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The effects of cholecystokinin octapeptide on human isolated alimentary muscle

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1 We studied cholecystokinin octapeptide (CCK-OP) for its motor effects and sites of action on human isolated muscle from stomach, small intestine and colon.

2 CCK-OP induced a concentration-dependent contraction of all the longitudinal muscles and of circular muscle from the stomach and large intestine. The peptide acted directly on these muscles at a site not involving muscarinic receptors.

3 CCK-OP relaxed the circular muscle of the small intestine and/or reduced the contractions to acetylcholine, by stimulating intramural postganglionic inhibitory neurones.

Introduction

Within the gut, cholecystokinin (CCK) has principal actions on gallbladder contraction (Ryan, 1981) and pancreatic secretion (Fried *et al.*, 1983), but it also affects gastrointestinal muscle. In isolated gut tissues from several species CCK can act directly on the smooth muscle, and indirectly through intramural postganglionic cholinergic neurones (Dockray, 1987). There are few studies on human isolated tissues. A purified CCK-pancreozymin preparation increased the spontaneous contraction amplitude of human gastric muscle *in vitro* with little effect on the resting tone (Cameron *et al.*, 1970). CCK-octapeptide (CCK-OP) often produced contractions of gastric strips by a direct action on the muscle, but did not affect circular muscle from the duodenum (Lüdtke *et al.*, 1988). CCK and CCK-OP contracted human isolated taenia coli, also by a direct action (Egberts & Johnson, 1977). The aim of our study was to investigate further the effects and sites of CCK-OP action on human isolated gastrointestinal muscle.

Methods

Specimens of human tissue, free from any macroscopically visible lesion, were obtained at operation for benign or malignant conditions, placed in Krebs solution at room temperature, and studied either immediately or after storage at 4°C for up to 24 h. The tissue was laid flat in Krebs solution and the mesentery, mucosa and submucosa were carefully cut away. Depending on the specimen size, 6–16 strips 3–4 mm wide and 20–30 mm long, were cut through the muscle coat parallel to either the circular or longitudinal muscle fibres, and set up in tissue baths (5–7 ml), containing Krebs solution maintained at 37°C and bubbled with 95% O_2:5% CO_2. The composition of the Krebs solution was as follows (mM): NaCl 121.5, $CaCl_2 \cdot 6H_2O$ 2.51, K_2HPO_4 1.18, KCl 4.7, $MgSO_4 \cdot 7H_2O$ 1.17, $NaHCO_3$ 25 and dextrose 5.55. The strips were placed under a load of 1 g and allowed to equilibrate for at least 1 h. Responses, magnified 10–15 times, were registered on pen recorders using isotonic transducers.

Each experiment started with a concentration-response curve to acetylcholine (ACh, 0.01–1 μM). Consistent ACh-induced submaximal contractions were obtained with ACh 0.01–0.2 μM that gave 40–80% of the maximal response. The contact time was always 30 s but although the cycle time was constant in each experiment it varied between experiments, usually from 7–10 min depending on the amount of spontaneous activity, the time taken for the tissue to relax after the ACh was washed out and the amount of time needed to study all of the strips used. CCK-OP was then added for 2 min followed, without washing out, by ACh for a further 30 s. This procedure allowed us to examine the effect on the response to ACh, and was particularly important in the tissues showing an inhibitory response to the peptide. Each strip was tested with repeated doses of CCK-OP, given at intervals of 1 h to avoid tachyphylaxis. Since it was therefore often not possible to generate a full CCK-OP concentration-response curve in a single muscle strip, neighbouring strips from the same specimen sometimes received different concentrations of CCK-OP. Contraction to CCK-OP after the 2 min contact time was measured in mm from the resting baseline, and expressed as a percentage of the maximal contraction to ACh obtained in the absence of CCK-OP. The results obtained from different specimens were then used to build up an average concentration-response curve to which was fitted a 3-variable logistic function, giving estimates of EC_{50}, slope at half of maximal response, and the upper asymptote (Black *et al.*, 1985).

