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Effect of desamino-cholecystokinin-octapeptide (CCK-7) on the intraluminal pressure and myoelectrical activity of the gall-bladder, stomach, and small intestine in conscious dogs

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1 Desamino-cholecystokinin-octapeptide (CCK-7) increased the gall-bladder pressure and decreased the gastric pressure following i.v. infusion in conscious dogs.

2 In small intestine and Heidenhain pouch CCK-7 exerted an initial excitatory effect followed by an inhibitory effect on intraluminal pressure and peristalsis.

3 In all organs the effect of CCK-7 on pressure was correlated with a change of spike discharge.

4 CCK-7 is more potent on gastrointestinal motor activity than a Boots preparation of pancreozymin.

5 The results suggest that CCK-7 at doses within the physiological range could be involved in the regulation of gastrointestinal motility.

Introduction

Desamino-cholecystokinin (CCK-7) is an analogue of cholecystokinin and consists of 7 amino acid residues (Figure 1). Cholecystokinin has been shown to be responsible for two effects: secretion of pancreatic enzymes and contraction of the gall-bladder (Bertaccini, 1976). Originally, it was believed that two hormones were involved: cholecystokinin, which induced gall-bladder contraction, and pancreozymin, which increased secretion of pancreatic juice. Now it is known that the two effects are brought about by one and the same hormone, which was thus given the name cholecystokinin-pancreozymin (Jorpes & Mutt, 1961). The C-terminal heptapeptide is essential (just as in caerulein) as well as the sulphate radical bound to tyrosine. Removal of the sulphate radical leads to disappearance of the gall-bladder concractile response (Mutt & Jorpes, 1968). Cholecystokinin also participates in modulation of gastrointestinal motility. A partially pure preparation of cholecystokinin when injected i.v. as a bolus, inhibits the motility of the stomach in dogs and humans (Johnson & Magee, 1965; Johnson, Brown & Magee, 1966; Sugawara, Tsara, Curt & Woodward, 1969; Chey, Mitanant, & Hendricks, 1970; Kwong, Brown, Wittaker & Duthie, 1972). Cholecystokinin and its carboxyl-terminal octapeptide produce a significant, dose-dependent decrease in intragastric pressure in conscious dogs during i.v. infusion (Valenzuela, 1976). Cholecystokinin also produces an inhibition of gastric emptying in conscious dogs during i.v. infusion (Debas, Faroog & Grossman, 1975).

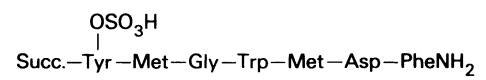

Figure 1 Amino acid residues of desamino-cholecystokinin-octapeptide (CCK-7).

The effect of CCK on the motor function of the small intestine has been studied extensively in man. CCK accelerates the passage of X-ray contrast media through the small intestine (Monod, 1964; Morin, Berancon, Grall, Jouve & Debray, 1965; Backlund, 1970). It also stimulates motility (Dolringer, Berz, Raptis, von Uexkull & Goebell. 1975) and the spike potential in the ileum (Mukhopadhyay, Thor, Copeland, Johnson & Weisbrodt, 1977; Wingate, Thomson, Pearce, & Dand, 1978).

According to Oigaard, Dorph, Christensen &

Table 1 Scheme of distribution of electrodes and fistulas in different organs of the dogs examined

dogs	*Balloon in gall-bladder*	*Fistula in stomach*	*Fistula in ileum*	*Electrodes in stomach*	*Electrodes in jejunum and ileum*	*Electrodes in stomach and Heidenhain pouch*
1	+		+	+	+	
2	+	+		+	+	
3		+		+	+	
4		+		+	+	
5			+	+	+	
6			+	+	+	
7						+
8						+

Christensen (1975) i.v. injection of CCK decreases the amplitude of spike potentials in the human duodenum but exerts no effect on the spike potentials of the distal ileum.

The objectives of the present study were: (1) to evaluate the effect of CCK-7 on the intraluminal pressure in the gall-bladder, stomach, and small intestine in conscious dogs; (2) to examine the effect of CCK-7 on the spike potentials as well as on the frequency and velocity of propagation of slow waves in the gastric and intestinal muscle and (3) to obtain information about the potency and the mechanism of the action of CCK-7 in comparison with a preparation of pancreozymin (PZ).

