Lingual Factors Enhance the Increase of Ornithine Decarboxylase Activity in Rat Jejunal Mucosa After Feeding

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Luminal nutrients are the main factors that stimulate ornithine decarboxylase (ODC) activity in rat intestinal mucosa following feeding. The aim of the present study was to determine whether lingual (oral) factors are related to the increase in jejunal ODC activity after feeding. ODC activity in the jejunum and liver was measured 3 hours after refeeding of 48-hour fasted rats. In the first experiment, rats were refed with a regular pellet, powder, or liquid diet. In the second experiment, rats were infused with the liquid diet through a gastric infusion tube following 48 hours' fasting. In the third experiment, the experimental rats had a gastric fistula that allowed free drainage from the stomach of all ingested liquid diet. In the fourth experiment, a truncal vagotomy was performed 1 week before the experiment. The increase of ODC activity in the jejunum of rats fed with the liquid diet was less than that of rats fed with the pellet diet or powder diet. The increase of ODC activity in the jejunal mucosa of rats infused through the gastric tube was less than that of rats fed per os, and the increase of ODC activity in the liver did not differ between these experimental groups. ODC activity did not increase in rats with a gastric fistula. Vagotomy did not affect the increase of jejunal ODC activity after feeding. In conclusion, the increase of ODC activity after feeding was attenuated in rats in which the diet was given by bypassing the mouth. This indicates that lingual factors enhance the increase of ODC activity in the jejunal mucosa after feeding, but the lingual factors alone do not increase ODC activity in the jejunum.

ORNITHINE DECARBOXYLASE (ODC), a first rate-limiting enzyme for polyamine synthesis, is important for mucosal growth of the small intestine. ODC activity in the small intestine is stimulated by many factors, including luminal nutrients,[1-3] epidermal growth factor,[4-7] insulin,[2] gut peptides,[8,9] histamine,[10-12] and intestinal mucosal injury.[7,13-15] This indicates that local factors in the intestinal mucosa are important for the increase in ODC activity.

Previous reports demonstrated that feeding is one of the most potent stimulant factors for ODC activity in the small intestine.[1-3,16,17] It has been demonstrated that local factors in the intestinal mucosa are important for the mechanism of the increase in ODC activity after feeding, since luminal nutrients stimulate intestinal ODC activity markedly.[1-3] We have previously demonstrated that the increase of ODC activity in the intestinal mucosa is due, in part, to a signal from the central nervous system,[18-20] which suggests that the increase in ODC activity after feeding may be stimulated by factors other than local nutrient factors. In the present experiments, we aimed to demonstrate whether lingual factors, in addition to nutrient factors, are important for the increase in intestinal ODC activity following feeding.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (280 to 330 g) were used in this study. They were housed in wire-bottomed cages placed in a room illuminated from 8 AM to 8 PM (12-hour light-dark cycle) and maintained at 21° ± 1°C. The rats were allowed free access to a standard rat pellet diet (3.4 kcal/g, caloric ratio of protein:lipid:carbohydrate = 29.4:11.8:58.5; Clea, Tokyo, Japan), standard rat powder diet (3.4 kcal/g, caloric ratio of protein:lipid:carbohydrate = 29.4:11.8:8.58.5; Clea), or standard rat liquid diet (1.0 kcal/mL, caloric ratio of protein:lipid:carbohydrate = 28.5:19.4:52.1; Oriental Yeast, Tokyo, Japan).

Collection of Intestinal Mucosa and Liver for Determination of ODC Activity

ODC activity of the intestinal mucosa and liver was measured. The animals were anesthetized under halothane anesthesia and then euthanized by exsanguination. The liver and intestine were separated. The small intestine was divided into four equal segments, the proximal 5 cm of the second intestinal segment being the jejunum. Mucosa from the jejunum was obtained by scraping with a glass slide over an ice-cold glass plate. ODC activity was measured immediately after this step.

