Acta Veterinaria (Beograd), Vol. 57, No. 2-3, 113-122, 2007.

DOI: 10.2298/AVB0703113R UDK 619:612.014.42

THE EFFECT OF CHOLECYSTOKININ OCTAPEPTIDE UPON THE MIGRATING MYOELECTRIC COMPLEX IN THE OVINE SMALL BOWEL

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(Received 15. September 2006)

There is no evident and precise data regarding the effect of cholecystokinin octapeptide (CCK-OP) on the migrating myoelectric complex (MMC) in sheep. Thus, in five rams seven bipolar platinum electrodes were implanted in the abomasal antrum and entire small intestine. The strain gauge force transducer was also attached near the duodenal electrode in three of these animals. During the experiments the myoelectric and motor activity was continuously recorded in fasted and non-fasted sheep. During the recordings two normal consecutive phases 3 of the MMC were recorded. Then, slow injections of CCK-OP of 0.02; 0.2 and 2.0 μg/kg of body weight were given intravenously during phase 1, 2a or 2b of the MMC, identified in the duodenum, until next two consecutive phases 3 MMC were recorded. The moderate dose of CCK-OP administered during phase 2a in non-fasted animals shortened the MMC cycles significantly while the highest dose of the hormone increased the cycle duration and inhibited phase 3 MMC in the duodenum. No such effect was observed in the jejunum. However, duration of phase 3 in this region was decreased mostly by the highest dose of CCK-OP. It can be concluded that CCK inhibits the MMC in ovine small bowel and its effect in the duodenum is most pronounced.

Key words: sheep, small intestine, migrating myoelectric or motor complex, phase 3, cholecystokinin

INTRODUCTION

Cholecystokinin (CCK) is one of the most important gastrointestinal hormones and exerts multiple physiological and pharmacological actions (Crawley and Corwin, 1994). It is one of the major regulators of gastrointestinal motility (Thomson *et al.*, 2003). Its effect on small intestine motility is well established, at least in monogastric species, and its regulatory effect on peristalsis is included to the physiological actions of the hormone (Walsh, 1994). The presence of specific CCK receptors creates the basis for these actions (Walsh, 1994; Miyasaka and Funakoshi, 2003). However, the spectrum, character and mechanism of CCK effects have not been recognized in details. CCK is still

the subject of extensive investigations (Dong *et al.,* 2005, Feltrin *et al.,* 2004). Thus, its expected effect upon the principal interdigestive motor pattern in the small bowel, i.e. the migrating motor or myoelectric complex (MMC) might be inhibitory.

The studies on dogs first indicated that CCK administration switches the gastrointestinal MMC into the irregular digestive pattern motor activity (Mukhopadhyay *et al.*, 1977; Schang and Kelly, 1981). Wingate *et al.* (1978a) showed that this effect was observed only in the proximal small intestine. In sheep, CCK also exerts its effects on gastrointestinal motility. These effects were demonstrated for CCK-peptides and for cerulein, the amphibian CCK analogue (Bueno and Praddaude, 1979, Ormas *et al.*, 1984). The inhibitory effect of CCK on MMC in ruminants was first suggested by Gregory but not documented (Cottrell and Gregory, 1991). Further study showed that CCK antagonist L-364,718 could induce the premature phase 3 in ovine duodenum thus confirming indirectly the previous suggestion (Onaga *et al.*, 1997). The opposite effect after central administration of CCK antagonist was obtained by Kania *et al* (1999). Thus, the aim of this study was to assess the precise effect of CCK-octapeptide (CCK-OP) on the MMC in the entire small intestine of conscious sheep.

MATERIALS AND METHODS

Five adult rams of Polish Merino Breed with individual body weight of 38-42 kg were used. Before surgery animals were fed with good-quality hay and were supplemented with a standard grain mixture. Drinking water was not limited.

Animal preparation

In 24 h fasted animals right lateral laparotomy was performed under general and local anesthesia as described previously (Romanski, 2003). Seven electrodes were implanted onto the antral (one electrode), bulbar (one electrode), duodenal (one electrode), jejunal (two electrodes) and ileal (two electrodes) wall. The strain gauge force transducer was attached near the duodenal electrode. The marked wires were exteriorized and were connected with the recorder during the experiment.