A brief report of the activity of CCK-7 on guinea-pig gall bladder has been published previously (Niedrich, Hartel, Bienert & Bergmann, 1979).

Methods

Animals

Eight dogs weighing 10–12 kg were used. Laparatomy along the linea alba was performed under anaesthesia and six pairs of silver ball-shaped electrodes were implanted in the muscle wall of the stomach (corpus and antrum) and the small intestine (jejunum and ileum). A fistula was made in the stomach or small intestine for recording motor activity. The details of the method have been described in detail elsewhere (Papasova & Milenov, 1965). Additionally, a Heidenhain pouch was prepared in two dogs from the corpus/fundus region of stomach, so that this region of the stomach was without vagal innervation. A pair of electrodes was implanted in this muscle-wall. In two dogs a balloon (volume 2 ml) was chronically implanted into the gall-bladder.

Recording

The intraorgan pressure was measured by a Statham transducer (P 23 Db) and recorded on a Varioscript pen recorder (Schwarzer). Simultaneously, the myoelectical activity was recorded on an encephalograph ('Bioskript' VEB Messgeratewerk. Zwonitz).

Protocol

The dogs were not used before the twentieth day after surgery. The animals were starved 18 h before the beginning of the experiment and each recording lasted for 2 to 4 h. Those records lacking any 'periodic fasting activity' were discarded and the experiments were repeated. We used the first 40 min of each recording as the control record. CCK-7 or PZ were administered for 30 s and recording was continued for 30 to 50 min (test period). The next dose was not administered until normal activity had been restored.

Substances

Pancreozymin (PZ) (Boots), atropine (Cascan), phentolamine (Ciba), and propranolol (ISIS-Chemie) were used. One unit of PZ has been taken as equivalent to 20 ng CCK-7. CCK-7 was synthesized by Dr Henklein in our laboratories using the method we have previously published (Niedrich *et al.*, 1979).

Table 2 Frequency of slow waves in the stomach of conscious dogs before and after CCK-7 administration

		CCK-7		
Control	*20 ng kg^{-1}*	*40 ng kg^{-1}*	*80 ng kg^{-1}*	*100 ng kg^{-1}*
4.20 ± 0.19	5.17 ± 0.17	5.31 ± 0.15	5.72 ± 0.13	6.05 ± 0.14

The mean (±s.e.mean) of 5 experiments is given.

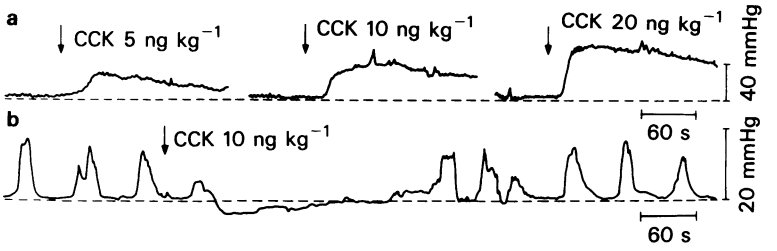

Figure 2 (a) Effect of different doses of CCK-7 on the intraluminal pressure in gall bladder. (b) Effect of CCK-7 on the intraluminal pressure of stomach. Both recordings (a) and (b) were made in the same dog.

All substances were injected intravenously. In some experiments, 3–5 min before infusion of CCK-7 and PZ (Boots) atropine (0.1 mg kg^{-1}) propranalol (1 mg kg^{-1}) or phentolamine (30 μg kg^{-1}) was injected.

Data analysis

The intraorgan pressure was measured in mmHg and expressed as percentage of the maximal pressure evoked by the substances. The frequency and velocity of propagation of the basic electrical rhythm (BER) were calculated from 30 waves before and after administration of each dose. The spike activity was expressed as the ratio of the number of spikes during peptide infusion and the number of the spikes during the control period.

Statistical analysis

Statistical significance of the differences between control and test period at a particular time was assessed using Student's t test. A value of $P<0.01$ was required for significance. P was obtained for a one-tailed test when an experiment was to detect an effect in one direction on theoretical or empirical grounds.

