Effects of cholecystokinin-octapeptide and cerulein on small-intestinal motility in sheep

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ABSTRACT: Cholecystokinin (CCK) affects the intestinal motility but in ruminants the question has not been entirely explored. The aim of this study was to examine the precise effects of CCK-octapeptide (CCK-OP) and its amphibian analogue, cerulein, on duodenal motor activity in unfasted rams in the course of chronic experiments. Five rams underwent the implantation of a strain gauge force transducer to the duodenal wall, and – additionally – the bipolar platinum electrodes to the duodenal bulb, distal duodenum, near the strain gauge force transducer, and proximal jejunum. During continuous motor recordings, 0.15M NaCl or CCK peptides were administrated intravenously. Injections of CCK-OP at doses of 20 (over 30 s), 200 (over 30 or 60 s), and 2 000 (over 30, 60, or 120 s) ng/kg of body weight and injections of cerulein at doses of 1, 10, or 100 ng/kg (given over the same periods) were each administered in the course of duodenal phase 1, 2a, or 2b of the migrating motor complex (MMC), i.e. 5 min after the onset of each phase. Injections of the smallest doses of CCK peptides exerted a slight and mostly insignificant effect on the duodenal areas under contraction (AUC). In the duodenum, the moderate doses of the hormones evoked short stimulatory effects followed by longer inhibitory biphasic effects on AUC. These effects were inversely related to the duration of the hormone injection. It is concluded that CCK evokes stimulatory and inhibitory (biphasic) physiological effects on duodenal motility in sheep.

Keywords: sheep; duodenum; motor activity; cholecystokinin octapeptide; cerulein; migrating motor complex

Duodenal motility is a very complex function, crucial for normal digestion, absorption, and chyme distribution and is controlled by manifold neurohormonal mechanisms. Cholecystokinin (CCK) is one of the main gastrointestinal hormones involved in this control (Dockray, 2006). The role of the hormone is broad and also contributes to the control of other physiological functions. In man and monogastric animals, CCK affects motor activity in the whole gastrointestinal tract (Botella et al., 1992; Kusano et al., 2005; Fornai et al., 2006). The recognized effect of CCK on small-intestinal motility is evident. The hormone, usually applied as CCK-octapeptide (CCK-OP) as the natural CCK form present in sheep, inhibits the arrival of the migrating motor complex (MMC) and induces a fed-like pattern in the upper small bowel (Heppell et al., 1982; Titchen, 1984; Mączka et al., 1993). Thus CCK rather stimulates the duodenal digestive motility; it may increase spiking activity *in vivo* and *in vitro* as well as it hastens the intestinal transit time (Xu et al., 1998; Lin et al., 2002). Pretreatment with CCK receptor antagonist reverses this action (Niederau and Karaus, 1991; Xu et al., 1998). However, the opposite and dual effects of CCK-OP and its amphibian analogue, cerulein, have been reported (Giuliani et al., 1990; Martins et al., 2006). In sheep, CCK peptides also inhibit the arrival of the MMC in the upper small intestine, but their effect on intestinal motility has not been fully elucidated so far in spite of several studies performed (Bueno and Praddaude, 1979; Cottrell

and Iggo, 1984; Ormas et al., 1984; Onaga et al., 1997; Romański, 2004, 2007b). Thus the dual effect of CCK upon the small-intestinal motility can also be expected in sheep. It still remains uncertain what the character of the duodenal motor response to CCK is like and this is the goal of this study.

MATERIAL AND METHODS

Animal preparation

Five healthy adult rams of the Polish Merino breed weighing 38–43 kg each were used. The rams were fed good-quality hay, 1 kg daily, and a grain mixture (Dolpasz, Wrocław) with 250 g daily, then fasted for 24 h before surgery. Drinking water was not limited. After general and local anaesthesia (Romański, 2007a), right lateral laparotomy, utilizing a diagonal incision, was performed and a strain gauge force transducer (RB Products, Madison, WI, USA), calibrated individually before the surgery, was attached near the duodenal electrode. Two bipolar platinum electrodes were implanted at the serosal side to the duodenal bulb, 5.5–6 cm distally to the pyloric ring and to the distal duodenum, 50 cm below the bulbar electrode and next two electrodes were placed in the jejunum, 200 and 300 cm from the duodenal electrode to confirm the presence of the MMC cycles. Other details of this procedure were described elsewhere (Romański, 2004, 2007a). Marked wires of the strain gauge force transducer and electrodes were exteriorized through the stab incision, soldered to the plug and fixed to the integument. Within three days the animals returned to normal feeding. The skin sutures were removed about 10 days after the surgery.

Experimental design

A total of 210 experiments lasting 5–6 h each were conducted.