The influences of drug antagonists were tested on consistent submaximal responses to CCK-OP, chosen on the basis of the composite concentration-response curve and giving contractions 30–70% of maximum, as demonstrated at the end of the experiment by giving a 3 fold higher CCK-OP concentration. On the two occasions when the test responses were >70% of maximum the experiments were omitted from the analysis. The contact times for the antagonists were: atropine, one cycle time (7–10 min) before the next addition of ACh; tetrodotoxin and hexamethonium, at least two cycle times before the addition of nicotine. Drug antagonist contact with the tissues before addition of CCK-OP was therefore substantially longer than these times.

The drugs used were: CCK-OP, acetylcholine hydrochloride, atropine sulphate, hexamethonium bromide, nicotine hydrogen tartrate, and tetrodotoxin (all from Sigma). They were added to the bathing fluid dissolved in 154 mM NaCl and washed out at the fixed time intervals found to be optimal in preliminary experiments.

Results

There were 177 strips of longitudinal muscle and 140 strips of circular muscle from 56 patients. All contracted with ACh 0.01–1 μM, in agreement with the findings of Bennett & Whitney (1966a,b). Many strips (174/317) showed spontaneous activity, as described in the latter papers, with amplitudes usually less than 10% of the maximum response to ACh.

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Table 1 The regions from which the muscle strips were taken, and a summary of the responses to various concentrations of cholecystokinin octapeptide (CCK-OP)

	Total studied	*Circular muscle (Contracting total)*		*Longitudinal muscle (Contracting/total)*	
Stomach	59 (12)	24/28	(8/9)	19/31	(8/10)
Jejunum	10 (1)	0/6*	(0/1)	3/4	(1/1)
Ileum	52 (12)	0/38*	(0/11)	10/14	(6/10)
Ascending colon	17 (4)	7/14	(2/4)	2/3	(1/2)
Transverse colon	33 (4)	1/18	(3/4)	14/15	(4/4)
Sigmoid colon	146 (23)	33/73	(14/19)	41/73	(17/21)

Some strips received the same concentration of CCK-OP repeatedly, others from the same specimen received different amounts, and others received a range of concentrations that produced partial or full sigmoidal log concentration-response curves. The numbers of strips studied are shown first, with the numbers of specimens given in parentheses.
* Relaxation or inhibition of the contraction to ACh.

When the spontaneous activity was greater than 30% of the maximum ACh-induced contraction, the strips were rejected (8 strips from two different specimens). Although the spontaneous activity varied among strips from different specimens, neighbouring strips from the same specimen showed a similar pattern. During the experiment the size of the spontaneous activity showed a tendency to reduce in 147/174 strips; the remainder showed little or no change.

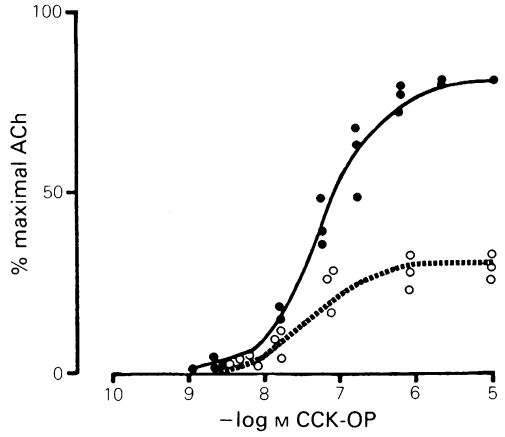

Figure 1 Derived concentration-response curve of cholecystokinin octapeptide (CCK-OP) using responses from 3 specimens of human transverse colon circular muscle. Cumulative addition (○) gave a smaller maximum response compared with non-cumulative (●) administration.

CCK-OP produced a contraction or no response in strips from all regions except the circular muscle from the small intestine which was inhibited by the peptide (Table 1). Not all strips from the same specimen responded. Most of the gastric strips responded (73% of circular and longitudinal muscle strips; 84% of specimens), compared with 50% of strips (80% of specimens) from the colon (Table 1). Storage at 4°C overnight (maximum 24 h) did not appear to affect the tissue responses, as shown previously with this and various other agonists. We did not analyse the effect of storage quantitatively, but the sensitivity to ACh and the response to CCK-OP in strips from two specimens were similar when used fresh or after overnight storage. We have obtained similar results with CCK-OP on four other specimens used immediately and after storage (unpublished observations). Results from fresh and stored tissues were therefore pooled.

