

Inhibition of Pancreaticobiliary Secretion by Loperamide in Humans

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Loperamide, a peripherally acting opiate receptor agonist with antidiarrheal action, inhibits ileal and colonic motor function. It was determined whether loperamide also affects gallbladder emptying and pancreatic enzyme secretion in humans. Plasma cholecystokinin (radioimmunoassay), gallbladder volume (ultrasonography), and intraduodenal bilirubin and amylase output (spot sampling) were measured at regular intervals before and during intraduodenal perfusion of an amino acid meal in 8 healthy subjects: once without and once with pretreatment of 8 mg loperamide, ingested 13 and 4 hours before the start of the meal. Loperamide decreased basal amylase output from 3.2 ± 0.5 to 1.0 ± 0.5 kU/h ($P < .005$) and abolished basal bilirubin output (21 ± 5 vs. 0 ± 0 $\mu\text{mol/h}$; $P < .005$) into the duodenum. Loperamide increased basal gallbladder volume from 28 ± 4 to 39 ± 4 mL ($P < .0001$) but was without effect on basal plasma cholecystokinin (2.7 ± 0.3 vs. 3.0 ± 0.3 pmol/L). During the amino acid meal, pretreatment with loperamide inhibited amylase output from 5.1 ± 0.8 to 1.6 ± 0.4 kU/h ($P < .001$), bilirubin output from 39 ± 6 to 18 ± 6 $\mu\text{mol/h}$ ($P < .0005$) and gallbladder contraction from $47\% \pm 3\%$ to $26\% \pm 6\%$ ($P < .05$), whereas loperamide enhanced amino acid-stimulated plasma cholecystokinin from 4.5 ± 1.6 to 7.6 ± 1.0 pmol/L ($P < .05$). It is concluded that loperamide inhibits basal and amino acid-stimulated gallbladder motility and intraduodenal output of bilirubin and amylase, despite an enhanced postprandial cholecystokinin release. (HEPATOL-OGY 1997;26:256-261.)

Loperamide is widely used for the treatment of patients with diarrhea resulting from a variety of diseases.^{1,2} Dosages used to treat acute or chronic diarrhea vary from 2 to 16 mg daily.¹ Recently, even higher dosages have been recommended.² Loperamide probably exerts its antidiarrheal action by a change in motor function of the intestine, resulting in an increased capacitance of the gut and a delay in the passage of fluid through the intestine.^{1,3} Some studies also suggest antisecretory effects of the drug at the intestinal level.^{4,5} The

effects of loperamide on pancreaticobiliary functions are poorly investigated. Inhibition of gallbladder emptying or pancreatic enzyme secretion by loperamide may have important clinical implications because stasis of bile is a major factor contributing to the formation of gallstones⁶⁻¹⁰ and because impairment of pancreatic enzyme secretion may induce or aggravate maldigestion.¹¹⁻¹³

The aim of this study, therefore, was to determine the effect of loperamide on basal and meal-stimulated gallbladder motility and pancreatic enzyme secretion in healthy volunteers. Gallbladder emptying and pancreatic enzyme secretion were studied in response to an intraduodenal amino acid meal to circumvent possible influences of loperamide on gastric emptying or on the digestion of nutrients.¹⁴

PATIENTS AND METHODS

Subjects. Eight healthy volunteers (3 women and 5 men; age range, 19-27 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 Nijmegen, and all subjects gave their written informed consent before entering the study.

Reagents. Loperamide chloride was obtained from Janssen Pharmaceutica (Beerse, Belgium). Radioiodinated porcine pancreatic polypeptide was obtained from Novo Nordisk AS (Bagsvaerd, Denmark). Synthetic CCK₃₃ was from Peninsula Laboratories Europe, Ltd. (St. Helens, England). ¹²⁵I-hydroxyphenyl propionic acid-succinimide ester (Bolton-Hunter reagent) was obtained from New England Nuclear Corp. (Boston, MA). Pharmacia Decanting Suspension no. 3 was from Pharmacia Diagnostics, AB (Uppsala, Sweden). Human pancreatic polypeptide and L-amino acids were from Sigma Chemical Co. (St. Louis, MO). Polyethylene glycol 4000 (PEG-4000) from BDH, Ltd. (Poole, England). All other materials were obtained from Merck (Amsterdam, The Netherlands).

