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Metabolism of exogenous histamine in rainbow trout (*Oncorhynchus mykiss*)

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Abstract

Information on the metabolism of exogenous histamine in fish is of much concern regarding the effect of dietary histamine on fish. Histamine catabolic enzymes, diamine oxidase and histamine N-methyl transferase were measured in the tissues of rainbow trout. Diamine oxidase was detected in the stomach, pylorus caeca and intestine. Histamine N-methyl transferase was detected only in the liver.

A change in the contents of histamine and its metabolites was observed in the tissues of rainbow trout after oral administration of histamine. A large amount of imidazole acetic acid was observed in the serum, kidney, liver and muscle. On the other hand, 1-methyl histamine was detected only in the liver. Histamine and its metabolites, imidazole acetic acid and 1-methyl histamine were metabolized and diminished within 48 hr in all tissues.

These results showed that histamine was metabolized by two metabolic routes in rainbow trout. One is the main pathway producing imidazole acetic acid by intestinal diamine oxidase and the other is the complementary pathway producing 1-methyl histamine by liver N-methyl-transferase.

Introduction

Brown fishmeal has now become important for fish aquaculture. A large amount of L-histidine contained in brown fishmeal can be easily converted to histamine by bacterial decarboxylase activity. Sometimes, a large amount of histamine and gizzerosine are detected in brown fishmeal. There have been some reports on the effects of histamine on fish, such as gastric mucosa damage (Watanabe et al. 1987) and the distention of the stomach of rainbow trout (Fairgrieve et al. 1994). It usually requires a large amount of histamine to cause gastric abnormalities in fish. Information on the metabolism of exogenous histamine in fish is not available.

Histamine is derived from L-histidine by histidine decarboxylase after decarboxylation. There are two main metabolic pathways of histamine in mammals; one is the oxidation by diamine oxidase (EC 1.4.3.6) producing imidazole acetic acid; and the other is methylation by histamine N-methyltransferase (EC 2.1.1.8) producing 1-methylhistamine. Diamine oxidase converts histamine to 4-imidazole carboxyaldehyde, and then 4-imidazole acetic acid is immediately derived from 4-imidazole carboxyaldehyde by aldehyde dehydrogenase with oxidation. Imidazole acetic acid was conjugated with ribose and then excreted. On the other hand, histamine methyltransferase converts histamine to 1-methylhistamine, and then 1-methylhistamine was converted to 1-methylimidazole acetic acid by monoamine oxidase. Enzyme acetylase, which converts histamine to N-acetyl histamine, has been identified from enterobacteria (Oguri and Yoneya 2002).

The metabolism of exogenous histamine in humans, guinea pigs, rabbits, rats and cats has been discussed in the literature (Granerus 1968; Wetterqvist and White 1968; Wetterqvist 1968; Irman-Florjanc

and Erjavec, 1994). In mammals, the small intestine is an important site for diamine oxidase (Waton 1956) and the brain is an important site for histamine N-methyl transferase (Prell and Green 1994).

On the other hand, diamine oxidase was detected in eel intestine (Holstein 1975), in mackerel liver, stomach, pylorus caeca and intestine, in tuna liver, stomach, pylorus caeca and intestine, and in yellowtail intestine (Matsumiya and Otake 1981). It is presumed that diamine oxidase plays a role in the detoxification of biogenic amines in the digestive organ in fish. Reports on histamine N-methyltransferase activity in fish are very rare. Few reports on the metabolism of exogenous histamine in fish are known, such as the case of eel that was administered histamine with intraperitoneal injection (Holstein 1971). But there is no information regarding the metabolism of orally administered histamine in fish.

In this paper, the distribution of histamine catabolism enzymes, such as diamine oxidase and histamine N-methyltransferase, and the change in the concentration of histamine and its metabolites in the tissues of rainbow trout after a oral administration are discussed.

Materials and methods

Materials

Histamine dihydrochloride, sodium nitrite, sulfanilic acid, sodium nitrite, 4-hydroxy-3-methoxy-phenylacetic acid, sodium laurylsulfate and horseradish peroxidase were purchased from Wako Pure Chemical Industries (Osaka, Japan). 1-Methylhistamine, 3-amino-1, 2, 4-triazole, pargyline and aminoguanidine were obtained from Sigma (St. Louis, MO, U.S.A), and all other reagents used were of analytical grade.