Myoelectric and motor activities were continuously recorded using a multichannel electroencephalograph (Reega Duplex TR XVI, Alvar Electronics, Montreuil, Paris, France) also adapted for mechanical activity recordings. Food was removed from the cage twenty-four hours before each experiment. At least two consecutive phases 3 of the MMC including one full normal cycle of the MMC were recorded each time. During control recordings, injections of 5 ml 0.15 M NaCl were administered over 30 s into the jugular vein through a thin polyethylene catheter introduced each time the experiment. The saline injections were performed in the course of phase 1 (5 min after its start in the duodenum), 2a (5 min after its start in the duodenum) or phase 2b (5 min after its start in the duodenum) of the MMC. In the basic group of experiments, slow intravenous injections of CCK-OP (Sincalide, Squibb Institute, Princeton, NJ, USA) at the small, moderate and high doses, i.e. 20, 200, or 2 000 ng/kg, and cerulein (Farmitalia Carlo Erba, Milan, Italy) at the small, moderate and high doses, i.e. 1, 10 or 100 ng/kg, were applied. The small doses of both CCK peptides were administered over 30 s, the moderate doses over 30 or 60 s, and the high doses over 30, 60, or 120 s. After saline or CCK peptide administration, the motor and myoelectric activities were recorded until the arrival of the first organized phase 3 of the MMC. After cessation of all the experiments, the animals were sacrificed and the localizations of the strain gauge force transducer and the electrodes were confirmed during autopsy.

Data analysis

The MMC cycles and their phases were identified in the duodenum according to the criteria proposed by Code and Marlett (1975) with a slight modification (Romański, 2002). The division of phase 2 into phases 2a and 2b of the MMC, described earlier by Dent et al. (1983), was performed according to more precise criteria (Romański, 2007a). The myoelectric and motor recordings were visually analysed and the areas under contraction curve (AUC) values were calculated. The duration of the periods was equal to one minute. The AUC values were calculated by multiplication of the width of the phasic contraction measured in the middle of the contraction height by the absolute contraction height and expressed as g/s/min (Romański, 2004). Contractions lasting more than 10 s (considered as tonic contractions), contractions exhibiting the amplitude smaller than 0.2 g, and contractions whose widths were equal to or greater than their heights were omitted. In the recordings obtained from the experiments with saline and CCK peptide injections, the AUC values were calculated in four one-minute periods, i.e. before the injection and ten one-minute periods after the injection. On

the mechanograms, the measurements were performed using a calliper with an accuracy of about 0.3 mm.

Statistical data processing

All the values were grouped and the means and standard deviations were calculated. Statistical significance, i.e. when $P < 0.05$, was calculated using Student's *t*-test for paired values, where appropriate, preceded by one-way analysis of variance (Snedecor and Cochran, 1971).

RESULTS

Saline injections evoked no effect and these data are not shown here.

The injection of CCK-OP at the low dose over 30 s during phase 1 of the MMC evoked a small but significant excitatory effect on duodenal motility in the first minute after cessation of the peptide injection (Table 1). The application of moderate doses of CCK peptide over 30 s caused a more evident increase in the duodenal motor activity in the first minute following the hormone injection. When CCK was administered over 60 s, the elevation of AUC values was even higher than after CCK given over 30 s. The administration of both CCK-OP at the high dose over 30 s during phase 1 of the MMC increased the AUC values significantly during the whole observation time but in the third minute following the hormone administration the effect was much smaller than during the remaining periods of observation. When CCK was applied over 60 s, a significant stimulatory effect was noted within 1, 2, 5–10 min periods. The administration of CCK at the high dose over 120 s in the course of phase 1 of the MMC produced a stimulatory response within 1, and 7–10 min of observation (Table 1). In the remaining periods the results were slightly higher than during the control period but did not reach the level of significance.

Slow injections of CCK-OP during phase 2a of the MMC at the low dose induced insignificant AUC values in 1, and 3–10 min of observation (Table 1). The moderate dose given over 30 s elevated AUC values significantly within the first minute of the observation period and these values decreased to the control level in the third minute following CCK. In the subsequent periods the AUC values gradually increased but did not become significant. When the moderate CCK dose was injected over 60 s in the course of phase 2a of the MMC, a significant rise in AUC values was observed within 1–3 min after the hormone administration while the highest stimulation was seen in the second minute. During the fourth minute an insignificant fall of motor activity was observed and the values were slightly higher than during the control period. The administration of CCK-OP at the high dose over 30 s during phase 2a of the MMC evoked a statistically significant rise in AUC values in the first minute and then within 2–5 min following the hormone injection. When CCK was given over 60 s, a significant rise in AUC values was observed within 2–4 min while in the next minute the AUC values significantly decreased. The administration of CCK-OP over 120 s in the course of phase 2a of the MMC brought similar results but the inhibitory period lasted two minutes and arrived within 4 to 5 min (Table 1).

The injection of CCK-OP during phase 2b of the MMC at the low dose produced an insignificant decrease in AUC values in the second minute following the hormone administration (Table 1). When the CCK peptide was given at the moderate dose over 30 s, a significant increase of motor activity was observed within the first minute while in 3 to 6 minutes the values were significantly lower than in the control. Given over 60 s, CCK-OP produced an insignificant elevation of the duodenal contractions in the first minute while within 3–6 min the changes were inhibitory and statistically significant (Table 1). After the high dose given over 30 s during phase 2b of the MMC, no stimulatory alterations occurred while significant inhibitory changes were observed within 2–9 min following the hormone administration. When CCK-OP was applied over 60 s, significant inhibition was extended between 1 and 9 minutes of the posthormonal part of the experiment. The longest application of the high CCK dose suppressed the motility during the entire observation period (Table 1).

