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OVINE MODEL FOR CLEAR-CUT STUDY ON THE ROLE OF CHOLECYSTOKININ IN ANTRAL, SMALL INTESTINAL AND GALLBLADDER MOTILITY

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Ovine model for clear-cut study on the role of cholecystokinin in antral, small intestinal and gallbladder motility. K.W. ROMAŃSKI. Pol. J. Pharmacol., 2004, 56, 247–256.

Cholecystokinin (CCK) is one of the major gastrointestinal hormones involved in the control of digestive tract and gallbladder motility. Its action involves several mechanisms. The ovine model was developed in order to further explore the role of CCK in gastric, small intestinal and gallbladder motility under various experimental conditions. Five Merino sheep were used with bipolar electrodes implanted to their antrum, entire small intestine and gallbladder as well as strain gauge force transducers were attached to the duodenum and gallbladder fundus, near the electrodes. In the course of chronic experiments, the myoelectric and motor activity were recorded by means of the adapted electroencephalograph. Among the variety of CCK-octapeptide or cerulein doses, three doses of each CCK peptide were selected and then applied for various time periods. Finally, the effects of the hormones administered within 30 s during phase 2 of the same or different migrating myoelectric complexes (MMCs) on gastrointestinal and gallbladder myoelectric and motor activity were studied in fasted and non-fasted animals. Injection of the highest dose inhibited rumination in four of the five sheep and inhibited phase 3 MMC in the antroduodenal region. Hormone administration inhibited dose-dependently antral myoelectric activity. The effects of moderate dose of both CCK peptides on myoelectric activity of the duodeno-jejunum was usually opposite (i.e. stimulatory) than that of the ileum. Gallbladder response to CCK peptides exhibited mostly the tonic character, and in some experiments, the slow wave frequency and amplitude were altered. It is concluded that CCK acts on several targets and different mechanisms underlie its multiple actions on gastrointestinal and gallbladder motility in sheep.

Key words: *antrum, small bowel, gallbladder, myoelectric and motor activity, cholecystokinin octapeptide, cerulein*

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Abbreviations: CCK – *cholecystokinin,* MAI – *myoelectric activity index,* MI – *motility index,* MMC – *migrating motor (myoelectric) complex,* OP-CCK – *cholecystokinin octapeptide*

INTRODUCTION

Cholecystokinin (CCK) is one of the most important gastrointestinal hormones profoundly involved in the control of several functions in the body, including gastric, intestinal and gallbladder motility [11]. It inhibits gastric myoelectric and motor activity and gastric emptying [4, 9], and stimulates intestinal motility and gallbladder contractions [7, 12, 13]. The hormone is active in the ruminant and non-ruminant animals and its action on gastrointestinal and gallbladder motility does not seem to vary considerably between both these groups of animal species; however, in ruminants, its action have not been precisely described in various physiological conditions. In sheep, the CCK peptides like CCK- octapeptide (OP-CCK) or cerulein (ceruletide) can reduce abomasal myoelectric and motor activity, affect interdigestive duodenal motility, and stimulate gallbladder motor function [15, 18, 22]. These actions appear to be similar to those in non-ruminant species. However, no integrative and systematic studies regarding actions of CCK peptides on gastrointestinal and gallbladder myoelectric and motor activity have been performed under various precise experimental conditions, which was the basic scope of this work. Initially, the clear-cut model to study this complex problem was developed and tested in detail in order to outline the range of CCK action on antral, small-intestinal and gallbladder myoelectric and motor activity in sheep.