Study Protocol. After a 12-hour fast, the volunteers presented at the laboratory at 7:30 am. In random order, two experiments were performed separated from each other by at least 1 week. At the beginning of each test a double-lumen polyvinyl tube with an OD of 5.7 mm was placed in the duodenum. The proximal lumen of the tube was positioned at the level of the papilla Vateri, and the distal lumen was positioned at the ligament of Treitz. The position of the tube was verified by fluoroscopy. In addition, an in-dwelling intravenous catheter was placed in a forearm. The catheter was kept patent by a heparine-saline solution and was used for the collection of blood samples. After an equilibration period of at least 30 minutes, the following tests were performed. In test 1, saline (300 mosmol/L) was continuously perfused intraduodenally for 2 hours (300 mL/h) together with the recovery marker PEG-4000 (6g/L). During the third test hour, an isoosmotic solution (300 mOsm; Fig. 1) of an amino acid mixture,¹⁵ containing L-valine (2.17 g), L-methionine (1.71 g), L-tryptophan (0.67 g), and L-phenylalanine (2.34 g) was perfused intraduodenally together with saline (140

Abbreviations: PEG-4000, polyethylene glycol 4000; CCK, cholecystokinin.

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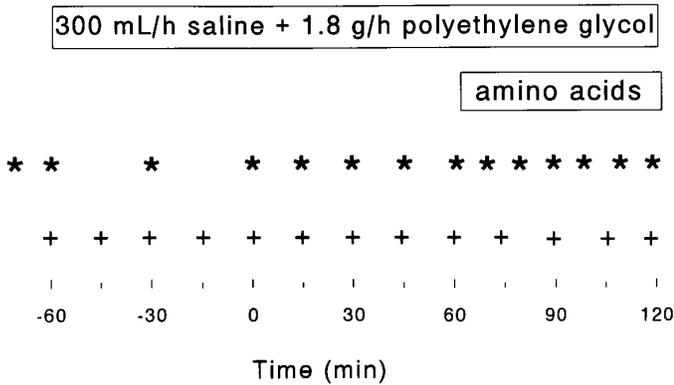


FIG. 1. Study protocol for the two tests performed in random order. In both tests, saline was continuously perfused intraduodenally together with the recovery marker PEG-4000 (1.8 g/h) from -60 to 120 minutes at a constant rate of 5 mL/h. During the last test hour, 6.89 g of an amino acid solution was also perfused intraduodenally. In one of the two tests, loperamide (8 mg) was ingested 13 and 4 hours before the start of the amino acid perfusion. *Time points when blood was drawn for the measurement of plasma CCK and pancreatic polypeptide levels, whereas gallbladder volume was determined by ultrasonography. +Time points when duodenal juice was sampled for the determination of bilirubin and amylase.

mL) and demineralized water (160 mL). Test 2 was performed according to the same protocol as test 1. However, 13 and 4 hours before the start of the amino acid perfusion, 8 mg of loperamide was administered orally (Fig. 1). Five-milliliter samples of duodenal contents were taken during 15-minute periods from the tip of the tube by spot-sampling¹⁶ and kept on ice. Blood samples were taken every 30 minutes during the first hour and subsequently every 15 minutes until the end of the test period (Fig. 1).