Results

Intraluminal pressure in gall-bladder

Both CCK-7 and PZ increased the gall-bladder pressure in a dose-dependent manner. The threshold dose was 5 ng kg^{-1} for CCK-7 (Figure 2) and 10 ng kg^{-1} for PZ ($n=10$). The effect lasted for 3 to 6 min depending on dose (Figure 2), CCK-7 (20 ng kg^{-1}) was 43 ± 6% ($n=5$) more affective than PZ (20 ng kg^{-1}) (Figure 3).

Intraluminal pressure and myoelectrical activity of the stomach

CCK-7 decreased the intragastric pressure and eliminated periodic fasting activity in the stomach (Figure 4). PZ had the same effect on intragastric pressure. The threshold dose for appearance of this effect was 10 ng kg^{-1} for CCK-7 and 20 ng kg^{-1} for PZ ($n=10$). Both substances induced changes in the myoelectrical activity of the stomach. CCK-7 (Figure 5) and PZ suppressed the spike discharges for 5 to 15 min. Simultaneously, CCK-7 increased the frequency of slow waves in a dose-dependent manner (Table 2) without affecting propagation velocity. CCK-7 at doses over 80 ng kg^{-1} led to vomiting accompanied by antiperistaltic propagation of the slow waves in antrum and corpus regions.

Intraluminal pressure and myoelectrical activity of Heidenhain pouch

Figure 6 demonstrates the intrapouch pressure changes before and after CCK-7 administration. Rhythmic contractions with a frequency of 120 Hz were recorded. CCK-7, injected i.v., increased the intrapouch pressure as well as the amplitude and frequency of rhythmic contractions (excitatory phase), and this effect was dose-dependent between

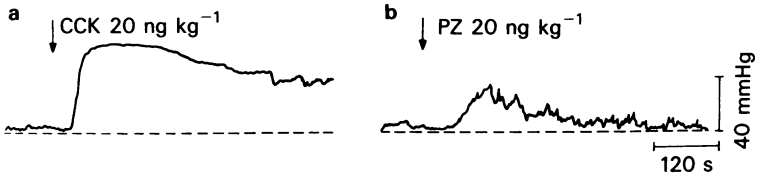

Figure 3 Intraluminal pressure in the gall bladder. Comparison between the effect of CCK-7 (a) and pancreozymin (PZ) (Boots) (b) on the intraluminal pressure in the gall bladder of one dog.

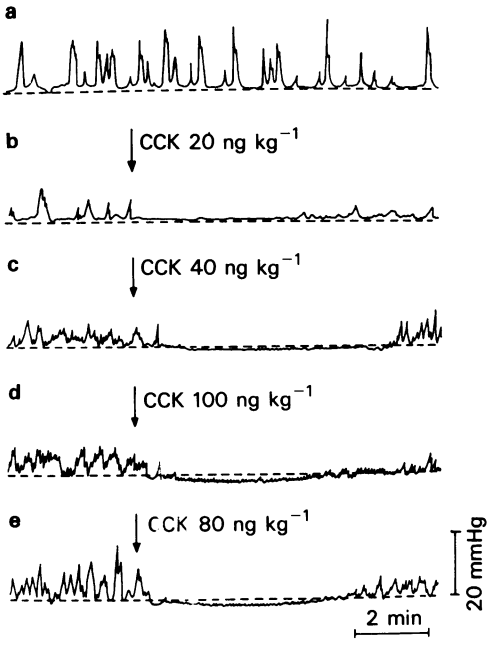

Figure 4 Intraluminal pressure in stomach. (a) Background activity of stomach consisting of periodic fasting activity with contractions of high amplitude. (b–e) Effect of CCK-7 at different doses on intraluminal pressure and periodic fasting activity. All records are from the same animal.