MATERIALS and METHODS

Five adult rams (Polish Merino Breed) weighing 38–43 kg each were used in the study. After a 24 h fasting period, the right lateral laparotomy was performed under general and local anesthesia and ten bipolar platinum electrodes embedded in Teflon coat and two strain gauge force transducers (RB Products, Madison) were attached from the serosal side to the antrum (1 electrode), duodenal bulb (1 electrode), duodenum (1 electrode and 1 strain gauge force transducer), jejunum (2 electrodes), ileum (2 electrodes) and gallbladder (3 electrodes and 1 strain gauge force transducer), as described previously [17]. The wires were exteriorized, soldered to the plug and fixed. After the surgery, the animals were allowed at least 10 days of recovery. Drinking water was given *ad libitum* and the rations of fodder were gradually increased during the post-surgical period. At least three days before the first experiment, rams were fed with the complete amount of standard fodder including good quality hay (1 kg per animal per day), supplemented with standard grain mixture (3–5 g/kg). During chronic experiments, the myoelectric and motor activity were continuously recorded using 10-channel electroencephalograph (Reega Duplex TR XVI, Alvar Montreuil, Paris), also adapted for mechanical recording. The time constant was adjusted to 0.01 s and the paper speed was 2.5 mm/s. The strain gauge force transducers were individually calibrated before the implantation. Two series of all types of experiments were performed. In the first series, 10-channel electromyographical recordings were performed. In the second series, the electrical activity was registered in 8 channels (without proximal jejunal and proximal ileal electrodes), and in 2 remaining channels, the mechanical activity from both strain gauge force transducers was monitored. Four phases of the migrating myoelectric complex (MMC) were identified in the small bowel (according to the criteria described in detail elsewhere [16]) and the gallbladder, while the duodenal MMC served as the reference point. A total of 360 experiments were performed, 72 experiments on every ram, lasting 4–6 h each, except 20 control experiments (10 with fasted and 10 with non-fasted rams) each lasting 3–4.5 h. Sixty five experiments were performed on animals fasted for 48 h and 285 experiments were performed on non-fasted animals. Before each experiment, a thin polyethylene tube was inserted to the jugular vein for slow injection of saline or hormone. During control experiments, after the recording of one full MMC cycle, 5.0 ml of 0.15 M NaCl was injected intravenously (*iv*) during phase 2 MMC within 30 s and the recording was continued until the arrival of two consecutive phases 3 MMC. In the course of experiments with hormone injections, two consecutive phases 3 MMC were recorded during the control period. Then, the OP-CCK (Sincalide, Squibb Inst., Princeton) or cerulein (Takus, Farmitalia Carlo Erba, Milan) were infused *iv* at various doses, and the next two phases 3 MMC were recorded. Each

dose was tested during separate experiments, except the series when three doses of the peptide hormones were injected during the same MMC cycle. Of a variety of doses (OP-CCK 5, 20, 100, 200, 500, 1000 and 2000 ng/kg or cerulein 0.2, 1, 5, 10, 20, 50 and 100 ng/kg) which were initially tested, only three doses of each peptide (OP-CCK 20, 200 and 2000 ng/kg or cerulein 1, 10 and 100 ng/kg) were used in further experiments. During other experiments, the selected moderate doses (OP-CCK 200 ng/kg or cerulein 10 ng/kg) were given *iv* at different time periods. In the next series of experiments, the effects of all three doses of both peptides were compared in fasted and non-fasted rams. During the next series of the experiments, the effects of hormonal peptide administration in the same or in the different MMC cycles were compared. Additionally, a moderate dose of CCK peptides was repeated during the same MMC cycle at 3–4 minute time intervals. The effect of the peptide hormones on rumination and on MMC cycle duration were also determined. The experiments were performed in random order. At least two days were allowed between two consecutive experiments, except the experiments in which peptide hormones were administered during the same MMC cycle. After termination of experiments in the given animal, the position of the electrodes and strain gauge force transducers was confirmed at autopsy. No inflammatory reactions or perforation of the gastrointestinal or gallbladder wall was observed. Only the experiments with normal phase(s) 3 MMC arriving during the control period were included in the analysis.

The myoelectric activity was assessed to measure the slow wave amplitude and slow wave frequency in the antrum, small bowel and, when possible, also in the gallbladder. The myoelectric activity index (MAI) for the antrum, small bowel and gallbladder was calculated as follows: the average of spikes amplitude in one spike burst measured exact to 0.5 mm (expressed in μV) was calculated and multiplied by the duration (expressed in s) of this spike burst. These values from all spike bursts during a two-minute recording period prior to (control) or just after the injection (treatment) were added. The result was then divided by two, thus, it was finally expressed in (μV × s)/min. For mechanical recordings, the motility index (MI) was calculated as follows: the area of each contraction was calculated (mean width of the contraction expressed in s was multiplied by its amplitude from the baseline to the top and expressed in g × 1000), expressed in s × 1000 g and the values of all contractions during the 2 min before (control) or 2 min just after the injection (treatment) were added. This value was divided by two, thus, the results were expressed in (s × 1000 g)/min. The MMC cycle duration was expressed in minutes. The slow wave frequency was expressed in cycles per minute (cpm) and slow wave amplitude was measured in μV.

The statistical significance between respective "control" and "treatment" values was calculated where appropriate using Student's *t*-test for paired values followed by analysis of variance [21].

