Mucosal mast cells are involved in CCK disruption of MMC in the rat intestine

MARISABEL MOURELLE, AND PATRI VERGARA
Department of Cell Biology and Physiology, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain

Juanola, Carme, Magda Giralt, Marcel Jiménez, Marisabel Mourelle, and Patri Vergara. Mucosal mast cells are involved in CCK disruption of MMC in the rat intestine. Am. J. Physiol. 275 (Gastrointest. Liver Physiol. 38): G63–G67, 1998.—Our aim was to determine if mucosal mast cells could be activated by endogenous CCK and, as a consequence, mediate CCK actions in the small intestine. Rats were prepared for electromyography to record electrical activity in the small intestine. In another group of animals, the duodenum was perfused to measure rat mast cell protease II (RMCP II) as indicative of mast cell degranulation. Endogenous CCK release was induced by administration of soybean trypsin inhibitor (SBTI) in conscious rats or by intraduodenal perfusion of ovalbumin hydrolysate (OVH) in anesthetized rats. CCK concentration was measured by bioassay on pancreatic acini. SBTI in control rats disrupted migrating motor complexes (MMC) for >40 min. In rats treated with the mast cell stabilizer ketotifen, SBTI did not induce any change in the MMC pattern. RMCP II concentration in the duodenal perfusate significantly increased after OVH. Perfusate from ketotifen-treated animals did not show any significant increase in RMCP II values during OVH perfusion, although CCK plasma concentration was not different from the control group. Furthermore, infusion of the CCK-B receptor antagonist L-365,260 significantly blocked the increase of RMCP II values during OVH. Our results indicate that mucosal mast cells participate in CCK intestinal actions.

**MATERIALS AND METHODS**

Animals. All experimental protocols were carried out under the supervision and regulations of the Ethics Committee of the Universitat Autònoma de Barcelona. In this study, male Sprague-Dawley rats, 8–10 wk old and weighing 300–350 g, were used. Animals had free access to water and a standard pellet diet containing (% total weight) 17.62% protein, 43.30% starch, and 2.50% fat (A-04 Panlab, Barcelona, Spain). Rats were kept in an environmentally controlled room in groups of 3–4 animals before surgery and individually after surgery and during ketotifen treatment.

Electromyography studies. Rats were surgically prepared for chronic electromyography (EMG) in aseptic conditions and under general anesthesia with Pentalath Sodium (35–40 mg/kg ip). Preamesthesia treatment consisted of atropine (0.1 mg/kg ip) and diazepam (3–5 mg/kg ip). Three pairs of electrodes (Ni/Cr, 80:20; 120-µm diameter; Microfil Industries, Lausanne, Switzerland) were implanted in the small intestine, as described previously (23, 25). The first electrode was placed 2 cm caudal to Treitz’s ligament and the second and third were implanted at 10 and 20 cm caudal to the first electrode, respectively. Electrodes were folded and fixed to the back of the animal to allow free movement in the cage.
the surgical procedure, rats were treated with antibiotic (penicillin G benzathine) and buprenorphine for 48 h. Electrode position was verified at the necropsy.

EMG recordings started 9 days after surgery, when rats had totally recovered and exhibited clear MMC. Electrodes were unfolded from the back of the animal and protected with a rubber tube that allowed the animal to move freely in the cage throughout the experiment. Electrodes were connected to the recording system. Before the experiment, rats were fasted overnight (12–16 h). After 60 min of recording a clear MMC pattern, we administered soybean trypsin inhibitor (SBTI) through an intragastric cannula to induce CCK release. Recordings lasted for at least another 60 min after treatment.

Plasma CCK measurement. CCK concentration was measured using a previously described technique (14). Briefly, the extracts of plasma obtained after 15 min of duodenal perfusion of either buffer saline or ovalbumin hydrolysate were passed through Sep-Pak cartridges and then tested for their CCK content, based on their ability to stimulate amylase release from isolated pancreatic acini. The specificity of the method was assessed by the blockade of the induced amylase release by the CCK-A receptor antagonist L-364,718. In addition, CCK concentration was measured after SBTI oral administration in conscious animals to compare our results with previous findings (15).

