

Interaction of pentagastrin and the octapeptide of cholecystokinin on the human lower oesophageal sphincter

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SUMMARY The intravenous infusion of the octapeptide of cholecystokinin inhibited the response of the lower oesophageal sphincter to graded doses of pentagastrin in normal men. The possibility that the effects of these peptides on the lower oesophageal sphincter are only pharmacological and not physiological is discussed.

Gastrin increases and cholecystokinin (CCK) decreases lower oesophageal sphincter pressure in normal man (Giles, Mason, Humphries, and Clark, 1969; Resin, Stern, Sturdevant, and Isenberg, 1973; Fisher, DiMarino, and Cohen, 1973). As the biologically active C-terminal peptide fragments of these two hormones have similar structures (Ondetti, Rubin, Engel, Plusec, and Sheehan, 1970), it has been suggested that they act on the same receptor on cell membranes (Grossman, 1970). This hypothesis is supported by reports of inhibition of pentagastrin-stimulated gastric acid secretion by CCK and the related peptide caerulein (Brooks and Grossman, 1970; Brooks, Agosti, Bertaccini, and Grossman, 1970). The purpose of this study was to determine if infusion of the octapeptide of CCK (OP-CCK) inhibited the response of the lower oesophageal sphincter to pentagastrin in normal man.

Methods

Seven adult male volunteer subjects without clinical evidence of gastric or oesophageal disease were studied. Each gave informed consent. Each subject was studied on two days. After an overnight fast, a motility tube assembly was passed into the stomach. The tube assembly consisted of four polyvinyl tubes (OD 1.2 mm, ID 0.8 mm) with 1.2 mm side openings. The distal three openings were spaced at 1.5 cm intervals along the tube assembly; the proximal opening was 5 cm proximal to the second opening. Each tube was attached to a pressure transducer (Statham Instruments). The transducers were

attached to an Electronics for Medicine amplifying and recording apparatus. The tubes were perfused with water at 0.8 ml/min. The assembly was withdrawn so that the second and third openings were within the lower oesophageal sphincter. With two openings within the lower oesophageal sphincter, it was possible to detect movement of the tube rapidly and reposition it easily and quickly if movement occurred.

After the tube assembly was positioned, an intravenous infusion of either 154 mM NaCl or OP-CCK 40 ng/kg-hr was begun. Lower oesophageal sphincter pressure was recorded for 10 minutes. Pentagastrin was then given through a three-way stopcock attached to the infusion in doses of 0.05, 0.1, 0.2, 0.4, and 0.6 µg/kg. Doses were given over 30 seconds at 10-minute intervals in random order; a control injection of 154 mM NaCl was included in the series of pentagastrin injections. Subjects had a NaCl infusion on one day and an OP-CCK infusion on the other day; this order was also randomized. The order of pentagastrin injections was the same for any one subject on both days, but differed between subjects. Lower oesophageal sphincter pressure was continuously recorded and later measured from the recording at points equivalent to 30-second intervals throughout the study. The records were coded so that the person who made the measurement did not know in what order the pentagastrin doses or the background infusions were given. Lower oesophageal sphincter pressure was recorded as the mean of end-inspiratory and end-expiratory pressures in mmHg greater than intragastric pressure. The higher of the two readings within the lower oesophageal sphincter at each time

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period was used. The basal lower oesophageal sphincter pressure was defined as the mean of the 20 measurements during the 10-minute basal period on the saline infusion day, and of the last 10 measurements during the basal period on the OP-CCK infusion day. Peak response was defined as the mean of the two highest consecutive measurements occurring within five minutes after an injection of pentagastrin.

In three of these same subjects, an abbreviated reverse experiment was performed. On separate days, pentagastrin, 0.5 µg/kg-hr, and 154 mM NaCl were infused, and OP-CCK 5 and 20 ng/kg was given over 30 seconds at 10-minute intervals. Again, orders of infusion and bolus injection were randomized, and measurements of the lower oesophageal sphincter pressure were made in identical fashion as above.

Student's *t* test for paired measurements and the sign test were used in the statistical evaluation of the data (Snedecor and Cochran, 1971).

Results

The figure records the two mean basal lower oesophageal sphincter pressure levels followed by the peak response to increasing doses of pentagastrin in the presence of a background infusion of either normal saline or OP-CCK. The OP-CCK infusion did not significantly decrease basal lower oesophageal sphincter pressure, but partially inhibited the

response to pentagastrin. The inhibition was significant ($P < 0.05$) by the *t* test for the 0.1, 0.2, and 0.4 µg/kg doses, and for all doses by the sign test. The figure also records the pentagastrin dose response curve of the sphincter in the presence of a normal saline infusion, obtained for an additional 20 subjects in a previous study. Comparison of this curve with the two curves from the present study suggests that the peak at 0.1 µg/kg in the dose response curves in the present study may be due to random variation in a small number of subjects.

An attempt was made to determine if the inhibition was competitive or not using a linear transformation of the Michaelis-Menten equation (Dowd and Riggs, 1965; Goldstein, Aronow, and Kalman, 1968). The data did not fit Michaelis-Menten kinetics sufficiently well to allow a definite conclusion.