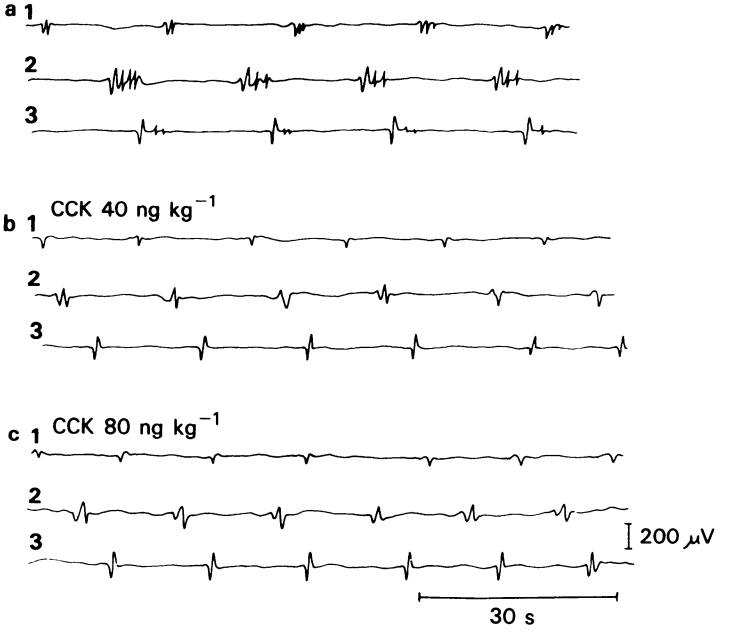

Figure 5 Myoelectrical activity of the stomach consisting of slow waves and spike discharges. (a) Background activity recorded from different parts of stomach as demonstrated in the scheme shown in Table 1. (b) and (c) CCK-7 (40 ng kg^{-1} and 80 ng kg^{-1}) suppresses the spike discharges in different parts of the stomach. Only slow waves were recorded after drug infusion.

20 and 100 ng kg^{-1}. The excitatory phase lasted for 2 to 3 min and was followed by a gradual restoration of tone with a total suppression of rhythmic contractions – the inhibitory phase (Figure 6).

The same effects, initial excitation followed by inhibition, was also observed after PZ injection. The threshold dose on this organ was 20 ng kg^{-1} for CCK-7 and 40 ng kg^{-1} for PZ ($n = 10$). CCK-7 and PZ increased the spike discharge during the excitatory phase and suppressed the spike discharges during the inhibitory phase. The restoration of tone was accompanied by a marked increase of the frequency of slow waves.

Intraluminal pressure and myoelectrical activity of small intestine

Figure 7 shows intraluminal pressure changes of ileum in a conscious dog which is typical for experiments on ileum. Rhythmic contractions with a frequency of 14 to 16 per minute were recorded. CCK-7 at doses of 10, 20, 40, 80 and 100 ng kg^{-1} i.v. caused an increase of tone for 2 to 3 min accompanied by an increase in the amplitude of rhythmic contractions (excitatory phase). This effect was dose-dependent within the range of 10 to 100 ng kg^{-1} (Figure 7) and lasted 2 to 3 min. After restoration of tone CCK-7 completely suppressed the rhythmic contractions (inhibitory phase). Complete restoration of the control trace pattern occurred within 15 to 20 min after starting an injection. A similar increase of tone followed by inhibition of rhythmic contractions was also observed after PZ (threshold dose 20 ng kg^{-1}). In order to determine the relative potency of the two substances, they were administered at the same dose level. It was observed that the excitatory effects as well as the inhibitory effect of CCK-7 were greater than those of PZ. Statistical analysis showed that the stimulation of tone by CCK-7 was greater (30%) than that by PZ, at 80 ng kg^{-1}.