Cerulein administration over 30 s at the low dose during phase 1 of the MMC increased the AUC values significantly during the first two minutes (Table 2). The administration of the hormone over 30 s but at the moderate dose in the course of the same MMC phase increased the contractile parameter significantly in the first minute while during the second minute of observation the increase did not exceed the statistical significance level. When cerulein was given over 60 s, the effect was similar

Table 1. Duration of the stimulatory and inhibitory effects of prolonged injections of the various doses of CCK-OP administered during phase 1, 2a and 2b of the MMC on motor activity index (AUC) of the duodenum in unfasted sheep

		0	1	2	3	4	5	6	7	8	9	10
Phase 1 of the MMC												
20 ng/kg	30 s	**0.02**	**0.09**[a]	**0.05**	**0.01**	**0.01**	**0.01**	**0.01**	**0.02**	**0.02**	**0.05**	**0.06**
		0.01	0.04	0.03	0.00	0.01	0.02	0.01	0.03	0.04	0.04	0.03
200 ng/kg	30 s	**0.01**	**0.24**[b]	**0.04**	**0.03**	**0.02**	**0.03**	**0.01**	**0.03**	**0.02**	**0.04**	**0.07**
		0.01	0.16	0.02	0.02	0.01	0.03	0.02	0.02	0.02	0.03	0.05
	60 s	**0.01**	**0.38**[c]	**0.06**	**0.01**	**0.00**	**0.01**	**0.03**	**0.03**	**0.02**	**0.06**	**0.09**
		0.02	0.21	0.04	0.01	0.00	0.02	0.02	0.03	0.04	0.03	0.07
2 000 ng/kg	30 s	**0.01**	**0.48**[c]	**0.23**[c]	**0.07**[a]	**0.34**[c]	**0.39**[c]	**0.36**[c]	**0.31**[c]	**0.45**[c]	**0.88**[c]	**1.29**[c]
		0.00	0.19	0.09	0.05	0.15	0.23	0.24	0.22	0.20	0.37	0.64
	60 s	**0.02**	**0.69**[c]	**0.13**[a]	**0.03**	**0.08**	**0.14**[a]	**0.20**[b]	**0.26**[c]	**0.32**[c]	**0.46**[c]	**0.89**[c]
		0.00	0.35	0.06	0.02	0.07	0.09	0.11	0.13	0.17	0.22	0.34
	120 s	**0.01**	**0.56**[c]	**0.08**	**0.04**	**0.05**	**0.04**	**0.06**	**0.10**[a]	**0.10**[a]	**0.15**[b]	**0.17**[b]
		0.01	0.21	0.07	0.02	0.03	0.03	0.04	0.06	0.06	0.10	0.09
Phase 2a of the MMC												
20 ng/kg	30 s	**0.11**	**0.17**	**0.11**	**0.12**	**0.17**	**0.21**	**0.20**	**0.21**	**0.20**	**0.31**	**0.35**
		0.05	0.10	0.06	0.08	0.07	0.11	0.14	0.10	0.14	0.16	0.23
200 ng/kg	30 s	**0.13**	**0.70**[c]	**0.24**	**0.12**	**0.16**	**0.17**	**0.28**	**0.29**	**0.29**	**0.35**	**0.39**
		0.06	0.29	0.18	0.04	0.09	0.06	0.13	0.11	0.10	0.15	0.19
	60 s	**0.12**	**0.52**[c]	**0.99**[c]	**0.76**[c]	**0.06**	**0.23**	**0.21**	**0.17**	**0.14**	**0.16**	**0.17**
		0.05	0.18	0.40	0.24	0.07	0.09	0.12	0.05	0.05	0.06	0.06
2 000 ng/kg	30 s	**0.10**	**0.87**[c]	**0.19**	**0.40**[b]	**0.51**[b]	**0.78**[c]	**0.90**[c]	**0.10**	**0.10**	**0.17**	**0.22**
		0.04	0.28	0.10	0.17	0.23	0.16	0.15	0.04	0.07	0.09	0.08
	60 s	**0.12**	**0.94**[c]	**0.60**[c]	**0.31**[b]	**0.04**[a]	**0.20**	**0.24**	**0.30**	**0.27**	**0.15**	**0.24**
		0.06	0.33	0.21	0.04	0.01	0.08	0.09	0.15	0.17	0.06	0.08
	120 s	**0.13**	**0.61**[c]	**0.68**[c]	**0.31**	**0.04**[a]	**0.04**[a]	**0.06**	**0.11**	**0.09**	**0.12**	**0.14**
		0.07	0.24	0.26	0.18	0.03	0.03	0.03	0.04	0.03	0.07	0.05
Phase 2b of the MMC												
20 ng/kg	30 s	**1.24**	**1.38**	**0.57**	**1.19**	**1.25**	**1.30**	**1.32**	**1.34**	**1.40**	**1.44**	**1.41**
		0.51	0.42	0.24	0.46	0.33	0.39	0.28	0.36	0.41	0.38	0.42
200 ng/kg	30 s	**1.20**	**1.96**[a]	**0.87**	**0.47**[b]	**0.38**[b]	**0.52**[a]	**0.66**[a]	**0.88**	**1.14**	**1.32**	**1.38**
		0.32	0.41	0.56	0.31	0.33	0.34	0.24	0.35	0.47	0.56	0.51
	60 s	**1.29**	**1.68**	**0.97**	**0.54**[a]	**0.51**[b]	**0.58**[a]	**0.62**[a]	**0.98**	**1.22**	**1.41**	**1.42**
		0.35	0.42	0.48	0.27	0.22	0.33	0.32	0.45	0.47	0.50	0.60
2 000 ng/kg	30 s	**1.31**	**0.29**[c]	**0.18**[c]	**0.12**[c]	**0.12**[c]	**0.16**[c]	**0.17**[c]	**0.25**[c]	**0.30**[c]	**0.81**	**0.85**
		0.34	0.13	0.06	0.05	0.06	0.07	0.08	0.10	0.14	0.38	0.33
	60 s	**1.25**	**0.24**[c]	**0.13**[c]	**0.18**[c]	**0.17**[c]	**0.19**[c]	**0.19**[c]	**0.23**[c]	**0.34**[c]	**0.34**[b]	**0.75**
		0.30	0.11	0.06	0.08	0.07	0.07	0.09	0.09	0.18	0.21	0.42
	120 s	**1.18**	**0.18**[c]	**0.11**[c]	**0.10**[c]	**0.15**[c]	**0.15**[c]	**0.18**[c]	**0.20**[c]	**0.19**[c]	**0.20**[c]	**0.22**[c]
		0.26	0.08	0.03	0.04	0.07	0.06	0.10	0.08	0.06	0.08	0.07