In contrast to infusion of OP-CCK, bolus injections of OP-CCK lowered the lower oesophageal sphincter pressure (table). This action was inhibited by a background infusion of pentagastrin ($P = 0.03$, sign test).

	Lower Oesophageal Sphincter Pressure (mm Hg)	
	Saline	Pentagastrin (0.5 µg/kg-hr)
Before injection	15.3 ± 2.2	17.9 ± 2.2
Mean minimum after		
5 ng/kg OP-CCK	9.5 ± 0.5	17.0 ± 5.5
20 ng/kg OP-CCK	2.2 ± 1.2	7.3 ± 2.2

Table Mean ± SEM basal and minimum lower oesophageal sphincter pressure in three normal subjects after bolus injections of the octapeptide of cholecystokinin (OP-CCK) with background intravenous infusion of either 154 mM NaCl or pentagastrin

Discussion

This study demonstrated that pentagastrin and OP-CCK each inhibited the other's action on the lower oesophageal sphincter pressure. This inhibition is consistent with the hypothesis that these two structurally similar peptides act via a common receptor. Fisher *et al* (1973) have recently reported that studies *in vitro* of the interaction of these peptides on opossum lower oesophageal sphincter muscle strips demonstrated similar inhibition and indicated competitive kinetics. Our data did not allow a definite conclusion as to the nature of the inhibition, but are consistent with competitive kinetics.

An important consideration is whether the demonstrated interaction of OP-CCK and pentagastrin is physiological or only pharmacological (Grossman,

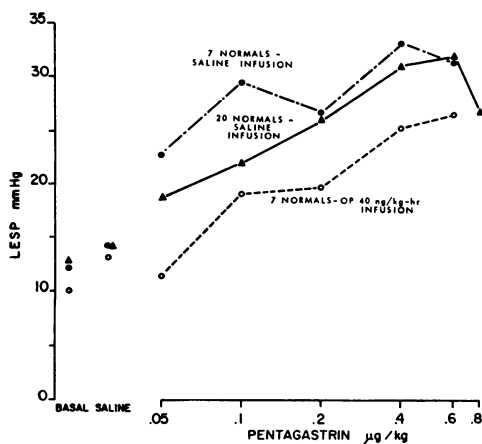


Fig Mean peak lower oesophageal sphincter pressure (LESP) after intravenous injection of pentagastrin, with background intravenous infusion of either 154 mM NaCl or octapeptide of cholecystokinin (OP). The open and closed circles represent data from seven subjects studied with both NaCl and OP; the triangles represent data from 20 subjects with NaCl infusion only.

1973; Cohen, 1974). Rehfeld and Stadil (1973) recently reported that intravenous bolus injections of gastrin in doses which cause contraction of the lower oesophageal sphincter (Cohen and Lipshutz, 1971) produced unphysiologically high serum concentrations of gastrin in man. Presumably bolus injections of OP-CCK and pentagastrin also produce serum concentrations greater than physiological levels of the parent hormones. If so, then the results of the bolus injections in the present study must be considered pharmacological rather than physiological effects.

Conversely, OP-CCK given as an intravenous infusion at 40 ng/kg-hr may be physiological, as this is approximately equivalent to the infusion rate of CCK which produced gallbladder contraction in man (Ondetti *et al.*, 1970; Malagelada, Go, and Summerskill, 1973). Thus our data showing no significant decrease in lower oesophageal sphincter pressure by this dose of OP-CCK do not support the hypothesis that CCK is important in the physiological regulation of lower oesophageal sphincter pressure. However, these data do not disprove this hypothesis, as larger amounts of CCK than required for gallbladder contraction may be released by meals, and CCK may potentiate the effect of some other physiological inhibitor or stimulant of lower oesophageal sphincter pressure. Our data on pentagastrin infusion alone are not extensive enough to draw conclusions regarding possible increases in lower oesophageal sphincter pressure, but Frank, Walker, and Fordtran (1973) reported that 0.9 µg/kg-hr pentagastrin did not increase the lower oesophageal sphincter pressure significantly, despite producing maximum gastric acid secretion.

Changes in lower oesophageal sphincter pressure have been reported after manipulations of gastric and duodenal contents, eg, food, glycine, acid, alkali, which may alter serum concentrations of gut hormones (Castell and Harris, 1970; Babka and Castell, 1973; Lipshutz, Gaskins, Lukash, and Sode, 1973; Roszkowski, Guillou, and Giles, 1973). Although the observed changes in lower oesophageal sphincter pressure have been said to be due to these hormones, the experimental methods produce physiological changes other than increase or decrease in blood concentration of one hormone, and intravenous infusions of active peptide fragments in presumably physiological doses have not produced significant changes in the lower oesophageal sphincter pressure. Our current view is that gastrin and CCK clearly have pharmacological effects upon lower oesophageal sphincter pressure but that their roles, if any, in its physiological control have not been established. If gastrin is not involved in regulation of basal lower oesophageal sphincter pressure,

then the decrease in lower oesophageal sphincter pressure produced by bolus injection of OP-CCK cannot be explained by inhibition at the postulated CCK-gastrin receptor; this action could be due to direct activation of an inhibitory receptor rather than to blocking of a stimulatory receptor.

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