

Dose-related effects of cerulein short infusions on proximal small bowel motility in sheep

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ABSTRACT

The effect of cholecystokinin (CCK) upon the intestinal motility has not been entirely explored in ruminants. The aim of this study was to examine the precise effects of CCK amphibian analogue, cerulein, on small-intestinal myoelectric activity in rams in the course of chronic experiments. Five rams underwent implantation of bipolar platinum electrodes to the duodenal bulb, the distal duodenum and jejunum. During continuous myoelectrical and motor recordings, 0.15 M NaCl or the various doses of cerulein were administered intravenously. Short infusions of the smallest dose of cerulein exerted a slight and mostly insignificant effect on the duodenal bulb and the duodenal myoelectric activity index (MAI) values. In the duodenal bulb, the effects of cerulein on myoelectric activity were dose-dependent and closely related to the phase of the MMC. In the duodenum, the higher doses of the hormone evoked short stimulatory response followed by longer inhibitory biphasic effects on MAI. These effects were inversely related to the duration of hormone injection. Infusions of hormones at the higher doses caused a less pronounced biphasic effect. It is concluded that cerulein exerts an inhibitory effect upon the myoelectric activity of the duodenal bulb and a strong stimulatory and inhibitory (biphasic) effect on duodenal motility in sheep.

Keywords: cerulein, duodenal bulb, duodenum, migrating myoelectric complex, myoelectric activity, sheep

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INTRODUCTION

Duodenal motility is a very important intestinal phenomenon, crucial for normal digestion and absorption, and its motor functions are controlled by many neuro-hormonal mechanisms. Cholecystokinin (CCK) is one of the principal gastrointestinal hormones involved in this control. The role of the hormone is not limited to motility and it also participates in the control of other physiological functions.⁵⁴ In humans and monogastric animals, CCK affects the motor activity of the whole gastrointestinal tract *in vivo* and *in vitro*.^{2,11,56} The recognised effect of CCK on small-intestinal motility is clear. The hormone inhibits the arrival of the migrating motor complex (MMC) in the upper small bowel.¹⁹ Furthermore, it evokes the specific spike burst pattern and may increase spiking activity as well as hasten intestinal transit time.^{29,36,56} Its amphibian analogue, cerulein, is also active in mammals. In

sheep, CCK peptides are also active in modulation of gastrointestinal motility, inhibit the arrival of the MMC in the upper small intestine and affect contractile activity, but their influence on intestinal motility has not been fully elucidated although several studies have been undertaken.^{38,45,46,53} The dual effect of CCK on small-intestinal motility can also be expected to occur in sheep. The duodenal spiking activity response to CCK and whether there is a difference in this response between the duodenal bulb and the duodenum in conscious sheep have still to be elucidated.

MATERIALS AND METHODS

Animal preparation

Five healthy adult rams of the Polish Merino breed weighing 38–43 kg each were used. The rams were fed with good-quality hay, 1 kg daily, and a grain mixture (Dolpasz, Wrocław). They were fasted for 24 h before surgery, but allowed unlimited access to drinking water. The experimental procedure was approved by the Ethical Committee of the Veterinary

School, Wrocław University of Environmental and Life Sciences, Poland.

After general and local anaesthesia⁴⁵, right lateral laparotomy (diagonal incision) was performed and 2 bipolar platinum electrodes were implanted, one on the serosal side of the duodenal bulb, 5.5–6 cm distal to the pyloric ring and the other on the distal duodenum 50 cm below the bulbar electrode. A strain gauge force transducer (RB Products, Madison), calibrated individually before surgery, was attached near the duodenal electrode to verify the myoelectric activity tracings. Details of this procedure have been described elsewhere.^{43,45} Marked wires were externalised through the stab incision, soldered to the plug and fixed to the wool. Within 3 days the animals returned to normal feeding. The skin sutures were removed 10 days after surgery.