Experiments

A total of 180 trials were performed. The scheme of the experiment was similar as previously published by Romanski (2002). During the control recordings two normal consecutive phases 3 of the MMC were recorded. Then, CCK-OP was administered at doses of 0.02, 0.2 and 2.0 μg/kg of body weight during 30 (two lower doses) and 60 seconds (Romanski, 2004) into the jugular vein through the indwelling polyethylene catheter inserted before the experiment. The hormone was injected randomly during phase 1, 2a or 2b of the MMC identified in the duodenum. The myoelectric and motor recordings were continued until two consecutive phases 3 of the MMC were observed following drug administration. After the termination of the experiment the animals were

sacrificed and the positions of the electrodes and of the transducer were confirmed.

Analysis of tracings and calculations

The recordings were visually analysed and MMCs and their phases were identified in the small intestine. The onset and duration of an activity front was estimated, as well as the duration of the whole pattern. The percentage of the activity fronts initiated on each segment of the small intestine was also calculated. The results of the duration of phase 3 MMC and of the whole MMC pattern underwent statistical analysis. For this purpose Student t-test for paired and unpaired values, where appropriate, followed by analysis of variance, was applied (Snedecor & Cochran, 1971).

RESULTS

Duration of the MMC cycle in fasted animals did not vary significantly before and after CCK-OP administration except at the highest given dose during phase 2b of the MMC (Table 1). When the hormone was introduced during phase 2a MMC, an increasing tendency was observed. A similar tendency was seen in non-fasted sheep after the moderate dose of CCK-OP was given during phase 2b MMC. When CCK was given during phase 2a MMC, it shortened the cycle duration significantly since in three of five sheep it induced the premature phase 3 of the MMC (Table 1). The highest dose of CCK offered during phase 2a or 2b MMC significantly elongated the MMC cycle.

The increase in MMC cycle duration in response to CCK administration was accompanied by inhibition of phase 3 MMC in the duodenum as the first phase 3 after the higher doses of CCK started usually from the jejunum (Table 2). In spite of it, their occurrence was also delayed. These changes were similar in fasted and non-fasted sheep.

Duration of phase 3 in the jejunum also differed markedly depending on CCK dose both in fasted and non-fasted animals. After the higher CCK doses, phases 3 shortened in the jejunum significantly and a decresing tendency was observed (Table 3).

First phase 3 of the MMC arriving after the highest dose of the hormone was often abnormal. It was found to be abortive, not fully developed, less regular or in two cases even retropropagated (Fig. 1).

Table 1. Duration of the migrating myoelectric complex (MMC) before (control) and after administration of three doses of cholecystokinin octapeptide (CCK-OP) during various MMC phases in fasted and non-fasted sheep

			CCK-OP 0.02 μg/kg			CCK-OP 0.02 μg/kg			CCK-OP 0.02 μg/kg		
			ph . 1	ph. 2a	ph. 2b	ph. 1	ph. 2a	ph. 2b	ph. 1	ph. 2a	ph. 2b
F	Ctr	mean	60.0	61.6	61.4	60.8	60.8	64.8	64.4	63.4	62.2
		± S.D.	31.5	22.9	28.0	27.4	28.0	22.4	23.7	21.4	23.6
F	CCK	mean	69.2	67.2	63.6	73.4	65.6	56.4	63.2	81.4	106.0[a]
		± S.D.	18.4	20.2	22.6	28.2	17.6	31.0	21.0	30.1	26.7
NF	Ctr	mean	57.8	60.8	48.8	58.0	60.8	58.0	61.8	61.0	62.4
		± S.D.	22.7	25.1	26.5	25.2	22.8	22.0	30.2	21.6	24.5
NF	CCK	mean	52.2	55.8	60.4	61.6	21.8[a]	80.2	74.4	115.8[a]	140.2[c]
		± S.D.	27.3	27.7	20.4	24.8	20.4	29.6	23.6	31.0	30.8

Legend: values in minutes, F – fasted, NF – non-fasted, CCK – CCK-OP, ph. 1, ph 2a, ph 2b – MMC phases, Ctr – control values. Statistical significances vs. relevant control value: student t-test for paired values followed by variance analysis, [a]$p<0.05$, [b]$p<0.01$, [c]$p<0.001$, n=5.