The explanations for Table 1

values expresseding/s/min; 0–10 – one-minute consecutive periods; 0 – control period, 1–10 – period safter hormone administration; dose of the hormone expressed in ng/kg;

duration of hormone administration expressed in seconds (s); the values of three previous control periods, insignificantly different from period 0, are not shown; statistical significances: $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$ vs. relevant value of period 0; other explanations see the text

to that after the low cerulein dose (Table 2). The injection of the high dose of cerulein in the course of phase 1 of the MMC over 30, 60, and 120 s produced a significant increase in AUC values in all observation periods except 3, 2–4, and 2–5 min, respectively, while after the hormone administration over 120 s insignificant increases were observed also in the 7th and 9th min following the drug administration (Table 2).

The introduction of cerulein at the low dose in the course of phase 2a of the MMC did not evoke a significant effect while in the last minutes of the experiment the increase of AUC values was noted (Table 2). When the moderate cerulein dose given over 30 s was used, a significant increase of contraction was observed during the first two minutes following cerulein administration. The injection of the hormone over 60 s caused a significant elevation of AUC values within 1–3 min afterwards (Table 2). The high dose of cerulein given over 30 s produced a similar excitatory effect while during 4–5 min periods significant inhibition of the contractility arrived. The administration of the high dose of cerulein over 60 s significantly increased the duodenal motility during 2–3 min and insignificant inhibition was observed during the next minute. The longest application of the high cerulein dose during phase 2a of the MMC stimulated motility significantly during 2–4 min, in the next minute the AUC values were similar to those during the control period and during the 5th min a significant inhibitory response was noted. Insignificant suppression was still observed in the next two minutes (Table 2).

Cerulein injection at the low dose during phase 2b of the MMC (Table 2) caused an insignificant effect on duodenal contractility although during the last three minutes of observation a slight elevation of AUC values were present. The moderate dose applied over 30 s increased significantly the duodenal contractions within the first minute after the hormone administration and during the next three minutes the inhibitory effect, statistically significant, was noted. Cerulein given over 60 s at this dose evoked a roughly similar response (Table 2). When the hormone was applied at the high dose over 30 s during phase 2b of the MMC, only an inhibitory effect was observed. Statistically significant inhibition of AUC values occurred during the remaining part of the experiment. The administration of cerulein at the same dose over 60 and 120 s produced similar inhibition (Table 2).