Perfusion studies. Rats were anesthetized by inhalation of halothane to allow cannulation with a polyethylene tubing of the right jugular vein. Stage III anesthesia was maintained with Pentothal Sodium bolus infusions in the jugular vein as required. The intestine was exposed through an abdominal midline incision, and polyvinyl tubing was inserted in the duodenal lumen by an incision in the stomach wall and passed through the pylorus. Another plastic tube was inserted at the end of the duodenum next to Treitz’s ligament, to allow drainage and perfusate collection. The total length of the duodenum exposed to perfusion was 7–8 cm. After surgery, the abdomen was covered with a gauze soaked in liquid Vaseline to avoid tissue dryness. Animals were killed at the end of the experiment with an overdose of anesthetic.

Rat mast cell protease II (RMCP II) concentration in the duodenal perfusate was measured by ELISA. A monoclonal antibody against RMCP II raised in mouse was diluted to a concentration of 1 μg/ml in 0.1 M carbonate buffer (pH 9.6). Coated plates were incubated with the antibody at 4°C for 21–24 h before use. A 30-min incubation with 4% (wt/vol) BSA was done before loading standard and unknown samples to avoid unspecific reaction. The sample incubation was 1 h at room temperature. A sheep anti-RMCP II and horseradish peroxidase conjugate was added afterward and incubated for 1 h. Plates were developed using o-phenylenediamine as substrate and read at 492 nm after reaction was stopped. RMCP II concentration was quantified against RMCP II standard curve. All these products were obtained from More
dun Animal Health (Edinburgh, UK).

Substances. The mast cell membrane stabilizer ketotifen, dissolved in drinking water (0.075 mg/ml), SBTI (type II-S), dissolved in distilled water containing carboxymethyl cellulose (0.4%), and albumin chicken egg hydrolysate, dissolved in buffer saline solution (5% wt/vol), were obtained from Sigma Chemical (St. Louis, MO). CCK-8, dissolved to 10−4 M in 1% bicarbonate solution and further diluted in saline solution, was obtained from Peptide Institute (Osaka, Japan). The CCK-A receptor antagonist L-364,718 and the CCK-B receptor antagonist L-365,260 were generously provided by Merck Sharp & Dohme. Antagonists were diluted at 3 mM in ethanol, then to 0.3 mM in 10% DM SO and further diluted in saline solution.

Experimental protocols. EMG and perfusion studies as well as CCK bioassay were done in both control and ketotifen-treated rats. In treated animals, ketotifen (10 mg·kg−1·day−1) was given to each individually caged rat in drinking water for 48 h before the experiment. The amount of ketotifen ingested by each rat was controlled daily. Only those animals that ingested ≥10 mg·kg−1·day−1 were included in the experiment.

In perfusion studies buffer saline was perfused into the duodenum at a rate of 12 ml/h. After 30 min this saline perfusion was changed by the ovalbumin hydrolysate solution for 1 h to induce endogenous secretion of CCK. Perfusate was collected in ice-chilled tubes at 15-min intervals for RMCP II analysis. To evaluate CCK involvement on mast cell activation, CCK antagonists were infused intravenously in another series of perfusion studies. In this case, the antagonist was given as a bolus of 3 × 10−7 mol/kg plus an infusion from the beginning to the end of the experiment of a solution of 2 × 10−7 mol·kg−1·h−1. In another group of animals, CCK-8 at a concentration of 3 × 10−9 mol·kg−1·h−1 was infused for 1 h.

Data are presented as means ± SE (expressed as ng/ml of RMCP II or pM concentration of CCK). Statistical analysis for significant differences was performed according to ANOVA for paired data in RMCP II results and unpaired data in CCK bioassay, followed by a post hoc Bonferroni test. Differences were considered significant when P < 0.05. MMC disruption was evaluated visually from EMG recordings.

RESULTS

Intestinal motor patterns. Fasting rats showed an organized pattern of MMC with a duration of 14.3 ± 1.8 min (at electrode E2) as described previously (22, 24). Oral administration of SBTI disrupted the MMC pattern, causing an irregular spiking activity similar to the postprandial state that lasted >40 min (n = 5) (Fig. 1A). Ketotifen-treated rats showed a regular MMC pattern during fasting. The mean duration of the MMC did not differ from the control group (13.0 ± 1.5 min). In five of six ketotifen-treated rats, SBTI did not disrupt the fasting MMC pattern (Fig. 1B). The mean duration of these MMC after SBTI was 12.5 ± 0.6 min, similar to those before SBTI infusion.

