Cholecystokinin in the control of gastric acid secretion in man

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Abstract
This study was designed to determine the role of cholecystokinin in the control of gastric acid secretion in men using loxiglumide, a specific cholecystokinin receptor blocker. Three groups of healthy subjects (A, B, and C) were used; group A – for studies with postprandial gastric secretion, group B – for studies with exogenous gastric secretagogues and group C – for 12 hour intragastric pH-meter. Cephalic phase stimulated by modified sham feeding in group A subjects increased gastric acid secretion to about 50% of pentagastrin maximum and the treatment with loxiglumide in a standard dose (20 μmol/kg iv loading dose plus infusion of 20 μmol/kg/h afterwards) failed to affect this secretion. Gastric acid response to a 5% peptone meal instilled intragastrically greatly enhanced gastric acid secretion and plasma gastrin concentration but the addition of loxiglumide in the standard dose resulted in further increase in both gastric acid and plasma gastrin responses to peptone meal. Infusion of caerulein in gradually increasing doses (15–120 pmol/kg/h) and gastrin releasing peptide (25–200 pmol/kg/h) resulted in a dose dependent stimulation of gastric acid secretion reaching about 35% and 25% of maximum attained with pentagastrin. When loxiglumide was added in a standard dose, the acid responses to caerulein and gastrin releasing peptide were further increased to two to three fold attaining the peak reaching, respectively, about 100% and 50% of pentagastrin maximum. In group C subjects, 12 hour pH-meter revealed the usual increase in gastric pH after each meal in tests with placebo. Loxiglumide (1200 mg tablets tid, po) resulted in significantly lower pH after each meal and this was accompanied by significantly higher gastrin responses than in placebo tests. We conclude that cholecystokinin released by peptone meal, ordinary meals or gastrin releasing peptide exerts a potent inhibitory influence on gastric acid secretion and gastrin release in men and this inhibition involves subtype A cholecystokinin receptors.

Gastric acid secretion depends upon the interplay of many stimulatory and inhibitory influences that arise within the central nervous system and the gastrointestinal tract resulting in the cephalic, gastric, and intestinal phases of this secretion. 1

Cholecystokinin is one of major gut hormones 2 that has also been implicated in the control of gastric secretion. Exogenous cholecystokinin or its natural analogue, caerulein, administered alone in fasted humans or in dogs 3, 4 caused partial stimulation of gastric acid secretion but when combined with gastrin it inhibited gastrin induced secretion. The inhibitory action of cholecystokinin could not be, however, confirmed on the isolated canine parietal cells which respond to gastrin and cholecystokinin with equal stimulation of acid production. 5 This discrepancy between the in vivo inhibition and in vitro stimulation by cholecystokinin of acid secretion has been explained that cholecystokinin in vivo may release a local inhibitor of parietal cells such as somatostatin. 6, 7 Indeed, using a highly selective and potent cholecystokinin receptor blocker such as L-364,718, it was recently found in dogs 8 that cholecystokinin released by a protein or fat meal exerts a tonic inhibitory influence on gastric acid secretion and this effect may be mediated, at least in part, by somatostatin. 9

This study was designed to use the selective antagonist of type A cholecystokinin receptors, loxiglumide, 10, 11 to evaluate the role of cholecystokinin in the control of gastric secretion and gastrin release in man under physiological conditions such as cephalic or gastrointestinal phase of this secretion as well as following stimulation with exogenous secretagogues including pentagastrin, caerulein or gastrin releasing peptide.

Methods

SUBJECTS
Studies were carried out on three groups (A, B, and C) of young healthy men (mean age 22 years, range 19–37; mean weight 71 kg, range 59–76). Group A subjects underwent modified sham feeding and peptone meal tests with or without administration of loxiglumide. Group B subjects were used in tests with the infusion of pentagastrin, caerulein or gastrin releasing peptide with or without addition of loxiglumide. Group C subjects were used for the intragastric recording of pH during 12 hours of normal activities with or without oral administration of loxiglumide. This study was approved by the Human Research Review Committee and informed consent was obtained from each subject.

