# Histamine Effect on Ornithine Decarboxylase of Rat Intestine in Cases of Ischemia-Reperfusion Compared with Refeeding (43873)

TAKANORI KOYAMA,* KAZUMA FUJIMOTO,*,[1] MASAHIRO KANG,† HIRONOBU YOSHIMATSU,† TOSHIIE SAKATA,† SEIJI TSUNADA,* HIDESUKE MORITA,* RYUICHI IWAKIRI,* TAKAHIRO SAKAI*

*Division of Gastroenterology,* Department of Internal Medicine, Saga Medical School, Saga 849, Japan and Department of Internal Medicine I,† Oita Medical College, Oita 879-55, Japan*

*Abstract.* **Our previous study suggested that histamine might enhance the increase of ornithine decarboxylase activity in injured intestinal mucosa. To test this hypothesis, we measured histamine content in mesenteric lymph and ornithine decarboxylase activity in intestinal mucosa after ischemia-reperfusion in the rat. We examined the effect of α-fluoromethylhistidine, a suicide inhibitor of histidine decarboxylase, on ornithine decarboxylase activity after ischemia-reperfusion and compared this with its effect on the rat after refeeding. Ischemia-reperfusion was performed by 15-min occlusion of the superior mesenteric artery. After ischemia-reperfusion, histamine content in mesenteric lymph increased, and this increase was completely suppressed by α-fluoromethylhistidine pretreatment. In contrast to ischemia-reperfusion, histamine content in mesenteric lymph did not change after refeeding. Ornithine decarboxylase activity increased markedly 3 and 6 hr after ischemia-reperfusion and refeeding, whereas α-fluoromethylhistidine attenuated the increase in ornithine decarboxylase activity only in the ischemia-reperfusion group. These results indicate that increase in histamine synthesis in the intestinal mucosa plays an important role in the increase of ornithine decarboxylase activity after ischemia-reperfusion but that histamine is not related to the increase in ornithine decarboxylase activity after refeeding.**
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Polyamines are necessary for normal cell growth in gastrointestinal mucosa (1, 2). In the biosynthesis of polyamines, ornithine decarboxylase (ODC) is the first rate-limiting enzyme that catalyzes the formation of putrescine, a precursor of other polyamines (1, 3–5). ODC activity in the small intestine increases markedly in response to a number of physiological factors, such as feeding (6, 7), insulin (6), epidermal growth factor (8–11), adaptation to lactation (12), and circadian rhythm (13, 14). These elevations in mucosal ODC activity are associated with increased mucosal growth of the small intestine (1, 2).

It has also been demonstrated that intestinal ODC activity increases markedly when the intestinal mucosa is injured by ischemia-reperfusion (I/R) (15), arabinosylcytosine (2), methotrexate (11), or stress (16). The increase of ODC activity under these pathophysiological situations enhances restoration of injured intestinal mucosa (1, 2, 11, 15, 16).

We have previously demonstrated that the activity of histidine decarboxylase, a synthesizing enzyme of histamine, increases markedly after I/R, leading to an elevation in histamine output into mesenteric lymph (17). Histamine synthesized after I/R facilitates repair of small intestine injured by ischemic insult (17). We have also demonstrated that ODC activity, elevated after I/R, is involved in intestinal repair (15), and that the increase of ODC activity after I/R is, at least in part, mediated by an increase in histamine-synthesis (17).

[1] To whom requests for reprints should be addressed at Department of Internal Medicine, Saga Medical School, 5-1-1 Nabeshima, Saga 849, Japan.

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The aim of the present study was to compare the stimulatory effect of histamine on the increase in ODC activity under two different conditions: intestinal repair after I/R, a pathophysiological situation, and cell growth after refeeding, a physiological situation. For that purpose, we examined: (i) histamine output in mesenteric lymph; and (ii) effect of pretreatment with α-fluoromethylhistidine (α-FMH), a suicide inhibitor of histidine decarboxylase, on the increase in ODC activity.

## Materials and Methods

**α-FMH Pretreatment.** Male Sprague-Dawley rats, weighing 270–320 g, were injected intraperitoneally (ip) with α-FMH dissolved in physiological saline (100 mg, 200 mg, or 400 mg/kg body wt) twice daily for 2 days prior to the experiment. Control rats were injected ip with physiological saline (vehicle). α-FMH was kindly supplied by Dr. J. Kollonitsch, Merck Sharp & Dohme Research Laboratories.

