CHOLECystokinIN OCTAPEPTIDe AND CERULEIN INHIBIT OvINE DUDENAL MOTILITY IN A DOSE- AND REGION-SPECIFIC MANNER

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Abstract

The aim of this study was to examine the influence of cholecystokinin (CCK)-octapeptide (OP) and its amphibian analogue cerulein infusions on duodenal myoelectric and motor activities, as well as to compare the effects of CCK peptides on duodenal bulb and duodenal motility in non-fasted conscious rams. Five rams underwent implantation of bipolar platinum electrodes to the duodenal bulb, and distal duodenum, as well as a strain gauge force transducer near the duodenal electrode. During continuous myoelectrical and motor recordings, 0.15 M NaCl or CCK peptides were administered intravenously. Infusions of CCK-OP at doses of 5 and 50 ng/kg/min and infusions of cerulein at doses of 0.5 and 1.5 ng/kg/min were applied for 60 min and started 15 min after the onset of the duodenal phase 2b of the migrating motor complex. The higher infusion dose of the CCK-OP in the duodenal bulb triggered the strong inhibitory response within few minutes following the start of infusion while in the duodenum its inhibitory effect was shorter and arrived within 40-50 min following the onset of the infusion. The higher dose of cerulein evoked a reaction similar to CCK-OP response in the duodenal bulb while in the duodenum the clear inhibitory response arrived about 20 min earlier than after CCK-OP. A lower infusion dose of CCK peptides evoked less pronounced effects. It is concluded that CCK-OP inhibits ovine duodenal motility in a dose-and region-specific manner. This effect seems to be physiological.

Key words: sheep, duodenum, myoelectric activity, motor activity, cholecystokinin octapeptide, cerulein.

Duodenal motility is essential for normal digesta transport from the stomach and is controlled by several neurohormonal mechanisms. Cholecystokinin (CCK) is one of the main gastrointestinal hormones involved in this control. The hormone, usually applied as CCK-octapeptide (CCK-OP) as the natural CCK form present in sheep, has been reported to inhibit the arrival of the migrating motor complex (MMC) and induce a feed-like pattern in the upper small bowel (8). Some reports indicate that CCK might also stimulate duodenal digestive motility. It evokes the specific spike burst pattern, increases spiking activity, and accelerates the intestinal transit time (9, 14). However, opposite and dual effects of CCK-OP and its amphibian analogue cerulein have been reported (6, 10). 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 having been carried out (16, 19, 21, 22). Thus in sheep, either an inhibitory or excitatory effect of CCK upon the small-intestinal motility can be expected, although it is not known, which effect can be physiological. Furthermore, it still remains uncertain what is the difference in response to CCK between duodenal bulb and duodenum and what is the sensitivity of these regions to CCK. Thus, the aim of this study was to assess the character of duodenal motility response to physiological doses of CCK-OP in non-fasted sheep and to compare it with cerulein.

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, Wroclaw), then fasted for 24 h before surgery but allowed unlimited access to water. After general and local anaesthesia (21), right lateral laparotomy was performed and 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 bulb electrode. An additional electrode was placed in the jejunum, 200 cm from the duodenal electrode to verify myoelectric activity.

A strain gauge force transducer (RB Products, Madison, USA), calibrated individually before the surgery, was attached near the duodenal electrode to verify myoelectric activity.
readings. The obtained motor recordings reflected the incidence of spike bursts. Other details of this procedure have been described elsewhere (19, 21). Marked wires were exteriorised through a stab incision, soldered to the plug and fixed to the surface. The animals returned to normal feeding within 2-3 d. The skin sutures were removed 10 d after the surgery.

**Experimental design.** A total of 50 experiments lasting 5-6 h each were conducted. In the course of the experiments, myoelectric and motor activities were continuously measured using a multichannel electroencephalograph (Reega Duplex TR XVI, Alvar Electronics, Montreuil) also adapted for mechanical activity recordings. Food was removed from the cage 20 h before each experiment. At least two consecutive phases three of the MMC including one full normal cycle of the MMC were measured each time. During separate control recordings, infusions of saline at a rate of 1 ml/min for 60 min were conducted in each animal. The saline infusions were performed during the course of phases 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 entire group of basic experiments, CCK-OP was administered at the rates of 5 (small dose) and 50 ng/kg (high dose) over 60 min and cerulein was given at the rates of 0.5 (small rate) and 1.5 ng/kg (high dose) over 60 min. The infusions of CCK-OP and cerulein were started at the same times as the infusions of saline. Each dose of CCK peptide was given in separate randomised experiments at the same periods as the saline injections. After saline or CCK peptide administration, the myoelectric and motor activities were measured until the complete termination of the CCK-related motility response, i.e. at least 60 min. After termination of all the experiments, the animals were slaughtered 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 and Marlett (3) with a slight modification (17). The myoelectric and motor readings were visually analysed and the myoelectric activity index (MAI) values were calculated (see 19). The MAI values were calculated by multiplying the average amplitude of each spike burst by its duration period, as described previously (20), and calculated as the sum of the areas and sum of MAI values during 10 min. The myoelectric data were recalculated for 1 min period (µV x s x 10 min⁻¹) and expressed as µV s min⁻¹. Spike bursts with amplitudes below 3 µV were ignored. Thus the duration of each considered periods was equal to 10 min. On the tracings, the measurements were performed using a calliper with an accuracy of about 0.3 mm.

