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HISTIDINE DECARBOXYLASE IN THE TRANSPLANTABLE ARGYROPHILIC GASTRIC CARCINOID OF *PRAOMYS (MASTOMYS) NATALENSIS*

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Abstract—A transplantable argyrophilic gastric carcinoid of *Praomys (Mastomys) natalensis* contained appreciable activities of specific histidine decarboxylase and aromatic L-amino acid decarboxylase which may relate to the inherent properties of its parent cells, probably histamine-containing argyrophil cells distributed in the gastric mucosa. Specific histidine decarboxylase free from aromatic L-amino acid decarboxylase was purified 10-fold by acid plus heat treatment and subsequent ammonium sulfate precipitation; it was not essentially different from that prepared from other mammalian sources.

IN MAN, the carcinoid tumor arises in the mucosa of the alimentary tract and tracheobronchial tree. While the gastric and bronchial carcinoid (foregut origin) are less likely to give an argentaffin reaction than those of the ileum, appendix and cecum (midgut origin), some gastric carcinoids give an argyrophil reaction, and several gastric carcinoids have been reported to contain a high level of histamine. Elevated histamine levels were present in the urine of the patients also.[1,2]

Mastomys are a distinct subgenus of rodent, intermediate in size between the mouse and the rat. The spontaneous occurrence of cancers in the body of the glandular stomach of these animals was first reported in 1957 by Oettle.[3] These tumors, which are found in approximately 40 per cent of old Mastomys, have been characterized as malignant argyrophilic carcinoids, since they contain argyrophil but not argentaffin granules and are transplantable to other Mastomys.[4,5] To our knowledge, they are the only animals other than man to develop carcinoid tumors of the stomach. Animals in which the transplanted tumors grow after being transplanted into the muscles of the thigh are often found to have massive hemorrhage into the gastrointestinal tract or perforated ulcers of the duodenum due to hypersecretion of gastric acid. Recently we have found that a transplantable argyrophilic gastric carcinoid of Mastomys contained appreciable amounts of histamine, and that the microsome-free supernatant of the tumor tissue produced significant amounts of histamine in the presence of L-histidine.[6] These findings may account for the hypersecretion of gastric acid and formation of multiple ulcers in the stomach and duodenum. The present studies were undertaken to examine some properties of specific histidine decarboxylase purified from the argyrophilic carcinoids which had been transplanted into the thigh muscles of Mastomys.

MATERIALS AND METHODS

Preparation of histidine decarboxylase

Mastomys bearing the eleventh generation transplant of the carcinoid were sacrificed 2 months after the tumor had been transplanted into the thigh muscle. The tumor nodules, usually 5 mm in diameter, were removed, frozen, and stored at −80° for 10 months. A 775 mg amount of the tumor tissue was added to 10 ml of 0·01 M potassium phosphate buffer, pH 7·0, and homogenized in a Teflon homogenizer. The homogenate was centrifuged at 135,000 *g* for 60 min. The clear supernatant was added to the same volume of 0·1 M sodium acetate buffer, pH 4·4, resulting in pH 4·5, and was heated at 52° for 5 min. After centrifugation, the impurities were discarded and the supernatant was brought to 40% saturation with ammonium sulfate. The precipitate obtained by centrifugation was dissolved in an aliquot of 0·01 M phosphate buffer, pH 7·0, and dialyzed against 2 l. of the same buffer solution with three changes of external fluid.

Enzyme assays

Histidine decarboxylase activity was routinely determined by measuring the formation of histamine from L-histidine. Each test, unless stated otherwise, was carried out in a final volume of 1 ml containing 0·15 M potassium phosphate buffer, pH 6·5, 5×10^{-3} M L-histidine neutralized with 0·1 N NaOH, 1×10^{-5} M pyridoxal-5-phosphate, 1×10^{-4} M aminoguanidine sulfate and an enzyme (50–435 μg protein). All incubations were performed at 37° for 1 hr, and the amount of histamine produced was determined fluorometrically as described previously.[6] Enzyme activity was expressed as micrograms of histamine (free base) produced per milligram of protein in 1 hr, and the smallest amount of histamine which could be detected was 0·1 μg/ml of the reaction mixture.

