J. Pharm. Pharmac., 1966, **18**, 769–774 Received May 31, 1966

Histidine decarboxylase in experimental tumours

ROLF HÅKANSON

Histidine decarboxylase activity has been demonstrated in some experimental tumours by direct enzyme assay. The kinetic properties of semi-purified preparations of the histamine-forming enzyme from Rous rat sarcoma and Walker rat mammary carcinoma were similar to those of the "specific" histidine decarboxylase of the foetal rat. Transplants from a malignant hamster melanoma had no "specific" histidine decarboxylase but high dopa decarboxylase activity. The lack of histidine decarboxylase in this tumour seems to indicate that a high histamine-forming capacity is not indispensable for tumour growth.

A high rate of histamine biosynthesis has been demonstrated in some tissues characterized by rapid cell multiplication (cf. Kahlson, 1961); the liver of the foetal rat is a conspicuously rich source of histidine decarboxylase (Kahlson, Rosengren, Westling & White, 1958; Burkhalter 1962). Apart from neoplastic mast cells (Hagen, Weiner, Ono & Lee, 1960) certain experimental tumours are potent in forming histamine; Mackay, Marshall & Riley (1960) found a high histidine decarboxylase activity in a rat hepatoma and Håkanson (1961) demonstrated a considerable histamine-forming capacity in transplants from a rat mammary carcinoma, an observation which was later confirmed by Hallenbeck & Code (1962).

At least two enzymes are capable of catalysing the biosynthesis of histamine in mammalian tissues: the non-specific aromatic L-amino-acid decarboxylase (Udenfriend, Lovenberg & Weissbach, 1960; Lovenberg, Weissbach & Udenfriend, 1962)—also referred to as dopa decarboxylase (Rosengren, 1960)—and the more specific variety, which occurs in high amounts in tissues of the foetal rat (Ganrot, Rosengren & Rosengren, 1961; Burkhalter, 1962; Håkanson, 1963) and mouse (Håkanson, to be published). This enzyme has also been demonstrated in the bone marrow of the rat (Håkanson, 1964) and in the gastric mucosa of the rat (Håkanson & Owman, 1966) and mouse (Håkanson, to be published); in the latter species high activities of "specific" histidine decarboylase appear also in the renal cortex of pregnant animals (Håkanson, to be published, cf. Rosengren, 1963). The kinetic properties of histidine decarboxylase from the foetal rat have been the object of extensive investigation (Håkanson, 1963, 1966a).

Some data on the characteristics of the histamine-forming enzyme of rat hepatoma have been reported by Mackay, Riley & Shepherd (1961). The possibility of a connection between histamine formation and tumour growth (cf. Kahlson, 1961) prompted a more detailed study on the properties of histidine decarboxylase from some experimental non-mast-cell tumours.

Material and methods

The following experimental tumours were examined: Rous sarcoma of the rat (Ahlström & Jonsson, 1962), Walker rat mammary carcinoma

From the Department of Pharmacology, University of Lund, Lund, Sweden.

256 (cf. Håkanson, 1961) and a malignant hamster melanoma (type M, Mel 1 of Fortner) described by Salamon & Storck (1961). Fresh tumour tissue (approx. 5 g) was collected and homogenized in 2 volumes of ice-cold 0·1 M phosphate buffer, pH 7·0. The particulate material was spun down (20,000 × *g*, 20 min, 0°) and discarded. The supernatant was used as enzyme source. In some experiments the enzyme was precipitated with ammonium sulphate as specified in 'Results'; the precipitate was taken up in buffer and dialysed overnight. Incubations were in an atmosphere of nitrogen at 37°. As a rule pyridoxal-5-phosphate (1 μg/ml) was added to the incubation medium which consisted of the enzyme extract diluted with 0·1 M phosphate buffer to a final volume of one ml. Dopa decarboxylase activity was determined at pH 7·0 by a radiometric method (Håkanson & Owman, 1965; Håkanson, 1966b); 3 μg ^{14}C-DL-dopa (5·75 mc/mM, Radiochemical Centre, Amersham) was added as substrate. Histidine decarboxylase activity was examined at various pH values and with various concentrations of substrate by fluorometric determination of the amount of histamine produced (Shore, Burkhalter & Cohn, 1959, cf. Håkanson, 1963); when a high sensitivity was needed a radiometric method was employed, in which 2 μg ^{14}C-L-histidine (32 mc/mM, Radiochemical Centre) was added as substrate (Håkanson, 1966b); the incubation was at pH 7. Specimens of each tumour were stained for the demonstration of mast cells by the histochemical technique of Bloom & Kelly (1960).