STUDIES ON THE EFFECTS OF LOXIGLUMIDE ON MODIFIED SHAM FEEDING AND PEPTONE MEAL INDUCED Gastric SECRETION
In group A (eight subjects), modified sham feeding and intragastric peptone meal were used to induce, respectively, cephalic vagal and gastrointestinal phases of gastric secretion as described before. 10, 12 Briefly, for modified sham feeding, a double lumen Drelling tube was
STUDIES ON THE EFFECTS OF LOXIGLUMIDE ON CAERULEIN, PENTAGASTRIN AND GAstraIN RELEASING PEPTIDE INDUCED GASTRIC SECRETION

Group B subjects were also intubated with a double lumen Drägering tube and gastric acid secretion was stimulated using caerulein (Farmitalia, Milano, Italy) infused iv in gradually increasing doses (15–120 pmol/kg/h), each dose being infused for 30 minutes and then doubled with or without addition of loxiglumide (20 μmol/kg in a loading dose at the start of the study followed by 20 μmol/kg/h) given throughout the periods of caerulein administration. After withdrawal of infusion of caerulein, the gastric aspiration was continued for 60 minutes and then pentagastrin (2-6 nmol/kg/h) was infused for 60 minutes with or without administration of loxiglumide (20 μmol/kg/h) to achieve maximal secretory response to this peptide. In six group B subjects, pentagastrin was also infused in separate test day in a constant dose (0.65 μmol/kg/h) to elicit submaximal secretory response. When gastric acid output in response to pentagastrin reached a well sustained plateau, caerulein was added to iv infusion in a constant dose of 30 pmol/kg/h with or without addition of loxiglumide (20 μmol/kg/h). In control tests, pentagastrin alone was administered for the entire 150 minutes.

The same six subjects of group B were also used in different occasions in tests with iv infusion of gradually increasing doses (25–200 pmol/kg/h) of gastrin releasing peptide (gift of Professor N Yanaihara, Shizuoka, Japan) with or without the administration of loxiglumide in a standard dose as used in the feeding experiments.

STUDIES ON THE EFFECT OF LOXIGLUMIDE ON 12 HOUR INTRAGASTRIC pH

Subjects of group C (eight men) underwent two 12 hour recording tests in a placebo controlled randomised crossover study. Each subject was passed under fluoroscopic control for the aspiration of gastric juice as described previously.14 Residual gastric contents were discarded and the gastric aspirates were collected by a suction pump in 15 minute periods. After 45 minutes basal period, the subjects were served an appetising meal of 250 g beef steak, 150 g french potatoes, with about 250 ml water to drink the food was tested, chewed and spat out for 15 minutes. The aspiration of gastric juice was carried out during modified sham feeding and during consecutive 105 minute period. Then pentagastrin (2 nmol/kg/h) was infused for 60 minutes and the aspiration of gastric juice was continued to determine maximal acid output in these subjects. All meals were prepared in a separate room so that subjects could not see or smell the food until the time of modified sham feeding and each subject was trained in a preliminary study not to swallow food during chewing. In different test day the modified sham feeding was performed while loxiglumide (kindly provided by Dr L Rovati, Rotta Labs, Milano, Italy) was infused intravenously starting with a loading dose of 20 μmol/kg given during five minutes at the beginning of the test followed by a constant iv dose of 20 μmol/kg/h throughout the study period.

Six subjects of group A were then used for intragastric peptone meal test (to mimic the gastrointestinal phase of gastric secretion) performed alone or in combination with loxiglumide. On the first test day, 5% peptone solution (Bactoprotein, Difco Labs, Detroit, Michigan, USA) adjusted to pH 5-5 (with 1 M NaOH) and osmolarity of about 300 mOsm/l (with 1 M NaCl) was administered during six consecutive 30 minute meals. On a second test day, loxiglumide was administered iv in a loading dose of 20 μmol/kg followed by a constant dose of 20 μmol/kg/h starting with the fourth 30 minute peptone meal.