Compared with the response to ACh, the contraction to CCK-OP was smaller, developed more slowly and was much more sustained. Repeated contractions were consistent, provided that the strip was not exposed to CCK-OP for more than 2.5 min or with a cycle time of less than 1 h. Preliminary experiments showed that longer contact times or shorter intervals between doses caused tachyphylaxis which was irreversible and complete for at least 12 h. Figure 1 shows the comparison between non-cumulative and cumulative administration of CCK-OP to strips of transverse colon circular

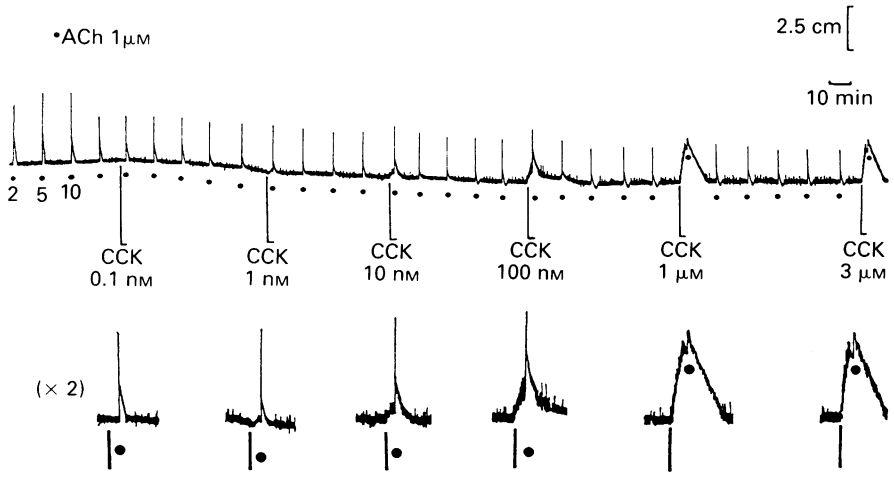

Figure 2 Representative tracing of the response to increasing concentrations of cholecystokinin octapeptide (CCK) applied to a gastric circular muscle strip. The interval between applications was 1 h, interspersed with submaximal contractions to ACh (1 μM, except at the start of the trace where 2, 5 and 10 μM were given).

muscle. Figure 2 shows concentration-dependent contractions of gastric circular muscle that began after at least 20 s, and gradually increased to a maximum at about 1 min. After washout, the tissue recovered its resting tone over about 20 min, mainly regardless of the size of the CCK-induced contraction.

Figure 3 shows the composite concentration-response curve obtained from different concentrations in different strips of taeniae from the sigmoid colon. Similar results, summarised in Table 2, were obtained with tissues from all other regions except small bowel circular muscle which was inhibited. In both layers from all the different regions of the gut the threshold for contraction was similar (range 2–20 nM CCK-OP), and the maximum contraction (about 80% of the maximum to ACh) was usually produced by 1 μM CCK-OP. In 85/164 strips contracted by CCK-OP, the response to ACh added in the presence of the peptide increased by a mean of 18% (range 5–39%). In one circular muscle strip from a sigmoid colon and one longitudinal muscle strip from the only specimen of jejunum, only the response to the subsequent addition of ACh increased. The other strips showed little or no change in the contraction to ACh added in the presence of CCK-OP.

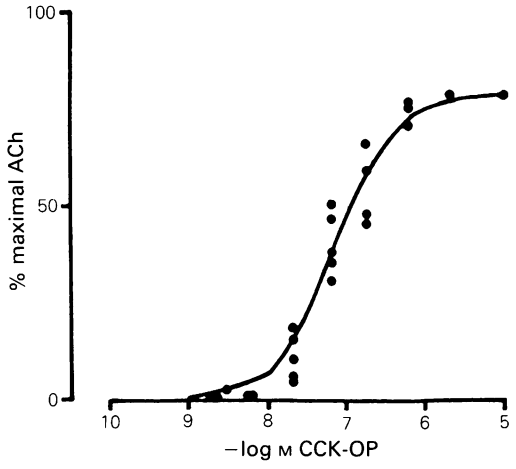

Figure 3 The derived concentration-response curve of cholecystokinin octapeptide (CCK-OP) in human sigmoid colon longitudinal muscle (*taenia coli*). Responses are expressed as percent maximal contractions induced by acetylcholine (ACh).