Activities of Dopa decarboxylase and 5-hydroxytryptophan decarboxylase were assayed by the method of Lovenberg *et al.*[7] The amounts of dopamine and 5-hydroxytryptamine produced were determined fluorometrically after separation from an Amberlite IRC 50 column (0·5 × 3·0 cm).[7,8] It is possible to determine as little as 0·25 μg dopamine and 0·3 μg 5-hydroxytryptamine in each standard reaction mixture.

Protein concentration was estimated by the method of Lowry *et al.*[9]

RESULTS AND DISCUSSION

Activities of histidine decarboxylase and aromatic L-amino acid decarboxylase in malignant cells

Håkanson[10] reported that the particle-free supernatant of the Rous sarcoma and the Walker mammary carcinoma of the rat was devoid of Dopa decarboxylase but produced 10 and 2 ng of histamine per 10 mg of tissue in 1 hr, respectively, while the supernatant of a malignant melanoma of the hamster had high Dopa decarboxylase activity but no histidine decarboxylase activity. We also compared the activities of histidine decarboxylase and aromatic L-amino acid decarboxylase of the present argyrophilic carcinoid with those of several ascites tumors. After cell disruption by freeze-thawing, a dialyzed microsome-free supernatant was made from each tumor cell strain and used as a source for these decarboxylases. The argyrophilic carcinoid cells had an appreciable activity of specific histidine decarboxylase and powerful activities

of Dopa decarboxylase and 5-hydroxytryptophan decarboxylase, while cells of rat ascites hepatoma 62[11] had only aromatic L-amino acid decarboxylase, which was about 10 per cent that of normal rat liver.

Mouse Ehrlich ascites tumor cells were below the limit value of the assay technique in both enzyme activities (Table 1). Since the argyrophilic carcinoid contained 1 mg protein per 10 mg of wet tissue, the activities of histidine decarboxylase as well as Dopa decarboxylase of this tumor can be calculated to be 137 times higher than the histidine decarboxylase activity of the Rous rat sarcoma and 6625 times higher than the Dopa decarboxylase activity of the malignant melanoma of the hamster (Table 1).[10] Thus, the high amine-forming capacity of the argyrophilic carcinoid seems to relate to the inherent properties of its parent cells rather than to rapid cell multiplication during fetal development[12] and tumor growth.[13]

TABLE 1. AMINE-FORMING ACTIVITIES IN VARIOUS CELLS*

Cell	Amine formed (μg/mg protein/hr)			
	Histamine		Serotonin	Dopamine
	pH 6·5	pH 9·0†	pH 9·0†	pH 7·0‡
Argyrophilic carcinoid	1·37	0·27	23·9	212
Rat ascites hepatoma 62§	undetectable	undetectable	1·4	13·5
Mouse Ehrlich ascites tumor‖	undetectable	undetectable	undetectable	undetectable
Adult rat liver	undetectable	undetectable	16·7	100

* Standard reaction mixture and conditions used as described in text.

† Tris–HCl buffer was used.

‡ Potassium phosphate buffer was used.

§ Transplantable ascites hepatoma cells were supplied in June, 1966 by Dr. S. Odashima of the Medical Institite of Sasaki Foundation, Tokyo, Japan, and maintained in Donryu strain rats in our laboratories.

‖ Hypotetraploid Ehrlich/4N ascites tumor cells were supplied in January, 1963 by Dr. K. Kaziwara of the Takeda Research Laboratories, Osaka, Japan, and maintained in dd strain mice in our laboratories.