RESULTS

The highest dose of OP-CCK or cerulein inhibited the rumination in four of five sheep. Inhibition began 11 s before the end of the peptide hormone administration and lasted 17 s after hormone injection. No inhibition of rumination was observed in response to the moderate and smallest doses of CCK peptides. OP-CCK and cerulein altered the

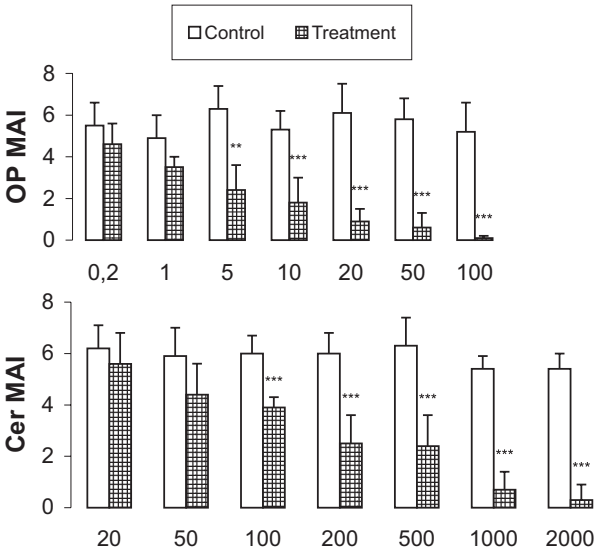

Fig. 1. The effect of various doses of OP-CCK (OP) and cerulein (Cer) on ovine antral myoelectrical activity. The data are expressed in (mV × s) per 1 min of the spike bursts (the myoelectric activity index, MAI) calculated during 2-minute periods before (control) and after CCK peptide administration (treatment) in non-fasted rams. CCK peptides were given *iv* during 30 s in phase 2 MMC identified in the duodenum. Doses are given in ng/kg. Means ± SD, n = 5. Statistical significances: ** $p < 0.01$, *** $p < 0.001$ as compared with the respective control value. For other explanations see the Materials and Methods section

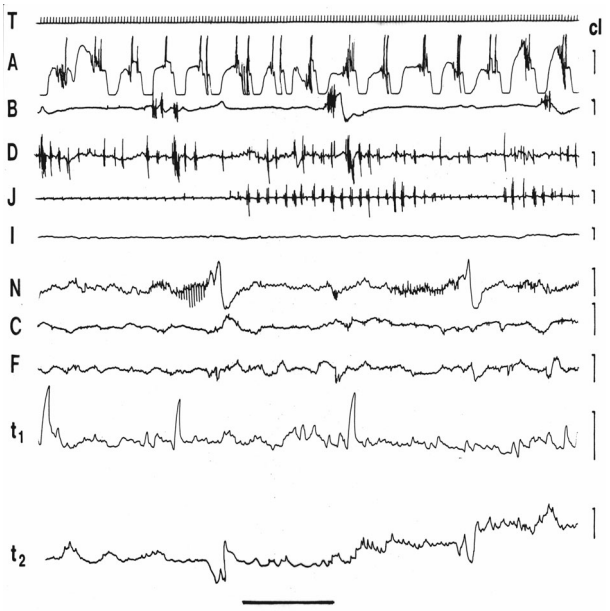

Fig. 2. The effect of *iv* administration of cerulein at the dose of 1 ng/kg during the duodenal phase 2 MMC on antral, small intestinal and gallbladder motility in non-fasted rams. Note the minimal effect on gastrointestinal motility and the effect on gallbladder tonus. Abbreviations: T – time in seconds, cl – calibration 100 μV (electrical recordings) or 5 g (mechanical recordings) – vertical bars. Electrodes: A – pyloric antrum, B – duodenal bulb, D – duodenum, J – jejunum, I – ileum, N – gallbladder neck, C – gallbladder corpus, F – gallbladder fundus. Strain gauge force transducers: t_1 – duodenum, near electrode D, t_2 – gallbladder, near electrode F. Horizontal bar: cerulein administration

myoelectric and motor activity of the gastrointestinal tract and gallbladder. The clearest, reproducible and dose-dependent changes were observed in the antral spike burst activity, which served as the reference for the selection of representative doses of both hormones and for comparison of the effects of OP-CCK with these of cerulein (Fig. 1). As it can be read from these results, antral motility (inhibitory) effects of OP-CCK at the dose of 20 ng/kg were at the border of statistical significance and these effects were roughly similar to those after cerulein at the dose of 1 ng/kg. The effects of the smallest selected dose of the hormones on the antral, small intestinal and gallbladder motility are shown in Figure 2. The second selected (moderate) dose of OP-CCK was 200 ng/kg and its effect was similar to cerulein dose of 10 ng/kg (Fig. 1 and 3). The highest dose of OP-CCK (2000 ng/kg) was clearly more effective, thus, this dose was selected as the maximal dose in this study. The effect exerted by this dose of OP-CCK was similar to the ef-