Animals

Rainbow trout (*Oncorhynchus mykiss)* about 35 g in average weight were obtained from a commercial hatchery and distributed randomly to 6 fiberglass tanks (40 L). A commercial diet (Oriental yeast industries, Tokyo, Japan) was given to the fish until the start of the experiments. The fish were fasted for 24 h before the experiments.

Enzyme activity

Diamine oxidase. Rainbow trout were anesthetized by immersion in MS-222 solution (100 mg/l). The liver, kidney, muscle, spleen, stomach, pylorus caeca, intestine and blood were quickly removed. The tissues were homogenized with a cold 0.1 M sodium phosphate buffer pH 7.4 by a Polytron homogenizer. The homogenate was centrifuged at 18 000 *g* for 1 h at 4 °C, and the supernatant was used as the enzyme solution. Protein contents of the enzyme solutions were measured by the method of Bradford (1976).

Diamine oxidase activities were assayed by the fluorometric method (Kumazawa and Suzuki 1987). The assay mixture contained 0.1 ml of the enzyme solution, 0.1 ml of 4- hydroxy-3- methoxy-phenylacetic acid (final concentration 1 mg/ml), 0.1 ml of horseradish peroxidase (0.5 mg/ml), 3 ml of the 0.1 M sodium phosphate buffer (pH 7.4) and 0.1 ml of 3-amino-1, 2, 4-triazole (1 mg/ml) as an inhibitor of catalase. The assay mixture was incubated at 30 °C for 10 min and 0.1 ml of the histamine solution (0.3 mM) was added to the mixture. The mixture was incubated for more than 30 min and 2 ml of 1 N NaOH was added to terminate the reaction. The fluorescence intensity was measured with excitation at 323 nm and emission at a 426 nm by a Spectrofluorometer (JASCO FP777). As a blank test, the assay mixture without histamine was incubated and histamine was added after adding a sodium hydroxide solution. Hydrogen peroxide was used to make calibration curves.

Histamine N-methyltransferase activity. An enzyme solution was prepared for the diamine oxidase method. Histamine N-methyltransferase activity was detected by the method of Fukuda et al. (1991). The assay mixture contained 0.1 ml of the enzyme solution, 0.1 ml of the histamine solution (final concentration 0.1 mM), 0.1 ml of S-adenosyl methionine (0.25 mM), and 0.7 ml of the 0.1 M sodium phosphate buffer pH 7.4 containing 0.1 mM pargyline and 0.1 mM aminoguanidine. The assay mixture was incubated at 30 °C for 30 min. After incubation, 50 μl of 60% perchloric acid was added to the mixture to terminate the reaction. The mixture was centrifuged at 18 000 *g* for 10 min and the supernatant was adjusted to about pH 7 by 2 N KOH. The mixture was centrifuged again at 18 000 *g* for 10 min and the supernatant was applied to *o*-phthalaldehyde (OPA) derivatization. As a blank test, the assay mixture without histamine was incubated and histamine was added after the perchloric acid addition.

1-Methylhistamine was detected by the HPLC method of Saito et al. (1994) with a slight modification. *O*-phthalaldehyde reagent was prepared by

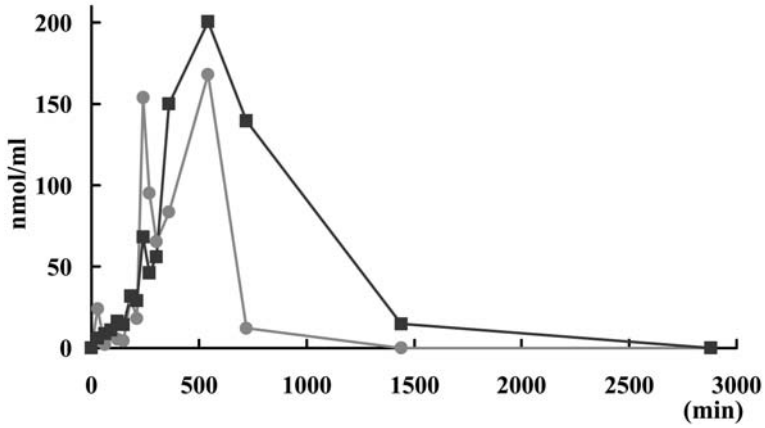

Figure 1. Concentrations of histamine (●) and imidazoleacetic acid (■) in serum after oral histamine administration. Histamine and imidazole acetic acid were detected by HPLC. 1-Methyl histamine was under the detection limit. Each data point represents the mean ($n = 3$).