All tests with peptone meal started after an overnight fast. A double lumen tube was introduced into the stomach and basal gastric acid secretion was quantified by suction aspiration for a 30 minute period. Peptone meal was introduced into the stomach in the volume of 300 ml at the start of each of six consecutive 30 minute periods. The stomach was emptied completely before the administration of the next 300 ml meal. Gastric content was continuously mixed with the aspiration perfusion system and the meal stimulated acid secretion was measured by intragastric titration with the end point of pH 5-5 using 50 mM Na(OH) as described previously.14
given either placebo or loxiglumide (1200 mg) three times daily 30 minutes before standard liquid meals (Fresubin, Fresenius, Münster, Germany) taken at fixed times: breakfast at 0830 hours, lunch at 1330 hours and supper at 1800 hours. The composition (protein, 3·8%; amino acids, 0·6%; fat, 3·4%; carbohydrates, 14%; minerals, vitamins and water 84%) of each meal was similar and contained about 1 kcal/ml. The volume of breakfast and supper meal was 300 ml and that of lunch meal was 500 ml. Recording of intragastric pH was assessed during 12 hours starting early morning (prebreakfast) by means of an intraluminal system, including an antimony electrode (Monocrystal model 91–0215, Synectics AB, Sweden) connected to the portable apparatus, which permitted the pH recording to be sampled every four seconds (Digitrapper MKII, 6200, Synectics AB, Sweden). The antimonie electrode used an external reference on thorax with contact jelly (Hellige 217, Fritz Hellige, FRG). At the beginning and at the end of each examination the antimonite electrodes were accurately calibrated at 21°C with pH 7·01 and pH 1·07 (buffers 5001 and 5002, Synectics AB, Sweden) and a temperature correction for intragastric reading (37°C) was performed. The pH electrodes were passed through an anaesthetised nostril and were positioned in the gastric corpus under fluoroscopic control approximately 5 cm below the lower oesophageal sphincter. The connecting wires were fixed to the nares with adhesive tape. The data recorder of intragastric minielectrodes was carried in a small bag so that it did not interfere with normal daily life. The pH recordings started at 0810 and lasted for 12 hours. Patients were instructed not to lie down during the day or to take additional meals or alcoholic drinks and carbonated beverages during the examination period.

The data of intragastric pH monitoring were transferred to IMB compatible computer (PC AT12) programmed with Gastrogram version 5·50 serial No E1024 (Gastrosoft Inc, Sweden) for calculating median pH values for each 12 hour test. Data from all eight subjects treated with placebo and loxiglumide were analysed with the use of program STAT pHAC II/PHARM, version 2·16 D3 (Gastrosoft Inc, Sweden). Gastric acidity was expressed as pH and values from each subject were transferred into 10 minute median values. The median and mean 12 hour intragastric pHs were predefined between study days using the Wilcoxon’s signed rank test. Box whisker plots of median and mean intragastric pH in eight subjects were performed for 12 hour period and separately for the 120 minute period after each standard meal. Statistics of pH profile were calculated and compared in medians and means with significance level of less than 0·05.

RADIOIMMUNOASSAY

Venous blood samples were obtained from peripheral vein under basal conditions and at 30 minute intervals in tests with modified sham feeding, pentagastrin, and gastrin releasing peptide infusion. In tests with 12 h pH-metry, the blood samples were taken 30 and 60 minutes
before and 30, 60, and 90 minutes after each meal. Plasma gastrin was determined using gastrin antiserum 4562 kindly donated by Professor J F Rehfeld of Aarhus, Denmark, and used in a final dilution of 1:140 000. Each sample was assayed in duplicate. The sensitivity of the gastrin measurement in the present assay was 2.5 pmol/ml serum equivalent to human G-17 as described previously.

STATISTICAL ANALYSIS
Results are expressed as means (SEM). Statistical significance was determined by both the Wilcoxon’s signed-rank test and the paired t test. Significance was accepted with p value less than 0.05.

Results
Figure 1 shows gastric acid response to modified sham feeding in group A subjects with or without infusion of loxiglumide. The mean acid output reached peak in the first 30 minute period after the start of modified sham feeding reaching about 50% of pentagastrin induced maximal acid output (about 26.4 (2.8) mmol/15 minutes) in these subjects and then declined towards the basal value within consecutive three 30 minute periods of examination. In tests with iv infusion of loxiglumide both basal and modified sham feeding induced peak acid outputs tended to increase above the control values but this increase was not statistically significant. Mean plasma gastrin concentrations also tended to increase after modified sham feeding, particularly in tests with loxiglumide but, again, this rise over premodified sham feeding values did not reach statistical significance.