ODC Assay

ODC activity was assayed by a radiometric technique in which the amount of $^{14}CO_2$ liberated from L-[1-^{14}C]ornithine (52.3 mCi/mmol; New England Nuclear, Boston, MA) was measured.[3] Either mucosal scrapings (200 mg) or samples of liver (200 mg) were placed in 2 mL 0.1-mol/L Tris hydrochloride buffer (pH 7.4) containing 1 mmol/L EDTA, 50 μmol/L pyridoxal 5-phosphate, and 5 mmol/L dithiothreitol. The tissues were homogenized twice with a Polytron tissue homogenizer for 15 seconds and centrifuged at 30,000 × *g* for 30 minutes. Protein content was 1 to 2 mg in a 200-μL aliquot of the supernatant, which was incubated in stoppered vials in the presence of 3.5 nmol L-[1-^{14}C]ornithine for 15 minutes at 37°C. $^{14}CO_2$ liberated by the decarboxylation of ornithine was trapped on a piece of filter paper impregnated with 20 μL 2N NaOH, which was suspended above the reaction mixture. The reaction was terminated by addition of 0.3 mL 10% trichloroacetic acid. Radioactivity of $^{14}CO_2$ trapped in the filter paper was measured in an aqueous miscible scintillant (Opti-Fluor; Packard Instrument, Downers Grove, IL). The samples were counted for 5 minutes in a liquid scintillation spectrometer (460 CD; Packard Instrument). Results are expressed as picomoles of CO_2 produced per milligram protein per hour.

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Experiment 1: ODC Activity Following Refeeding With the Pellet, Powder, or Liquid Diet

Procedure. Rats were fasted for 48 hours before the experiment and then refed for 30 minutes at 10 AM. Food consumption during the 30 minutes was measured. ODC activity was measured 3 hours after refeeding at 1 PM. ODC activity was measured in rats refed with the pellet, powder, or liquid diet. Control rats were fasted throughout the experiment. Ten rats were examined from each group tested.

Experiment 2: ODC Activity in Rats Fed Through a Gastric Tube

Surgery. After 24 hours of fasting, a silicon infusion tube was introduced into the stomach through the fundus. Postoperatively, the animals were infused through the gastric tube at 3 mL/h with a saline solution containing 145 mmol/L NaCl and 4 mmol/L KCl while in restraining cages.

Procedure. After 24 hours of recovery following surgery, the rats were refed at 10 AM. The rats were fasted for 48 hours (24 hours before and 24 hours after surgery). Rats in the first group were infused with 15.0 mL liquid diet for 30 minutes through the gastric tube. The amount of the liquid diet infused through the gastric tube was determined by results of the first experiment. Rats in the second group were refed orally by free access to the liquid diet for 30 minutes. Control rats were infused with saline for 30 minutes through the gastric tube. ODC activity was measured 3 hours after refeeding at 1 PM. Ten rats were tested from each group.

Experiment 3: ODC Activity in Rats With a Gastric Fistula

Surgery. Under halothane anesthesia, a cannula was implanted in the anterior wall of the forestomach just above the transverse ridge, according to the method previously described.[21-23] The cannula allowed free drainage from the stomach of all ingested liquid diet when it was opened during the experiment. After surgery, rats were allowed to recover for 5 days with the gastric cannula closed, to allow free access to the liquid diet. By testing time, the body weight of the rats had returned to normal.

Procedure. After 48 hours of fasting, rats were refed with the liquid diet for 30 minutes at 10 AM. During the refeeding period, the gastric cannula was opened in the experimental rats to drain the ingested liquid diet. We ascertained that all ingested liquid diet was drained from the gastric fistula during the refeeding period. The gastric cannula in the control rats was closed during the refeeding period. ODC activity was measured 3 hours after refeeding at 1 PM. Seven rats were tested from each group.

Experiment 4: ODC Activity in Truncally Vagotomized Rats After Refeeding

Surgery. Truncal vagotomy was performed under halothane anesthesia as previously described.[18,24,25] The esophagus was gently lifted just below the liver and diaphragm, and the mesentery and visible vagal fibers were cut within 2 cm of the esophagus. Transection of the vagal fibers on both sides was microscopically verified in each rat after the experiment. In the sham animals, vagal fibers were isolated in a similar fashion but were not cut. Surgery was performed at least 1 week before testing. By testing time, the body weight of the rats had returned to normal.

Procedure. Vagotomized rats or sham-treated rats were refed with the pellet diet or liquid diet for 30 minutes at 10 AM. Food consumption during the 30 minutes was measured. ODC activity was measured 3 hours after refeeding at 1 PM. Six rats were tested from each group.