Release of endogenous CCK. Previous to perfusion studies we measured CCK concentration during duodenal perfusion of SBTI. The results (data not shown) did not reveal a significant increase of CCK in plasma. Consequently, we checked our assay by measuring CCK concentration both during fasting and after oral administration of SBTI as described previously by Liddle et al. (15). We found results similar to those already reported [1.8 ± 0.3 pM (n = 3) during fasting and 1.9 ± 2.6 pM (n = 5) 7 min after SBTI oral administration]. Because SBTI seemed unable to release CCK in our anesthetized model, we tested CCK release after infusion of ovalbumin hydrolysate. This substance had been reported to induce the highest CCK release in an ex vivo model (4). Perfusion of the duodenum with saline solution gave CCK concentration values similar to those reported during fasting (2.6 ± 0.3 pM; n = 7). During perfusion with ovalbumin hydrolysate, CCK concentration reached 10.4 ± 0.9 pM (n = 6). Conse-
quently, we used ovalbumin hydrolysate instead of SBTI to induce CCK release in the perfusion experiments.

In ketotifen-treated rats, CCK concentrations were not different from the control rats (4.01 ± 0.7 pM (n = 6) during buffer saline perfusion and 11.2 ± 2.7 pM (n = 7) during ovalbumin hydrolysate).

Mast cell activation measured by RMCP II concentration. Basal concentration of RMCP II in the duodenal perfusate was 10.2 ± 1.1 ng/ml. RMCP II concentration significantly increased to 21.6 ± 4.6 ng/ml (n = 5) during ovalbumin hydrolysate perfusion (Fig. 2). In ketotifen-treated animals, there was no difference between basal and peptide hydrolysate perfusion (n = 4) (Fig. 2).

CCK involvement in mast cell activation. Intravenous infusion of either L-364,718 or L-365,260 did not modify basal concentration of RMCP II. The CCK-A receptor antagonist L-364,718 did not block the increase in RMCP II after ovalbumin hydrolysate perfusion (21.05 ± 1.13 vs. 10.10 ± 0.59 ng/ml during basal conditions; n = 4, P < 0.05). In contrast, the CCK-B receptor antagonist L-365,260 significantly reduced the increase in RMCP II concentration observed after ovalbumin hydrolysate perfusion (n = 5) (Fig. 3). However, a slight increase in RMCP II concentration was still observed during peptide hydrolysate perfusion in the presence of L-365,260 (from 9.41 ± 0.40 ng/ml in basal conditions to 12.44 ± 1.23 ng/ml during ovalbumin hydrolysate perfusion). Infusion of CCK-8 at a dose of $3 \times 10^{-9}$ mol·kg$^{-1}$·h$^{-1}$ slightly increased RMCP II concentration from 7.2 ± 0.3 to 10.6 ± 1.7 ng/ml (n = 4).

**DISCUSSION**

This study demonstrates that degranulation of mast cells is involved in the disruption of MMC by endog-
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Fig. 3. RMCP II concentration (ng/ml) both in basal conditions and after OVH duodenal perfusion in the presence of CCK receptor antagonists L-364,718 and L-365,260 given intravenously. Bars are means ± SE. *P < 0.05 vs. OVH response in control. +P < 0.05 vs. respective OVH response.

In addition, CCK-B receptors are involved in mucosal mast cell activation. We intend to correlate these findings with the CCK control mechanisms of intestinal motor patterns. Ingested protein is the major dietary stimulant of CCK release in rats (15), although other nutrients, such as lipids, might also contribute to postprandial CCK levels (8). A complex mechanism involving a pancreatic monitor peptide and an intestinal CCK-releasing factor has been described previously (13). In conscious rats, trypsin inhibitors such as SBTI are potent stimulants of CCK release (15). We corroborated this finding in conscious animals, but in our perfused model SBTI was unable to increase CCK plasma concentration. In contrast, albumin hydrolysate significantly increased CCK concentration in this model. These results indicate that ovalbumin hydrolysate is a strong releaser of endogenous CCK in this model. This finding suggests that the experimental model may modify CCK-releasing mechanisms. The fact that our results are similar to those found in isolated duodenal segments (23) indicates that either the surgical procedure or, more likely, the constant washout of the intestinal content by perfusion is responsible for the lack of effect of SBTI in these conditions. Because our objective was to induce endogenous CCK release in both experimental protocols, we used SBTI in conscious animals and ovalbumin hydrolysate in perfusion studies with the animal anesthetized to ensure maximal induction of CCK release in each protocol. The concordance of the results obtained in both types of experiments strengthen, in our opinion, the conclusions of this study.