Intragastric administration of 5% peptone caused several fold increase in gastric acid secretion over basal value and after 60 minutes acid secretion reached a well sustained plateau throughout the consecutive 30 minute periods (Fig 2). Loxiglumide added to iv infusion resulted in a significant augmentation of acid response to peptone meal and this increase averaged about 30% of the control value obtained with peptone meal alone. Plasma gastrin rose from basal value of about 25 pmol/l to the plateau of about 80 pmol/l after peptone meal and this was further significantly increased when loxiglumide was added to iv infusion.

Caerulein infused iv in gradually increasing doses ranging from 15 to 120 pmol/kg/h resulted in a small but significant stimulation of gastric acid secretion reaching peak at a dose of 30 pmol/kg/h and amounting to about 35% of pentagastrin induced maximal acid outputs in these subjects (Fig 3). At a dose of 60 and 120 pmol/kg/h, acid outputs tended to decline but remained significantly higher at basal acid secretion. When infusion of caerulein was combined with a constant background dose of loxiglumide, gastric acid outputs were significantly higher at all doses of caerulein. The maximal acid response to caerulein combined with loxiglumide was attained at a dose of 120 pmol/kg/h and it was not significantly different from that induced with pentagastrin in these subjects. For comparison,
CCK and gastric secretion

![Graph of gastric pH with Placebo and Loxiglumide](image)

**Figure 6:** Median intragastric pH in subjects treated with placebo (upper panel) and loxiglumide (lower panel). Medians of eight tests on eight subjects. The shaded area represents the range of pH monitoring.

The maximal acid response to pentagastrin alone, was not significantly different from that to pentagastrin combined with loxiglumide in the same subjects (Fig 3).

When caerulein (30 pmol/kg/h) was added to infusion of a constant dose of pentagastrin at a dose 0-62 nmol/kg/h producing submaximal acid secretion, a significant reduction in acid response was observed. This reduction was completely reversed when the infusion of caerulein was combined with loxiglumide (Fig 4).

In the third series of tests with gastrin releasing peptide in group B subjects, infusion of this peptide in gradually increasing doses (25-200 pmol/kg/h) resulted in a dose dependent stimulation of acid secretion reaching peak of about 25% of pentagastrin maximum (Fig 5). The addition of a standard dose of loxiglumide augmented acid outputs at all doses of gastrin releasing peptide and the maximal response to gastrin releasing peptide was achieved at a dose of 100 pmol/kg/h reaching about 50% of pentagastrin maximum.

Gastrin releasing peptide infusion caused a dose dependent increment in plasma gastrin concentrations and the peak gastrin response to gastrin releasing peptide alone was achieved at a dose of 200 pmol/kg/h. Addition of loxiglumide caused further significant increase in plasma gastrin concentrations at all dose levels of gastrin releasing peptide reaching peak at a dose of 50 pmol/kg/h (Fig 5).

Figures 6 and 7 show the median daytime pH profile in the same eight subjects treated with placebo or with loxiglumide (1200 mg tid po). For most of the time, the pH was between 1 and 2 during the interdigestive periods and the median pH was not significantly different between placebo and loxiglumide treatments. With meals, the pH rose to 4–6 and this rise lasted for about 90–180 minutes in subjects treated with placebo. In subjects ingesting loxiglumide, however, the rise in pH after each of the standard meals was significantly smaller and lasted only about 60–90 minutes. The median and mean postprandial pH values (for a two hour period after the meal) were significantly lower in tests with loxiglumide as compared with placebo treatments (Fig 8). Plasma gastrin levels between meals in placebo-treated subjects were similar to those in loxiglumide treated subjects but the postprandial increments in plasma gastrin with loxiglumide were significantly higher than in placebo controls (Table).