Statistics

Results are expressed as the mean ± SE. All data were evaluated by Student's t test or by one-way ANOVA in which multiple comparisons were made with the method of least-significant difference.[26] Differences were considered significant if their probability of chance occurrence was less than five in 100 ($P < .05$).

RESULTS

Experiment 1: ODC Activity Following Refeeding With the Pellet, Powder, or Liquid Diet

The amount of food ingested during the 30-minute refeeding period was 15.3 ± 0.8 kcal in rats refed with pellet diet, 16.3 ± 0.6 kcal in rats refed with powder diet, and 15.4 ± 1.3 kcal in rats refed with liquid diet. Food consumption during the 30 minutes did not differ among rats refed with the pellet, powder, or liquid diet. ODC activity in the jejunal mucosa and liver is shown in Table 1. ODC activity in jejunal mucosa increased markedly 3 hours after refeeding in rats refed with any of the diets compared with fasted rats ($P < .01$ for each). However, the increase of jejunal ODC activity in rats refed with the liquid diet was less than that in rats refed with the pellet or powder diet ($P < .05$ for each), whereas no differences in food consumption during the refeeding period were seen among the groups tested. ODC activity in the liver increased significantly after refeeding ($P < .01$ for each), and the increase did not differ among the groups refed with any of three diets.

Experiment 2: ODC Activity in Rats Fed Through a Gastric Tube

Rats with a gastric cannula were infused with 15 mL liquid diet, which was determined by results of the first experiment. ODC activity of the jejunal mucosa in rats infused with the liquid diet through the gastric tube increased significantly compared with the activity in fasting controls, whereas this increase was significantly lower than that in rats refed for 30 minutes. The rats consumed 14.6 ± 1.6 mL liquid diet during the refeeding period. These results indicate that the increase of ODC activity in the jejunum after refeeding was attenuated when the diet was given by bypassing the mouth. In contrast to the jejunum, the increase of ODC activity in the liver did not differ between the two groups (Table 2).

Experiment 3: ODC Activity in Rats With a Gastric Fistula

ODC activity in the jejunal mucosa increased markedly 3 hours after refeeding when the gastric cannula was closed

Table 1. ODC Activity (pmol CO_2/mg protein) in Jejunal Mucosa and Liver 3 Hours After Refeeding in 48-Hour Fasted Rats

Site	Fasting Controls	Rats Fed Diet		
		Pellet	Powder	Liquid
Jejunal mucosa	1.8 ± 0.6	142.2 ± 15.3*†	132.7 ± 17.7*†	98.2 ± 8.9*
Liver	1.1 ± 0.5	12.1 ± 1.7*	14.5 ± 2.3*	15.3 ± 2.1*

NOTE. Values are the mean ± SE.

*$P < .01$ v fasting control.

†$P < .05$ v rats refed with liquid diet.

Table 2. ODC Activity (pmol CO_2/mg protein) in Jejunal Mucosa and Liver of Rats Infused With Liquid Diet Per Gastric Tube (15 kcal/30 min) Versus Rats Refed With Liquid Diet

Site	Fasting Controls	Rats Fed Per Gastric Tube	Rats Fed Per Os
Jejunal mucosa	2.1 ± 0.4	38.8 ± 5.0*†	91.6 ± 5.9*
Liver	0.9 ± 0.5	13.9 ± 2.2*	14.8 ± 2.1*

NOTE. Values are the mean ± SE.
*$P < .01$ *v* fasting control.
†$P < .01$ *v* per os.

($P < .01$ *v* fasting controls). In contrast, ODC activity did not increase at all in refed rats compared with fasting controls when the gastric cannula was opened to drain all the ingested liquid diet from the gastric fistula. These results indicate that the oral factors themselves had no effect on ODC activity in the jejunal mucosa (Table 3).

Experiment 4: ODC Activity in Truncally Vagotomized Rats After Refeeding

ODC activity increased in rats refed with the pellet or liquid diet after sham-vagotomy (pellet diet, 140.5 ± 22.2 pmol CO_2/mg protein; liquid diet, 83.6 ± 14.4 pmol CO_2/mg protein). Truncal vagotomy had no effect on jejunal ODC activity (pellet diet, 148.2 ± 20.1 pmol CO_2/mg protein; liquid diet, 91.1 ± 11.3 pmol CO_2/mg protein). This indicates that lingual factors that enhanced the stimulatory effect of the diet on jejunal ODC activity were not mediated through the vagal nerve.