**Statistical analysis of data.** All the values were grouped and the means and standard deviations were calculated. Statistical significances, i.e. when P<0.05, P<0.01, or P<0.001, were calculated using the Student’s t-test for paired values, preceded by one-way analysis of variance (25). Statistical significances of the periods 5-16 were compared with the relevant pooled periods 1-4, i.e. expressed as the single values.

**Results**

The motor readings reflected the incidence of the spike bursts (Fig. 1). Injections or infusions of saline evoked no effect and these data are not shown.

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**Fig. 1.** The effect of the high dose of cerulein administration on myoelectric and motor activity of the ovine upper small bowel. A reading of almost 2 min and 5 min after the onset of cerulein infusion is shown. Note the high correlation between spiking activity and contractions in the duodenum and the presence of two types of the spike bursts in the duodenal bulb.

B – the myoelectric activity in the duodenal bulb; D – the myoelectric activity in the duodenum, T – the motor activity in the duodenum, strain gauge force transducer located near the duodenal electrode; C – calibration, 50 µV; s – time in seconds. Single arrow indicates the small-amplitude spike burst; doubled arrow indicates the normal (high-amplitude) spike burst in the duodenal bulb.
Fig. 2. The effects of infusions of cerulein and CCK-OP at two different doses on the myoelectric activity index of the duodenal bulb in sheep. MAI – the myoelectric activity index. Ordinate: MAI values expressed in µV s min⁻¹. Abscissa: 1-10 – consecutive periods of MAI calculation; periods 1-2 – control one-minute periods, 3-8 – six ten-minute consecutive periods during infusions of CCK peptides, 9-10 – two ten-minute postinfusion periods. Means ± S.D. * P<0.05, ** P<0.01, *** P<0.001 vs. the relevant value of the period 2. Student’s t-test for paired values preceded by ANOVA I was applied here. Other explanations as in the section Material and Methods.
Fig. 3. The effects of infusions of cerulein and CCK-OP at two different rates on the myoelectric activity index of the duodenum in sheep.

Explanations as in Fig. 2
During the low rate of CCK-OP infusions performed in the course of phase 2b of the MMC, the inhibitory tendency of spiking activity (MAI) in the duodenal bulb was short and not significant (Fig. 2), arrived about 40 min following start of the infusion, lasted 1.9 ±0.7 min, and after termination of peptide infusions partial inhibition lasting 4-5 min was observed. After the small dose of infused cerulein (Fig. 2), the duration of the inhibitory period was significantly longer, and arrived just after peptide infusion onset, lasted 43.8±13.6 min (as measured from the start of inhibition till the end of infusion), and, following cessation of peptide infusion, partial inhibition still lasted for 1-3 min in the duodenal bulb. The high dose of CCK-OP infusion evoked strong inhibitory response in the duodenal bulb (Fig. 2) 2-4 min following the infusion onset, lasting till the end of the infusion (57.5±18.5 min, P<0.001 vs. the lower dose, as measured from the start of inhibitory effect till the end of peptide infusion), and even over 50 min following the end of infusion. The higher dose of cerulein evoked a similar response, lasting 58.8±17.5 min (P<0.001 vs. the lower dose) (Fig. 2). Mainly during the inhibitory period, a series of small amplitude-spike bursts evoked by CCK peptides were observed in the duodenal bulb (Fig. 1). These series lasted 40-110 s each and during 60 min recording two-three series were observed. In the control recordings single small amplitude-spike bursts were occasionally observed as well.