**Ischemia-Reperfusion (I/R).** Rats were fasted for 24 hr. Under halothane anesthesia, a laparotomy was performed. The superior mesenteric artery was occluded for 15 min with a micro-bulldog clamp. At the end of the ischemic period, the clamp was released, and a few drops of 2% lidocaine were applied directly onto the superior mesenteric artery to facilitate reperfusion. In the sham-operated controls, the superior mesenteric artery was isolated in a similar fashion but was not occluded. In both groups of animals, the intestinal lymph duct was cannulated with a small vinyl tube according to the method of Bollman *et al.* (18). A silicon infusion tube was introduced about 2 cm down the duodenum through the fundus of the stomach (19). Postoperatively, the animals were infused intraduodenally at 3 ml/hr with a saline solution containing 145 m*M* NaCl and 4 m*M* KCl in restraining cages.

**Refeeding After 48 hr Fasting.** Under halothane anesthesia, the intestinal lymph duct was cannulated (18, 19) and the animals were allowed to recover in restraining cases. The animals were fasted postoperatively, but allowed access to tap water. After 48 hr of fasting, each rat was refed with normal pellet rat chow (CE-2, Clea, Japan). Control rats were not allowed access to rat chow.

**Histamine Output in Intestinal Lymph After I/R or Refeeding.** Intestinal lymph samples were collected from lymph-fistula rats 1 hr (0–1 hr), 3 hr (2–3 hr), and 6 hr (5–6 hr) after I/R or refeeding to measure histamine output in intestinal lymph. The lymph sample (1 ml) was treated in 0.3 ml of 0.4 *M* perchloric acid containing 0.32 μmole/l pros-methylhistamine as the internal standard. After centrifugation, 0.25 ml of clear supernatant was used for determination of histamine content by the high-performance liquid chromatography method of Tsuruta *et al.* (20) as modified by Oishi *et al.* (21). Three subgroups of animals were studied in both the I/R and refeeding groups as follows: experimental group pretreated with vehicle, control group pretreated with vehicle, and experimental group pretreated with α-FMH (100 mg/kg). Five rats were tested in each group.

**Effects of α-FMH on ODC Activity After I/R or Refeeding.** ODC activity in rat jejunal mucosa was measured in four subgroups consisting of I/R and refeeding groups as follows: experimental group pretreated with vehicle, control group pretreated with vehicle, experimental group pretreated with α-FMH (100 mg/kg) and experimental group pretreated with α-FMH (100 mg/kg). ODC activity was measured 1 hr, 3 hr, and 6 hr after I/R or refeeding. Additionally, ODC activity was measured 6 hr after I/R in rats pretreated with 200 mg/kg or 400 mg/kg α-FMH. Five or six rats were studied in each group at each time point.

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/mmole, New England Nuclear, Boston) (7). Rats were anesthetized under halothane anesthesia and then euthanized by exsanguination. The small intestine was divided into four equal segments. The proximal 5 cm of the second intestinal segment was the jejunum. Mucosa was obtained by scraping with a glass slide over an ice cold glass plate, and ODC activity was measured immediately. Mucosal scrapings from jejunum were placed in 2 ml of 0.1 *M* Tris buffer (pH 7.4), containing 1 m*M* EDTA, 50 μ*M* pyridoxal 5′ phosphate, and 5 m*M* dithiothreitol. The tissues were homogenized and centrifuged at 30,000*g* for 30 min. Protein content was determined, and a 200 μl aliquot of supernatant was incubated in stoppered vials in the presence of 3.5 nmole of L-[1-$^{14}$C]ornithine for 15 min at 37°C. The $^{14}CO_2$ liberated by the decarboxylation of ornithine was trapped on a piece of filter paper impregnated with 20 μl of 2 *N* NaOH, which was suspended above the reaction mixture. The reaction was terminated by the addition of 0.3 ml of 10% trichloroacetic acid. Radioactivity of the $^{14}CO_2$ trapped in the filter paper was measured in an aqueous miscible scintillant (Poly-Flour, Packard Instrument, Downers Grove, IL).

**Statistics.** Results are expressed as means ± SEM. Data were evaluated by one-way analysis of variance and multiple comparisons were carried out with the method of least significant difference. Differences were considered significant if the probability of the difference occurring by chance was less than 5 in 100 ($P < 0.05$).

## Results

**Histamine Content in Mesenteric Lymph After I/R or Refeeding.** Histamine content in mesenteric

lymph after I/R is shown in Table 1. Histamine content did not increase 1 hr after I/R, however, in mesenteric lymph there was a marked increase 3 and 6 hr after I/R compared with sham-treated animals ($P < 0.01$ in each). This increase in histamine content in mesenteric lymph after I/R was attenuated by suppression of histidine decarboxylase activity by pretreatment of α-FMH ($P < 0.01$ for each time point).