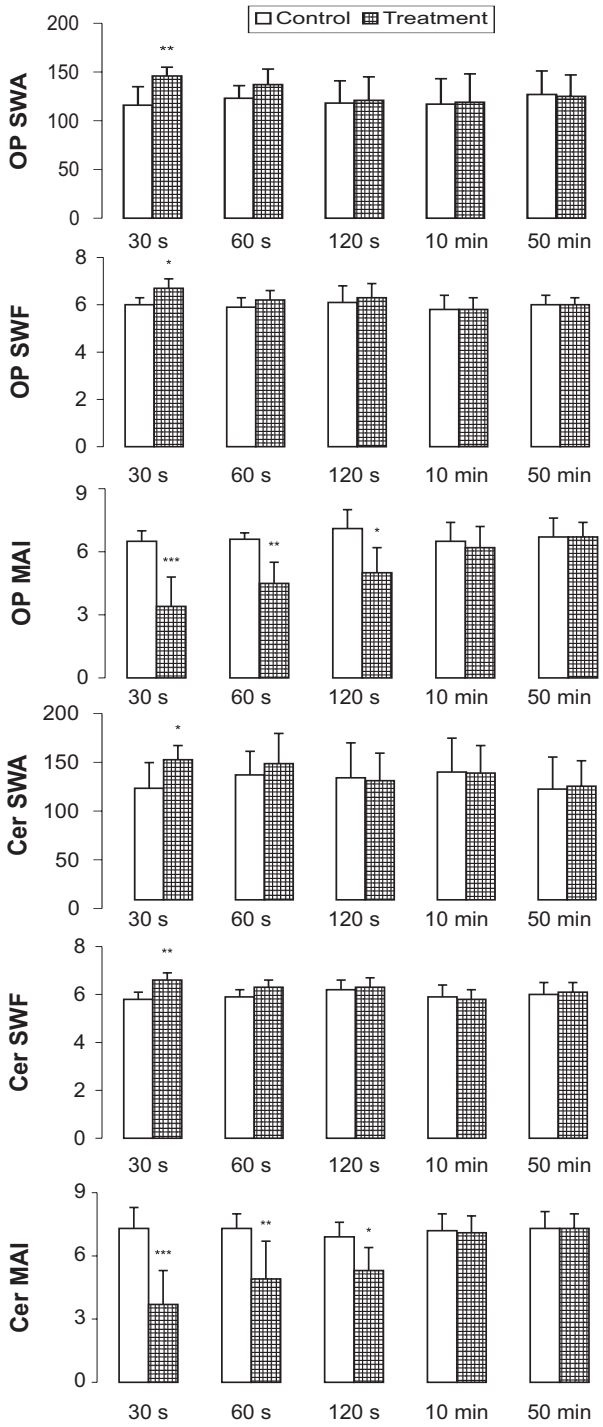

Fig. 3. The myoelectrical activity of antrum before and after the selected moderate (antrum) dose of OP-CCK and cerulein given during the various time periods of phase 2 MMC in non-fasted rams. Abbreviations: SWA – slow wave amplitude (data expressed in μV and calculated during two-minute recording period), SWF – slow wave frequency (data calculated during two-minute recording period and expressed in cycles per minute), MAI – myoelectrical activity index (calculated during 2 min period and expressed in μV × s/min. Statistical significances: * $p < 0.05$, ** $p < 0.01$. For other explanations see Figure 1

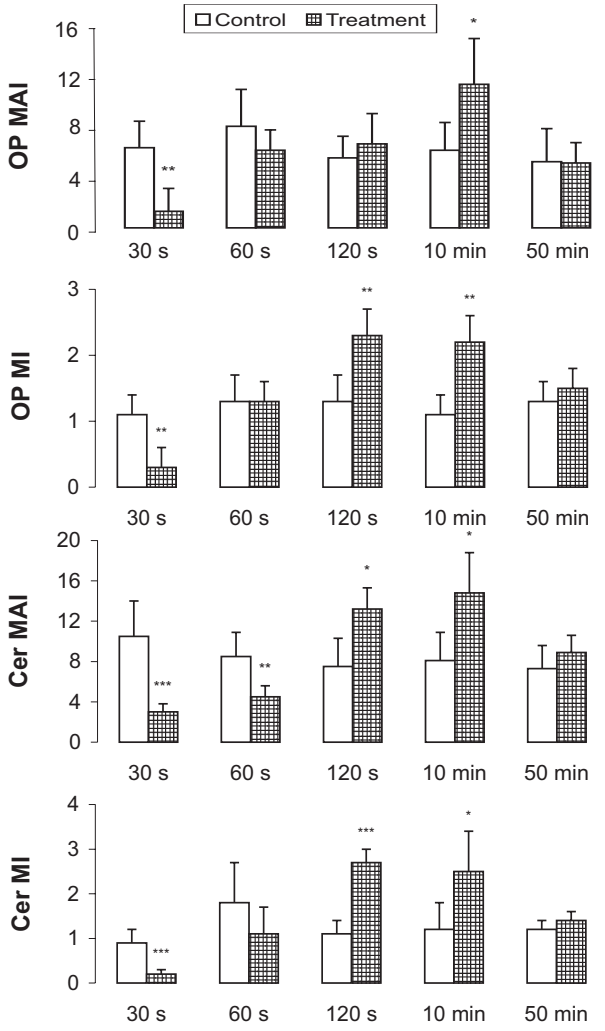

Fig. 4. The myoelectrical and motor activity of the duodenum before and after the high selected doses of CCK peptides administered during duodenal phase 2 MMC in non-fasted rams in various time periods. Abbreviations: MI – motility index (data expressed in 1000g × s/min). Statistical significances: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. For other explanations see Figure 1