In the duodenum, a small dose of infused CCK-OP induced no significant inhibitory response, starting 32-38 min following infusion onset and lasting till the end of the infusion, i.e. 11.8±3.2 min, and then still over 70 min following the cessation of peptide infusion (Fig. 3). Infusion of a small dose of cerulein also evoked a partially-delayed significant inhibitory response in the duodenal MAI, starting 14-18 min following infusion onset and lasting 31.9±6.4 min, and still was maintained over 60 min after end of infusion (Fig. 3). The high dose of infused CCK-OP induced the inhibitory response 31-34 min after start of infusion, lasting 13.1±3.4 min during hormone infusion and still over 60 min after the end of infusion (Fig. 3). The higher dose of cerulein (Fig. 3) produced an inhibitory response 9-19 min after infusion onset lasting 41.0±6.9 min (P<0.001 vs. the smaller dose) and terminated over 60 min after the end of infusion. During the inhibitory period the duodenal spike bursts contained irregular spiking amplitude and reduced duration.

There were no marked differences between the effects of CCK-OP and cerulein infusions started in the course of phases 2a and 2b of the MMC thus the former data are not shown here.

**Discussion**

Infusions of CCK-OP and cerulein elicited marked inhibitory alterations in the myoelectric activity of the ovine duodenum. The onset time of the inhibition elicited depended on the peptide, dose, and region of the duodenum.

As it has been confirmed several times, CCK plays a crucial role in the control of small intestinal motility (24). This may also be true in sheep (15, 19). However, its effect may be different in the various regions of the small bowel (21). The role of duodenal bulb motility in the transport of digesta is mainly related to gastric emptying because of the importance of pressure gradient between the antrum and the duodenum (23). In ruminants, the flow of digesta is relatively constant, suggesting that the role of the duodenal bulb may be even greater in ruminants than in monogastrics. Thus, duodenal bulb motility differs from duodenal motor function in sheep, i.e. fewer phasic contractions and spike bursts can be observed in the duodenum and the duration of phase 3 of the MMC is often shorter or phase 3 is absent (18). In the present study, CCK peptides evoked pronounced inhibition of high amplitude-spike bursts in the duodenal bulb. These spike bursts apparently originated from the circular smooth muscle layer. Furthermore, during inhibitory period the low-amplitude spike bursts, most probably originating from the longitudinal muscle, were measured. In the duodenum, the effect of CCK peptide infusions was inhibitory. The longitudinal smooth muscle layer is much thinner than the circular muscle layer - thus it can evoke only spike bursts with relatively low amplitude. However, it has been reported that the effect of CCK on duodenal motility in man, dog, rabbit, rat and sheep is excitatory (4, 7, 8, 12, 19). Thus, the stimulatory effect of a given dose of CCK (10 ng/kg) on duodenal motility seems to be primary (13), and this conclusion cannot be drawn from the present study. Similar effects were reported for cerulein (1, 14, 16, 19). However, other reports indicate that the effect of CCK action on duodenal motility can be inhibitory or no effect can be observed while some authors observed simultaneous stimulatory effect in the jejunum (2, 5, 10).

The small dose of CCK-OP infusion induced no effect on the duodenal bulb, while cerulein evoked a stronger effect. On the contrary, the higher doses of both peptide infusions induced a greater effect in the duodenal bulb than in the duodenum. As it was discussed earlier (21), moderate doses of CCK-OP and cerulein, as well as the highest doses, administered over 120 s, remain within the physiological range. Thus the only reasonable inference that can be drawn from these findings is that endogenous CCK may also inhibit myoelectric activity in the duodenal bulb and that the inhibitory effect of CCK represents the primary response in this region. In the rat duodenum, the small doses of CCK peptides elicited transient stimulation in the myoelectric and motor activities and moderate doses evoked the dual effect (6). Other authors observed the dual effect of gastrin peptides in ovine stomach (11). These observations may suggest that in the stomach and 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. In the present study no dual effect was observed. Thus the inhibitory action of CCK might be considered primary since the infusion of the hormone appears to be more physiologically relevant form of...
administration than even slow injection. It is also possible that the effect of neuronal action of CCK can differ from its endocrine effect. Thus the question whether the CCK can be classified as a stimulatory hormone still remains unresolved.

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 (19) 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 (22). This remains in concert only with that part of the present study where CCK-OP, administered at 20-fold higher dose than cerulein, exerted a stronger effect and comprised inhibition of myoelectric activity in the duodenal bulb and the duodenum, but not stimulatory changes, although the differences were not consistent.

Thus, it can be concluded that in sheep physiological doses of CCK peptides evoked an inhibitory effect on duodenal myoelectric activity. This effect was different in part from those on the duodenal bulb. Thus in sheep, as in monogastric species, CCK plays a major role in the control of this function. CCK might thus be considered as a physiological regulator of the duodenal motility in sheep.

References