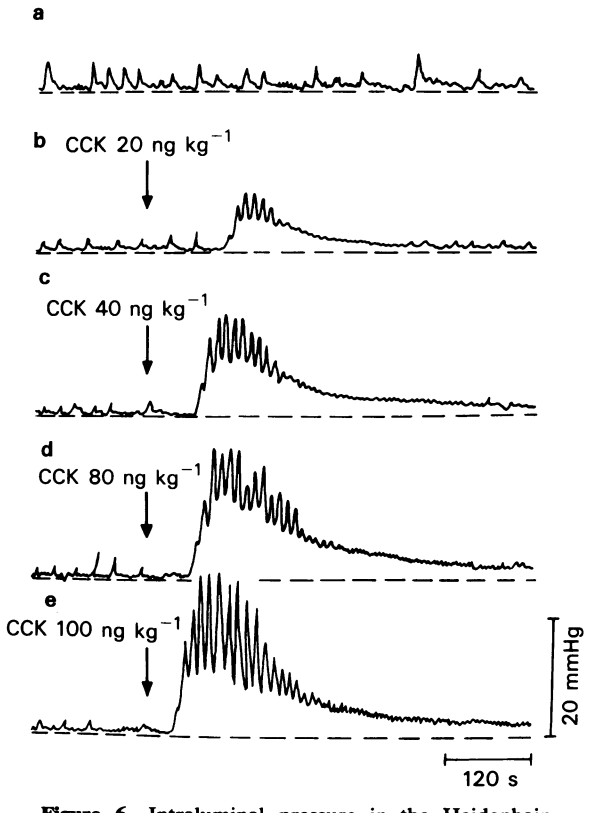

Figure 6 Intraluminal pressure in the Heidenhain pouch before and after CCK-7 infusion. (a) Background activity of the Heidenhain pouch consisting of rhythmic contractions. (b–e) influence of CCK-7 in different doses on the motility of the Heidenhain pouch, CCK-7 increased the tone and amplitude of rhythmic contractions in a dose-dependent manner. During restoration of the tone CCK-7 inhibited the rhythmic contractions.

Simultaneous measurement of intraluminal pressure and myoelectrical activity showed that each spike complex was connected with one event of periodic fasting activity. From 6 pairs of electrodes positioned along the gut wall the velocity of propagation of slow waves (BER) in stomach and small intestine was determined. CCK-7 and PZ did not change the velocity of propagation of slow waves.

The excitatory phase in the recording of pressure induced by CCK-7 and PZ was accompanied by an increase in spike discharges. As can be seen from Figure 8 only 32.5% ($n = 8$) of the slow waves were accompanied by groups of spikes during periodic fasting activity but CCK-7 increased to 96.6% ($n = 8$) the number of slow waves that were accompanied by groups of spikes (Figure 8). The inhibitory effect induced by both drugs was accompanied by partial or complete suppression of spike discharges. However, the inhibitory effect was accompanied by an increase in the frequency of slow waves.

It is possible that the bolus injection used in the experiments produced an activity front of regular spiking activity followed by a period of motor quiescence. However, we do not think it likely that the bolus injection had this effect because in control recordings similar intense spikes accompanied by contractions of high amplitude were observed.

Side effects of CCK-7 and PZ

CCK-7 and PZ at doses of 10 to 100 ng kg^{-1} exerted no effect on the pulse rate, respiration or on the behaviour of the dogs used.

Effects of various drugs on CCK responses

Pretreatment with propranolol (1 mg kg^{-1}) did not alter the CCK-7-induced responses in jejunum,

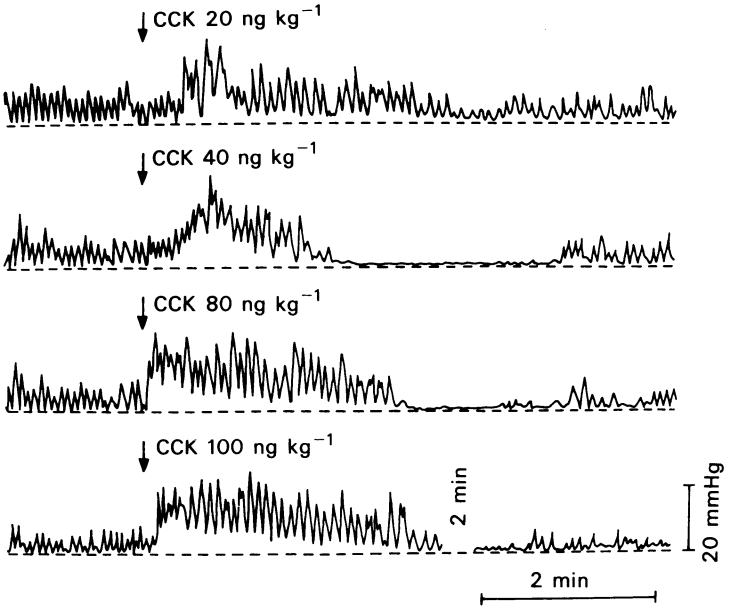

Figure 7 Intraluminal pressure in the ileum of a conscious dog before and after CCK-7 infusion. CCK-7 increased the tone in a dose-dependent manner. The restoration of tone was accompanied by elimination of peristaltic contractions.