fect obtained after the highest dose of cerulein (100 ng/kg, Fig. 1 and 4). All these doses were administered during 30-second periods, which were found to be most effective and simultaneously long enough to avoid any inconsistent results (Fig. 3 and 4). Antral slow wave frequency and amplitude, antral spike bursts and duodenal contractions were analyzed for this purpose. Three selected doses of OP-CCK and cerulein were applied during the same MMC cycle and during the other MMC cycle in a separate experiment in order to compare the strength of these effects (Fig. 5). The jejunal myoelectric response was attenuated when all three doses of both hormones were given during the same MMC cycle a few minutes apart in succession. When the hormones were administered during the separate MMC cycles, their action was more effective than administered during the same MMC cycle (Fig. 5). The smallest dose of cerulein, unlike OP-CCK, triggered significant response. Additionally, the effect of a moderate dose of cerulein, given twice at the 3–4 minute intervals, on gastrointestinal myoelectric activity, was assessed. The results (MAI) for antrum were as follows: 7.0 ± 0.8, 5.2 ± 0.5 ($p < 0.05$) and 6.4 ± 0.8 (N.S.) for control, and after two consecutive doses of cerulein, respectively. In the duodenum the results were: 6.2 ± 2.4, 2.3 ± ($p < 0.05$) and 3.1 ± 0.8 ($p < 0.05$) for control, and two moderate doses of cerulein, respectively. Thus, in the next series of experiments, each dose of the hormones was given during 30 s, in separate experiments. In the second part of the study, some effects of OP-

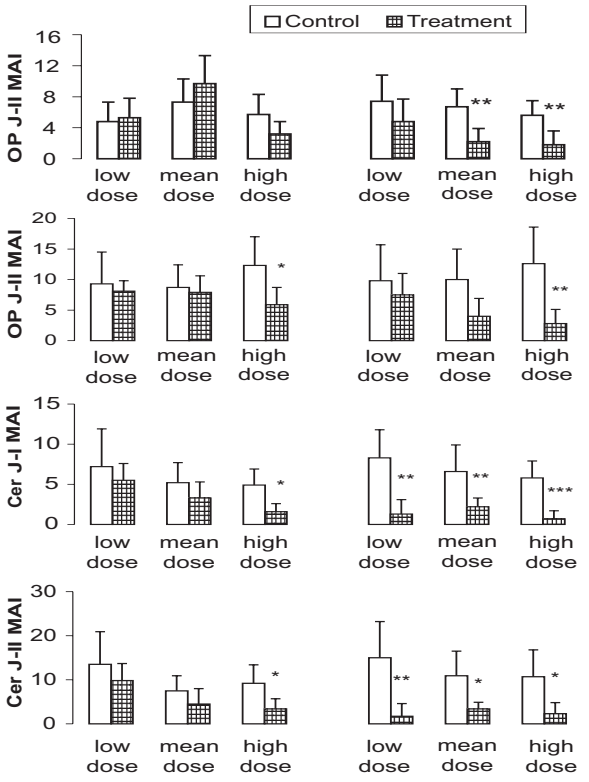

Fig. 5. The effect of three selected doses of CCK peptides administered during phase 2 of the same MMC cycle (left bar series) or during phases 2 of different MMC cycles (right bar series) on the jejunal myoelectrical activity in non-fasted rams. Abbreviations: J-I data from the recordings obtained from the first jejunal electrode. J-II data from the recordings obtained from the second (distal) jejunal electrode. Means ± SD, n = 5. Statistical significances: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 1. The effects of three doses of cerulein and OP-CCK on the site of phase 3 origin and delay in fasted and non-fasted sheep

		Fasted			Non-fasted		
		site of origin		phase 3 delay	site of origin		phase 3 delay
		Du	Gb	Du	Du	Gb	Du
OP	20 ng/kg	1	8	0	1	8	0
	200 ng/kg	2	4	3	4	3	4
	2000 ng/kg	6	1	7	7	2	5
Cer	1 ng/kg	2	6	0	0	9	0
	10 ng/kg	4	1	4	6	5	4
	100 ng/kg	8	0	6	8	3	6