DISCUSSION

Injections of CCK-OP and cerulein elicited marked excitatory and inhibitory alterations in the motor activity parameters of the ovine duodenum. As it has been confirmed several times, CCK plays a crucial role in the control of the small intestinal motility (Smout, 2004). This may also be true in sheep (Onaga et al., 1997; Romański, 2004). However, its effect may be different in the various regions of the small bowel (Fargeas et al., 1989; Romański, 2007a). In the duodenum, the moderate doses of CCK peptides had a clear dual effect. A prompt increase in spiking and contractile activity followed by inhibitory alterations was seen. Biphasic and even triphasic responses of gastric and duodenal motility to CCK-OP and other peptides acting through CCK receptors have already been observed (Cottrell and Iggo, 1984; McLeay and Wong, 1989; Giuliani et al., 1990). These findings suggest that the mechanism of CCK action on duodenal motility is composed and may exhibit even the adaptive features. However, it seems to be amply confirmed that the effect of CCK on duodenal motility in man, dog, rabbit, rat and sheep is excitatory (Gutiérrez et al., 1974; Heppell et al., 1982; Elbrønd et al., 1994; Merle et al., 2000; Romański, 2004). Thus, the stimulatory effect of a moderate dose of CCK on duodenal motility seems to be primary (Mizumoto et al., 1992), and this conclusion can be inferred also from the present study. Similar effects were reported for cerulein (Bertaccini et al., 1968; Ormas et al., 1984; Niederau and Karaus, 1991; Romański, 2004). Other reports indicate that the

Table 2. Duration of the stimulatory and inhibitory effects of injections of the various doses of cerulein during phase 1, 2a and 2b of the MMC on the motor activity index (AUC) of the duodenum in unfasted sheep

		0	1	2	3	4	5	6	7	8	9	10
Phase 1 of the MMC												
1 ng/kg	30 s	**0.02**	**0.50**^{c}	**0.06**^{a}	**0.03**	**0.00**	**0.02**	**0.02**	**0.01**	**0.03**	**0.01**	**0.01**
		0.01	0.21	0.02	0.02	0.01	0.03	0.00	0.02	0.01	0.00	0.01
10 ng/kg	30 s	**0.00**	**0.56**^{c}	**0.14**	**0.04**	**0.02**	**0.02**	**0.01**	**0.02**	**0.03**	**0.05**	**0.07**
		0.02	0.22	0.09	0.03	0.01	0.02	0.00	0.02	0.02	0.03	0.02
	60 s	**0.01**	**0.47**^{c}	**0.10**^{b}	**0.05**	**0.03**	**0.02**	**0.00**	**0.03**	**0.06**	**0.10**	**0.12**
		0.01	0.24	0.03	0.04	0.01	0.02	0.01	0.02	0.04	0.06	0.06
100 ng/kg	30 s	**0.01**	**1.28**^{c}	**0.10**^{a}	**0.14**	**0.20**^{a}	**0.34**^{b}	**0.86**^{c}	**1.36**^{c}	**1.52**^{c}	**1.47**^{c}	**1.48**^{c}
		0.00	0.63	0.07	0.16	0.15	0.28	0.49	0.51	0.63	0.71	0.66
	60 s	**0.02**	**1.26**^{c}	**0.11**	**0.06**	**0.10**	**0.17**^{a}	**0.26**^{a}	**0.38**^{c}	**0.68**^{c}	**1.17**^{c}	**1.42**^{c}
		0.00	0.52	0.09	0.05	0.08	0.12	0.22	0.24	0.43	0.51	0.53
	120 s	**0.01**	**1.41**^{c}	**0.07**	**0.04**	**0.07**	**0.09**	**0.08**^{a}	**0.11**	**0.15**^{a}	**0.14**	**0.16**^{a}
		0.02	0.76	0.05	0.02	0.05	0.06	0.05	0.09	0.11	0.12	0.12
Phase 2a of the MMC												
1 ng/kg	30 s	**0.14**	**0.18**	**0.20**	**0.15**	**0.16**	**0.17**	**0.19**	**0.20**	**0.23**	**0.31**	**0.38**
		0.08	0.10	0.12	0.08	0.07	0.12	0.13	0.10	0.12	0.15	0.17
10 ng/kg	30 s	**0.11**	**0.84**^{c}	**0.24**^{a}	**0.17**	**0.08**	**0.10**	**0.12**	**0.14**	**0.18**	**0.26**	**0.30**
		0.06	0.33	0.07	0.13	0.06	0.06	0.07	0.09	0.07	0.12	0.14
	60 s	**0.12**	**0.54**^{c}	**0.48**^{c}	**0.46**^{c}	**0.18**	**0.07**	**0.15**	**0.19**	**0.18**	**0.24**	**0.32**
		0.04	0.17	0.19	0.14	0.10	0.04	0.06	0.09	0.06	0.10	0.12
100 ng/kg	30 s	**0.13**	**0.96**^{c}	**0.57**^{c}	**0.43**^{c}	**0.04**^{a}	**0.05**^{a}	**0.08**	**0.12**	**0.18**	**0.25**	**0.33**
		0.06	0.34	0.18	0.11	0.04	0.03	0.03	0.04	0.07	0.13	0.14
	60 s	**0.10**	**0.87**^{c}	**0.57**^{c}	**0.57**^{c}	**0.06**	**0.11**	**0.08**	**0.09**	**0.12**	**0.20**	**0.18**
		0.05	0.28	0.14	0.21	0.10	0.05	0.05	0.05	0.04	0.08	0.06
	120 s	**0.11**	**0.70**^{c}	**0.46**^{c}	**0.60**^{c}	**0.12**	**0.05**^{a}	**0.07**	**0.08**	**0.11**	**0.09**	**0.13**
		0.05	0.23	0.13	0.26	0.04	0.02	0.03	0.04	0.09	0.04	0.05
Phase 2b of the MMC												
1 ng/kg	30 s	**1.18**	**1.23**	**1.14**	**1.27**	**1.19**	**1.17**	**1.24**	**1.29**	**1.37**	**1.43**	**1.41**
		0.31	0.40	0.42	0.58	0.38	0.30	0.35	0.40	0.45	0.44	0.42
10 ng/kg	30 s	**1.24**	**2.40**^{a}	**0.60**^{c}	**0.41**^{c}	**0.75**^{a}	**1.01**	**1.18**	**1.22**	**1.30**	**1.35**	**1.37**
		0.38	0.71	0.08	0.13	0.23	0.34	0.41	0.27	0.31	0.38	0.35
	60 s	**1.22**	**2.10**^{a}	**0.70**^{a}	**0.60**^{b}	**0.91**	**1.18**	**1.34**	**1.27**	**1.40**	**1.38**	**1.44**
		0.33	0.50	0.21	0.17	0.32	0.46	0.58	0.46	0.52	0.41	0.38
100 ng/kg	30 s	**1.32**	**0.32**^{c}	**0.12**^{c}	**0.03**^{c}	**0.06**^{c}	**0.09**^{c}	**0.17**^{c}	**0.11**^{c}	**0.15**^{c}	**0.18**^{c}	**0.19**^{c}
		0.41	0.11	0.07	0.01	0.04	0.05	0.08	0.06	0.09	0.06	0.06
	60 s	**1.28**	**0.39**^{c}	**0.23**^{c}	**0.15**^{c}	**0.17**^{c}	**0.21**^{c}	**0.23**^{c}	**0.22**^{c}	**0.24**^{c}	**0.36**^{c}	**0.51**^{c}
		0.35	0.16	0.14	0.12	0.11	0.10	0.14	0.13	0.12	0.20	0.23
	120 s	**1.23**	**0.36**^{c}	**0.12**^{c}	**0.08**^{c}	**0.11**^{c}	**0.17**^{c}	**0.17**^{c}	**0.19**^{c}	**0.20**^{c}	**0.18**^{c}	**0.19**^{c}
		0.32	0.12	0.07	0.03	0.06	0.08	0.09	0.10	0.08	0.08	0.11