Recently, we demonstrated that postprandial disruption of the MMC pattern is a complex reflex induced by endogenously released CCK through peripheral CCK-B receptors, in which vagal afferents are also implicated (23, 24). However, despite the existence of CCK-B receptors in the rat vagus nerves (3, 18), only responses mediated by CCK-A receptors have been localized in vagal afferents (5, 20). Therefore, an intermediate step in CCK actions should be considered, particularly as it has been demonstrated that vagal afferents respond to mast cell products, such as 5-hydroxytryptamine (5-HT), and that CCK responses are reduced by 5-HT antagonists (2, 5, 12). Moreover, in the small intestine of the rat, vagal afferents are in very close proximity to mucosal mast cells (27). Finally, it has been demonstrated that mucosal mast cells respond to gastrin (19). All these precedent data led us to consider the hypothesis that CCK could modify mast cell activity and that maybe a secretory product of mast cells could be responsible for modifying vagal afferent activity.

As a first step, we focused on the study of the possible correlation between CCK release and mast cell degranulation. The finding that SBTI was ineffective in ketotifen-treated rats means that MMC disruption induced by endogenous CCK release (23, 24) can be blocked by stabilization of mast cell membranes. Furthermore, by means of perfusion studies we have demonstrated that CCK degranulates mucosal mast cells. This action is mediated by CCK-B receptors, allowing us to suggest that CCK-B receptors responsible for MMC disruption by endogenous CCK could be located in mucosal mast cells.

Other evidence reinforces this conclusion. RMCP II is a specific marker of mucosal mast cell degranulation (17). The fact that the CCK-A receptor antagonist was inefficient also suggests that the mechanism might be specific for postprandial disruption of the MMC pattern and not for other physiological actions mediated by CCK-A receptors (11, 20).

Although there is controversy regarding the specificity of ketotifen to stabilize mucosal mast cell membranes (6, 7, 10), the fact that in ketotifen-treated rats there was not an increase in the specific mucosal mast cell enzyme RMCP II indicates that ketotifen stabilizes rat mucosal mast cells in our conditions. It has been also suggested that ketotifen might have other nonspecific actions (1). Moreover, ketotifen did not modify CCK concentration in either of the two protocols, indicating that it did not modify endocrine cells producing CCK and that the results we observed are consequences of its action on mucosal mast cells.

Mucosal mast cells are very abundant along the whole gastrointestinal tract, but the duodenum shows a higher concentration of cells, together with the stomach and ileum (16). Endocrine type I cells responsible for secreting CCK are also concentrated at the duodenum (22).

Exogenous infusion of CCK-8 induced only a slight mast cell degranulation at the same concentration that induced MMC disruption (23). However, there are several examples in which CCK-8 does not reproduce endogenous CCK actions. For instance, peripheral CCK-A receptors are involved in CCK-8 disruption of MMC, whereas only peripheral CCK-B receptors are involved in postprandial disruption of MMC in the rat (23). A paracrine effect of CCK on afferent fibers has been suggested as the mechanism of action of CCK to
control both small intestinal motility (24) and pancreas secretion (11). Recently, it has been demonstrated that large forms of CCK (CCK-58), predominantly in the intestine of several species, have less affinity for CCK-A receptors than CCK-8 (21), which could explain differences between endogenous CCK and exogenous CCK-8 actions. The release of a large form of CCK in the intestine might be responsible for mast cell degranulation.

In conclusion, mast cell degranulation is involved in the mechanism of action of endogenous CCK controlling the MMC disruption and thus mast cells are actively involved in the physiological control of intestinal motility. This relationship may aid understanding of the mechanisms responsible for motor disorders resulting from inflammatory disease in the gastrointestinal tract.

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Address for reprint requests: P. Vergara, Unidad de Fisiología, Facultad de Veterinaria, Universitat Autònoma de Barcelona, 08193, Bellaterra, Barcelona, Spain.

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