**Discussion**

This study provides evidence that cholecystokinin exerts a tonic inhibitory action on gastric acid secretion and gastrin release in response to ordinary meals, gastric peptone or gastrin releasing peptide in man and that these effects are mediated by the type A cholecystokinin receptors.

Cephalic phase of gastric secretion elicited in the present study by modified sham feeding is assumed to be mediated by vagal nerves stimulating directly the oxyntic cells and activating the release of gastrin and histamine. The reports concerning the rise in plasma gastrin after modified sham feeding in man are controversial, confirmed by some investigators and questioned by others. The present study shows only a small and insignificant increment in plasma hormone concentration after the modified sham feeding. The release of cholecystokinin was observed only in anaesthetised dogs with electrically stimulated vagal nerves but usual cephalic stimulation in conscious dogs attained by sham feeding or insulin hypoglycaemia failed to result in any significant change in plasma cholecystokinin concentrations. Our results in man show that the pretreatment with loxiglumide did not affect significantly gastric acid secretion or plasma gastrin response to modified sham feeding indicating that cephalic vagal excitation does not involve cholecystokinin. This is in keeping with the recent finding
showing that vagotomy in man did not influence the release of cholecystokinin but only increased the sensitivity of target organs to this hormone. In this study loxiglumide failed to affect basal gastric acid outputs and plasma gastrin concentrations suggesting that cholecystokinin does not play any role in the control of basal gastric secretion.

The gastrointestinal phase of gastric acid secretion was reproduced in this study by the intragastric instillation of 5% peptone meal kept at a constant pH 5.5 in the stomach by intragastric titration technique. Such a procedure prevented the usual fall in the intragastric pH after a meal and resulted in a well sustained stimulation of gastric acid secretion reaching about 50% of pentagastrin maximum in these subjects. It was accompanied by several fold increase in plasma gastrin concentrations probably reflecting an excessive release of antral hormone as a result of gastric distention by the meal and the action of peptic digests at high pH on the antral G-cells.

The question remains whether such artificial gastrointestinal meal composed of protein digests stimulates the release of cholecystokinin in man and whether the amounts of hormone released are sufficient to affect gastric acid secretion. In our previous study with a similar gastric peptone meal in man, plasma cholecystokinin rose several fold over basal value and exogenous cholecystokinin in a dose as low as 20 pmol/kg/h caused significant inhibition of gastric acid secretion in response to this meal. As the pretreatment with loxiglumide completely abolished the inhibitory effect of exogenous cholecystokinin on meal induced gastric acid secretion we assumed that cholecystokinin induced gastric acid secretion was mediated by type A cholecystokinin receptors. The gastric acid secretion induced by peptone meal itself, however, was not affected by the loxiglumide in that study probably because of the relatively low dose of loxiglumide (800 mg po plus iv infusion of 1 mg/kg/h) used. Also the failure of the MK-329, another potent type A cholecystokinin receptor blocker, to affect the peptone meal induced gastric acid secretion in dogs was probably related to the insufficient dose of the blocker used intragastrically. In the present study we decided to use loxiglumide in larger intravenous dose (20 μmol/kg loading dose plus 20 μmol/kg/h infused iv afterwards) because our dog experiments revealed that the bioavailability of loxiglumide from the gastrointestinal tract was only about 40% and because other reports showed that higher doses of this blocker in humans are safe and well tolerated. Such large dose of loxiglumide applied iv did not influence basal gastric acid secretion or basal plasma gastrin concentration (see Figure 6) but greatly augmented the peptone stimulated gastric acid secretion and significantly raised the postprandial plasma gastrin concentrations indicating that, indeed, cholecystokinin released by this peptone has a tonic inhibitory influence on both gastric acid secretion and gastrin release in man.