The explanation see Table 1

effect of CCK action on duodenal motility can be inhibitory or no effect can be observed while some authors observed a simultaneous stimulatory effect in the jejunum (Fleckenstein and Öigaard, 1977; Bueno and Praddaude, 1979; Giralt and Vergara, 2000; Martins et al., 2006). This confirms the view that the character of CCK action can be different in the various small intestinal segments.

The biphasic motor response to CCK observed here raises a question regarding the possible mechanism of dual CCK action on gastrointestinal motility. It is well known that the motor action of CCK is mediated by at least two peripheral subtypes of CCK receptors present in the gastrointestinal tract, including duodenal smooth muscles, enteric neurons, vagal afferent fibres and the brain, and that CCK can exert its modulatory actions, including motility, within these structures (Bueno, 1993; Grider, 1994; Mantyh et al., 1994; Li et al., 1999; Noble et al., 1999; Beinfeld, 2001; Sayegh and Ritter, 2003). Therefore, the three most plausible mechanisms are presented below. One possibility is that at the smaller doses CCK stimulates a given CCK receptor subtype and at the higher dose it further activates another CCK receptor subtype in the gut. If it is the case, the effect of CCK peptide can be due rather to the greater CCK_1 receptor density in the duodenum than CCK_2 receptor density than due to the greater affinity of CCK_1 receptors than of CCK_2 receptors to CCK peptide. Thus, the primary stimulatory response can be followed by inhibition. It is well known that CCK is a gut hormone and it is also a neuronal modulator. Its action on intestinal motility can be either direct or can be mediated by several factors including the cholinergic system. This is probably also the case in sheep. Therefore, the second possibility is that the excitatory response to the direct action of CCK on smooth muscles can be followed by an inhibitory neuronal response. Since some gastrointestinal hormones can interact with CCK, the most convincing mechanism of the dual action of CCK on small intestinal motility is that the first stimulatory response is elicited by the rapid (possibly direct) action of CCK and the subsequent inhibitory action is induced by one of the inhibitory hormones including somatostatin, which is released by CCK (Miyasaka and Funakoshi, 2003). This is the third possibility. An immense task should be undertaken to explore the mechanisms of various possible actions of CCK on intestinal motility more precisely.