Blood was collected into ice-chilled glass tubes containing 2 g/L of ethylenediaminetetraacetic acid. After the experiments, the blood samples were centrifuged at 4°C for 15 minutes (3,000g). In test 2, an additional blood sample was taken just before the start of the amino acid perfusion for measurement of plasma loperamide levels. Plasma and duodenal samples were stored at -20°C until assayed for cholecystokinin (CCK), pancreatic polypeptide, loperamide, PEG-4000, bilirubin, and amylase. Each time that a blood sample was taken, 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 as described previously.^{17,18} The antibody used (T204) binds to biologically active CCK peptides containing the sulfated tyrosine region with almost equal affinity. On a molar base, sulfated gastrins cross-reacted <2% in the assay, whereas no cross-reactivity with unsulfated 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 pancreatic polypeptide levels were also determined by radioimmunoassay.¹⁹ The antibody used showed no cross-reactivity with structurally related gastrointestinal regulatory peptides, such as peptide YY or neuropeptide Y, 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 pancreatic polypeptide levels were performed in one run.

Plasma loperamide levels were determined at the department of Drug, Metabolism and Pharmacokinetics, Janssen Pharmaceutica, Beerse, Belgium, as described previously.²⁰

Duodenal Samples. Duodenal samples were analyzed for PEG-4000,²¹ bilirubin,²² and amylase activity.²³ Flow rates passing the

distal duodenal sampling site were calculated based on known perfusion rates and PEG-4000 concentrations at the perfusion and sampling ports.¹⁶ Outputs of bilirubin and amylase were calculated from the product of concentrations or activity and flow rates.

Gallbladder Ultrasonography. Longitudinal and transverse images of the gallbladder were obtained by real-time ultrasonography (Sonolayer Sal 77-B; Toshiba, Tokyo, Japan) using a 3.75-MHz transducer.^{24,25} Gallbladder volume was calculated from these images by the sum of cylinders method using a computer system.²⁶ 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 milliliters and as percentage of the mean basal value at 45 and 60 minutes. Integrated plasma CCK, pancreatic polypeptide, and gallbladder responses were determined by calculating the area under the plasma CCK concentration-time curves, pancreatic polypeptide concentration-time curves, or gallbladder volume time curves. Subsequently, incremental plasma CCK, pancreatic polypeptide concentrations, and gallbladder volume responses to the meal were calculated by subtracting the integrated response in the basal period (0-60 minutes) from the integrated response in the period of stimulation (60-120 minutes) in each experiment. Similarly, incremental bilirubin and amylase outputs were calculated by subtraction of the total output in the basal period (0-60 minutes) from the total output in the period of stimulation (60-120 minutes). Results are expressed as means \pm SEM. Statistical analysis was performed by Student's *t* test for paired results or by two-way analysis of variance when appropriate. Differences with a two-tailed *P* value of .05 were considered significant.

RESULTS

Plasma Loperamide Levels. Plasma levels of loperamide at the start of the amino acid perfusion in test 2 were 2.5 \pm 0.3 ng/mL.

Plasma Concentrations of CCK and Pancreatic Polypeptide. Plasma CCK and pancreatic polypeptide time curves are shown in Figs. 2 and 3. Loperamide significantly (*P* < .05) inhibited basal pancreatic polypeptide levels from 25 \pm 5 to 20 \pm 4 pmol/L (Fig. 3 and Table 1) but was without significant effect on basal CCK (2.7 \pm 0.3 pmol/L without loperamide vs. 3.0 \pm 0.3 pmol/L with loperamide; Fig. 2 and Table

