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Cholestyramine Influences Meal-Stimulated Pancreaticobiliary Function and Plasma Cholecystokinin Independent of Gastric Emptying and Food Digestion

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Background: Cholestyramine enhances gallbladder emptying and plasma cholecystokinin responses to oral ingestion of a mixed meal. It is not known whether this effect occurs independently of alterations in gastric emptying or maldigestion of nutrients. Methods: We perfused 15 g of an amino acid meal intraduodenally for 60 min in seven healthy volunteers, once with and once without cholestyramine. Intraduodenal perfusion of saline with or without cholestyramine (6 g/h) was started 60 min before the amino acid meal and continued for 2 h. Results: Cholestyramine markedly enhanced the incremental plasma cholecystokinin response to the meal from 36 ± 12 to 139 ± 25 pmol/l · 60 min (P < 0.005), incremental amylase output from 2.4 ± 0.7 to 5.7 ± 0.7 kU/h (P < 0.05), and incremental integrated gallbladder contraction from 1948 ± 235 to 2840 ± 189% · 60 min (P < 0.05). Conclusion: The enhancing effect of cholestyramine on postprandial gallbladder contraction, pancreatic enzyme secretion, and plasma cholecystokinin release is not dependent on gastric emptying rates or appropriate digestion of nutrients.

Key words: Amino acids; cholecystokinin; gallbladder motility; pancreatic enzyme secretion; pancreatic polypeptide

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The anion exchanger cholestyramine precipitates bile salts. Cholestyramine is a drug used in clinical practice to lower plasma cholesterol levels, to treat obstructive liver diseases, or to decrease diarrhoea induced by bile salt overflow into the colon. Recently, cholestyramine has also been used as a tool in physiologic studies to investigate the role of bile salts in the regulation of plasma cholecystokinin (CCK) release and pancreaticobiliary secretion (1-5).

In physiologic studies that used cholestyramine to precipitate bile acids, cholestyramine enhanced plasma CCK levels and gallbladder responses to an orally or intraduodenally administered mixed meal (1-4) and to bombesin infusion (5). Direct effects of cholestyramine on these functions are unlikely, since colestipol, a bile salt-binding resin with a different molecular structure from cholestyramine, has comparable effects on CCK release and gallbladder motility (5). Nevertheless, cholestyramine may indirectly affect gallbladder motility and plasma CCK release by enhancing gastric emptying (6) or by delaying the digestion of fatty nutrients (1). To investigate these possibilities, we have studied the effect of cholestyramine on plasma CCK and pancreatic polypeptide (PP) levels, gallbladder motility, and pancreatic enzyme secretion in response to a meal stimulus. To avoid indirect effects induced by gastric emptying, both cholestyramine and the meal stimulus were administered intraduodenally. To avoid effects of cholestyramine on the hydrolysis of nutrients induced by bile salt precipitation, an amino acid mixture was used as meal stimulus.

MATERIALS AND METHODS

Subjects
Seven healthy volunteers (three women and four men; median age, 23 years; range, 19-26 years) participated in the studies. None of the volunteers was taking any medication or had a history of gastrointestinal symptoms or surgery. The study protocol was approved by the ethical committee of the University Hospital of Nijmegen, and all subjects gave their written informed consent before entering the study.

Reagents
Cholestyramine (Questran®) was obtained from Bristol-Myers, Woerden, The Netherlands, as packets containing 4 g of the resin; radioiodinated porcine pancreatic polypeptide
Test 2 was performed in accordance with the same protocol as test 1. However, 1 h after the start of saline perfusion cholestyramine (6 g/h) was perfused intraduodenally during the last 2 test hours (Fig. 1). This dosage of cholestyramine was chosen because previous studies indicated that this amount was sufficient to enhance plasma CCK and pancreaticobiliary responses to a meal in humans (2, 3). On the basis of in vitro binding studies we calculated that 6 g of cholestyramine could bind approximately one pool of bile salts in humans (8, 9). Five-millilitre samples of duodenal contents were taken from the tip of the tube during 15-min periods by spot-sampling (10) and kept on ice. Blood samples were taken every 30 min during the 1st h and subsequently every 15 min until the end of the test period (Fig. 1).

Blood was collected in ice-chilled glass tubes containing 2 g/l of ethylenediaminetetraacetate (EDTA). After the experiments the blood samples were centrifuged at 4°C for 10 min at 3000 g. Plasma and duodenal samples were stored at −20°C until assayed for CCK, PP, PEG-4000, and amylase. Each time a blood sample was drawn, two longitudinal and two transverse images of the gallbladder were obtained by real-time ultrasonography.