10 experiments on 5 sheep were analyzed. Site of origin – number of phases 3 MMC originating below the duodenum (Du) and observed in the gallbladder (Gb). Phase 3 delay – number of first phases 3 arriving in the duodenum later than within 60 min following hormone administration. OP – OP-CCK, Cer – cerulein. For other explanations see Materials and Methods section

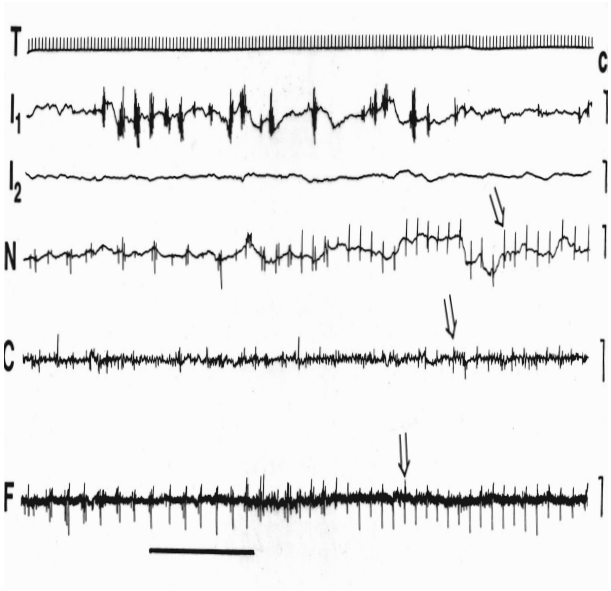

Fig. 6. The effects of *iv* administration of cerulein at the dose of 1 ng/kg on the ileal and gallbladder myoelectrical activity in fasted sheep. Single slow waves in the gallbladder are indicated with arrows. Abbreviations: T – time in seconds, cl – calibration 100 μV (vertical bars). Electrodes: I_1 – first (proximal) ileum, I_2 – second (distal) ileum, N – gallbladder neck, C – gallbladder corpus, F – gallbladder fundus. Horizontal bar: cerulein administration

Table 2. The initial effect of CCK peptides on the duodenal myoelectric (MAI) and motor activity (MI) in fasted and non-fasted sheep

		Fasted						Non - fasted					
		low dose		mean dose		high dose		low dose		mean dose		high dose	
		c	t	c	t	c	t	c	t	c	t	c	t
OP	$\bar{x}$	1.0	0.6	1.0	0.5	1.0	0.4*	7.7	6.1	7.6	4.7*	8.0	5.9
	± SD	0.5	0.2	0.6	0.5	0.5	0.3	3.2	2.1	2.1	1.0	3.1	2.5
Cer	$\bar{x}$	0.8	0.7	0.9	0.4*	0.9	0.3*	8.8	7.6	9.3	5.0*	9.2	6.3
	± SD	0.4	0.3	0.5	0.1	0.5	0.1	2.1	1.7	3.0	1.3	2.7	2.8
OP	$\bar{x}$	0.4	0.8*	0.7	1.7**	0.8	2.5***	0.8	1.3	1.3	2.2*	1.0	2.1
	± SD	0.1	0.2	0.3	0.4	0.3	0.8	0.2	0.6	0.4	0.7	0.2	0.9
Cer	$\bar{x}$	0.4	0.6	0.6	1.0*	0.8	1.7*	0.8	1.0	0.9	1.5*	0.8	1.7*
	± SD	0.1	0.2	0.1	0.3	0.2	0.7	0.3	0.3	0.2	0.6	0.2	0.8

Upper panel presents the myoelectric activity (MAI). Lower panel shows motor activity (MI). The doses of peptides: OP – 20, 200 and 2000 ng/kg, Cer – 1, 10 and 100 ng/kg. Peptides were given *iv* during 30 s, c – control, t – treatment. n = 5. Statistical significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. For other explanations see Table 1

CCK and cerulein on gastrointestinal and gallbladder motility were demonstrated. Initially, the effects of three doses of both hormones on the arrival of phase 3 MMC in the duodenum and gallbladder were studied and are presented in Table 1. It can be seen that CCK exerted inhibitory effect on phase 3 of the MMC. The inhibitory effect of both hormones was more evident in the duodenum since phase 3 MMC often originated from the jejunum after hormone administration or its arrival in the

Table 3. The effects of moderate and high doses of CCK peptides on the myoelectric activity (MAI) of the small intestine in sheep

