Effect of cholecystokinin on myoelectric activity of small bowel of the dog

ARUN K. MUKHOPADHYAY, PIOTR J. THOR, EDWARD M. COPELAND, LEONARD R. JOHNSON, AND NORMAN W. WEISBRODT

Department of Physiology and Department of Surgery, University of Texas Medical School, Houston 77030, and Experimental Surgery Unit, University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77025

Mukhopadhyay, Arun K., Piotr J. Thor, Edward M. Copeland, Leonard R. Johnson, and Norman W. Weisbrodt. Effect of cholecystokinin on myoelectric activity of small bowel of the dog. Am. J. Physiol. 232(1): E44-E47, 1977 or Am. J. Physiol.: Endocrinol. Metab. Gastrointest. Physiol. 1(1): E44-E47, 1977.—The effect of cholecystokinin on the myoelectric activity of the small intestine was determined in conscious dogs. Six animals were implanted with electrodes along the small intestine, and a cannula was placed in the stomach. A second cannula was inserted into the duodenum in three animals, and a pancreatic fistula was prepared in three animals. Recordings were made in the fasted state, during the intravenous infusion of either saline or cholecystokinin-octapeptide (CCK-OP), during the intraduodenal infusion of either saline or L-tryptophan, and during the fed state. CCK-OP disrupted the fasted pattern of myoelectric activity, caused a dose-dependent increase in spike potentials, and caused a dose-dependent increase in pancreatic protein secretion. Stimulation of myoelectric activity occurred at doses that produced submaximal protein secretion; however, the stimulation was not identical to that seen with feeding. Intravenous infusion of L-tryptophan increased pancreatic protein secretion, interrupted the fasted pattern of motility, and induced a pattern similar to that seen with feeding. We conclude that CCK alters small intestinal motility and may play a role in the changes in small-bowel motility caused by the ingestion of food.

spike potential; motility; tryptophan

In recent years gastrointestinal hormones have been shown to influence gastrointestinal secretory functions (3, 4, 6, 7) and to alter bowel motility patterns (8, 11-13, 15, 18). In most of these studies, the hormones were administered by rapid intravenous injections or short-term infusions. Methods of separating pharmacological from physiological responses are not clearly established: nevertheless, prolonged intravenous infusion of relatively low doses of hormone is more physiologic than rapid intravenous bolus injection. We have shown previously (11, 18) that pentagastrin and secretin alter in vivo electrical activity of the small intestine of the dog. Infusion of pentagastrin interrupts the interdigestive myoelectric complex and produces a pattern similar to that seen with feeding (18). Infusion of secretin, on the other hand, inhibits the overall small intestinal contractile activity and the focus of origin of the interdigestive myoelectric complex (11). In addition, Ruckebusch and Fioramonti have found that insulin as well as pentagastrin can induce a fed pattern (13).

The purpose of the present investigation was to evaluate the role of cholecystokinin in the control of small intestinal motility in healthy, conscious dogs. Myoelectric activity of the small intestine was recorded during the intravenous administration of varying doses of cholecystokinin octapeptide (CCK-OP) and during the endogenous release of cholecystokinin as a result of the intraduodenal infusion of L-tryptophan.

METHODS

Experiments were performed in six mongrel dogs. Each dog was anesthetized with thiopental sodium, 20 mg/kg, and methoxyflurane. A laparotomy was performed under aseptic conditions in order to insert a Thomas cannula into the most dependent portion of the stomach in each animal and to insert a duodenal cannula in the anterior aspect of the second part of the duodenum in three of the animals. In the other three dogs, a pancreatic fistula was prepared as described by Herrera et al. (5). The fistula was drained by a cannula with a side arm that was inserted into the duodenum. The cannula was so constructed that when closed, all pancreatic secretions would flow into the duodenum, and when opened, pancreatic secretion could be collected externally. The cannula also had a second lumen for perfusion of the duodenum during collection of pancreatic secretion. Fourteen monopolar, silver wire electrodes were sewn on the serosal surface of the small bowel in all animals. The electrodes were spaced about 25 cm apart, between the gastroduodenal junction and the ileocecal junction. A coil of silver wire was placed subcutaneously in the right flank to serve as a reference electrode. Details of constructing and implanting similar electrodes have been reported (10). The recorded electrical potential changes represented potential differences between individual electrodes and the reference electrode. Recordings were made with an Offner type R-411 dynagraph through the RC input of 9806 A couplers at a time constant of 1 s. The high-frequency cutoff control was set at 100 Hz. Recordings were started 2 wk
after surgery. Data were obtained from conscious dogs which were either fasted for 24 h or fed 454 g of canned dog food (Prescription Diet, Riviana Foods, Topeka, Kan.) immediately prior to the recording.