Results

Rous rat sarcoma. The tumour was found to contain about 10 μg histamine (free base) per gram fresh tissue. Only few mast cells occurred in the tumour. The tissue was devoid of dopa decarboxylase but a high histidine decarboxylase activity could be demonstrated (Table 1). The

TABLE 1. MAST CELLS, HISTAMINE CONTENT AND ENZYME ACTIVITIES OF SOME EXPERIMENTAL TUMOURS

Tumour	Mast cells	Histamine, μg/g	Dopa decarboxylase activity*	Histidine decarboxylase activity
Rous rat sarcoma	very few	9	0	10
Walker rat mammary carcinoma ..	absent	2·5	0	2
Malignant hamster melanoma ..	absent	0·6	32	0

* Enzyme activities were determined by radiometric micromethods (Håkanson, 1966b) and expressed as ng amine produced per 10 mg tissue in 1 hr.

histamine-forming enzyme was almost quantitatively recovered in the fraction precipitated with ammonium sulphate at between 25 and 40% saturation. The properties of this enzyme were very similar to those of histidine decarboxylase from the foetal rat: the optimum pH of the reaction changed with the substrate concentration (Fig. 1); the Michaelis-Menten constants, which were derived from Lineweaver-Burk (1934) plots of initial velocities, varied with pH (Fig. 2). Michaelis constants defined with respect to certain specific ionized species of histidine were fairly

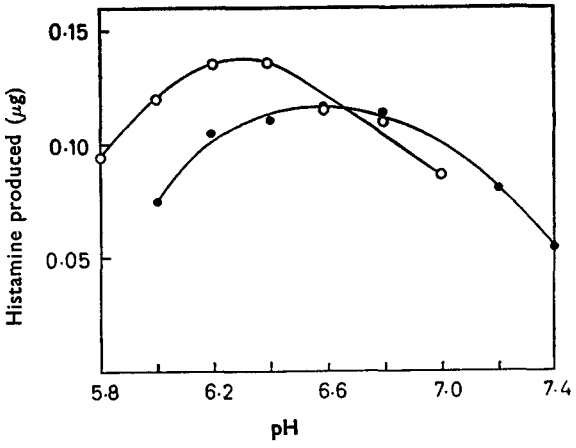


FIG. 1. Variation of the optimum pH of histidine decarboxylase from Rous sarcoma of the rat with the substrate concentration. Enzyme activity is expressed as μg histamine produced in 1 hr. The incubation medium was made up to a total volume of 1 ml with 0·1 M phosphate buffer. Incubations were under nitrogen at 37°. ○——○, 5×10^{-3} M histidine; pyridoxal-5-phosphate 1 μg/ml. ●——●, 5×10^{-4}M histidine; pyridoxal-5-phosphate 1μg/ml.

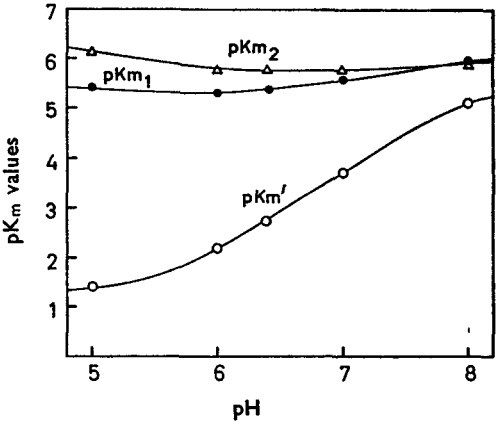


FIG. 2. Apparent pK_m (pK_m') (○——○) versus pH. The pK_{m_1} (●——●) and pK_{m_2} values (▲——▲) were calculated from equations 1 and 2 respectively.

constant within the experimental pH region (Fig. 2). These "true" Michaelis constants (K_m) were derived from the apparent Michaelis constants (K_m') at given pH by the equations:

$$K_m' = K_m\left(1 + \frac{(H^+)}{10^{-9}}\right) \quad .. \quad .. \quad .. \quad (1)$$

or

$$K_m' = K_m\left(1 + \frac{(H^+)}{10^{-6}} + \frac{(H^+)}{10^{-9}} + \frac{(H^+)^2}{10^{-15}}\right) \quad .. \quad .. \quad (2)$$

(The K values of histidine were approximated to 10^{-6} and 10^{-9}. The carboxyl-group was assumed to be ionized throughout). Equation 1 refers to the ionic form of histidine having an ionized carboxyl-group and a non-ionized α-amino-group (see appendix). The corresponding species in equation 2 is the one with ionized carboxyl-group and non-ionized imidazole- and α-amino-groups. A similar mathematical treatment of other species of histidine produced K_m values which varied widely within the experimental pH region. The consequences and implications of similar observations on the properties of foetal rat histidine decarboxylase have been discussed elsewhere (Håkanson, 1963, 1966a).

