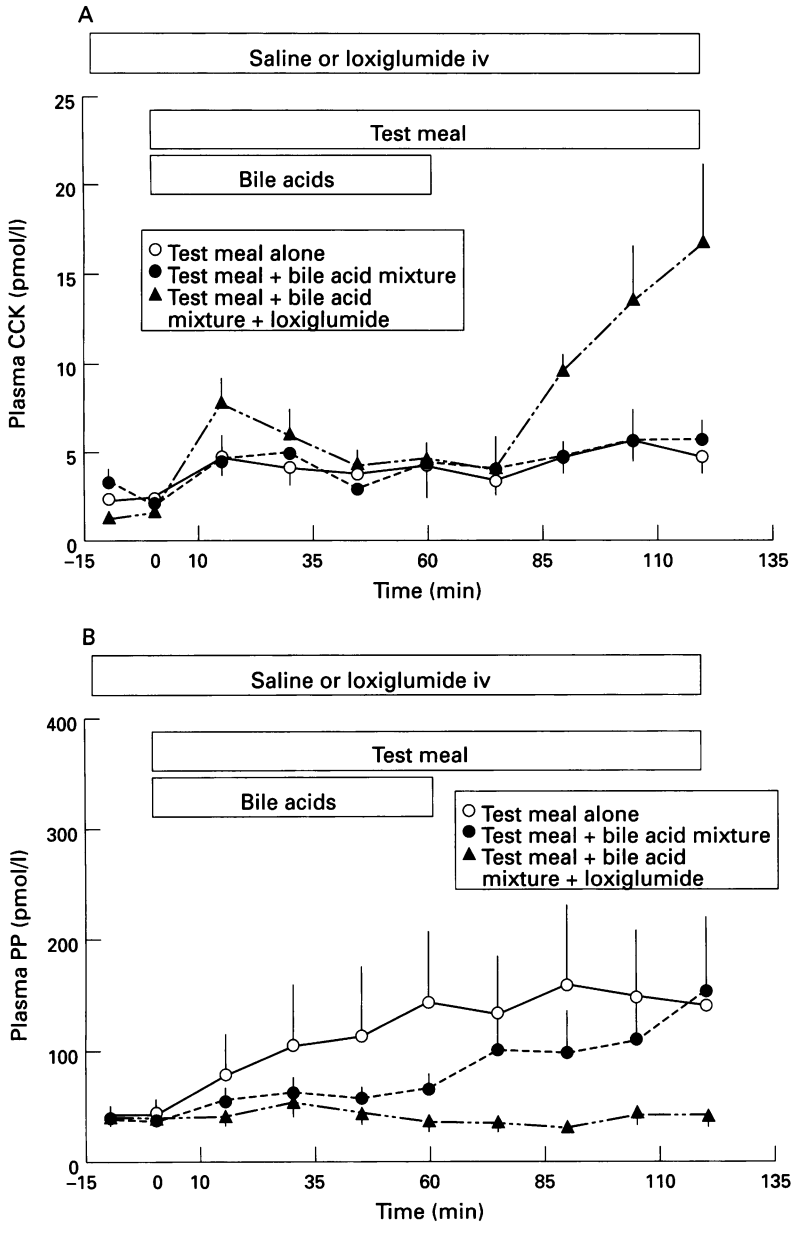

Figure 4: CCK (A) and PP (B) output during continuous duodenal perfusion of a test meal (day 1), a test meal plus an individual bile acid mixture as determined on day 1 in a quantity that equalled 30% of the output measured on day 1 during the first hour of stimulation, with saline infused intravenously (iv) (day 2), and a test meal plus an individual bile acid mixture as determined on day 1 in a quantity that equalled 30% of the ouput measured on day 1 during the first hour of stimulation, with loxiglumide infused iv at a concentration of 10 mg/h per kg; this infusion was started at least 60 minutes before the test meal period and was continued throughout the study.

As seen in acute bile diversion induced by external biliary drainage in patients with obstructive jaundice[25] or by oral or duodenal application of the bile acid binding resin cholestyramine,[9 26] CCK-A receptor blockade, which reduced intraluminal bile acid content to almost zero, substantially stimulated plasma CCK release. This increase in plasma CCK could be diminished to near control values (test meal perfusion alone, intravenous saline) by the addition of only 30% of the individual bile acid mixture to the test meal. This is in agreement with the inhibitory effect of chenodeoxycholic acid on cholestyramine induced CCK stimulation.[4] It can be concluded that the presence of one third of the amount of bile acids released during a test meal perfusion is suficient to suppress excess release of CCK.

Our results clearly demonstrate a negative feedback control of CCK release by intraduodenal bile acids. The CCK producing cell is under constant suppression by intraduodenal bile acids which cannot be further enhanced by the addition of a physiological bile acid mixture. However, removal of duodenal bile acids by inhibition of gall bladder contraction unmasks this suppression leading to a dramatic increase in plasma CCK levels, which in turn can be reversed by intraduodenal bile acid application. It remains open whether a threshold of intraduodenal bile acid concentration (or amount) exists at which suppression of CCK release is turned off or whether gradual reduction of bile acids causes a reciprocal rise in plasma CCK levels. Interestingly, during CCK-A receptor blockade, plasma CCK levels rose only when luminal stimuli of CCK release, such as protein or fatty acids, were concomitantly perfused through the duodenum. Basal values were unaffected. It may be speculated that luminal stimuli of CCK act via release of a CCK releasing factor which under physiological postprandial conditions is partly inactivated by intraluminal bile acids.

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