**Exogenous administration of CCK-OP.** Recordings were taken from conscious dogs before any infusions were given. Ten minutes prior to recording from fasted dogs, the gastric and duodenal or pancreatic fistula cannulas were open. The stomach was rinsed with distilled water, and the cannulas were cleaned with a metal probe to insure patency. The gastric and duodenal cannulas were opened during all recordings from fasted dogs. They were closed when recording from fed dogs. The lumen of the cannula from the pancreatic fistula was open during all recording periods. A polyethylene catheter (PE-50) was inserted into a peripheral vein and was connected to a syringe adapted to fit an infusion pump (Harvard Apparatus). CCK-OP (Squibb Pharmaceutical Co., N.Y., SQ 19,844 batch NN005NA) was diluted with sodium chloride solution (154 meq/liter) and infused intravenously in doses of 125, 250, 500, and 1,000 ng/kg-h. Experiments with different doses were carried out on separate days. Each recording session lasted from 2 to 4 h.

**Duodenal infusion of L-tryptophan.** All recordings were performed on conscious fasted dogs. The gastric cannula was opened and the stomach was rinsed with distilled water. The duodenal cannula (or the lumen of the pancreatic cannula which entered the duodenum) was cleaned and connected to polyethylene tubing (ID 0.2, OD 0.4 cm) to facilitate intraduodenal infusion. The other end of the tubing was connected to a syringe adapted to fit an infusion pump. Initial recordings were obtained with intraduodenal infusion of saline (154 meq/liter) at a rate of 126 ml/h. Subsequently, L-tryptophan was infused at the same rate in a concentration to yield a dose of 2 or 4 mmol/h.

The recordings were analyzed to determine the temporal distribution of spike potential activity. For each animal, tracings from three electrodes (one from the proximal, one from the mid, and one from the distal small intestine) were analyzed. Each recording was divided into 2-min intervals, and the number of slow waves with superimposed spike potentials during each interval was determined for each of the three tracings chosen. This number was then divided by the total number of slow waves present during the same interval and expressed as a percent. The percentages for the 2-min intervals were combined for each condition, and the average percent and variance of the percentages determined. The patterns of spike potential activity under any two conditions were compared by determining the ratio of their variances (14). Patterns were considered different when the ratio between the variances of any two conditions exceeded the critical value when the probability level was 0.05 or less.

Secretions from the pancreatic fistula were collected during each 15-min interval of the experiment. The volume was recorded to the nearest 1.0 ml. Protein concentration in an aliquot of each sample was estimated by measuring the absorbance at 280 nm in a Spectronic 600 (Bausch & Lomb, Inc., Rochester, N.Y.) spectrophotometer with human serum albumin as a standard. Protein output was calculated and expressed as milligrams of protein secreted in 15 min. When recordings were made from fed animals, the remainder of the protein secretion was injected into the duodenum.

**RESULTS**

Figure 1 illustrates the effect of intravenous administration of 125 ng CCK-OP/kg-h on the myoelectric pattern of a fasted dog. CCK-OP infusion promptly increased the number of slow waves with superimposed spike potentials. This increase in spike potential activity occurred during the normally quiescent phase of the interdigestive myoelectric complex and was produced by all doses of cholecystokinin. Cessation of CCK-OP infusion was followed promptly by a decrease in spike potential activity. Progression of phase III of the interdigestive myoelectric complex from the proximal to the distal small bowel was uninterrupted by this dose, whereas higher doses abolished the complex.