Walker rat mammary carcinosarcoma 256. The tumour had no dopa decarboxylase and considerably less histidine decarboxylase activity than the Rous sarcoma (Table 1). Mast cells were absent and the histamine content was low (cf. Håkanson, 1961). The histamine-forming enzyme of this tumour was similar to that of the Rous sarcoma; the apparent K_m changed with pH in an identical manner.

Hamster melanoma. This tumour had high dopa decarboxylase activity (cf. Håkanson, Möller & Stormby, 1965) but no histidine decarboxylase activity could be detected by any of the methods used (Table 1). The histamine content was low and mast cells were absent from the tumour tissue.

Discussion

The present results suggest that the Michaelis constant of histidine decarboxylase from some experimental tumours must be defined in terms of a specific ionized species of histidine, presumably the one with the ionized carboxyl group and non-ionized imidazole- and α-amino-groups (cf. Håkanson, 1963). Apparent K_m values (K_m values with respect to total amount of histidine present) were determined from Lineweaver-Burk (1934) plots over a wide range of pH values and were noted to decrease as the pH of the incubation medium was increased. Michaelis constants defined with respect to "true" substrate were derived from these apparent K_m values by relations 1 or 2 and were found to be fairly constant throughout the pH range examined; this observation seems to support the assumption that the enzyme attacks a certain ionic species of histidine. It appears that the species of histidine that predominates at the pH region where enzyme activity is optimal does not necessarily correspond to the species of histidine that constitutes the enzyme-substrate complex. A more detailed analysis of the kinetics of histamine formation, which considers also the ionization of the enzyme, will be published elsewhere (Håkanson, 1966a, and to be published).

The present results clearly show that the histamine-forming enzyme demonstrated in the Rous sarcoma and in the Walker rat mammary carcinoma is different from the aromatic L-amino-acid decarboxylase and very similar to the more specific variety of the histamine-forming isoenzymes.

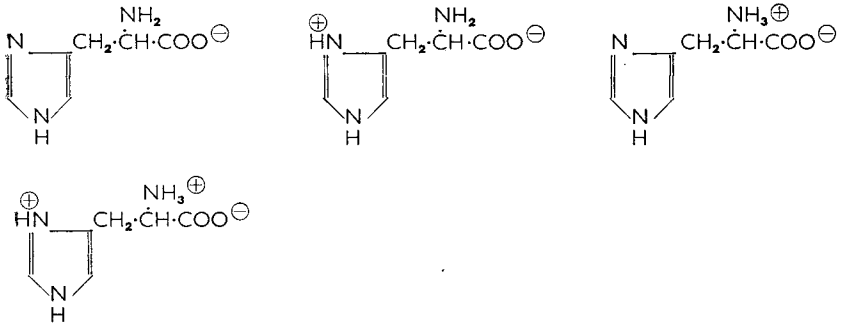
Kahlson has postulated a connection between a high rate of histamine

formation and rapid tissue growth. This view has been challenged by several authors (Kameswaran, Telford & West, 1961, Mackay, Reid & Shepherd, 1961; Kameswaran & West, 1962, Rosengren, 1966). An active biosynthesis of histamine may be a common phenomenon in experimental tumours of the rat and mouse but the lack of histidine decarboxylase in the hamster melanoma seems to indicate that a high histamine-forming capacity is not a necessary feature of tumour growth.

Acknowledgements. Supported by grants from Riksföreningen mot Cancer, The Royal Physiographic Society and the Medical Faculty of Lund. Experimental tumours were provided by Dr. N. G. Stormby, Department of Pathology, Lund, Sweden.

APPENDIX

Histidine occurs in various ionized species within the experimental pH region:



The corresponding conservation equation for histidine is

$$(S_t) = (S_1) + (S_2) + (S_3) + (S_4)$$

The concentration of each ionic variety of histidine is governed by the law of mass action and expressed by the following equations, which are derived from the conservation equation. (The K values are approximated to 10^{-6} and 10^{-9}.)