		Cerulein						OP – CCK					
		duodenum		jejunum		ileum		duodenum		jejunum		ileum	
		c	t	c	t	c	t	c	t	c	t	c	t
M	$\bar{x}$	4.1	1.0**	7.3	2.5**	4.8	10.0*	6.1	2.9*	7.3	2.7*	6.9	11.4*
	± SD	1.6	0.6	2.7	1.5	1.3	4.2	2.3	1.1	3.1	1.5	1.8	2.9
H	$\bar{x}$	4.9	0.8**	10.2	0.3***	6.6	20.7***	6.2	0.3***	10.7	0.3***	7.7	26,5***
	± SD	2.2	1.0	3.4	0.5	1.8	6.1	3.4	0.4	4.2	0.4	2.4	7.7

Abbreviations: c – control, t – treatment, M – moderate dose: cerulein 10 ng/kg, OP-CCK 200 ng/kg. H – high dose: cerulein 100 ng/kg, OP-CCK 2000 ng/kg. For other explanations see Tables 1 and 2. Statistical significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

duodenum was delayed. Phase 3 MMC in the gallbladder arrived usually at the beginning of the duodenal phase 1 MMC and was also inhibited by higher doses of the hormones. Both substances exerted dose-dependent stimulatory effect on the gallbladder motor activity exhibiting rather tonic character (Fig. 2), and in a part of experiments it increased the gallbladder slow wave frequency and amplitude (Fig. 6). However, these changes were not reproducible. The effect of three doses of both peptides on duodenal myoelectric and motor activity was also studied in fasted and non-fasted rams (Tab. 2). The effects on motor activity seemed to be more evident in fasted animals, but generally, the results in fasted and non-fasted rams were similar. Finally, the effects of both higher doses of the peptides on the myoelectric activity of duodenum, jejunum and ileum were compared (Tab. 3). While the effects on duodeno-jejunum were rather inhibitory, the response of the ileum was mostly stimulatory. After the smallest dose of CCK peptides, this effect was occasionally delayed and of inhibitory character (Fig. 6). Therefore, the smallest dose of the hormone was able to exert the noticeable effects not only in the gallbladder but also in the small bowel.

DISCUSSION

The results showed that the applied doses of OP-CCK (20, 200 and 2000 ng/kg) clearly induced different responses and that usually very similar effects were obtained when cerulein was given at the doses of 1, 10 and 100 ng/kg. The biggest difference between OP-CCK and cerulein's action upon the MAI was observed in the jejunum when the peptide hormones were administered during separate MMC cycles suggesting the existence of desensitization. Cerulein was up to 20 times more effective than OP-CCK. When their effects were analyzed, the most evident antral inhibition was obtained following the highest dose, therefore, the OP-CCK dose up to 2000 ng/kg could be considered as physiological dose for this region. It also seemed likely that the lowest dose was physiological, at least for the gallbladder, while the moderate dose could be physiological mainly for the small intestine. The used range of doses was similar as in other studies in sheep, only 10-fold higher doses of OP-CCK than of cerulein were earlier used [10, 18]. The proposed physiological doses are in concert with the reported doses exerting evident responses. Ruckebusch and Soldani [18] observed that cerulein dose of 2 ng/kg was sufficient to stimulate gallbladder contraction, but antroduodenal motility remained unaffected. In another report [3], OP-CCK at the dose of 100 ng/kg did not evoke any response in the ovine antroduodenal region, while again, the stimulatory changes in the gallbladder were observed. Further confirmation regarding the relatively small antral sensitivity to CCK can be derived from the report of Cottrell and Reynolds [5]. To activate the antral tension receptors in sheep for 2 min, these authors used the intraarterial bolus dose of OP-CCK as high as 4000 ng. As it was found in the dog [13], cerulein at the dose of 10 ng/kg elevated plasma CCK immunoreactivity to postprandial values. Therefore, in the present study, the effect of the selected moderate dose of the hormone can be physiological and the question arises whether the plasma CCK level can exceed the postprandial values following the application of the highest dose of hormone used in the study. However, the smallest dose of both CCK peptides exerted short-term effects on the small intestine in most cases. The meal is considered as the strongest

physiological stimulus. Thus, after the highest dose of CCK peptide, plasma CCK level exceeds the postprandial value, which may mean that this dose is pharmacological. If the effect of moderate dose of CCK on gallbladder, and even on small intestinal motility is physiological, it should be concluded that the effect of this dose on antral motility is partly physiological since the antral inhibition in response to the moderate dose of OP-CCK or of cerulein was incomplete. It is possible that under the physiological conditions, the inhibitory effects on antral motility is exerted not by CCK alone but may be the result of hormonal interaction. This view was supported by Schmidt et al. [20] who concluded that meal-induced gallbladder contraction and fasting tone were primarily controlled by CCK while the gastrointestinal motility was only partially controlled by this hormone.