Atropine 500 nM always completely blocked the contraction to ACh, but there was no significant change with CCK-OP (mean contraction height ± s.e.mean 95.5 ± 5.3% (n = 12) of that in the absence of atropine: stomach, 2 longitudinal and 1 circular strip; jejunum, 1 longitudinal strip; ileum, 2 longitudinal strips; large bowel, 3 longitudinal and 3 circular strips). Tetrodotoxin 50 nM always completely blocked the response to nicotine, but had no effect on CCK-OP (mean contraction height 99.9 ± 3.4%; n = 12) of that in the absence of tetrodotoxin: stomach, 2 longitudinal and 2 circular strips; jejunum, 1 longitudinal strip; ileum, 2 longitudinal strips; large bowel, 1 ascending colon circular strip, 2 sigmoid taeniae and 2 circular strips). An experiment illustrating the influence of atropine and tetrodotoxin in one longitudinal muscle strip from the sigmoid colon is shown in Figure 4.

In contrast, none of the 27 ileal or 6 jejunal circular muscle strips (from 12 and 1 patients respectively) contracted to CCK-OP (0.02–2 μM); instead, inhibitory responses were obtained in 16/33 (11 ileal and 5 jejunal) strips. Figure 5 shows concentration-dependent relaxations and a reduction of the superimposed ACh-induced contraction in one ileal strip; this inhibitory effect was unchanged by atropine 500 nM, but was blocked by tetrodotoxin 50 nM. In another experiment CCK-OP (0.15–0.18 nM) relaxed each of the three strips from the same specimen and in two other experiments the peptide

Table 2 EC_{50} values for contractions to cholecystokinin octapeptide (CCK-OP) in human tissues (given as −log EC_{50}, mean value ± s.e.mean)

	Circular muscle	*Longitudinal muscle*
Stomach	6.71 ± 0.07 (5)	6.43 ± 0.06 (5)
Small intestine	—*	7.55 ± 0.10 (5)
Ascending colon	6.95 ± 0.80 (2)	7.60 (1)
Transverse colon	7.15 ± 0.06 (3)	7.00 ± 0.06 (4)
Sigmoid colon	7.10 ± 0.07 (6)	7.15 ± 0.07 (6)

The numbers of specimens are given in parentheses.
* Relaxation or inhibition of the contraction to ACh (one jejunal and four ileal specimens).

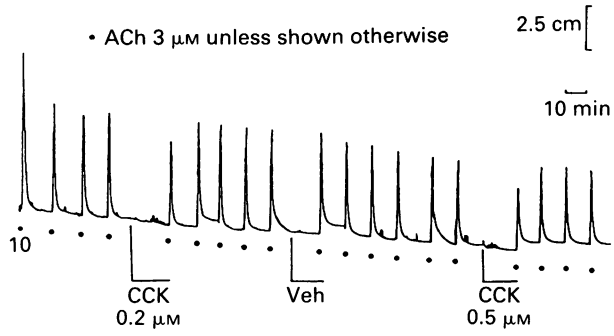

Figure 5 A concentration-dependent inhibition by cholecystokinin octapeptide (CCK) in human ileal circular muscle. Veh = vehicle.

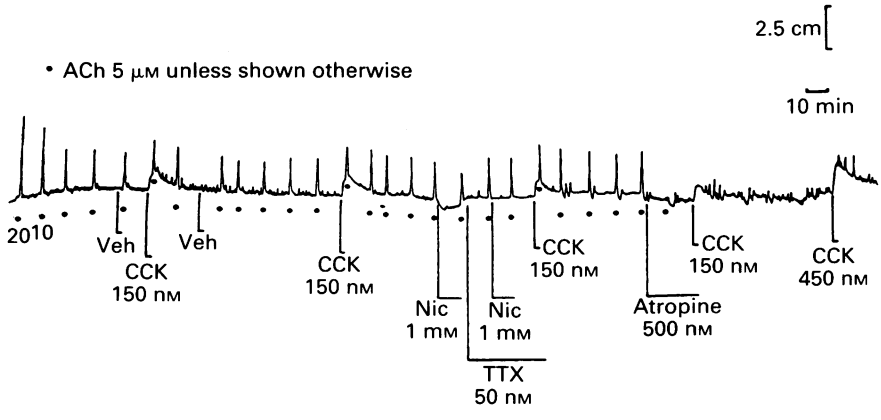

Figure 4 Representative tracing showing no effect of tetrodotoxin (TTX) and atropine on the contractile response induced by cholecystokinin octapeptide (CCK) in a longitudinal strip from the sigmoid colon. Veh = vehicle; Nic = nicotine.