The final issue is to compare the potency and specificity of the actions of CCK-OP and cerulein in the designed ovine model. This problem was briefly discussed earlier (Romański, 2004) and it was clear that this question should be dealt with separately for each gastrointestinal region. The essential corollaries emerging from the previous study concerning the ovine duodenum pointed out that the equipotent doses of cerulein and CCK-OP oscillate between 1:8 and 1:15 (Romański, 2007b). This remains in concert only with that part of the present study where CCK-OP, administered at a 20 times higher dose than cerulein, exerted a stronger effect and comprised the inhibition of myoelectric activity in the duodenal bulb and the duodenum, but not stimulatory changes, although the differences were not consistent. It was also noticed that the inhibitory effects of CCK peptides observed in the duodenal bulb were more jointly related to the peptide dose than those in the duodenum where the changes after the highest doses of the peptides given within 120 s were more distinct than after the same dose administered in the shorter time lag. This finding is accountable to the more natural action of the peptides administered at the submaximal doses which simulate the normal dynamics of hormone release and whose magnitude was also more physiological.

Thus, one is tempted to conclude that in sheep physiological doses of CCK peptides evoked a vast effect on duodenal myoelectric and motor activities, suggesting that in sheep, like in monogastric species, the role of CCK in the control of this function is remarkable. CCK might thus be considered as a physiological regulator of duodenal motility in sheep.

REFERENCES

Beinfeld M.C. (2001): An introduction to neuronal cholecystokinin. Peptides, 22, 1197–1200.

Bertaccini G., de Caro G., Endean R., Erspamer V., Impicciatore M. (1968): The actions of caerulein on the smooth muscle of the gastrointestinal tract and the gall bladder. British Journal of Pharmacology, 34, 291–310.

Botella A., Delvaux M., Berry P., Frexinos J., Bueno L. (1992): Cholecystokinin and gastrin induce cell contraction in pig ileum by interacting with different receptor subtypes. Gastroenterology, 102, 779–786.

Bueno L. (1993): Involvement of brain CCK in the adaptation of gut motility to digestive status and stress: a review. Journal of Physiology, 87, 301–306.

Bueno L., Praddaude F. (1979): Electrical activity of the gallbladder and biliary tract in sheep and its relationships with antral and duodenal motility. Annales de Biologie Animale, Biochimie, Biophysique, 19, 1109–1121.

Code C.F., Marlett J.A. (1975): The interdigestive myoelectric complex of the stomach and small bowel of dogs. The Journal of Physiology, 246, 289–309.

Cottrell D.F., Iggo A. (1984): The responses of duodenal tension receptors in sheep to pentagastrin, cholecystokinin and some other drugs. The Journal of Physiology, 354, 477–495.

Dent J., Dodds W.J., Sekiguchi T., Hogan W.J., Arndorfer R.C. (1983): Interdigestive phasic contractions of the human lower esophageal sphincter. Gastroenterology, 84, 453–460.

Dockray G.J. (2006): Gastrointestinal hormones: gastrin, cholecystokinin, somatostatin, and ghrelin. In: Johnson L.R. (ed.): Physiology of the Gastrointestinal Tract. Elsevier Inc., Amsterdam, The Netherland, 91–120.

Elbrønd H., Østergaard L., Huniche B., Skovgaard Larsen L., Bondo Andersen M. (1994): Rabbit sphincter of Oddi and duodenal pressure and slow-wave activity. Scandinavian Journal of Gastroenterology, 29, 537–544.

Fargeas M.J., Bassotti G., Fioramonti J., Bueno L. (1989): Involvement of different mechanisms in the stimulatory effects of cholecystokinin octapeptide on gastrointestinal and colonic motility in dogs. Canadian Journal of Physiology and Pharmacology, 67, 1205–1212.

Fleckenstein P., Öigaard A. (1977): Effects of cholecystokinin on the motility of the distal duodenum and the proximal jejunum in man. Scandinavian Journal of Gastroenterology, 12, 375–378.

Fornai M., Coluzzi R., Antonioli L., Baschiera F., Ghisu N., Tuccori M., Gori G., Blandizzi C., Del Tacca M. (2006): CCK (2) receptors mediate inhibitory effects of cholecystokinin on the motor activity of guinea-pig distal colon. European Journal of Pharmacology, 557, 212–220.

Giralt M., Vergara P. (2000): Inhibition by CCK of ascending contraction elicited by mucosal stimulation in the duodenum of the cat. Neurogastroenterology and Motility, 12, 173–180.

Giuliani S., Lippe I.T., Maggi C.A., Meli A. (1990): Dual effects of cholecystokinin-octapeptide on duodenal motility of urethane-anesthetized rats. The Journal of Pharmacology and Experimental Therapeutics, 252, 1312–1317.

Grider J.R. (1994): Role of cholecystokinin in the regulation of gastrointestinal motility. Journal of Nutrition, 124, 1334S–1339S.

Gutiérrez J.G., Chey W.Y., Dinoso V.P. (1974): Actions of cholecystokinin and secretin on the motor activity of the small intestine in man. Gastroenterology, 67, 35–41.

Heppell J., Blinks S., Kelly K.A., Go V.L.W. (1982): Inhibition of small intestinal interdigestive motility by cholecystokinin octapeptide (CCK-OP). In: Wienbeck M. (ed.): Motility of the Digestive Tract. Raven Press, New York, USA, 207–214.