Experimental design

A total of 105 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) also adapted for mechanical activity recordings. Twenty-four hours before each experiment, food was removed from the cage. At least 2 consecutive phases of the MMC including 1 full normal cycle of the MMC were recorded each time. During control recordings, slow injections of 5 ml 0.15 M NaCl were administered over 30 s into the jugular vein through a thin polyethylene catheter introduced before the experiment commenced. The saline injections were administered during the course of phases 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 course of basic experiments, slow intravenous injections of cerulein (Farmitalia Carlo Erba, Milan, Italy) at small, moderate, and high doses, *i.e.* 1, 10 and 100 ng/kg were applied. The small dose of cerulein was administered over 30 s, the moderate dose over 30 or 60 s, and the high dose over 30, 60 or 120 s. Each dose was given in separate randomised experiments at the same periods as the saline injections.

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After saline or cerulein administration, the myoelectric and motor activities were recorded until the arrival of the 1st organised phase 3 of the MMC. After cessation of all the experiments, the animals were sacrificed humanely and the positions of the electrodes and the strain gauge force transducer were confirmed during autopsy.

Analysis of data

The MMC cycles and their phases were identified in the duodenum according to the criteria proposed by Code & Marlett⁵ with a slight modification.⁴¹ The division of phase 2 into phases 2a and 2b of the MMC, proposed earlier by Dent *et al.*,⁷ was performed according to more precise criteria.⁴⁵ The myoelectric and motor recordings were visually analysed and the myoelectric activity index (MAI) values were calculated as describe previously⁴³). The MAI values were calculated by multiplying the average amplitude of each spike burst within the period examined, as described previously⁴⁴, by the duration of this spike burst and expressed as the sum of the areas of all the spike bursts during 1 minute ($\mu\text{V}/\text{s}/\text{min}$). The spike bursts with amplitudes below $3 \mu\text{V}$ were omitted. The duration of the periods was equal to 1 minute. In the recordings obtained from the experiments with saline and cerulein injections, the MAI values were calculated in 4 periods (each lasting 1 min), *i.e.* 1 period before the injection and 10 similar periods after the injection. On the tracings, the measurements were performed using a calliper with an accuracy of about 0.3 mm.

All the values were grouped and the means and standard deviations were calculated. Statistical significance, *i.e.* when $P < 0.05$, was calculated using the Student's *t*-test for paired and unpaired values, where appropriate preceded by 1-way analysis of variance.⁵¹

RESULTS

Short infusions of saline evoked no effect and these data are not shown here.

Short infusions of the small dose of cerulein administered during phase 1 of the MMC induced no changes, while short infusions during phases 2a and 2b of the MMC induced the weakest alterations in the spike bursts in the duodenal bulb (Table 1). Infusions of moderate doses of cerulein inhibited spike bursts in the duodenal bulb for longer periods than after infusion of the small doses although only the value obtained during phase 2b was significant when the hormone was infused over 30 s and during phase 2a and 2b when the hormone was infused over 60 s (Table 1). In these experiments no

Table 1: Duration of the inhibitory effects of the injections of cerulein on the myoelectric activity index (MAI) of the duodenal bulb in non-fasted sheep.

			Phase 1 of the MMC Mean \pm SD (n = 5)	Phase 2a of the MMC Mean \pm SD (n = 5)	Phase 2b of the MMC Mean \pm SD (n = 5)
Cerulein	1 ng/kg	30 s	286 \pm 78 [#]	228 \pm 51	62 \pm 13
		10 ng/kg	30 s	387 \pm 113	315 \pm 94
		60 s	426 \pm 97	461 \pm 136 ^c	328 \pm 76 ^c
100 ng/kg	30 s	30 s	685 \pm 184 ^c	621 \pm 235 ^c	688 \pm 243 ^c
		60 s	716 \pm 212 ^c	786 \pm 248 ^c	849 \pm 275 ^c
		120 s	847 \pm 256 ^c	894 \pm 286 ^c	1187 \pm 349 ^c

[#]Duration (in s) of the inhibition owing to the presence of phase 1 of the MMC, *i.e.* lack of inhibitory effect of the injection of CCK peptide.