reduced the contraction to ACh without altering the muscle tone. The reduction of the jejunal ACh-induced contraction was blocked by tetrodotoxin 50 nM but not by hexamethonium 20 μM ($n = 2$; Figure 6).

Discussion

The aim of the present study was to investigate the effects and sites of CCK-OP action in human isolated gastrointestinal muscle. CCK-OP has similar activity to CCK on human gut muscle (Egberts & Johnson, 1977) and is present in the human bloodstream (Walsh *et al.*, 1982). We studied both the circular and the longitudinal muscle because various substances affect these two layers differently (e.g. prostaglandin E_2, Bennett *et al.*, 1968; vasoactive intestinal peptide, Bennett *et al.*, 1984).

In agreement with the studies of Lüdtke *et al.* (1988) on human gastric strips, those regions of the gut that contracted to CCK-OP did so with nanomolar concentrations. Human gut *in vivo* is sensitive to CCK-OP at the normally circulating picomolar amounts (Kellow *et al.*, 1987). Lower sensitivity *in vitro* may be due to the difficulty of diffusion from the bath fluid into muscle strips, compared with diffusion from the blood, and to the absence of other factors that might augment motility *in vivo*.

Since the contractile effect of CCK-OP was not inhibited by atropine or tetrodotoxin, in agreement with Lüdtke *et al.* (1988), we conclude that the peptide stimulates human isolated alimentary muscles by a direct action at sites not involving muscarinic receptors.

The relaxation and/or inhibition of ACh-induced contraction in the circular muscle from the small bowel contrasts with the rest of the alimentary tract. Lüdtke *et al.* (1988) studied only duodenal circular muscle, apart from the stomach, and obtained no duodenal excitatory responses to CCK-OP; it seems that they did not look for inhibition by determining the effect on ACh-induced contraction. In our experiments the inhibition by CCK-OP in jejunal and ileal circular muscle was blocked by tetrodotoxin but not hexamethonium, suggesting an action on postganglionic inhibitory neurones.

Both direct muscle and neurally mediated effects of CCK have been described in isolated gut from laboratory animals. A direct contractile effect has been shown in canine antral muscle (Morgan *et al.*, 1978) and guinea-pig stomach (Gerner & Haffner, 1977). Evidence for a neurally mediated effect of CCK in the longitudinal muscle of guinea-pig ileum is that ACh is released (Vizi *et al.*, 1972; 1973), and that without the myenteric plexus the tissue responded to ACh but not to CCK (Hutchinson & Dockray, 1980; 1981). These authors considered that release of substance P may account for the atropine-resistant component of the CCK-induced contraction in this tissue. Grider & Makhlouf (1987) confirmed that CCK contracts guinea-pig ileum longitudinal and circular muscles both directly and by activation of cholinergic pathways, and they showed different sensitivities of the muscle cells and neurones. With regard to inhibitory effects, CCK activates receptors on nonadrenergic-noncholinergic neurones in dog stomach (Schmalz *et al.*, 1983; Schmalz & Szurszewski, 1983), cat sphincter of Oddi (Behar & Biancani, 1980) and lower oesophageal sphincter (Rattan & Goyal, 1983). It is therefore clear that species, regional, and muscle layer differences exist in the gastrointestinal responses to CCK. There also seem to be differences in the types of CCK receptors in human and guinea-pig intestine (unpublished).

Consistent with our *in vitro* findings, CCK *in vivo* inhibited human small bowel motility (Osnes, 1975) but increased colonic motor activity (Dinoso *et al.*, 1973). Thus, perhaps the pattern of motility affected by CCK released in response to a meal tends to reduce the propulsion in the small bowel, so increasing the time for digestion and absorption of the food, while the increased colonic motility stimulates defaecation (the 'gastrocolic reflex'). However, it may not be valid to extrapolate from *in vitro* to *in vivo* activity (Bennett, 1968). Nevertheless, our results are consistent with a possible regulatory role for CCK in human gastrointestinal motility.