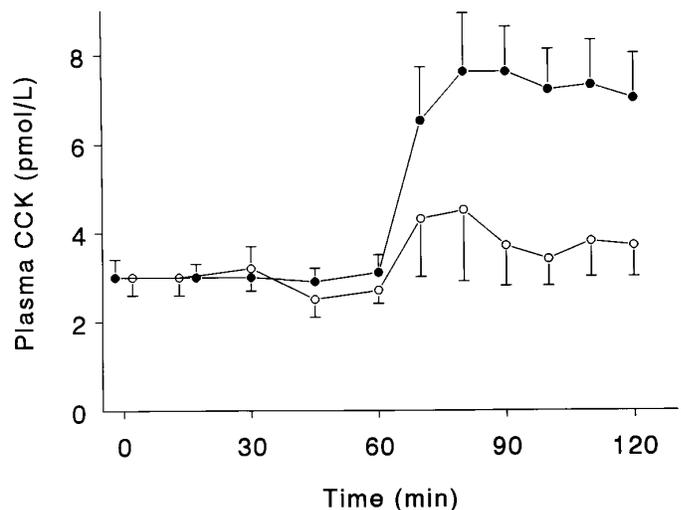


FIG. 2. Basal and amino acid-stimulated plasma CCK concentrations (in pmol/L) in 8 healthy subjects with (●) or without (○) prior ingestion of loperamide. Values are expressed as means \pm SEM.

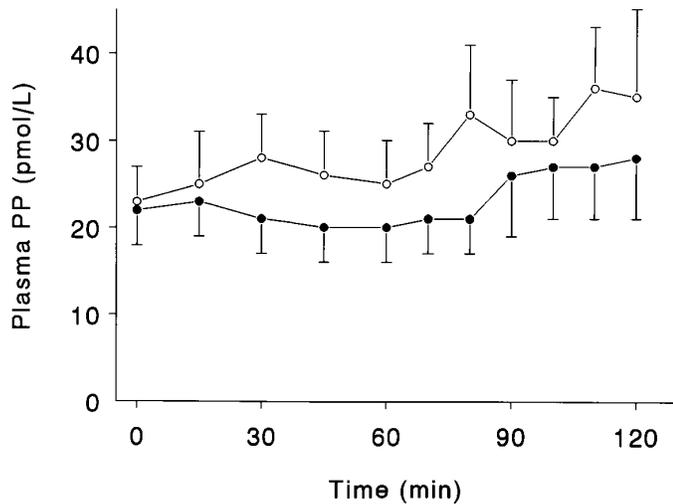


FIG. 3. Basal and amino acid-stimulated plasma pancreatic polypeptide concentrations (in pmol/L) in 8 healthy subjects with (●) or without (○) prior ingestion of loperamide. Values are expressed as means \pm SEM.

1). Intraduodenal administration of the amino acid meal induced a significant ($P < .05$) increase of plasma pancreatic polypeptide from 25 ± 5 to 36 ± 7 pmol/L (Fig. 3) and of plasma CCK from 2.7 ± 0.3 to 4.5 ± 1.6 pmol/L (Fig. 2). Loperamide induced a statistically significant ($P < .05$) enhancement of the amino acid-stimulated plasma CCK level from 4.5 ± 1.6 to 7.6 ± 1.0 pmol/L (Fig. 2 and Table 1) but inhibited ($P < .05$) amino acid-stimulated plasma pancreatic polypeptide levels from 36 ± 7 to 28 ± 7 pmol/L (Fig. 3 and Table 1).

Gallbladder Volume and Bilirubin Output. After pretreatment with loperamide, basal gallbladder volume was significantly greater than in the control experiment (39 ± 4 mL vs. 28 ± 4 mL; $P < .0001$; Fig. 4 and Table 1). This increase in gallbladder volume was accompanied by complete inhibition of bilirubin output into the duodenum under basal conditions (Fig. 5 and Table 1). Intraduodenal perfusion of amino acids resulted in significant gallbladder contraction ($P < .0001$) and bilirubin output ($P < .01$) into the duodenum (Fig. 4 and Table 1). Loperamide significantly ($P < .05$) attenuated maximum gallbladder contraction in response to