^{a,b,c}Statistical significance: ^a $P < 0.05$, ^b $P < 0.01$, ^c $P < 0.001$ vs the corresponding value obtained after administration of the small dose of cerulein. Student's *t*-test for unpaired values preceded by 1-way ANOVA.

excitatory changes were observed except the 'small' spike bursts (Fig. 1). When the high dose of cerulein was infused, duration of the inhibition of spiking activity

was significant regardless of the duration of infusion and MMC phase (Table 1).

In the duodenum, infusion of cerulein at the small dose during phase 1 of the

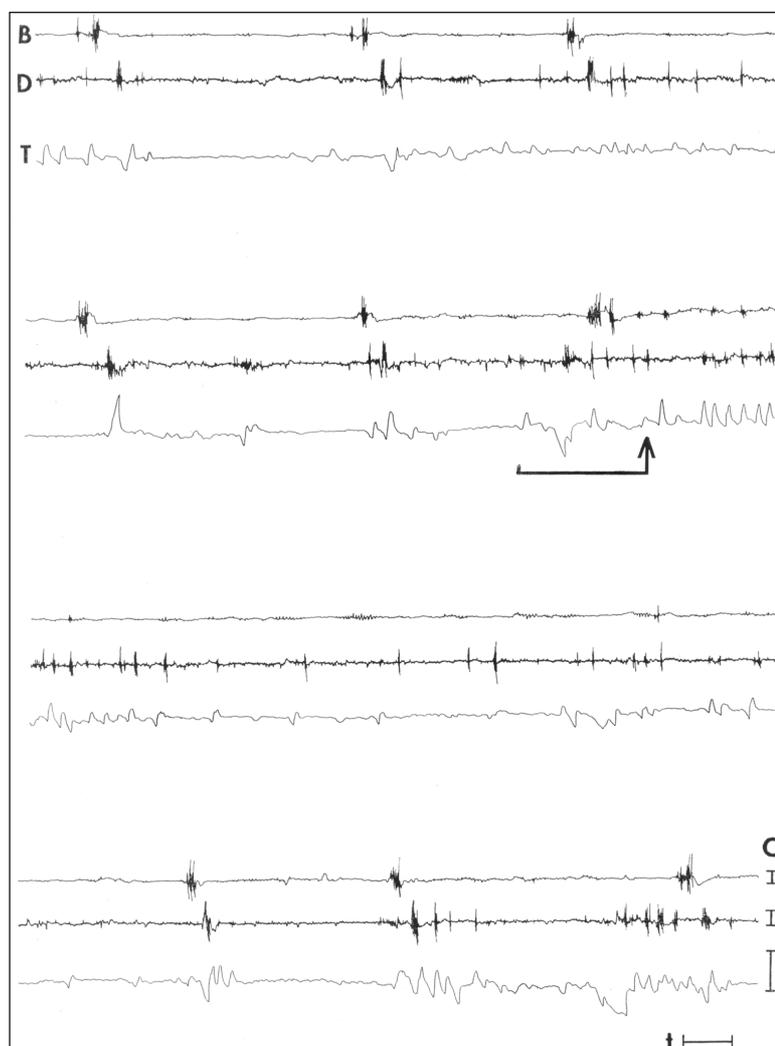


Fig. 1: The effect of a moderate dose of cerulein administered during late phase 2a of the MMC on the duodenal motility in sheep: 10-minute consecutive recording. Note the inhibitory effect in the duodenal bulb and the dual effect in the duodenum. Cerulein also elicited few 'small' spike bursts in the duodenal bulb. Myoelectric activity from the duodenal bulb (B) and the duodenum (D), or the mechanical activity from the duodenum (T); the bar ending with an arrow indicates 30 s cerulein infusion; C = calibration (50 μV), calibration of the strain gauge force transducer expressed in g; t = time. Time bar, 10 s.

Table 2: Duration of the stimulatory effects of slow injections of cerulein during phase 1 of the MMC on the myoelectric activity index (MAI) of the duodenum in non-fasted sheep.