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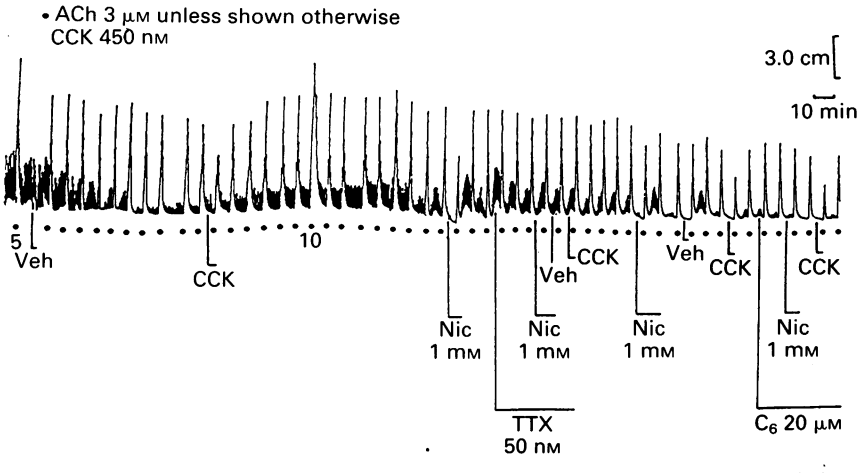

Figure 6 Block by tetrodotoxin (TTX), but not hexamethonium (C6), of the inhibitory response to cholecystokinin octapeptide (CCK-OP) in human jejunal circular muscle. Veh = vehicle; Nic = nicotine.

References

BEHAR, J. & BIANCANI, P. (1980). Effect of cholecystokinin, the octapeptide of cholecystokinin on the feline sphincter of oddi and gall bladder. *J. Clin. Invest.*, **66**, 1231–1239.

BENNETT, A. (1968). The relationship between *in vitro* studies of gastrointestinal muscle, and motility of the gastrointestinal tract *in vivo*. *Am. J. Dig. Dis.*, **13**, 410–414.

BENNETT, A., BLOOM, S.R., CH'NG, J., CHRISTOFIDES, N.D., PEACOCK, L.E. & RENNIE, J. (1984). Is vasoactive intestinal peptide an inhibitory transmitter in the circular but not the longitudinal muscle of guinea pig colon? *J. Pharm. Pharmacol.*, **36**, 787–790.

BENNETT, A., MURRAY, J.G. & WYLLIE, J.H. (1968). Occurrence of

prostaglandin E_2 in the human stomach, and a study of its effects on human isolated gastric muscle. *Br. J. Pharmacol. Chemother.*, **32**, 339–349.

BENNETT, A. & WHITNEY, B. (1966a). A pharmacological investigation of human isolated stomach. *Br. J. Pharmacol. Chemother.*, **27**, 286–298.

BENNETT, A. & WHITNEY, B. (1966b). A pharmacological study of the motility of the human gastrointestinal tract. *Gut*, **7**, 307–316.

BLACK, J.W., LEFF, P. & SHANKLEY, N.P. (1985). Further analysis of anomalous pK_B values for histamine H_2-receptor antagonists on the mouse isolated stomach assay. *Br. J. Pharmacol.*, **86**, 581–587.

CAMERON, A.L., PHILLIPS, S.F. & SUMMERSKILL, W.H.J. (1970). Comparison of effects of gastrin, cholecystokinin-pancreozymin, secretin and glucagon on human stomach *in vitro*. *Gastroenterology*, **59**, 539–545.

DINOSO, V.P., MESHKINPUR, H., LORBER, S.H., GUTIERREZ, J.G. & CHEY, W.Y. (1973). Motor responses of the sigmoid colon and rectum to exogenous cholecystokinin and secretin. *Gastroenterology*, **65**, 438–444.

DOCKRAY, G.J. (1987). Physiology of enteric neuropeptides. In *Physiology of the Gastrointestinal Tract*. Second Edition. ed. Johnson, L.R. pp. 41–66. New York: Raven Press.