Table 2. Initiation of phase 3 MMC before and after administration of CCK-OP in fasted and non-fasted sheep

			CCK-OP 0.02 μg/kg			CCK-OP 0.02 μg/kg			CCK-OP 0.02 μg/kg		
			ph . 1	ph. 2a	ph. 2b	ph. 1	ph. 2a	ph. 2b	ph. 1	ph. 2a	ph. 2b
F	Ctr	duod. bulb	40	70	50	60	40	20	80	40	20
		duodenum	50	30	40	40	50	80	10	60	60
		jejunum	10	0	10	0	10	0	10	0	20
F	CCK	duod. bulb	40	60	40	60	40	20	20	0	0
		duodenum	60	20	40	20	40	40	60	40	0
		jejunum	0	20	20	20	20	40	20	60	100
NF	Ctr	duod. bulb	50	60	40	30	40	30	20	10	40
		duodenum	50	30	50	70	60	60	60	90	50
		jejunum	0	10	10	0	0	10	20	0	10
NF	CCK	duod. bulb	40	40	20	40	20	0	20	0	0
		duodenum	60	40	60	60	60	60	40	20	0
		jejunum	0	20	20	0	20	40	40	60	100

Legend: values in % of the total number of phases 3 MMC originating from the small bowel, duod. bulb – data from the recording from duodenal bulb electrode, duodenum – data from duodenal electrode confirmed partially by mechanical recordings, jejunum – data from both jejunal electrodes.

Table 3. Duration of phase 3 MMC before and after CCK-OP administration in fasted and non-fasted sheep

		CCK-OP 0.02 μg/kg			CCK-OP 0.02 μg/kg			CCK-OP 0.02 μg/kg		
		ph . 1	ph. 2a	ph. 2b	ph. 1	ph. 2a	ph. 2b	ph. 1	ph. 2a	ph. 2b
F	Ctr mean	6.0	5.8	6.1	6.3	6.0	5.9	6.2	6.0	5.8
	± S.D.	0.7	0.9	0.8	1.0	0.8	0.6	0.9	0.6	0.7
F	CCK mean	5.8	5.0	5.7	5.9	5.1	5.0[a]	5.5	4.6a	3.9[b]
	± S.D.	0.8	0.7	1.1	0.8	0.7	0.4	0.8	0.5	0.7
NF	Ctr mean	5.6	5.5	5.8	5.4	5.7	5.6	5.9	5.6	5.7
	± S.D.	0.6	0.5	0.9	0.8	0.6	0.9	0.7	0.6	0.7
NF	CCK mean	5.8	5.6	5.6	5.3	5.2	5.1	5.2	4.2[a]	3.4[c]
	± S.D.	0.7	0.6	0.8	0.6	0.7	0.6	0.6	0.7	0.5

Legend: duration of phase 3 MMC calculated from the recordings obtained from the lower jejunal electrode before (control, n=10) and after (n=5) CCK-OP administration. Statistical significance: Student t-test for unpaired values followed by analysis of variance.

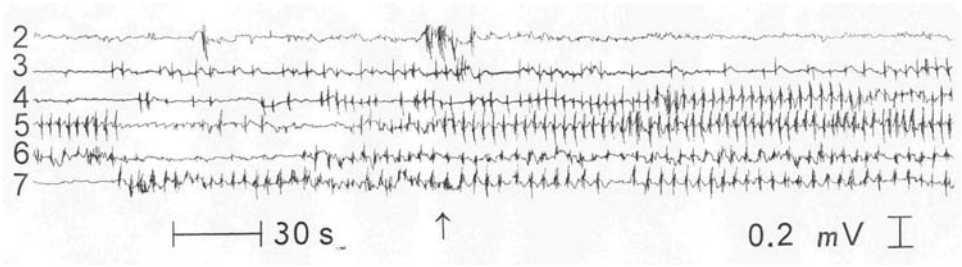

Figure 1. The retropropagated phase 3 of the MMC following CCK-OP administration (arrow) at the dose 2,0 ìg/kg in non-fasted sheep.
Legend: arrow indicates four minutes following the termination of CCK-OP injection. Electrode localization: 2 – duodenal bulb, 3 – duodenum, 4 and 5 – jejunum, 6 and 7 – ileum; c – calibration 100 μV, t – time (10 s)