		0	1	2	3	4	5	6	7	8	9	10
1 ng/kg	30 s	0.03	0.26^c	0.09	0.05	0.01	0.00	0.03	0.02	0.01	0.02	0.01
		0.02	0.14	0.05	0.03	0.01	0.01	0.01	0.00	0.01	0.00	0.02
10 ng/kg	30 s	0.04	0.42^c	0.16	0.06	0.01	0.02	0.00	0.02	0.03	0.06	0.08
		0.03	0.18	0.12	0.02	0.01	0.01	0.00	0.01	0.04	0.04	0.03
	60 s	0.05	0.40^c	0.18	0.07	0.02	0.04	0.01	0.05	0.09	0.14	0.19
		0.02	0.21	0.10	0.05	0.03	0.02	0.02	0.02	0.03	0.05	0.07
100 ng/kg	30 s	0.03	1.86^c	0.25	0.16	0.28^a	0.55^c	0.64^c	1.25^c	1.27^c	1.19^c	1.21^c
		0.04	0.34	0.22	0.23	0.18	0.27	0.33	0.47	0.48	0.41	0.44
	60 s	0.04	1.93^c	0.16	0.12	0.14	0.16^a	0.24^b	0.56^c	0.75^c	1.33^c	1.20^c
		0.02	0.27	0.09	0.07	0.09	0.07	0.11	0.22	0.34	0.65	0.57
	120 s	0.04	1.90^c	0.14	0.17	0.11	0.13	0.10	0.13	0.18	0.16^a	0.19^b
		0.03	0.45	0.08	0.12	0.08	0.11	0.07	0.07	0.12	0.08	0.11

Values expressed in $\mu\text{V s/min}$. 0–10 = 1 min consecutive periods: 0 = control period, 1–10 = periods after hormone administration. Statistical significances: vs corresponding value of period 0. Numbers in bold = mean values; numbers not in bold = $\pm\text{SD}$ values. For further explanation see Table 1.

MMC evoked a transient excitatory effect in the 1st minute, lasting 5–15 s after cessation of the peptide infusion (Table 2). The application of a moderate dose of cerulein during phase 1 of the MMC caused more evident, but also short alterations in the duodenal myoelectric activity when the hormone was injected either over 30 s or 60 s. Administration of cerulein at the high dose in the course of phase 1 of the MMC induced an evident (the greatest) stimulatory response in the duodenal MAI during the 1st minute and then after 1–3 minutes of the attenuated response the stimulation became significant again. This effect was partially related to the duration of hormone injection (Table 2).

Infusion of the small cerulein dose during phase 2a of the MMC produced a transient, non-significant response in the next and 2 last minutes of observation (Table 3). Application of the moderate dose over 30 s increased MAI significantly in the next minute, while administration of cerulein over 60 s increased MAI significantly for 3 minutes. The increase of MAI

in the last 2 minutes was observed but it did not reach statistical significance. Representative examples of the stimulatory and inhibitory effect of a moderate dose of cerulein are shown in Fig. 1. Following infusion of the high dose of cerulein in the course of phase 2a of the MMC, a significant increase in the duodenal MAI values was observed for 3 consecutive minutes and then a decrease in the MAI values, lasting 1–2 min, occurred. However, this inhibition was not significant. No stimulatory response was observed in the last minutes (Table 3). The correlation between the spike bursts and contractions is shown in Fig. 1.

Infusion of the small dose of cerulein during phase 2b of the MMC induced no changes in MAI although a stimulatory tendency (not significant) was observed in the 1st minute following hormone infusion (Table 4). The moderate dose of cerulein caused clear stimulatory effect in the 1st minute followed by inhibition of MAI lasting 5–6 min. When cerulein was infused at the high doses in the course of phase 2b of the MMC, initially partial then

strong inhibition of MAI followed again by partial inhibition was observed. The total duration of this inhibitory period in the duodenum lasted 12–25 min, while complete inhibition began 1–7 min after cessation of the peptide injection and lasted 3–8 min. No stimulatory effect in all 3 rates of the high dose of cerulein was observed (Table 4).