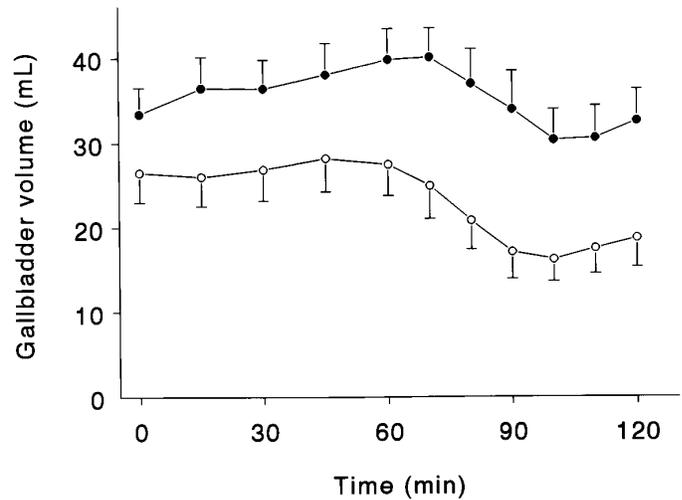


FIG. 4. Basal and amino acid-stimulated gallbladder volume (in mL) in 8 healthy subjects with (●) or without (○) prior ingestion of loperamide. Values are expressed as means \pm SEM.

the amino acid meal from $47\% \pm 3\%$ to $26\% \pm 6\%$ (Fig. 4 and Table 1) and intraduodenal bilirubin output from 39 ± 6 to 18 ± 6 μ mol/h ($P < .0001$; Fig. 5 and Table 1).

Pancreatic Enzyme Output. Loperamide inhibited basal amylase output from 3.2 ± 0.5 to 1.0 ± 0.5 kU/h ($P < .005$) and amino acid stimulated output from 5.1 ± 0.8 to 1.6 ± 0.4 kU/h ($P < .001$; Fig. 6 and Table 1).

DISCUSSION

Loperamide is a synthetic opiate receptor agonist that selectively interacts with peripheral opiate receptors in the digestive tract.^{1,27-29} Enkephalins are natural ligands for these receptors. Enkephalins have not only been shown in the colon, where loperamide competes with these ligands to inhibit bowel movements, but also in other places of the digestive system, such as the gastric antrum, duodenum, pancreas, cystic duct, and bile duct.^{27,30} The results of the present study (with the administration of loperamide causing plasma levels in the therapeutic range¹) provide evidence that loperamide not only interacts with peripheral opiate receptors in the colon but also with opiate receptors in the proximal part of

TABLE 1. Integrated Responses

Test	Basal (0-60 min)	Amino Acids (60-120 min)	Incremental	
CCK (pmol/L \cdot 60 min)	Without loperamide	174 \pm 24	229 \pm 55	55 \pm 35
	With loperamide	181 \pm 18	411 \pm 52*	230 \pm 41*
PP (pmol/L \cdot 60 min)	Without loperamide	1,606 \pm 279	1,886 \pm 384	280 \pm 130
	With loperamide	1,258 \pm 220*	1,446 \pm 295*	188 \pm 109
Gallbladder volume (mL \cdot 60 min)	Without loperamide	1,619 \pm 219	1,198 \pm 193	-421 \pm 56
	With loperamide	2,209 \pm 208*	2,077 \pm 223*	-132 \pm 76*
Bilirubin (μ mol)	Without loperamide	21 \pm 5	39 \pm 6	18 \pm 5
	With loperamide	0 \pm 0*	18 \pm 6*	18 \pm 6
Amylase (kU)	Without loperamide	3.2 \pm 0.5	5.1 \pm 0.8	1.9 \pm 0.6
	With loperamide	1.0 \pm 0.5*	1.6 \pm 0.4*	0.5 \pm 0.3

NOTE. Integrated responses under basal conditions (0-60 min) and in response to amino acids (60-120 min) in 8 healthy subjects with or without loperamide pretreatment. Incremental responses were obtained after subtraction of integrated values in the basal period (0-60 min) from integrated values during the period of stimulation (60-120 min). Results are expressed as means \pm SEM.

Abbreviation: PP, pancreatic polypeptide.