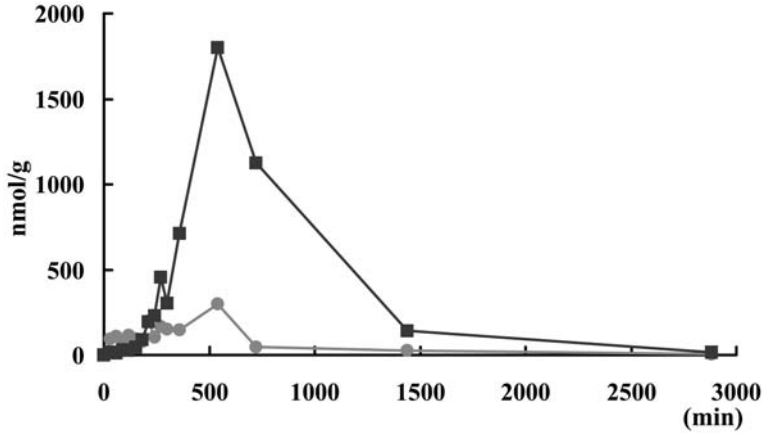

Figure 2. Concentrations of histamine (●) and imidazoleacetic acid (■) in kidney after oral histamine administration. Histamine and imidazole acetic acid were detected by HPLC. 1-Methyl histamine was under the detection limit. Each data point represents the mean ($n = 3$).

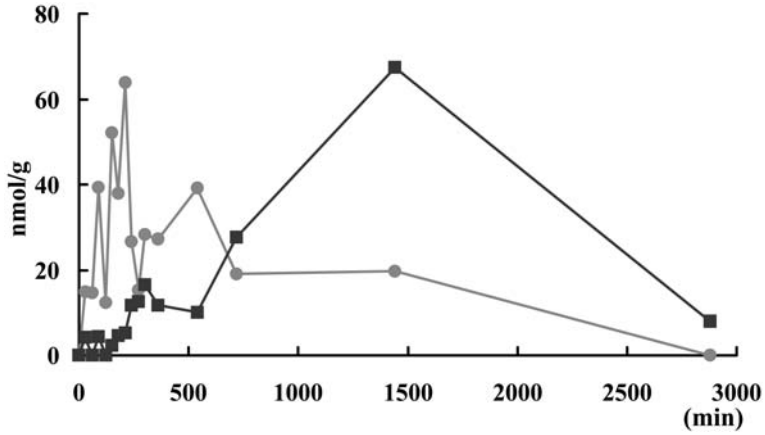

Figure 3. Concentrations of histamine (●) and imidazoleacetic acid (■) in muscle after oral histamine administration. Histamine and its metabolites were detected by HPLC. Each data point represents the mean ($n = 3$).

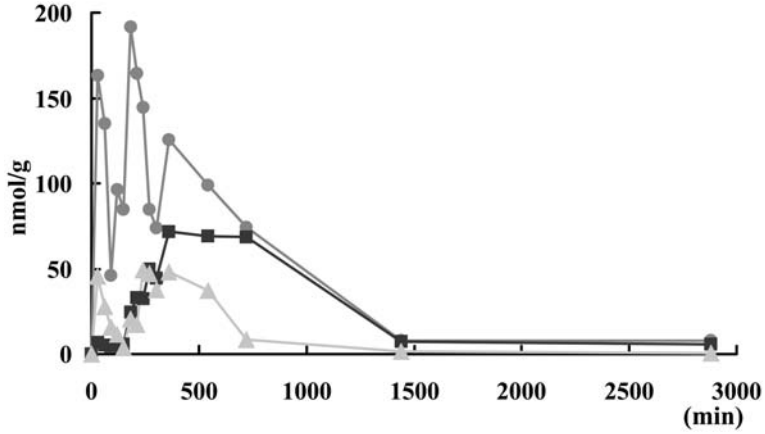

Figure 4. Concentrations of histamine (●), imidazoleacetic acid (■) and 1-methylhistamine (▲) in liver after oral histamine administration. Histamine and imidazole acetic acid were detected by HPLC. Each data point represents the mean ($n = 3$).