DISCUSSION

Infusions of cerulein elicited marked excitatory and inhibitory effects in the myoelectric and motor activity of the ovine upper small bowel. As found in the present study, it has also been demonstrated that the effect of CCK and cerulein may be different in the various regions of the small bowel.^{9,45} The role of duodenal bulb motility in the transport of digesta relies mainly on gastric emptying because of the importance of the pressure gradient between the antrum and duodenum.⁴⁷ Thus, the duodenal bulb motility differs from duodenal motor function in sheep, *i.e.* fewer phasic contractions and spike bursts can be observed in the duodenum

Table 3: Duration of the stimulatory effects of slow injections of cerulein during phase 2a of the MMC on the myoelectric activity index (MAI) of the duodenum in non-fasted sheep.

		0	1	2	3	4	5	6	7	8	9	10
1 ng/kg	30 s	0.22	0.33	0.24	0.16	0.20	0.18	0.23	0.22	0.27	0.35	0.40
		0.08	0.14	0.11	0.09	0.09	0.10	0.15	0.11	0.14	0.12	0.21
10 ng/kg	30 s	0.18	2.75^c	0.59^c	0.28	0.13	0.20	0.16	0.21	0.31	0.37	0.35
		0.07	0.81	0.22	0.13	0.09	0.08	0.11	0.07	0.12	0.14	0.16
	60 s	0.16	2.43^c	1.12^c	0.64^c	0.27	0.11	0.17	0.23	0.21	0.34	0.41
		0.07	0.66	0.43	0.19	0.14	0.07	0.08	0.12	0.09	0.13	0.15
100 ng/kg	30 s	0.25	3.17^c	1.63^c	0.58^b	0.12	0.10	0.13	0.18	0.23	0.27	0.42
		0.11	0.71	0.45	0.14	0.07	0.06	0.04	0.06	0.09	0.12	0.13
	60 s	0.19	3.41^c	1.38^c	0.64^b	0.07	0.12	0.11	0.10	0.15	0.22	0.21
		0.10	0.94	0.52	0.19	0.14	0.07	0.05	0.04	0.06	0.07	0.06
	120 s	0.21	3.32^c	0.93^c	0.66^c	0.16	0.10	0.12	0.12	0.14	0.18	0.17
		0.09	1.07	0.31	0.24	0.07	0.04	0.05	0.06	0.07	0.05	0.08

For explanation see Table 2.

Table 4: Duration of the stimulatory and inhibitory effects of slow injections of cerulein during phase 2b of the MMC on the myoelectric activity index (MAI) of the duodenum in non-fasted sheep.

		0	1	2	3	4	5	6	7	8	9	10
1 ng/kg	30 s	11.8 3.60	15.6 4.10	11.4 3.12	11.9 4.02	11.2 3.72	11.6 3.27	12.1 4.18	12.4 4.47	12.8 4.28	12.5 3.80	13.1 4.68
	60 s	11.3 3.40	23.6^c 2.10	0.50^c 0.12	0.26^c 0.18	0.41^c 0.27	0.73^c 0.41	3.96^a 2.30	5.78^a 2.11	8.30 2.13	10.2 2.86	11.0 3.17
10 ng/kg	30 s	11.9 4.20	21.7^b 3.32	0.41^c 0.16	0.31^c 0.14	0.37^c 0.23	1.94^c 0.81	4.12^a 3.36	6.28 3.82	9.70 3.61	10.3 3.43	10.9 4.11
	60 s	11.1 3.80	0.91^c 0.46	0.60^c 0.34	0.32^c 0.27	0.38^c 0.26	0.53^c 0.20	0.57^c 0.33	0.54^c 0.27	0.66^c 0.34	0.88^c 0.41	1.26^c 0.59
100 ng/kg	30 s	10.8 3.54	0.84^c 0.53	0.51^c 0.28	0.11^c 0.08	0.22^c 0.14	0.37^c 0.19	0.64^c 0.30	0.48^c 0.29	0.59^c 0.43	0.65^c 0.31	0.68^c 0.30
	60 s	11.7 4.00	0.87^c 0.32	0.33^c 0.18	0.18^c 0.07	0.24^c 0.13	0.40^c 0.16	0.43^c 0.21	0.46^c 0.23	0.51^c 0.18	0.47^c 0.15	0.49^c 0.28
	120 s											

For explanation see Table 2.

and phase 3 of the MMC is often of shorter duration or absent.⁴² In the present study cerulein evoked weaker and almost non-detectable excitation as well as pronounced inhibition of the normal (stronger) spike bursts in the duodenal bulb. These spike bursts apparently originated from the circular smooth muscle layer. Furthermore, stimulatory changes on low-amplitude spike bursts, most probably originating from the longitudinal muscle, were recorded. It has already been reported that cholecystokinin octapeptide (CCK-OP) exerts a direct contractile effect on the longitudinal muscle and inhibits circular muscle motility.⁶ However, reported data on the matter are scanty.