A quantitative analysis of the alterations in myoelectric activity produced by administration of CCK-OP is presented in Table 1. The average number of slow waves with spike potentials was increased by CCK-OP over the number seen during the fasted state. The increase was progressively greater as the dose of CCK-OP was raised (Fig. 2). The percent activity induced by 500 ng/kg-h approached that seen during feeding. Although there was an increase in the percentage activity toward that seen with feeding, the pattern seen during CCK infusion was different from that seen with feeding. During infusion of CCK, there was considerable minute-to-minute variation as depicted in Fig. 1. Thus, the variance of the distribution of slow waves with spike potentials was approximately twice as great during infusion of CCK-OP (at all doses) compared to the fed state (Table 1), and the variance ratios between the fed state

**FIG. 1.** Temporal distribution of slow waves with spike potentials during fasting and CCK OP infusion. Tracings from 3 electrodes, 1 in proximal, 1 in mid, and 1 in distal small bowel. Percent of slow waves accompanied by spike potentials is indicated on vertical axes; time in minutes on horizontal axes. During time denoted by arrows, CCK-OP (125 ng/kg-h) was infused intravenously. Similar results were obtained with all animals.
TABLE 1. Intestinal myoelectric and pancreatic secretory responses of CCK-OP, L-tryptophan, and food

<table>
<thead>
<tr>
<th>Dose</th>
<th>% Slow Waves with Spike*</th>
<th>Variance</th>
<th>Protein Output</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasted</td>
<td></td>
<td>16</td>
<td>110</td>
</tr>
<tr>
<td>CCK-OP</td>
<td></td>
<td>20</td>
<td>83</td>
</tr>
<tr>
<td>125 ng/kg-h (iv)</td>
<td></td>
<td>26</td>
<td>80</td>
</tr>
<tr>
<td>250 ng/kg-h (iv)</td>
<td></td>
<td>35</td>
<td>102</td>
</tr>
<tr>
<td>500 ng/kg-h (iv)</td>
<td></td>
<td>555</td>
<td>143</td>
</tr>
<tr>
<td>1,000 ng/kg-h (iv)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Tryptophan</td>
<td></td>
<td>33</td>
<td>44</td>
</tr>
<tr>
<td>2-4 mmol/h (id)</td>
<td></td>
<td></td>
<td>188 ± 14</td>
</tr>
<tr>
<td>Food</td>
<td></td>
<td>37</td>
<td>56</td>
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<tr>
<td></td>
<td></td>
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<td>440 ± 14</td>
</tr>
</tbody>
</table>

* Each value is the mean of the 2-min intervals for two experiments in each animal for each condition. Each recording analyzed was at least of 90 min duration. † Values from two experiments on each of three animals are expressed as mean ± SE in milligrams protein per 15 min interval. ‡ CCK OP, 1,000 ng/kg-h, was given to only two of the animals.

FIG. 2. Average percent of slow waves with spike potentials recorded from intestine and protein output from pancreas during intravenous infusion of various doses of CCK-OP, during intraduodenal infusion of L-tryptophan, 2-4 mmol/h (TRP), and after feeding (FOOD). Explanations of data analyses are presented in footnotes for Table 1.

and the states produced by the infusion of CCK-OP (at all doses) were significantly different (P < 0.05).

Infusion of CCK-OP stimulated protein secretion from the pancreas. The increase in secretion was dose dependent with the largest dose, 500 ng/kg-h, yielding a secretory rate similar to that seen with feeding (Fig. 2, Table 1). Thus, CCK-OP infusion caused a parallel increase in secretory and spike potential activity.

Intraduodenal infusion of L-tryptophan at a dose of 2-4 mmol/h also influenced motility (Figs. 2, 3). Before infusion of L-tryptophan, saline (154 meq/liter) was infused intraduodenally at the same flow rate as L-tryptophan. Saline did not alter the fasted pattern of small intestinal myoelectric activity. Infusion of L-tryptophan increased the number of slow waves accompanied by spike potentials during the normally quiescent period and also interrupted the passage of phase III of the interdigestive myoelectric complex from the proximal to the distal small bowel. The onset of activity induced by L-tryptophan was slower and the effect was more sustained compared to the activity induced by intravenous administration of CCK-OP. Quantitative analysis (Table 1) indicated that infusion of L-tryptophan increased the percentage of slow waves accompanied by spike potentials. This increase was comparable to that seen with feeding and with the infusion of 500 ng/kg-h of CCK-OP. The pattern of activity produced by L-tryptophan was similar to that seen with feeding but different from that seen with infusion of CCK-OP. The ratio of the variances of the distributions of slow waves with spike potentials produced by L-tryptophan and feeding was not significant, whereas the ratio of the variances of the distributions during L-tryptophan infusion and CCK-OP infusion was significant (P < 0.05).