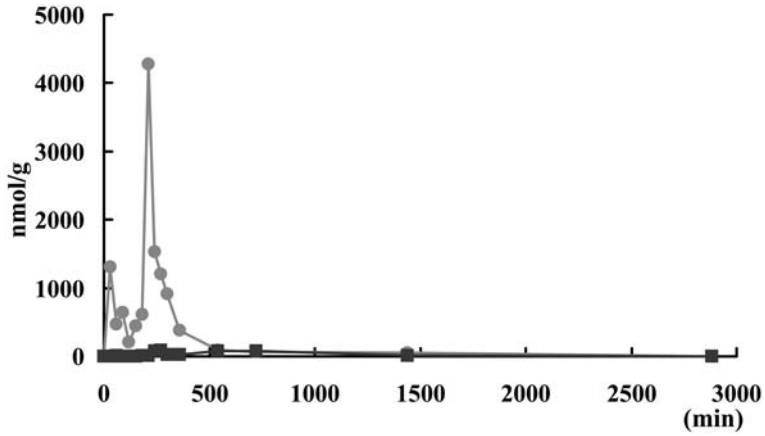

Figure 5. Concentrations of histamine (●) and imidazoleacetic acid (■) in spleen after oral histamine administration. Histamine and its metabolites were detected by HPLC. Each data point represents the mean ($n = 3$).

dissolving 8 mg of OPA and 10 mg of N-acetyl-L-cysteine in 1ml methanol. Ten μl of the enzyme reaction mixture, 20 μl of OPA reagent and 70 μl of 0.1 M sodium borate were mixed and 10 μl of the mixture was applied to HPLC.

The HPLC system consisted of two pumps (JASCO PU-980), a solvent mixing module (JASCO 880-30), an auto-sampler (JASCO AS-950), a column oven (JASCO CO-995), a fluorescence spectrophotometer (HITACHI F1000), and a data analyzer (BORWIN, JASCO). The mobile phases were (A) 50 mM sodium acetate buffer pH 5.3 and (B) methanol. The mobile phase was delivered at a flow-rate of 1.0 ml/min to a stainless steel column (250 × 4.6 mm i.d.) that was packed with TSK-ODS 80TM (5 μm particle size, Toso Co., Ltd, Tokyo, Japan) in our laboratory by the conventional slurry-packing technique. The linear gradient program for the separation procedure was from 0% of (B) to 60% (B) in 60 min. The fluorescence intensity was measured with the excitation wavelength at 348 nm and emission at 450 nm. Authentic 1-methylhistamine was used to make a calibration curve.

Histamine oral administration

The rainbow trout were anesthetized by immersion in MS-222; and 0.1 ml of a 10% (W/V) histamine solution in 0.9% saline was administrated by a sonde needle into the stomach.

They were put back into the tank. At 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 360, 540, 720, 1440 and 2880 min after administration, 3 fish were taken out from the tank each time, anesthetized again, their blood collected and the fish decapitated. They

Table 1. Diamine oxidase and Histamine N-methyltransferase activities in rainbow trout tissues

Enzyme activity (nmol/min/mg protein)	Liver	Kidney	Spleen	Muscle	Blood	Stomach	Pylorus caeca	Intestine
Diamine oxidase	N.D.	N.D.	tr.	N.D.	tr.	0.028	0.188	0.181
Histamine N-methyl transferase	0.057	0.007	N.D.	N.D.	0.002	0.001	0.001	0.002

were dissected to take out the liver, kidney, muscle and spleen. All tissues were kept at −80 °C until analysis. After coagulation the blood was centrifuged at 6000 *g* for 5 min and the supernatant was collected. Two mg of sulfosalicylic acid dihydrate was added to 100 μl of the supernatant and centrifuged at 18 000 *g* for 15 min. The supernatant was collected as a blood serum sample. Tissues were homogenized with the 4 times volume of 80% ethanol, and centrifuged at 18 000 *g* for 15 min. The supernatant was collected as tissue extract. As a blank test, 0.1 ml of 0.9% saline was administered instead of 10% histamine solution.

Histamine and imidazoleacetic acid were detected by the HPLC method of Sato et al. (1995) with a slight modification. The derivatization reagent was an equivolume mixture of 20 mM sulfanilic acid and in 1 N HCl and 200 mM sodium nitrite solution. Forty μl of the sample mixture, 20 μl of the derivatization reagent and 60 μl of 10% anhydrous sodium carbonate in a 5% ethanol solution were mixed and 10 μl of it was applied for the HPLC.