EGBERTS, E.H. & JOHNSON, A.G. (1977). The effect of cholecystokinin on human *taenia coli*. *Digestion*, **15**, 217–222.

FRIED, G.M., OGDEN, W.D., SWIERCZEK, J., GREELEY, G.H., RAYFORD, P.L.T. & THOMPSON, J.C. (1983). Release of cholecystokinin in conscious dogs: correlation with simultaneous measurements of gallbladder pressure and pancreatic protein secretion. *Gastroenterology*, **85**, 1113–1119.

GERNER, T. & HAFFNER, J.F.W. (1977). The role of local cholinergic pathways in the motor response to cholecystokinin and gastrin in isolated guinea pig fundus and antrum. *Scand. J. Gastroenterol.*, **12**, 751–757.

GRIDER, J.R. & MAKHLOUF, G.M. (1987). Regional and cellular heterogenity of cholecystokinin receptors mediating muscle contraction in the gut. *Gastroenterol.*, **92**, 175–180.

HUTCHINSON, J.B. & DOCKRAY, G.J. (1980). Inhibition of the action of cholecystokinin octapeptide on the guinea pig ileum myenteric plexus by dibutyryl cyclic guanosine monophosphate. *Brain Res.*, **202**, 501–505.

HUTCHINSON, J.B. & DOCKRAY, G.J. (1981). Evidence that the action of cholecystokinin octapeptide on the guinea-pig ileum longitudinal muscle is mediated in part by substance P release from the myenteric plexus. *Eur. J. Pharmacol.*, **69**, 87–93.

KELLOW, J.E., MILLER, L.J., PHILLIPS, S.F., HADDAD, A.C., ZINSMEISTER, A.R. & CHARBONEAU, J.N. (1987). Sensitivities of human jejunum, ileum, proximal colon and gallbladder to cholecystokinin octapeptide. *Am. J. Physiol.*, **252**, G345–G356.

LÜDTKE, F.E., GOLENHOFEN, K. & KÖHNE, C. (1988). Direct effects of cholecystokinin on human gastric motility. *Digestion*, **39**, 210–218.

MORGAN, K.G., SCHMALZ, P.F., GO, V.L.W. & SZURSZEWSKI, J.H. (1978). Electrical and mechanical effects of molecular variants of CCK on antral smooth muscle. *Am. J. Physiol.*, **235**, E324–E329.

OSNES, M. (1975). The effect of secretin and cholecystokinin on the duodenal motility in man. *Scand. J. Gastroenterol.*, (Suppl 35) **10**, 22–26.

RATTAN, S. & GOYAL, R.K. (1983). Pharmacological differences between neural (inhibitory) and muscle (excitatory) CCK receptors in cat lower esophageal sphincter. *Gastroenterol.*, **84**, 1281.

RYAN, J.P. (1981). Motility of the gallbladder and biliary tree. In *Physiology of the Gastrointestinal Tract*. ed. Johnson, L.R. pp. 473–494. New York: Raven Press.

SCHMALZ, P.F., MORGAN, K.C. & SZURSZEWSKI, J.H. (1983). Pentagastrin potentiates nonadrenergic inhibitory neuromuscular transmission in orad stomach of dogs. *Am. J. Physiol.*, **245**, G597–600.

SCHMALZ, P.F. & SZURSZEWSKI, J.H. (1983). The effect of proglumide on pentagastrin and nerve induced motility changes in the canine stomach. *Physiologist*, **26**, A82.

VIZI, S.E., BERTACCINI, G., IMPICCIATORE, M. & KNOLL, J. (1972). Acetylcholine-releasing effect of gastrin and related polypeptides. *Eur. J. Pharmacol.*, **17**, 175–178.

VIZI, S.E., BERTACCINI, G., IMPICCIATORE, M. & KNOLL, J. (1973). Evidence that acetylcholine released by gastrin and related polypeptides contributes to their effect on gastrointestinal motility. *Gastroenterol.*, **64**, 268–277.

WALSH, J.H., LAMERS, C.B. & VALENZUELA, J.E. (1982). Cholecystokinin-octapeptide immunoreactivity in human plasma. *Gastroenterol.*, **82**, 438–444.

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