The inhibition of rumination indicates that mostly the highest doses of CCK peptides apparently exhibited central action [6]. This effect was parallel to known inhibitory effects of CCK on food intake (satiety-inducing effect) in various animal species including sheep [1]. The inhibition of rumination, not observed following pentagastrin [2] could be linked with the suppression of reticular motility [1]. The involvement of CCK-A receptors in observed inhibitory effect of CCK on the MMC is possible since the CCK-A receptor antagonist, L364,718 given *iv* induced premature phase 3 MMC in duodenal electromyogram in conscious sheep [15].

The duration of CCK peptide administration seems to be important to assess its effect on the target organ because of the constant elimination of the hormone from the bloodstream. Several authors did not precisely record the time of hormone administration given either as the bolus dose or as the infusion [3, 4, 13]. Thus, because of the great variability of the models applied in the studies, it is often difficult to compare the effects of the same or similar dose of the hormone between two different protocols. To further examine the action of CCK on gastrointestinal and gallbladder motility, the effects of the same doses given in different time periods were studied. Then, to compare the different doses of the hormone used, the same time of their administration was always designed. The results showed that the shortest tested period was optimal for the determination of specificity of action of CCK peptides, especially when plasma CCK level was not monitored. This way of hormone administration seems to be more useful than the often reported prolonged hormone infusion. During hormone infusion protocol, only the results obtained during this infusion are usually considered. Thus, to obtain evident results, the infusion is sometimes prolonged and the total dose of the hormone is fairy large. At the end of infusion, the effect can be different than at the beginning of this period, what can lead to the misinterpretation of the data. Thus, pharmacological, rather than physiological effects, can be observed what did not take place in the present study. In this model, both the time of hormone administration and the overall dose were strictly controlled and reduced to the minimum, and the long-term effects induced by the given dose can be observed without the constant increase in the blood hormone concentration.

CCK is the ligand of membrane metabotropic receptors acting principally through two CCK-1 and CCK-2 receptors. The desensitization process of these receptors is quite well known [14] and the next problem tested in this model was to assess the extent of desensitization of CCK receptors, especially CCK-1 receptors when three increasing doses of the hormone were given in few 3–4 minute intervals. The slightly diminished response to two higher doses of OP-CCK and cerulein given during the same MMC cycle compared with the effect of the hormones administered during different MMC cycles suggests the existence of desensitization within CCK receptors and/or down-regulation of neural, probably cholinergic, transmission evoked by CCK peptide injection [19, 20]. The administration of two equal doses of the hormone within 3–4 min further confirmed these observations, at least partially, although the response to the second dose was significant as it was seen after the first dose. Thus, it can be concluded that desensitization of the response to CCK occurs in sheep, but its intensity is not marked.

CCK release is mainly induced by dietary fat and amino acids [11], i.e. its plasma level is enhanced after a meal [20]. Therefore, it can be expected that CCK action can be stronger when the hormone is administered in non-fasted animals rather than in fasted animals. However, the results did not confirm this opinion. Some possible reasons can be considered. There is a practical lack of digestive and interdigestive states in ruminant animals, thus, the differences between fasted and

non-fasted rams could be attenuated. It is also possible that there is an interaction between endogenous and exogenous hormone resulting in diminished or reversed response to the drug. Such an effect was observed in humans in response to loxiglumide, the CCK receptor antagonist [20]. Therefore, it can be stated that there is no meaningful difference in CCK action on intestinal and gallbladder motility between fasted and non-fasted sheep.

The action of CCK on gastrointestinal motility is complex and dependent on many physiological factors. The distance from pylorus seems also to be important. This view was confirmed here by the marked differences in the action of OP-CCK and of cerulein on two jejunal segments. In other experiments, an important difference in hormone action on duodenum, jejunum and ileum was observed. In the same experiment, the effect on ileum was opposite to the effect on duodeno-jejunum. The inhibitory effect was stronger in the duodenum than in jejunum, while in the ileum the effect was always stimulatory, especially when the higher doses of hormones were used. This difference can be caused by the variability in the smooth muscle CCK-receptor density along the small intestine, the spectrum of CCK action as neuropeptide [19], different hormonal interactions and up- and down-regulatory processes [8, 14].

The observed effects of CCK peptides on antral, small-intestinal and gallbladder motility in sheep did not vary considerably from the effects reported in other animal species, including man [11, 23]. These regions of the digestive system are not much different between ruminant and non-ruminant species and the proposed model of study of the CCK effects on motor function can be suitable in both groups of animal species. There are no reports concerning the existence of the slow waves in the gallbladder, at least *in vivo*. During control experiments of the present study, the slow waves were not always observed and were of variable frequency and amplitude. Thus, it was difficult to prove how these parameters are affected by CCK peptides. This study also showed that the effects of CCK peptides on gastrointestinal motility were complex, so they warrant further extensive investigations.

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