Table II shows histamine content in mesenteric lymph after refeeding. In contrast to I/R, histamine content did not increase 1, 3, or 6 hr following refeeding. In rats pretreated with α-FMH, histamine content in mesenteric lymph decreased slightly but not significantly compared to controls.

**Effect of α-FMH on an Increase in ODC Activity After I/R or Refeeding.** ODC activity 1, 3 and 6 hr following I/R is shown in Table III. ODC activity did not change in sham surgery rats of α-FMH pretreated rats, indicating that α-FMH itself had no effect on ODC activity. ODC activity did not change significantly 1 hr after I/R compared with sham-treated animals. ODC activity increased markedly 3 and 6 hr after ischemic insult compared with the corresponding sham-treated controls ($P < 0.01$ for each time point), and this increase peaked 6 hr after I/R. The increase in ODC activity was attenuated by α-FMH (100 mg/kg) pretreatment 3 and 6 hr after I/R ($P < 0.01$ in each). However, mucosal ODC activity in I/R animals pretreated with α-FMH was still significantly higher than the level in sham-treated rats ($P < 0.01$ for each time point), indicating that the increase in ODC activity after I/R was not completely suppressed by α-FMH pretreatment. Twice (200 mg/kg) or four times (400 mg/kg) doses of α-FMH could not suppress ODC activity 6 hr after I/R (200 mg/kg α-FMH: 40.5 pmole $CO_2$/hr/mg protein; 400 mg/kg α-FMH: 35.3 pmole $CO_2$/hr/mg protein).

The time course of ODC activity after refeeding is shown in Table IV. In contrast to the I/R group, ODC activity started to increase 1 hr after refeeding compared with the control group ($P < 0.01$). The increase in ODC activity was observed 3 and 6 hr after refeeding ($P < 0.01$ in each), and peaked 3 hr after I/R. The increase in ODC activity after refeeding in rats pretreated with α-FMH was the same level as in rats pretreated with vehicle only. This result indicated that pretreatment of α-FMH had no effect on the increase in ODC activity after refeeding.

## Discussion

As demonstrated in the previous study (17), histamine content increased in mesenteric lymph when synthesis of histamine in intestinal mucosa, which has elevated histidine decarboxylase activity, increased markedly following ischemic insult by 15-min occlusion of the superior mesenteric artery. We also demonstrated that the increase in histamine in mesenteric lymph after I/R was not due to degranulation of mucosal mast cells (17). In the present experiments, the increase of histamine in the mesenteric lymph after I/R was depressed by pretreatment of α-FMH, indicating that the increase in histamine after I/R was due to an increase in histamine synthesis in the intestinal mucosa. In contrast to I/R, histamine content in the mes-

**Table I.** Histamine Content in Mesenteric Lymph (ng/ml) After Ischemia by 15-min Occlusion of Mesenteric Artery Followed by Reperfusion

| | 1 hr After I/R | 3 hr After I/R | 6 hr After I/R |
|---|---|---|---|
| I/R | | | |
| Pretreated with vehicle | 21.1 ± 5.2 | 56.4 ± 6.0[a] | 61.1 ± 5.4[a] |
| Pretreated with α-fluoromethylhistidine | 12.3 ± 4.4 | 19.3 ± 3.4[b] | 14.5 ± 3.3[b] |
| Sham | | | |
| Pretreated with vehicle | 20.2 ± 4.6 | 16.9 ± 4.0 | 18.8 ± 3.2 |

*Note.* Values are means ± SEM. I/R = ischemia-reperfusion.
[a] $P < 0.01$, compared with sham-treated controls.
[b] $P < 0.01$, compared with I/R rats pretreated with vehicle only.

**Table II.** Histamine Content in Mesenteric Lymph (ng/ml) Following Refeeding in 48 hr-Fasted Rats

| | 1 hr After Refeeding | 3 hr After Refeeding | 6 hr After Refeeding |
|---|---|---|---|
| Refeeding | | | |
| Pretreated with vehicle | 25.2 ± 5.1 | 24.4 ± 4.6 | 23.5 ± 5.0 |
| Pretreated with α-fluoromethylhistidine | 18.1 ± 4.4 | 17.0 ± 4.0 | 17.0 ± 4.0 |
| Control | | | |
| Pretreated with vehicle | 24.1 ± 3.9 | 22.5 ± 3.9 | 24.0 ± 6.2 |

*Note.* Values are means ± SEM. Control rats were not refed.