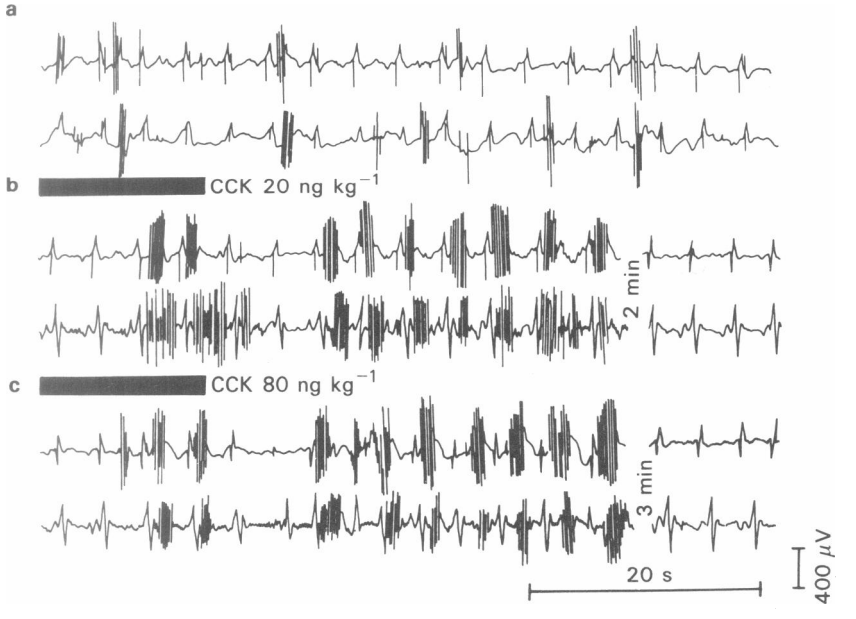

Figure 8 Myoelectrical activity in the ileum of a conscious dog. (a) Background activity, 32.5% ($n = 8$) of slow waves were accompanied by spike discharges during the periodic fasting activity. (b and c) CCK-7 (20 ng kg^{-1} and 80 ng kg^{-1}) increased the spike discharges during the excitatory phase and inhibited the spike discharges during the quiescent phase (right hand side).

ileum, or gall-bladder. Phenotolamine ($30\,\mu g\,kg^{-1}$) potentiated the excitatory effect of CCK-7 but atropine ($0.1\,mg\,kg^{-1}$) inhibited or even abolished the excitatory effects of CCK-7 (Figure 9).

Discussion

Effect of CCK-7 and CCK

The present results have shown that CCK-7 and the Boots preparation of pancreozymin (PZ), when injected i.v. in conscious dogs, increased the gall-bladder pressure and decreased the gastric pressure. Both substances exerted an initial excitatory effect followed by an inhibitory effect on the pressure and peristalsis of small intestine and Heidenhain pouch. The effect on motor activity correlated with excitation or inhibition of spike discharges. The inhibitory effect also influenced the frequency of slow waves. These results thus demonstrate that CCK-7 is capable of modulating the activity of the gall-bladder, stomach and small intestine. CCK-7 appears to be more potent than PZ at the concentrations tested.

Physiological or pharmacological action

The inhibitory effect of PZ on gastric motor activity in dog and man and also on gastric emptying has been described previously (Johnson & Magee, 1965; Johnson *et al.*, 1966; Sugawara *et al.*, 1969; Chey *et al.*, 1970; Kwong *et al.*, 1972; Debas *et al.*, 1975; Valenzuela; 1975). Debas *et al.*, (1975) described an inhibition of gastric emptying in conscious dogs after i.v. administration of PZ at doses which were submaximal for pancreatic protein secretion and gall-bladder contraction so that the authors came to the conclusion that inhibition of gastric emptying is a physiological rather than a pharmacological effect of CCK. According to Valenzuela (1976) CCK plays a physiological role in regulation of gastric pressure.

The threshold dose of both CCK-7 and PZ for modulation of gastric motility in our experiment was near to that for modulation of the gall-bladder pressure, and thus supports the view that inhibition of gastric pressure is one of the physiological actions of CCK and CCK-7.