The previous study stressed that the gastric carcinoid of Mastomys may be derived from the argyrophilic histamine-containing cells in the gastric mucosa, because both the tumor cells and the cells in the normal gastric mucosa gave the argyrophil but not the argentaffin reaction and contained appreciable amounts of histamine but no 5-hydroxytryptamine *in vivo*.[6] We further tried to measure the level of dopamine in the tumor tissue, since dopamine as well as 5-hydroxytryptamine could be demonstrated in the normal argyrophil cells of the gastric mucosa only when exogeneous monoamine precursors were supplied.[14] The level of endogeneous dopamine in the tumor tissue, however, was exceedingly low and below the sensitivity of the method used.[7] These findings indicate that the present tumor is a carcinoid secreting histamine rather than dopamine and 5-hydroxyindoles.

Effects of acid plus heat treatment or of inhibitors on activities of histidine decarboxylase and aromatic L-*amino acid decarboxylase in dialyzed microsome-free supernatant of argyrophilic carcinoid*

In order to remove aromatic L-amino acid decarboxylase from the tumor extract, the microsome-free supernatant was acidified to pH 4·5, heated at 52° for 5 min

according to the method of Håkanson,[15] and used as an enzyme source. When incubation was carried out at the optimum pH for aromatic L-amino acid decarboxylase activity, the formation of 5-hydroxytryptamine as well as dopamine by an enzyme after acid plus heat treatment disappeared completely. Histidine decarboxylase activity only could be demonstrated, and it was reduced to one-fifth of the original activity (Table 2). This result suggests strongly that there exist two amino acid decarboxylases in the tumor tissue.

TABLE 2. EFFECT OF ACID PLUS HEAT TREATMENT ON AMINE-FORMING ACTIVITIES IN EXTRACT OF ARGYROPHILIC CARCINOID*

Treatment	Amine formed (μg/hr)			
	Histamine		Serotonin	Dopa mine
	pH 6·5	pH 9·0	pH 9·0	pH 7·0
None	3·11	0·61	55	488
Acid + heat†	0·65	undetectable	undetectable	undetectable

* Standard reaction mixture and conditions used as described in text.

† Dialyzed microsome-free supernatant adjusted to pH 4·5 was heated at 52° for 5 min. After centrifugation, the supernatant was used as an enzyme.

Since aromatic L-amino acid decarboxylase purified from guinea pig kidney was known to be inhibited by α-methyl-dopa and stimulated by benzene,[7] the effect of these substances on histidine decarboxylase activity was tested. When the reaction mixtures containing 5×10^{-3} M L-histidine, 1×10^{-6} M pyridoxal-5-phosphate and an aliquot of the dialyzed supernatant were incubated at pH 6·5 in the presence and absence of 10^{-3} M α-methyl-dopa or $11{\cdot}3 \times 10^{-2}$ M benzene, the formation of histamine was not significantly different from control: 1·37 to 1·20 μg/mg of protein/hr in the presence of added α-methyl-dopa and to 1·17 μg/mg of protein/hr in the presence of added benzene. Thus, it is apparent that the argyrophilic carcinoid tumor elaborates histamine through a specific histidine decarboxylase reaction different from the aromatic L-amino acid decarboxylase reaction.

Some properties of specific histidine decarboxylase purified from argyrophilic carcinoid

As described in the text, the purification procedure of histidine decarboxylase was not essentially different from that of Håkanson,[15] who achieved 100- to 200-fold purification from fetal rat tissue. However, the histidine decarboxylase was purified only 10-fold from the present tumor (Table 3). The large loss of enzyme activity occurred after acid plus heat treatment to remove aromatic L-amino acid decarboxylase (see also Table 2), although the histamine-forming activity by the final enzyme fraction is comparable to that of the fetal rat enzyme (5–10 μg histamine/mg of protein/hr[15]). There was not sufficient material to permit further purification of the enzyme from the argyrophilic carcinoid of the present generation. The purified enzyme preparation had a slightly yellowish color even after extensive dialysis against 0·01 M phosphate buffer, pH 7·0. This dialysis resulted in marked dissociation of pyridoxal-5-phosphate from the holoenzyme, which was calculated to be 73 per cent