**Plasma samples**

Plasma CCK was measured by a sensitive and specific radioimmunoassay (RIA) as described previously (11, 12). The antibody used (T204) binds to biologically active CCK peptides containing the sulphated tyrosine region. On a molar base, sulphated gastrins cross-reacted <2% in the assay, while no cross-reactivity with unsulphated gastrins or structurally unrelated peptides was found. The detection limit of the assay was between 0.5 and 1.0 pmol/l CCK in plasma. The intra-assay precision ranged from 4.6% to 11.5% in the steep part of the standard curve. All measurements of plasma CCK levels were performed in the same run.

Plasma PP levels were also determined by RIA (13). The antibody used showed no cross-reactivity with structurally related gastrointestinal regulatory peptides like peptide YY (PYY) or neuropeptide Y (NPY) or with structurally unrelated peptides. The detection limit of the assay was 0.5 pmol/l of incubation mixture. The intra-assay variation ranged from 4% to 7% in the steep part of the standard curve. All measurements of plasma PP levels were performed in one run.

**Duodenal samples**

Duodenal samples were analysed for PEG-4000 (14) and amylase activity (15). Flow rates passing the distal duodenal sampling site were calculated on the basis of known perfusion rates and PEG-4000 concentrations at the perfusion and sampling ports (10). Outputs of amylase were calculated from the product of enzyme activity and flow rates. Furthermore, to exclude a possible direct effect of cholestyramine on the amount of free amino acid concentrations, duodenal samples taken during the last test hour were analysed for the amount of free amino acids by ion-exchange chromatography (7, 16).
Fig. 2. Basal and amino acid-stimulated (60-120 min) plasma cholecystokinin (CCK) concentrations (pmol/l) in seven healthy subjects either with (●) or without (○) intraduodenal administration (0-120 min) of cholestyramine at a dose of 6 g/h. Values are expressed as mean ± standard error (SEM). Statistical analysis was performed with Student’s t test for paired results. Differences with a two-tailed probability (P) value of less than 0.05 were considered significant (20).

RESULTS

Plasma CCK concentrations

Basal plasma CCK levels were unaffected by saline perfusion with or without cholestyramine and varied between 2.4 and 3.0 pmol/l during the first 2 h in both experiments (Fig. 2). Perfusion of the amino acid solution caused a rise in plasma CCK levels from 2.9 ± 0.2 to a maximum of 3.6 ± 0.5 pmol/l, achieved 15 min after the start of amino acid perfusion. Thereafter plasma CCK levels decreased to 3.1 ± 0.3 pmol/l during the last 45 min of the 3rd test hour. Perfusion of cholestyramine markedly enhanced amino acid-stimulated CCK levels to 5.2 ± 0.7 pmol/l 15 min after the start of amino acid perfusion. Furthermore, plasma CCK levels did not decrease in the last 45 min of the test hour but even tended to increase (Fig. 2). As a result, incremental integrated levels (Table I) during amino acid perfusion differed between the test without and with cholestyramine (36 ± 12 versus 139 ± 25 pmol/l · 60 min; P < 0.01).

Gallbladder ultrasonography

Longitudinal and transverse images of the gallbladder were obtained by real-time ultrasonography (Sonolayer Sal 77-B, Toshiba, Japan) using a 3.75-MHz transducer (17, 18). Gallbladder volume was calculated from these images by the sum of cylinders method using a computer system (19). The variation of volume measurements ranged from 6% to 22%.

Statistical analysis

All measurements were performed in duplicate, and the mean of these two measurements was used for further analysis of results. Gallbladder volume was expressed in millilitres and as a percentage of the mean volume obtained in the lst h of the experiment (−60 to 0 min). Integrated plasma CCK, PP, and gallbladder responses were determined by calculating the area under the CCK, PP, or gallbladder contraction time curves after subtraction of the mean of the values obtained in the first 60-min period (−60 to 0 min). Subsequently, incremental integrated CCK, PP, and gallbladder contraction to the amino acid meal was calculated by subtracting the integrated response in the basal period (0-60 min) from the integrated response in the period of stimulation (60-120 min) in each experiment. Similarly, incremental amylase outputs were calculated by subtraction of the total output in the basal period (0-60 min) from the total output in the period of stimulation (60-120 min). Results are expressed as mean ± standard error (SEM). Statistical analysis was performed with Student’s t test for paired results. Differences with a two-tailed probability (P) value of less than 0.05 were considered significant (20).