Peptone solution applied intragastrically is, however, highly artificial meal because it involves the constant neutralisation of secreted acid by intragastric titration and prevents the usual rise in the intragastric acidity after the meal. Therefore, we also used the most physiological assessment of intragastric acidity by continuous pHmetry using intraluminal pH electrode that has been recently validated in healthy subjects and peptic ulcer patients and used to monitor the action of various drugs on gastric acid secretion. Loxiglumide given orally three times daily before each meal did not influence the interdigestive pH but resulted in a significantly lower intragastric pH after each meal indicating an overall increase in the postprandial gastric acid secretion. Yet, despite the enhanced gastric acid secretion and lower intragastric pH, the plasma gastrin response to the

Figure 7: Median intragastric pH values in tests with placebo and loxiglumide before and after breakfast, lunch, and supper meals. Asterisk indicates significant (p<0.05) change as compared with placebo control.

Figure 8: Box-whisker plots of median with interquartile range and mean postprandial pH in eight subjects as determined for two hours after ingestion of standard meal. Asterisk indicates significant (p<0.05) difference as compared with placebo control.
standard meal in loxiglumide treated subjects reached significantly higher increments than in placebo treated subjects. Thus, the removal of the effects of endogenous cholecystokinin by the antagonism of its type A receptors with loxiglumide resulted in an increase in gastric acid secretion and gastrin release under physiological postprandial conditions.

The mechanism of the action of cholecystokinin on gastric acid secretion observed previously in dogs and in the present report in man has not been explained but it appears to be of dual character. One component of this action is a stimulatory effect but it is rather weak and probably involves the B type of cholecystokinin/gastrin receptors. This stimulatory component has been well documented under the in vitro conditions when cholecystokinin or its natural analog, caerulein, was found to be equipotent with that of gastrin in the stimulation of acid production. Under in vivo conditions, however, the stimulatory component is rather weak as shown in the present study by a small increase in acid secretion in response to iv infusion of graded doses of caerulein. When caerulein was added to pentagastrin infusion, a significant inhibition of gastric secretion was observed indicating that the predominant component of the action of caerulein is the inhibition of gastrin-stimulated gastric acid secretion. The antagonism of type A receptors with loxiglumide eliminated the inhibitory component as evidenced in this study by the conversion of caerulein from partial into the full gastrin like agonist of gastric secretion and by the reversal of the inhibitory effect of caerulein on the pentagastrin induced secretion. This effect of loxiglumide was probably mediated by type A cholecystokinin receptors probably localised on the somatostatin producing cells. The pretreatment with loxiglumide failed to affect the pentagastrin induced gastric acid secretion (see Figure 3) indicating that pentagastrin acts predominantly via the type B cholecystokinin/gastrin receptors and that loxiglumide is specific antagonist of the type A but not the type B receptors. Selective antagonism of type B cholecystokinin/gastrin receptors would be necessary to conclude that the stimulatory effect of cholecystokinin or caerulein on gastric acid secretion is caused by the type B receptors. In dogs, such blockade of type B cholecystokinin receptors with selective antagonist L-365,260 completely eliminated the stimulatory effects of gastrin and cholecystokinin on gastric acid secretion. Recent studies in man with the blocker of the type B receptors using L-365,260 revealed that such antagonism inhibited pentagastrin induced gastric acid secretion but no attempts were made to determine whether cholecystokinin or caerulein induced gastric acid secretion is affected by L-365,260.

The postprandial stimulation of gastric secretion and gastrin release can also be mimicked by infusion of gastrin releasing peptide. It is of interest that despite an excessive gastrin release as evidenced by the pronounced increment in plasma gastrin concentration, gastrin releasing peptide was rather weak stimulant of gastric acid secretion. The highest observed gastric acid response to gastrin releasing peptide reached only about 25% of pentagastrin maximum and this corresponded to about 60% of peptone meal induced acid output. After the blockade of the type A cholecystokinin receptors with loxiglumide, both gastric acid outputs and increments in plasma gastrin showed further significant increase. These results could be interpreted that cholecystokinin, that was shown to be released by gastrin releasing peptide, attenuates the stimulatory action of concurrently released endogenous gastrin on oxyntic glands so only a small increase in gastric acid secretion is observed after administration of gastrin releasing peptide. The antagonist action of type B component of cholecystokinin by the blockade of type A cholecystokinin receptors with loxiglumide, only the stimulatory action of cholecystokinin and gastrin persisted and this was probably mediated entirely by type B cholecystokinin/gastrin receptors on the oxyntic glands.


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