The HPLC system and column are the same as the method of detection of 1-methylhistamine with a UV-detector (JASCO UVIDEC-100-VI). The mobile phases are (C) 150 mM sodium acetate buffer (pH 6.0) ethanol = 95 : 5 containing sodium laurylsulfate (final concentration 5 mM) and (D) 60% acetonitrile. The supplement of sodium laurylsulfate in the mobile phase made it possible to make a good separation between histidine and imidazole acetic acid. The linear gradient program for the separation procedure was from 0% of (D) to 45% of (D) in 60 min at a flow rate of 1.0 ml/min. The optical density was a measured with a wavelength of 420 nm.

Results

Enzyme activity

The diamine oxidase activity and the histamine N-methyltransferase activity are shown in Table 1. The diamine oxidase activity was detected at stomach, intestine and pylorus caeca with histamine as a substrate. The enzyme activities in intestine and pylorus caeca are 6.4 times higher than in stomach. The histamine N-methyltransferase activity is detected in liver, kidney, blood and digestive organs. The liver has the highest activity of histamine N-methyl transferase in the tissues and slight activity in other tissues.

Concentration of histamine and its metabolites in rainbow trout tissues administered histamine

The injection of 0.9% saline into rainbow trout stomach did not cause the release of endogenous histamine into the tissues. After the administration of histamine solution in this experiment, none of the rainbow trout died but they showed an abnormal reaction until 48 h.

Histamine and imidazole acetic acid were immediately detected in all the analyzed organs after the histamine administration. The histamine concentration increased after administration and the highest concentration was observed at 540 min in serum and kidney, at 210 min in spleen and muscle and at 180 min in liver. The imidazole acetic acid was greatly increased in serum, kidney and muscle. There was very little imidazole acetic acid in spleen. Liver was the only organ in which 1-methyl imidazole acetic acid was detected whereas 1-methylhistamine was not detected in the other organs. Histamine, imidazole acetic acid and 1-methylhistamine almost disappeared during the 48 h.

Discussion

Diamine oxidase was detected in digestive organs such as stomach, pylorus caeca and intestine; and histamine N-methyltransferase was detected in liver. This result showed the exogenous histamine with oral administration was easily detoxified in digestive organs by diamine oxidase; and histamine that was not detoxified by diamine oxidase was carried to the liver and converted to 1-methylhistamine in rainbow trout.

A rapid increase of imidazole acetic acid content in the serum was observed after the oral administration of 10 mg of histamine was given to the trout. It showed that the most of the exogenous histamine was converted in the digestive organs and was carried by the blood stream. The imidazole acetic acid in the serum was carried to the muscle, liver and kidney and temporally accumulated there. The great increase of imidazole acetic acid content in the kidney shows that imidazole acetic acid has the tendency to accumulate in the kidney and might be converted to conjugate with ribose and then be excreted. The most 1-methyl histamine was detected in liver, in which histamine N-methyltransferase was confirmed to be present. The changes of histamine and its metabolites in rainbow trout are explained by the distribution of the histamine catabolism enzymes.

The histamine content in GE-positive meal is smaller than the content of histamine that was administrated trout in this study. A large amount of pure histamine is necessary to cause a gastric abnormality in fish (Watanabe et al. 1987). It can be seen that there will be a complex action by histamine and other components in fish meal.

Dietary histamine sometimes causes gastric abnormalities in trout (Watanabe et al. 1987; Fairgrieve et al. 1994). Otake et al. (1977) also observed that oral administration of histamine caused activation of pepsin and pathological changes in the stomach of mackerel. But in spite of these gastric abnormalities, the suppression of growth was not reported. On the other hand, a change of body color in rainbow trout fed dietary histamine has been reported and α-tocopherol was involved in the histamine abnormalities (Watanabe et al. 1987). We found that α-tocopherol content in tissues decreased in rainbow trout that were fed dietary histamine (under submission). α-Tocopherol is an essential vitamin, and α-tocopherol is used for the improvement of antioxidant activity (Huang et al. 2003, Tocher et al. 2002), tolerance for fish disease (Ortuno et al. 2000) and so on (Montero et al. 1999). The decrease of α-tocopherol by dietary histamine might cause other abnormalities in fish. Furthermore, histamine toxicity is said to be activated by the biogenic amines, which attenuate the activity of histamine catabolism enzymes (Taylor and Lieber 1979, Lyons et al. 1983). Stale fish meal contains not only a large amount of histamine but also many biogenic amines, such as putrescine, cadaverine, spermidine, spermine and others. Furthermore the food intake, vitamins and sex affect the metabolism of histamine (Imamura et al. 1984, Chatterjee et al. 1975, Wetterqvist and White 1968). We plan in the near future to study the factors that affect histamine metabolism and gastric damage in fish.

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