TABLE 3. PURIFICATION OF HISTIDINE DECARBOXYLASE*

Fraction and treatment	Volume (ml)	Protein (mg)	Histamine formed (μg/mg protein/hr)
Whole homogenate	10	87	0·46
135,000 *g* Supernatant	9	33	0·68
Acid + heat and 40% $(NH_4)_2SO_4$	4	2	4·5

* Standard reaction mixture and conditions used as described in text.

from the enzyme activity in the presence of sufficient pyridoxal-5-phosphate (Fig. 1). The activity of apoenzyme could be restored by the addition of pyridoxal-5-phosphate, and the maximum rate of histidine decarboxylation was attained at a pyridoxal-5-phosphate concentration of 1×10^{-6} M (Fig. 1), consistent with that of the fetal rat tissues.[16] The pH optimum of the reaction was found to vary with the substrate

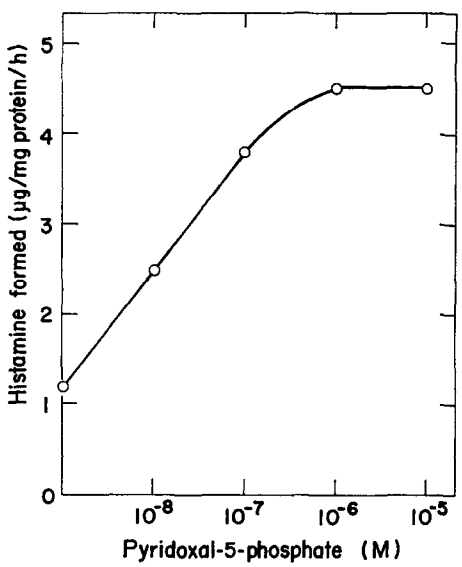

FIG. 1. Influence of coenzyme concentration on enzyme activity. The incubations were performed at pH 6·5 and at a substrate concentration of 5×10^{-3} M.

concentration (Fig. 2). When the substrate concentration was 5×10^{-3} M, the optimum pH of 6·4 was found for the histidine decarboxylation, and the decarboxylation was decreased gradually with increasing pH. At a decreased substrate concentration, 5×10^{-4} M, the maximal rate of decarboxylation occurred at a higher pH. Thus, kinetic properties of semipurified preparations of the histamine-forming enzyme from the argyrophilic carcinoid were very similar to those of the specific histidine decarboxylase prepared from fetal rat tissues or from the gastric mucosa of the rat and primates.[17–19]

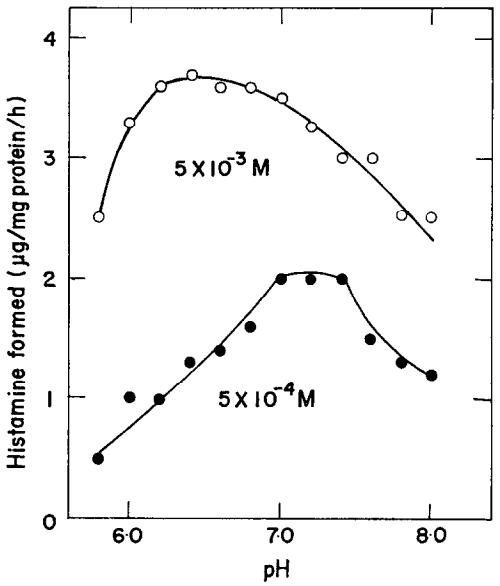

FIG. 2. Histidine decarboxylase activity plotted as a function of both substrate concentration and pH. The incubation was carried out in the presence of 10^{-6} M pyridoxal-5-phosphate. The substrate concentration is indicated for each curve.

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