**Table III.** Ornithine Decarboxylase Activity (pmole $CO_2$/hr/mg Protein) in Rat Jejunal Mucosa After Ischemia by 15-min Occlusion of Mesenteric Artery Followed by Reperfusion

| | 1 hr After I/R | 3 hr After I/R | 6 hr After I/R |
|---|---|---|---|
| I/R | | | |
| Pretreated with vehicle | 4.5 ± 3.0 | 60.8 ± 6.4[a] | 99.3 ± 8.1[a,c] |
| Pretreated with α-fluoromethylhistidine | 3.9 ± 2.2 | 30.4 ± 5.1[a,b] | 44.8 ± 5.9[a,b] |
| Sham | | | |
| Pretreated with vehicle | 2.7 ± 2.0 | 5.1 ± 2.4 | 4.0 ± 1.0 |
| Pretreated with α-fluoromethylhistidine | 3.0 ± 1.6 | 3.5 ± 2.2 | 4.4 ± 1.9 |

*Note.* Values are means ± SEM. I/R = ischemia-reperfusion.
[a] $P < 0.01$, compared with corresponding sham-treated controls.
[b] $P < 0.01$, compared with I/R rats pretreated with vehicle only.
[c] $P < 0.05$, compared with the value 3 hr after I/R.

**Table IV.** Ornithine Decarboxylase Activity (pmole $CO_2$/hr/mg Protein) in Rat Jejunal Mucosa Following Refeeding in 48 hr-Fasted Rats

| | 1 hr After Refeeding | 3 hr After Refeeding | 6 hr After Refeeding |
|---|---|---|---|
| Refeeding | | | |
| Pretreated with vehicle | 44.7 ± 6.3[a] | 140.1 ± 16.2[a] | 66.5 ± 7.9[a,b] |
| Pretreated with α-fluoromethylhistidine | 39.1 ± 5.1[a] | 128.7 ± 10.9[a] | 77.8 ± 8.5[a,b] |
| Control | | | |
| Pretreated with vehicle | 1.4 ± 0.8 | 0.9 ± 0.5 | 1.4 ± 0.8 |
| Pretreated with α-fluoromethylhistidine | 0.9 ± 0.5 | 1.2 ± 0.4 | 1.0 ± 0.6 |

*Note.* Values are means ± SEM. Control rats were not refed.
[a] $P < 0.01$, compared with corresponding controls.
[b] $P < 0.05$, compared with the values 3 hr after refeeding.

enteric lymph did not increase 1, 3, or 6 hr after refeeding. Namely, histamine synthesis did not increase after refeeding.

It is well demonstrated that ODC activity in small intestine increases markedly in response to physiological factors, such as feeding (6, 7), insulin (6), and adaptation to lactation (12), and that the increased ODC activity is important for mucosal growth of small intestine (1, 2). It has also been shown that ODC activity increases markedly in injured intestinal mucosa (2, 11, 15, 16), and that ODC activity under these pathophysiological situations enhances repair of injured intestinal mucosa (1, 2, 11, 15, 16).

In several rapidly proliferating tissues, such as experimental tumors (22), and mouse skin (23), and rat colon (24), after application of the tumor promotor tetradecanoylphorbol acetate, both the histamine-synthesis and ODC activity increase markedly, whereas the relationship between these two factors remains unclear. The present study showed that ODC activity increased markedly in both cases, following I/R and refeeding. But α-FMH, a suicide inhibitor of histidine decarboxylase, attenuated the increase of ODC activity only in the case of I/R, and pretreatment with α-FMH had no effect on the increase in ODC activity after refeeding. It has not yet been demonstrated which cells synthesize histamine in rat intestinal mucosa, although histamine synthesis increases after I/R (14). The results of the present investigation suggest that synthesis of histamine in the intestinal mucosa elevated after I/R and that this elevated histamine played, at least in part, a role in enhancing the increase in ODC activity after I/R. In contrast to I/R, luminal nutrient factors after refeeding directly increased ODC activity. This difference in mechanism should be supported by the difference in the time lag of the increase in ODC activity between I/R and refeeding groups. Namely, ODC activity started to increase 1 hr after refeeding and peaked 3 hr after refeeding, whereas ODC activity did not increase 1 hr after I/R but started to increase 3 hr after I/R.

Judging from these facts, histamine plays an important role in the increase of ODC activity after I/R under pathophysiological conditions whereby intestinal mucosa is injured and repaired, but histamine is not related to the increase in ODC activity after refeeding of intestinal mucosa under physiological conditions of normal cell growth.

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