<table>
<thead>
<tr>
<th>Test</th>
<th>0-60 min</th>
<th>60-120 min</th>
<th>Incremental</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCK (pmol/l  60 min)</td>
<td>Sal</td>
<td>-9 ± 6</td>
<td>27 ± 11‡</td>
</tr>
<tr>
<td></td>
<td>Sal + CH</td>
<td>-6 ± 8</td>
<td>133 ± 30†‡</td>
</tr>
<tr>
<td>PP (pmol/l  60 min)</td>
<td>Sal</td>
<td>-20 ± 90</td>
<td>648 ± 143‡</td>
</tr>
<tr>
<td></td>
<td>Sal + CH</td>
<td>3 ± 140</td>
<td>3172 ± 1006†‡</td>
</tr>
<tr>
<td>GBC* (% 60 min)</td>
<td>Sal</td>
<td>-121 ± 121</td>
<td>1827 ± 217†‡</td>
</tr>
<tr>
<td></td>
<td>Sal + CH</td>
<td>748 ± 352†‡</td>
<td>3589 ± 374†‡</td>
</tr>
<tr>
<td>Amylase (kU)</td>
<td>Sal</td>
<td>2.8 ± 0.9</td>
<td>5.2 ± 0.7†‡</td>
</tr>
<tr>
<td></td>
<td>Sal + CH</td>
<td>4.0 ± 1.3</td>
<td>9.7 ± 1.6†‡</td>
</tr>
</tbody>
</table>

Data are mean ± 1 SEM from seven healthy subjects; incremental responses were obtained by subtraction of integrated values in the basal period (0-60 min) from integrated values in the period of stimulation (60-120 min).

* GBC = gallbladder contraction.
† P < 0.05 versus saline perfusion.
‡ P < 0.05 versus 0-60 min.
of 37 ± 7 pmol/l 30 min after the start of amino acid perfusion. Ingestion of cholestyramine markedly increased plasma PP levels to a maximum of 90 ± 22 pmol/l 45 min after the start of amino acid perfusion. As a result, incremental integrated plasma PP levels (Table I) during amino acid perfusion were enhanced by cholestyramine (669 ± 216 versus 3169 ± 982 pmol/l · 60 min; \( P < 0.05 \)).

**Gallbladder volume**

Perfusion of saline did not affect basal gallbladder volume (37 ± 4 ml) (Fig. 4). Mean basal gallbladder volume decreased (\( P < 0.05 \)) from 35 ± 3 ml at the start to 25 ± 4 ml 1 h after perfusion of cholestyramine (\( P < 0.05 \) versus saline), while integrated gallbladder contraction increased (\( P < 0.05 \)) from −121 ± 121% · 60 min to 748 ± 352% · 60 min (Table I). Amino acid perfusion resulted in gallbladder contraction from 35 ± 3 ml to a minimum of 19 ± 2 ml 45 min after the start of amino acid perfusion. Cholestyramine further decreased gallbladder volume to a minimum of 7 ± 2 ml 60 min after the start of amino acid perfusion. Incremental integrated gallbladder contraction following amino acid perfusion (Table I) was markedly enhanced by cholestyramine (1948 ± 235 versus 2840 ± 189% · 60 min; \( P < 0.05 \)).

**Pancreatic enzyme output**

Basal amylase output (Fig. 5) during saline perfusion was not significantly changed by cholestyramine (2.8 ± 0.9 versus 4.0 ± 1.3 kU/h). Amino acid-stimulated amylase output was enhanced by cholestyramine (5.2 ± 0.7 versus 9.7 ± 1.6 kU/h; \( P < 0.05 \)). As a result, incremental amylase output was also enhanced (\( P < 0.05 \)) in the cholestyramine experiment when compared with amino acid perfusion alone (Table I).

**Amino acid analysis in duodenal juice**

Perfusion of cholestyramine together with amino acids did not affect free amino acid concentrations in duodenal juice at 90 min (Table II). Furthermore, similar amounts of free amino acids were found in the amino acid meal with and without cholestyramine at all other time points during the last test hour (60, 75, 105, and 120 min; data not shown).

**DISCUSSION**

The present study shows that pancreatic enzyme secretion and gallbladder contraction as well as plasma CCK and PP release in response to an intraduodenally administered amino acid meal are enhanced by duodenal perfusion of cholestyramine in humans.

The experimental protocol of the present study differs in several aspects from those of previous studies (1-5). First, a
Table II. Free essential amino acids present in duodenal juice (mmol/l) at 90 min when amino acids either without or with cholestyramine were perfused

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>Amino acids + cholestyramine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valine</td>
<td>7.6 ± 0.8</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>6.5 ± 0.6</td>
</tr>
<tr>
<td>Leucine</td>
<td>13.3 ± 1.5</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>2.6 ± 0.3</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td>Lysine</td>
<td>18.8 ± 1.5</td>
</tr>
<tr>
<td>Histidine</td>
<td>4.4 ± 0.4</td>
</tr>
</tbody>
</table>

Data are mean ± s for seven healthy subjects.

basal period of 60 min with or without intraduodenal administration of cholestyramine preceded the meal-stimulation period. Therefore, in contrast with previous studies, this protocol enables us to differentiate between effects of cholestyramine on unstimulated and on meal-stimulated functions. The results demonstrate that cholestyramine significantly induces gallbladder contraction under unstimulated conditions and tends to cause pancreatic enzyme secretion without affecting plasma CCK and PP release. Therefore, the enhancing effect of cholestyramine on meal-stimulated gallbladder contraction and pancreatic enzyme secretion, but not on CCK and PP release, might in part be explained by the effect of cholestyramine on these variables in the basal state.