DISCUSSION

In the second experiment of this study, we demonstrated that the increase of ODC activity in jejunal mucosa after infusion of diet through the gastric tube, bypassing the mouth, was less than the increase seen after refeeding per os, although the amount of diet consumed was the same in both conditions. This result indicates that lingual (oral) factors, in addition to nutrient factors, are important for enhancing the increase of ODC activity in the jejunum after feeding. A previous study[27] demonstrated that the influence of lingual factors on rat feeding behavior differed between rats fed with hard and soft diets, which is compatible with the results of our first experiment, whereby stimulation of jejunal ODC activity by lingual factors differed between rats fed with liquid diet and those fed with the other two diets. In contrast to the jejunum, lingual factors had no influence on ODC activity in the liver, indicating that the increase of ODC activity in the liver after feeding depended on the nutrients ingested.

Proprioceptive sensation from the oral cavity (lingual factors) regulates feeding behavior in rats through the central nervous system, including the hypothalamus.[27] Anatomically, the sensation from the oral cavity is transferred to the hypothalamus via the mesencephalic trigeminal nucleus.[28,29] The hypothalamic nuclei related to feeding behavior, including the lateral hypothalamus, send stimulatory signals for ODC activity in rat intestinal mucosa.[18-20] These facts, together with the results of the present experiments, suggest that lingual factors enhanced the postprandial increase in jejunal ODC activity through the central nervous system, including the hypothalamus.

Table 3. ODC Activity (pmol CO_2/mg protein) in Jejunal Mucosa of Rats With a Gastric Fistula 3 Hours After Refeeding With Liquid Diet

	Rats With a Gastric Fistula	
Fasting Controls	Closed	Open
2.4 ± 0.5	85.5 ± 14.5*	2.6 ± 0.8

NOTE. Values are the mean ± SE.
*$P < .01$ *v* fasting control.

The hypothalamus controls vagally mediated visceral functions such as gastric acid secretion, insulin secretion, and hepatic enzyme activity through direct neuronal connection to the dorsal motor nucleus of the vagal nerve.[30-32] We previously demonstrated that signals from the lateral hypothalamus stimulate ODC activity in small-intestinal mucosa, and that the signals are mediated, at least in part, through the vagal nerve.[18-20] It was demonstrated that stimulatory signals from the central nervous system for DNA synthesis in the small intestine are mediated through the vagal nerve in rats with ventromedial hypothalamus lesions.[33] These facts allow the hypothesis that lingual factors for the increase of jejunal ODC activity may be mediated through the vagal nerve. However, in the fourth experiment, we demonstrated that lingual factors that enhance the increase of jejunal ODC activity after feeding are not mediated through the vagal nerve. Insulin secretion induced by oral sensory stimulation through the central nervous system is well known as cephalic-phase insulin secretion.[34,35] Insulin is also known to induce ODC activity in the intestine.[2] The synthesis of epidermal growth factor in the salivary gland and/or submaxillary gland stimulates ODC activity in the small intestine.[4-7,36,37] These factors are candidates for lingual factors that enhance jejunal ODC activity, but determining the precise mechanism of enhancement of jejunal ODC activity by these factors requires further exploration.

The findings of the third experiment, ie, ODC activity in the jejunum did not increase after refeeding of the rats with a gastric fistula to drain all ingested liquid diet, demonstrated that the lingual factors themselves did not stimulate ODC activity in the jejunum after feeding. This result is compatible with the results of a previous report that the increase in ODC activity was attenuated in the bypassed jejunum, which did not make contact with ingested food after feeding.[3]

It is well known that feeding is the most potent stimulus for the increase of ODC activity in the intestinal mucosa, and that luminal nutrients are important for this increase.[1,38] This study indicates that lingual factors enhance the increase of ODC activity induced by luminal nutrients in rat jejunal mucosa after feeding, whereas the lingual factors themselves do not stimulate the enzyme activity.

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