L-Tryptophan also increased pancreatic protein secretion (Fig. 2, Table 1). Although protein secretion was approximately twice basal during infusion of L-tryptophan, stimulated levels were approximately half that induced by feeding. Protein output stimulated by L-tryptophan was less than that produced by infusion of CCK-OP at a dose of 250 ng/kg-h. Thus, L-tryptophan had a greater effect on motility than on pancreatic secretion when compared to the effects of CCK-OP on the same parameters.

FIG. 3. Temporal distribution of slow waves with spike potentials during fasting and perfusion of small intestine with L-tryptophan. Animals used and electrode tracings analyzed were same as in Fig. 1. During time denoted between arrows, L-tryptophan (4 mmol/h) was infused into duodenum. Saline was infused during rest of experiment.

DISCUSSION

The physiological regulation of small-bowel motility is complex. No doubt both neural and humoral factors are involved. The present study demonstrated that CCK-OP had the capacity to stimulate spike potential activity in the small bowel of the fasted dog. Since slow waves accompanied by spike potentials are directly related to contractions of smooth muscle, it follows that CCK-OP stimulates small-bowel contractions. Whether the stimulation of contractile activity by CCK is physiological, and whether the increased activity seen with eating is due to release of CCK will remain unknown until a reliable radioimmunoassay of CCK is available. Only with direct measurement of serum CCK can one know that sufficient hormone is released to stimulate a particular target and that effective exogenous doses of
hormone do not produce serum levels exceeding the physiological range. We have shown that doses of CCK-OP which are far below those needed for maximal pancreatic enzyme secretion do cause alterations of small-bowel motility. Debas and Grossman (3) have reported that the D_{50} for the effect of CCK-OP on pancreatic secretion was 130 ng/kg-h. Although we did not quantitatively determine the D_{50} for pancreatic secretion in our experiments, our results are comparable. Since we observed an increase in spike potential activity at a dose of 125 ng/kg-h, the effect on motility occurred at a dose below the D_{50} for pancreatic secretion. If by this criterion CCK is considered a physiological regulator of gastric emptying (2), then it must also be concluded that it physiologically regulates small-bowel motor activity.

Although CCK-OP stimulated spike potential activity, the minute-to-minute variability of the pattern made it qualitatively different from the pattern produced by feeding since feeding resulted in a relatively constant myoelectric pattern. Intraluminal infusion of L-tryptophan resulted in a pattern of spike potential activity similar to that produced by feeding. Consequently, neural and hormonal mechanisms other than cholecystokinin release might have been involved in the response to tryptophan. A further indication of the involvement of other factors was the observation that, compared to CCK-OP, L-tryptophan had a greater effect on small-bowel motility than it did on pancreatic secretion.

The functional perspectives of small-bowel motility have undergone some changes since the original description of the "interdigestive myoelectric complex" in fasted dogs (16) and since the demonstration that the interdigestive pattern is abolished by feeding (9). Since the pattern does change with feeding, there must be a physiologic regulatory mechanism which controls the conversion from one motility pattern to the other. Gastrointestinal hormones may take part in this conversion, and it is probable that more than one hormone is involved. Pentagastrin (18) and insulin (13) convert the fasted pattern to one that resembles that seen with feeding, and secretin (11) inhibits the genesis of the interdigestive myoelectric complex and reduces the overall contractile activity of the small bowel. The maintenance of the fed pattern of activity under physiologic circumstances is probably the net result of stimulatory and inhibitory influences. Results of this experiment indicate that CCK may be one of the stimulatory agents.

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Present address of A. Mukhopadhyay: Dept. of Medicine, Baylor College of Medicine, Houston, Texas.

Present address of P. Thor: Institute of Physiology, Medical Academy, Krakow, Poland.

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