The excitatory effect of CCK on the motility and spike discharges of small intestine has been described by several authors (Monod, 1964; Morin *et al.*,1965; Backlund, 1970; Dolringer *et al.*, 1975; Mukhopadhyay *et al.*, 1977; Wingate *et al.*, 1978). The excitatory effect in our studies, however, was short-lasting and was followed by restoration of tone and prolonged inhibition of peristaltic contractions

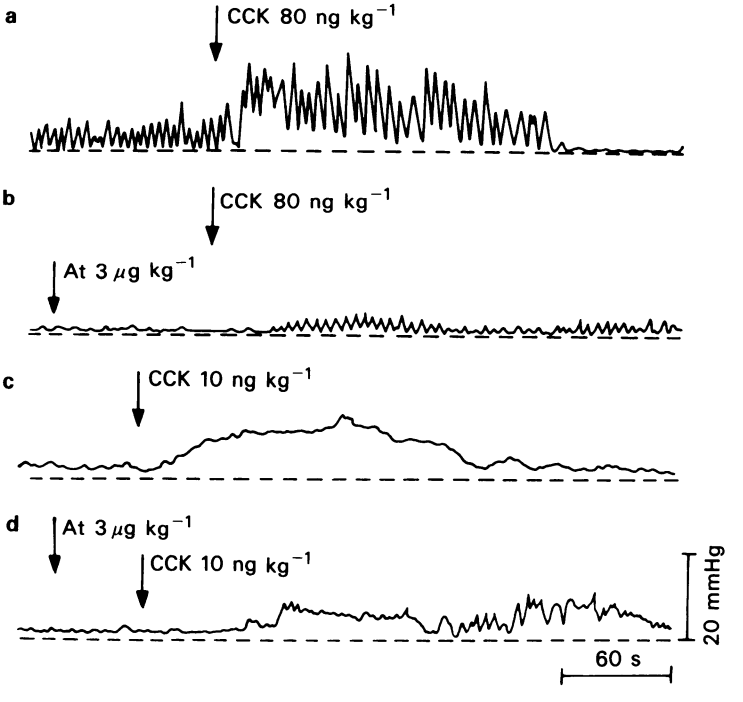

Figure 9 Effect of CCK-7 on the intraluminal pressure in ileum (a) and (b) and gall bladder (c) and (d) before and after administration of atropine (At) ($3\,\mu g\,kg^{-1}$). Atropine was injected 3 to 5 min before infusion of CCK-7.

(for 10 to 15 min). The threshold dose for these effects was also near to that for the effect on gall-bladder pressure. This suggests again that CCK, at doses within the physiological range, is possibly involved in the regulation of gastro-intestinal motility.

Mechanisms of action of CCK-7 and CCK

The mechanisms of action of CCK and CCK-7 on gastrointestinal motility seem to be complex. On the basis of our data and that published in the literature we propose the following mechanisms. The excitatory responses of the gall-bladder, small intestine, and Heidenhain pouch to CCK-7 and CCK were suppressed by atropine and ganglion blockers and were mediated, therefore, through release of acetylcholine, as has been demonstrated for isolated gall-bladder and gastrointestinal smooth muscle (Yau & Farrar, 1972; Fisher, DiMarino & Cohen, 1973; Vizi, Bertaccini, Impicciatore & Knoll, 1973). The inhibitory effects of CCK-7 and CCK on the motility and spike discharges in stomach and duodenum (Doringer *et al.*, 1975; Oigaard *et al.*, 1975) are mediated not by adrenergic transmission but probably by other hormonal factors, e.g secretin, somatostatin or VIP, and this is supported by the following observations:

(1) CCK induces an inhibitory effect on gastric motility *in vivo* (Johnson & Magee, 1965; Johnson *et al.*, 1966; Sugawara *et al.*, 1969; Chey *et al.*, 1970; Kwong *et al.*, 1972; Debas, *et al.*, 1975; Valenzuela, 1976). (2) CCK induces an excitatory effect on isolated gastric muscles (Comeron, Phillips & Summerskill, 1967; Comeron, Phillips & Summerskill, 1970; Fisher *et al.*, 1973; Gerner, Mahlumshagen & Hafner, 1976) (3) The inhibitory effect of CCK on gastric motility in conscious dogs appeared first in the stomach and duodenum, where the endocrine cells are localized, and only later, after 3 to 5 min, did it appear in the Heidenhain pouch.

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