In the duodenum, moderate doses of CCK peptides had a clear dual effect. A prompt increase in spiking and contractile activity followed by inhibitory alterations were 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.^{14,31} These findings suggest that the mechanism of CCK action on duodenal motility is complex and may exhibit adaptive features.

Cerulein action therefore appears to be similar to CCK-OP effect. Cerulein is considered as a CCK-OP analogue because of very similar structure.²⁵ The latter is known as one of existing forms of CCK in the body that has been confirmed in genetic studies. However, CCK-33, CCK-39, and CCK-59 appear to be synthesised in the 1st order.¹⁸ It is nevertheless known that CCK, as some other regulatory peptides, is processed to its biologically active forms by cleavage at single basic amino acids. The preprocholecystokinin is natural precursor of all forms of CCK.³² Synthesised CCK is released into the local environment directly or indirectly, *via* luminal CCK-releasing factor³⁴

and is bound to a specific receptor within a target cell. A more specific CCK-A receptor is located within the gastrointestinal tract where a CCK-B/gastrin receptor is also present. These receptors are cloned from a specific gene and are linked with G-protein within the cellular membrane.^{21,39} These receptors were found in the smooth muscle cells, endocrine cells, and vagal nerve.^{8,15,28} Binding of CCK (or cerulein) to the receptor evokes a cascade of intracellular processes involving second messenger systems, activation of protein kinase C, and protein phosphorylation.^{26,39} Thus, CCK can affect gastrointestinal motility owing to its direct action on the smooth muscles²⁷, through the peptidergic route^{3,40,50,55}, as well as in cooperation with other regulatory peptides.^{12,17,20,22,48,49} In sheep, the mode and mechanism of CCK action seems to be similar to those in monogastric species. CCK can modulate the interdigestive motor activity *via* CCK-A receptors³⁷ although its action *via* CCK-B/gastrin receptors cannot be excluded.⁵⁷ CCK can affect gastrointestinal function peripherally, but also centrally.^{16,23,24} Furthermore, direct or indirect actions of CCK in cooperation with other peptides are also possible.^{33,52,57} The composite mechanism of CCK action on ovine gastrointestinal motility explains its dual effect.

The observed stimulatory effect of a moderate dose of CCK on duodenal motility seems to be primary³⁵, and this conclusion can also be inferred from the present study. Similar effects have previously been reported for cerulein.^{1,36,38,43} Other reports indicate that the effect of CCK on duodenal motility can be inhibitory or no effect can be observed, while some authors observed a simultaneous stimulatory effect in the jejunum.^{4,10,13,30}

The small doses of CCK-OP and cerulein induced no effect in the duodenal bulb, although discernible inhibitory

changes were observed after moderate doses. As discussed earlier⁴⁵, the moderate doses of CCK-OP and cerulein as well as the highest doses administered over 120 s remained within the physiological range. Thus the only reasonable inference that can be drawn from these findings is that endogenous CCK may also inhibit the myoelectric activity in the duodenal bulb and that the inhibitory effect of CCK represents the primary response in this region. In the duodenum, the small doses of CCK peptides elicited transient stimulation in the myoelectric and motor activities and the moderate doses evoked the dual effect. These observations may suggest that in the duodenum the action of CCK can be physiological and comprise both excitatory and inhibitory responses, although the excitatory response can be regarded as the primary effect.

Finally, it is concluded that cerulein evokes an inhibitory effect in ovine duodenal bulb myoelectric activity, while its primary short-term effect on the duodenum is stimulatory and followed by longer inhibition. Thus, the mechanisms of cerulein action on these 2 parts of the duodenum are partially different.

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