DISCUSSION

The obtained results indicate that CCK may inhibit phase 3 MMC and increase the cycle duration in fasted and non-fasted sheep. CCK-OP used in this study represents the form of CCK present in sheep tissue (Titchen, 1986). Since OP-CCK does not cross the blood-brain barrier (Fioramonti and Bueno, 1988), its effect following intravenous administration can be considered peripheral. The effect of CCK-OP on MMC cycle length was significant at higher doses. Similar results were obtained in dogs (Wingate *et al.,* 1978a; Wingate *et al.,* 1978b; Thor *et al.,* 1990). In sheep, except the suggestion of Gregory (Cottrell & Gregory, 1991) no such studies have been reported, thus the comparison of the doses cannot be made. However, it is known that in sheep the dose of CCK-OP 150 pmol/kg/h, (i.e. doses below 2 pmol/kg/30 s) is the maximal physiological dose (Zavros and Shulkes, 1997). The higher doses used in the present study, which produced significant changes in MMC cycle duration and inhibited phase 3 MMC in the duodenum were greater than the physiological dose suggested previously although the total amount of CCK-OP infusion was not markedly different. Thus, this action can probably be interpreted as pharmacological.

In the previous studies it was suggested that CCK, as the typical digestive hormone, can change the interdigestive myoelectric activity to a postprandial pattern (Lee *et al.,* 1980). However, the myoelectric activity following CCK not always resembled this pattern (Mukhopadhyay *et al.,* 1977). In sheep the postprandial pattern formerly does not occur since the MMC is not inhibited by feeding (Ruckebusch, 1989). Even if some changes in myoelectrical activity occur after feeding, they are not precisely defined. Thus, it is difficult to state that in sheep CCK converts the interdigestive motor activity to the digestive pattern.

The inhibition of phase 3 MMC by CCK-OP administration was more efficient in the duodenum than in the jejunum. Though the distribution of CCK receptors along the small intestine is not well known in sheep it is possible that the mechanism of CCK action in the duodenum can be different than in the jejunum.

In the dog the concentration of CCK receptors in the duodenum is high (Mantyh *et al.,* 1994). Furthermore, the concentration of small forms of CCK is greater in the upper small bowel (Walsh, 1994). It has also been reported that the presence of this hormone in upper small intestine is greater than in the distal parts of the bowel (Bryant and Bloom, 1979). The differences in the regulatory mechanism of CCK action in the small bowel can comprise it role in somatostatin release (Herzig *et al.,* 1994; Zavros and Shulkes, 1997). It is known that somatostatin can be responsible for the inhibition of phase 3 MMC in the duodenum (Walsh, 1994). However, the small doses of CCK can be more active in the proximal jejunum than in the distal duodenum (Fleckenstein and Öigaard, 1977). Thus, the exact mechanism of CCK action on small intestine motility is far from fully elucidated.

It is concluded that CCK may increase the MMC cycle duration in the small bowel and inhibits phase 3 of the MMC in the duodenum of fasted and non-fasted sheep.

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Acta Veterinaria (Beograd), Vol. 57, No. 2-3, 113-122, 2007.
Romanski WK: The effect of cholecystokinin octapeptide upon the migrating myoelectric complex in the ovine small bowel

UTICAJ HOLECISTOKININ OKTAPEPTIDA NA MIGRIRAJUĆI MIOELEKTRIČNI KOMPLEKS SIRIŠTA I TANKIH CREVA OVACA

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SADRŽAJ

U ovom radu su opisani efekti holecistokinin - oktapeptida (HCK-OP) na formiranje mioelektričnog migrirajućeg kompleksa (MMK) u digestivnom traktu ovaca. U ogledima je korišćeno pet ovnova kod kojih je ugrađeno po sedam platinastih bipolarnih elektroda u antralni deo abomazusa i tanka creva. Kod tri ovna ugrađeni su i transdjuseri koji registruju stepen istezanja. U ogledima su registrovane promene MMK kod normalno hranjenih životinja kao i kod životinja koje su bile podvrgnute gladovanju. HCK-OP je bio aplikovan intravenski u tri različite doze (0,02, 0,2 i 2 mg/kg telesne mase). Na osnovu dobijenih rezultata autori su zaključili da HCK-OP inhibira formiranje MMK kod ovaca i da je njegov efekat najizraženiji u duodenumu.