Second, the meal and the cholestyramine were administered intraduodenally in this study. Intraduodenal administration excludes possible effects of cholestyramine on gastric factors like gastric emptying, which may interfere with the ultimate results (21). The persistence of the enhancing effects of cholestyramine after intraduodenal administration indicates that cholestyramine can exert its effects independently of gastric factors. Only one other study in humans investigated intraduodenal administration of a meal (3). However, in that study a mixed meal containing whole protein and fat was used.

Finally, in our study an amino acid mixture was perfused intraduodenally. This elemental meal has the advantage that cholestyramine cannot interfere with the digestion of fat or protein, which is essential for stimulation of gallbladder contraction and CCK release (7). This is important because it has been suggested that the effect of cholestyramine is mediated by insufficient formation of micelles as a result of intraluminal bile salt sequestration or by binding of fatty acids (1). This may result in malabsorption of fat and subsequently lead to the exposure of an increased surface area of the proximal intestinal mucosa to CCK-stimulating fatty nutrients for a prolonged time (1). The present data indicate that the enhancing effect of cholestyramine is not necessarily related to fatty nutrients but persists with an amino acid mixture and therefore indicates that other mechanisms are involved.

Similar to previous studies plasma PP responses paralleled plasma CCK responses to cholestyramine under basal and meal-stimulated conditions (1). Because circulating CCK may induce PP release (22-24), the effect of cholestyramine on PP can at least in part be attributed to the increased CCK release. Similarly, the enhanced pancreaticobiliary response to the amino acid meal with cholestyramine may be explained by the increased plasma CCK levels (18, 25-27). However, in the present study we also found that cholestyramine tended to increase pancreatic enzyme secretion and significantly stimulated gallbladder contraction under basal conditions. The mechanism by which cholestyramine affected pancreatic enzyme secretion and gallbladder motility in the present study was probably not related to plasma CCK, since plasma CCK levels were not significantly altered. Another possibility is that the effect of cholestyramine under basal conditions was mediated by activating vagal cholinergic pathways (28, 29). However, absence of PP secretion in response to cholestyramine does not support this hypothesis. PP is a hormone that is primarily regulated by vagal cholinergic mechanisms (30, 31). Therefore, other hormonal or neural mechanisms are probably involved in mediating the effect of cholestyramine under basal conditions.

The mechanism by which cholestyramine enhanced the plasma CCK response to the amino acid meal remains speculative. Theoretically, cholestyramine may bind amino acids and thereby delay the absorption of CCK-stimulating substances, which may in turn trigger CCK cells to increased secretion. This possibility was excluded by the presence of similar concentrations of free amino acids in both the study with and that without cholestyramine.

The results of several studies provide evidence that cholestyramine most probably augments CCK release by precipitating bile salts in the small bowel. Colestipol, another bile salt sequestran with a chemical structure that is not similar to cholestyramine, had similar effects (5). Total diversion of bile in dogs significantly increased the release of CCK in response to intraduodenal administration of amino acids, while replacement of the bile salt pool with intraduodenal administration of taurocholate reversed the enhancement effect (2). Release of cholecystokinin in response to oral amino acids in humans was significantly inhibited by oral taurocholate (2). Chenodeoxycholic acid inhibited CCK release in response to an intraduodenal mixed liquid meal in humans and prevented the enhancement effect of cholestyramine (3). Administration of CCK receptor antagonists in humans results in a marked exaggeration of meal-stimulated plasma CCK levels (25-27). Because CCK receptor antagonists inhibit gallbladder contraction to a greater extent than pancreatic enzyme secretion in humans, this suggests that the effect is primarily due to a decreased amount of bile salts in the small bowel (27, 32).

The mechanism by which bile acids inhibit amino acid-stimulated CCK release is not known. Bile acids may interfere with the stimulatory action of amino acids on CCK release by slowing down amino acid absorption, resulting in an increased
surface area of small-intestinal mucosa exposed to stimulating nutrients. However, Dimagno did not observe acceleration of amino acid absorption by bile salts (32, 33). Furthermore, the finding that cholesteramine also enhances bombesin-stimulated plasma CCK levels indicates that the effect is not dependent on the presence of nutrients in the gut. Whether the effect of bile acids is mediated by other factors such as a CCK releasing factor or by inhibitory hormones such as somatostatin remains to be established (34–36).

In conclusion, the present study demonstrates that the enhancing effect of cholesteramine on meal-stimulated gallbladder contraction, pancreatic enzyme secretion, and CCK and PP release is independent of gastric emptying and the hydrolysis of nutrients.

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