* Significantly different from test without loperamide pretreatment ($P < .05$).

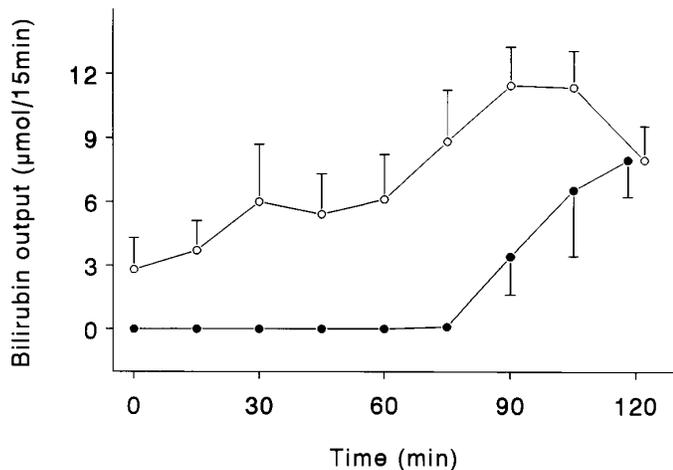


FIG. 5. Basal and amino acid-stimulated bilirubin output (in $\mu\text{mol}/15$ min) in 8 healthy subjects with (●) or without (○) prior ingestion of loperamide. Values are expressed as means \pm SEM.

the intestinal tract, because we have found that loperamide inhibits basal and amino acid-stimulated gallbladder motility and bilirubin output, as well as amylase output.

Several studies show that ultrasonography and the sum of cylinders method is a reliable, accurate, and precise method for quantification of gallbladder volume and gallbladder contraction in humans.^{24-26,31-34} However, it has to be noted that the changes in gallbladder volume as measured by ultrasonography do not necessarily reflect the total amount of bile emptied from the gallbladder. The gallbladder probably does not empty in a steady progressive fashion in response to a meal, but filling and emptying occur more or less simultaneously.³⁵⁻³⁷ Jazrawi et al. provided evidence that refilling of the gallbladder starts immediately after the ingestion of a meal and that the total volume of bile expelled by the gallbladder postprandially amounts to up to six times its basal volume.³⁶ They argued that the majority of hepatic bile enters the gallbladder before reaching the duodenum even in the postprandial period.^{35,36} This indicates that the cumulative bilirubin output into the duodenum may be a good estimate of the total amount of bile handled by the gallbladder. The data of the present study indicate that loperamide potently inhibited gallbladder motor function as measured both by ultrasonography and by intraduodenal bilirubin output.

In a previous study, it was shown that loperamide inhibited basal and meal-stimulated trypsin and bilirubin output into the duodenum of patients with a short bowel syndrome.³⁸ From that study, it was not clear whether loperamide inhibited these functions by inhibition of gastric emptying,³⁹ resulting in a diminished supply of food to stimulating receptors for gallbladder contraction and pancreatic enzyme secretion in the duodenum. To exclude effects of loperamide on gastric emptying, we administered the test meal into the duodenum. An elementary test meal was used to exclude possible confounding effects of loperamide on nutrient digestion, because we have shown previously that appropriate digestion of protein is essential for stimulation of CCK release and pancreaticobiliary secretion in humans.¹⁴

It has been suggested that the inhibitory effect of the opioid peptide enkephalin on meal-stimulated pancreatic protein secretion in dogs was mediated by a reduction in the release

of CCK.^{40,41} According to our data, the evidence in humans is otherwise because the release of CCK in response to a meal is enhanced by loperamide. A prolonged contact time between stimulating nutrients and CCK-secreting cells may be responsible for this enhanced release of CCK by loperamide because loperamide delays the passage of fluid through the intestine. In a previous study, we have shown that loperamide inhibits gallbladder contraction in response to intravenously administered CCK, resulting in plasma CCK concentrations as observed after a meal.⁴² We therefore postulate that the sensitivity of the pancreas and gallbladder to respond to an amino acid meal (causing increases of CCK in the physiological range as observed postprandially) is attenuated by loperamide because of interference with opioid receptors on the pancreas and gallbladder. Subsequently, according to the negative feedback concept between pancreaticobiliary output and CCK release,⁴³⁻⁴⁶ the diminished output of pancreaticobiliary products into the duodenum enhances the release of CCK.