$$(S_1) = \frac{(S_t)}{1 + \frac{(H^+)}{10^{-6}} + \frac{(H^+)}{10^{-9}} + \frac{(H^+)^2}{10^{-15}}} \qquad (S_2) = \frac{(S_t)}{1 + \frac{10^{-6}}{(H^+)} + \frac{10^{-6}}{10^{-9}} + \frac{(H^+)}{10^{-9}}}$$

$$(S_3) = \frac{(S_t)}{1 + \frac{(H^+)}{10^{-6}} + \frac{10^{-9}}{10^{-6}} + \frac{10^{-9}}{(H^+)}} \qquad (S_4) = \frac{(S_t)}{1 + \frac{10^{-6}}{(H^+)} + \frac{10^{-9}}{(H^+)} + \frac{10^{-15}}{(H^+)^2}}$$

However, the enzyme may attack several ionized varieties of histidine indiscriminately. These are defined by the following relations: $(S_5) = (S_1) + (S_2)$, $(S_6) = (S_1) + (S_3)$, $(S_7) = (S_3) + (S_4)$, $(S_8) = (S_2) + (S_4)$.

The concentration of these ionic varieties are given by the following equations:

$$(S_5) = \frac{(S_t)}{1 + \frac{(H^+)}{10^{-9}}}; \qquad (S_6) = \frac{(S_t)}{1 + \frac{(H^+)}{10^{-6}}}; \qquad (S_7) = \frac{(S_t)}{1 + \frac{10^{-9}}{(H^+)}}; \qquad (S_8) = \frac{(S_t)}{1 + \frac{10^{-6}}{(H^+)}}$$

Equations (1) and (2) in the text refer to S_5 and S_1 respectively.

References

Ahlström, C. G. & Jonsson, N. (1962), *Acta path. microbiol. scand.*, **54**, 145–172.
Bloom, G. & Kelly, J. W. (1960). *Histochimia*, **2**, 48–52.
Burkhalter, A. (1962). *Biochem. Pharmac.*, **11**, 315–322.
Ganrot, P. O., Rosengren, A. M. & Rosengren, E. (1961). *Experientia*, **17**, 263–264.
Hagen, P., Weiner, N., Ono, S. & Lee F. L. (1960). *J. Pharmac. exp. Ther.*, **130**, 9–12.
Håkanson, R. (1961). *Experientia*, **17**, 402.
Håkanson, R. (1963). *Biochem. Pharmac.*, **12**, 1289–1296.
Håkanson, R. (1964). *Experientia*, **21**, 501.
Håkanson, R. (1966a). *Europ. J. Pharmac.* In the press.
Håkanson, R. (1966b). *Acta pharmac. tox.*, **24**, 217, 231.
Håkanson, R., Möller, H. & Stormby, N. G. (1965). *Experientia*, **21**, 265.
Håkanson, R. & Owman, Ch. (1965). *J. Neurochem.*, **12**, 417–429.
Håkanson, R. & Owman, Ch. (1966). *Biochem. Pharmac.*, **15**, 489–499.
Hallenbeck, G. A. & Code, C. F. (1962). *Proc. Soc. exp. Biol. Med.*, **110**, 649–651.
Kahlson, G. (1961). *Perspectives in Biology and Medicine*, **5**, 179–197.
Kahlson, G., Rosengren, E., Westling, H. & White, T. (1958). *J. Physiol., Lond.*, **144**, 337–348.
Kameswaran, L., Telford, J. M. & West, G. B. (1961). *Ibid.*, **157**, 23 P.
Kameswaran, L. & West, G. B. (1962). *Ibid.*, **160**, 13 P.
Lineweaver, H. & Burk, D. (1934). *J. Am. chem. Soc.*, **56**, 658–662.
Lovenberg, W., Weissbach, H. & Udenfriend, S. (1962). *J. biol. Chem.*, **237**, 89–93.
Mackay, D., Marshall, P. B. & Riley, J. F. (1960). *J. Physiol., Lond.*, **153**, 31 P.
Mackay, D., Reid, J. D. & Shepherd, D. M. (1961). *Nature, Lond.*, **191**, 1311.
Mackay, D., Riley, J. F. & Shepherd, D. M. (1961). *J. Pharm. Pharmac.*, **13**, 257–261.
Rosengren, E. (1960). *Acta physiol. scand.*, **49**, 364–369.
Rosengren, E. (1963). *J. Physiol., Lond.*, **169**, 499–512.
Rosengren, E. (1966). *Acta Univ. Lund.*, II, No. 8, p. 20.
Salamon, T. & Storck, H. (1961). *Arch. klin. exp. Derm.*, **216**, 161–169.
Shore, P. A., Burkhalter, A. & Cohn, V. H. (1959). *J. Pharmac. exp. Ther.*, **127**, 182–186.
Udenfriend, S., Lovenberg, W. & Weissbach, H. (1960). *Fedn Proc. Fedn Am. Socs exp. Biol.*, **19**, 7.