Kusano M., Minashi K., Maeda M., Shimoyama Y., Kuribayashi S., Higuchi T., Sugimoto S., Kawamura O. (2005): Postprandial water intake inhibits gastric antral motility with increase of cholecystokinin in humans. Scandinavian Journal of Gastroenterology, 40, 1176–1181.

Li Y., Zhu J., Owyang C. (1999): Electrical physiological evidence for high- and low-affinity vagal CCK-A receptors. American Journal of Physiology, 277, G469–G477.

Lin H.C., Zaidel O., Hum S. (2002): Intestinal transit of fat depends on accelerating effect of cholecystokinin and slowing effect of an opioid pathway. Digestive Diseases and Sciences, 47, 2217–2221.

Mantyh C.R., Pappas T.N., Vigna S.R. (1994): Localization of cholecystokinin A and cholecystokinin B/gastrin receptors in the canine upper gastrointestinal tract. Gastroenterology, 107, 1019–1030.

Martins S.R., Oliveira R.B., Ballejo G. (2006): Activation of neural cholecystokinin-1 receptors induces relaxation of the isolated rat duodenum which is reduced by nitric oxide synthase inhibitors. Brazilian Journal of Medical and Biological Research, 39, 271–275.

Mączka M., Thor P., Lorens K., Konturek S.J. (1993): Nitric oxide inhibits the myoelectric activity of the small intestine in dogs. Journal of Physiology and Pharmacology, 44, 31–42.

McLeay L.M., Wong M.H. (1989): Excitatory and inhibitory effects of gastrin peptides on gastric motility in sheep. American Journal of Physiology, 257, R388–R395.

Merle A., Faucheron J.L., Delagrange P., Renard P., Roche M., Pellissier S. (2000): Nycthemeral variations of cholecystokinin action on intestinal motility in rats: effects of melatonin and S 20928, a melatonin receptor antagonist. Neuropeptides, 34, 385–391.

Miyasaka K., Funakoshi A. (2003): Cholecystokinin and cholecystokinin receptors. Journal of Gastroenterology, 38, 1–13.

Mizumoto A., Ueki S., Ohtawa M., Itoh Z. (1992): Endogenous CCK is not involved in the regulation of interdigestive gastrointestinal and gallbladder motility in conscious dogs. Regulatory Peptides, 41, 249–256.

Niederau C., Karaus M. (1991): Effects of CCK receptor blockade on intestinal motor activity in conscious dogs. American Journal of Physiology, 260, G315–G324.

Noble F., Wank S.A., Crawley J.N., Bradwejn J., Seroogy K.B., Hamon M., Roques B.F. (1999): International Union of Pharmacology. In: XXI. Structure, Distribution, and Functions of Cholecystokinin Receptors. Pharmacological Review, 51, 745–781.

Onaga T., Mineo H., Kato S. (1997): Effect of L364718 on interdigestive pancreatic exocrine secretion and gastroduodenal motility in conscious sheep. Regulatory Peptides, 68, 139–146.

Ormas P., Belloli C., Sagrada A., Arioli F., Tanzi G.B., Beretta C. (1984): Possible mechanisms of action of caerulein on intestinal motility of sheep. Annales de Recherches Vétérinaires, 15, 557–562.

Romański K.W. (2002): Characteristics and cholinergic control of the 'minute rhythm' in ovine antrum, small bowel and gallbladder. Journal of Veterinary Medicine Series A, 49, 313–320.

Romański K.W. (2004): Ovine model for clear-cut study on the role of cholecystokinin in antral, small intestinal and gallbladder motility. Polish Journal of Pharmacology, 56, 247–256.

Romański K.W. (2007a): Regional differences in the effects of various doses of cerulein upon the small-intestinal migrating motor complex in fasted and non-fasted sheep. Journal of Animal Physiology and Animal Nutrition, 91, 29–39.

Romański K.W. (2007b): The effect of cholecystokinin-octapeptide and cerulein on phasic and tonic components in ovine duodenum with special reference to the 'minute rhythm'. Acta Veterinaria Brno, 57, 113–122.

Sayegh A.I., Ritter R.C. (2003): Cholecystokinin activates specific enteric neurons in the rat small intestine. Peptides, 24, 237–244.

Smout A.J. (2004): Small intestinal motility. Current Opinion in Gastroenterology, 20, 77–81.

Snedecor G.W., Cochran W.G. (1971): Statistical Methods. The Iowa State University Press, Ames, USA.

Titchen D.A. (1984): Gastrointestinal peptide hormone distribution, release, and action in ruminants. In: Milligan L.P., Grovum W.L., Dobson A. (eds.): Control of Digestion and Metabolism in Ruminants. A Reston Book. Prentice-Hall, Englewood Cliffs, UK, 227–248.

Xu M.Y., Lu H.M., Wang S.Z., Shi W.Y., Wang X.C., Yang D.X., Yang C.X., Yang L.Z. (1998): Effect of devazepide reversed antagonism of CCK-8 against morphine on electrical and mechanical activities of rat duodenum *in vitro*. World Journal of Gastroenterology, 4, 524–526.

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