Recently, it was shown that enkephalin attenuates pancreatic enzyme secretion in the rat by inhibiting the release of acetylcholine.⁴⁷ A direct effect of opioids on pancreatic acini was unlikely.⁴⁸ In humans, loperamide may also attenuate pancreaticobiliary secretion by inhibiting the release of acetylcholine. The finding of decreased pancreatic polypeptide levels, a hormone that is primarily controlled cholinergically,^{49,50} after administration of loperamide supports the hypothesis that loperamide also interferes with the human cholinergic system to attenuate pancreaticobiliary secretion.

The results of the present study may also be explained by an increased outflow resistance at the level of the sphincter of Oddi.^{51,52} However, this is unlikely because basal amylase output was not inhibited, and amino acid-stimulated amylase and bilirubin output were not abolished.

A diminished gallbladder motility causes bile stasis, which is a major factor contributing to the formation of gallstones.⁶⁻¹⁰ Therefore, it may be speculated that patients undergoing long-term treatment with loperamide are at risk for the development of gallstone disease. The finding that loperamide also increased the cholesterol saturation index of bile in normal volunteers who took loperamide capsules sufficient to cause

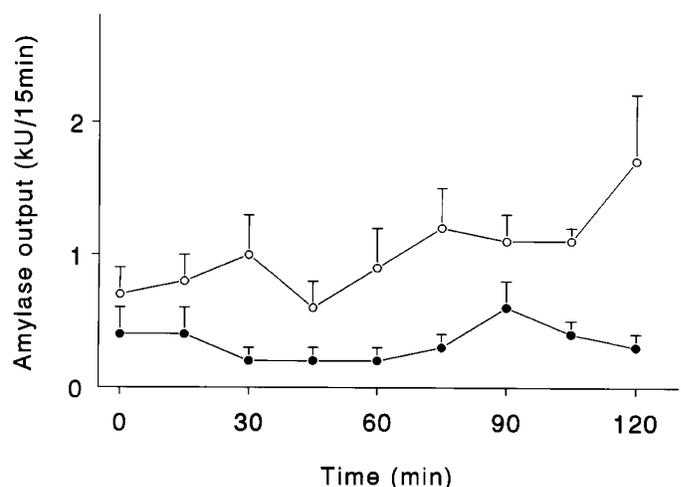


FIG. 6. Basal and amino acid-stimulated amylase output (in $\text{kU}/15$ min) in 8 healthy subjects with (●) or without (○) prior ingestion of loperamide. Values are expressed as means \pm SEM.

symptomatic constipation and slowing down of small intestinal transit supports this notion.⁵³ However, we are not aware of any study dealing with the incidence of gallstone disease in patients undergoing long-term treatment with loperamide.

The finding that the short-term administration of loperamide impairs pancreatic enzyme secretion in healthy subjects may also have clinical implication. As far as we know, there is one study in humans that showed an inhibition of pancreatic enzyme secretion by loperamide in patients with a short bowel syndrome.³⁸ Although loperamide is generally considered a safe drug, with few adverse reactions reported worldwide,^{54,55} further studies are needed to determine the clinical relevance of the inhibitory effects of loperamide on gallbladder motility and pancreatic enzyme secretion, particularly in patients undergoing long-term treatment with higher doses.

In conclusion, loperamide inhibits basal and amino acid-stimulated gallbladder motility and pancreatic enzyme secretion in humans, despite an enhanced amino acid-stimulated CCK release.

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