RESEARCH PAPERS

AMINO-ACID DECARBOXYLASE ACTIVITIES IN RAT HEPATOMA

BY D. MACKAY, J. F. RILEY AND D. M. SHEPHERD

From the Department of Pharmacology and Therapeutics, University of St. Andrews, Queen's College, Dundee, and the Radiotherapy Department, Dundee Royal Infirmary

Received January 26, 1961

The effects of pH, substrate concentration and addition of benzene on the rate of histamine production by extracts of rat hepatoma and guinea-pig kidney have been determined, and manometric studies of the DOPA and 5-HTP decarboxylase activities of these extracts have also been made. The histidine decarboxylases of the two tissues have quite different properties, and the histidine decarboxylase of the rat hepatoma is not associated with 5-HTP and DOPA decarboxylase activities. Whereas the urinary histamine output of hepatoma-bearing female August rats is greatly raised, the 5-HIAA output lies within the normal range.

MACKAY, Marshall and Riley (1960) have recently shown that the urine of female August rats implanted with a transplantable hepatoma, F-HEP, contains large amounts of histamine, and that extracts of these tumours are rich in histidine decarboxylase. These results fit in well with those of Kahlson (1960) on the histamine-forming capacity of regenerating rat liver. Telford and West (1960), who incidentally have been unable to confirm Kahlson's results for regenerating liver, have also reported that the histidine decarboxylase of the liver of the foetal rat differs in its pH optimum from that of the post-natal rat.

Further studies on the urine of hepatoma-bearing rats, on rat hepatoma extracts, and on extracts of guinea-pig kidney are now reported. The urinary output of 5-hydroxyindolyl-3-acetic acid (5-HIAA) by female August rats, implanted with F-HEP, has been followed simultaneously with the histamine output. The effects of pH, substrate concentration and the addition of benzene on the rate of histamine production by extracts of rat hepatoma and guinea-pig kidney have been determined, and manometric studies of the β-(3,4-dihydroxyphenyl)-α-alanine (DOPA) and 5-hydroxytryptophan (5-HTP) decarboxylase activities of rat hepatoma have also been carried out.

EXPERIMENTAL

Urinary Output of Histamine and 5-HIAA

Pooled 24-hr. urine samples were collected from a group of three female August rats implanted with F-HEP. The daily volume of urine (8–20 ml.) was collected in flasks containing 0·5 ml. of a mixture of toluene and glacial acetic acid (3 ml. toluene : 25 ml. glacial acetic acid), and was thus suitable for the determination of both 5-HIAA and histamine. The histamine was assayed directly on the atropinised guinea-pig ileum. The 5-HIAA was determined by the method of Macfarlane, Dalgliesh, Dutton, Lennox, Nyhus and Smith (1956) except that, instead of 5-HT creatinine sulphate, the standard solutions contained 5-HIAA. The standards consisted of appropriate dilutions of a stock solution (5 mg. 5-HIAA in 20 ml. glacial

acetic acid). To obtain reproducible results it was necessary to adjust the concentration of acetic acid in all samples, urines and standards, to a constant value.

Enzyme Studies

For measurement of histidine decarboxylase activity the tissue extracts and incubation media were prepared basically as described by Mackay and Shepherd (1960), the volume of saline used in the preparation of the extract being 3 ml./g. of tissue and not 30 ml./g. of tissue as erroneously stated. The iso-osmotic phosphate buffers were of various pH values, and the substrate concentrations were also varied. Aminoguanidine to give a

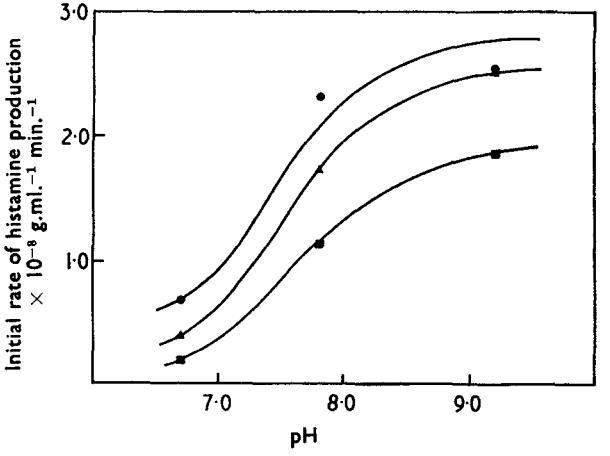

FIG. 1. The initial rate of production of histamine by the histidine decarboxylase of guinea-pig kidney as a function of pH and substrate concentration. Incubations at 37°. L-Histidine concentrations—● 15 mg./ml. ▲ 10 mg./ml. ■ 5 mg./ml.

final concentration of 6×10^{-5} M was added routinely to inhibit any histaminase, and in all experiments a final concentration of 40 μg./ml. of pyridoxal-5'-phosphate was also present. The rate of histamine production was followed by withdrawing samples at various times after the beginning of the reaction, and the histamine was assayed on the atropinised guinea-pig ileum. When benzene was added to the incubation medium the volume added was small (0·3 ml./25 ml. of medium). All incubations were carried out at 37°.

For the manometric studies the volume of saline used in the preparation of the tissue extracts was 2 ml./g. of tissue, and the Warburg flasks contained 1·0 ml. of extract in 2·0 ml. of final incubation medium. The concentration of 5-HTP or DOPA in the final media was always 0·5 mg./ml. Iso-osmotic phosphate buffers of various pH values were used and the reaction was stopped as required by tipping 0·3 ml. of 2N sulphuric acid into the flask from the centre well. The incubations were carried out in an atmosphere of oxygen-free nitrogen at 37°.

Results and Discussion

The effects of pH and substrate concentration on the initial rate of formation of histamine, from L-histidine, by extract of guinea-pig kidney are shown in Fig. 1. The optimum pH of this enzyme appears to be close to 9, and the concentration of histidine required to saturate the enzyme is at least 15 mg./ml. On the other hand, the optimum pH for histamine production by rat hepatoma extract is close to 7·0, as shown in Fig. 2. Preliminary experiments showed that the enzyme is practically saturated at a substrate concentration of 1 mg./ml. The effect of benzene on the

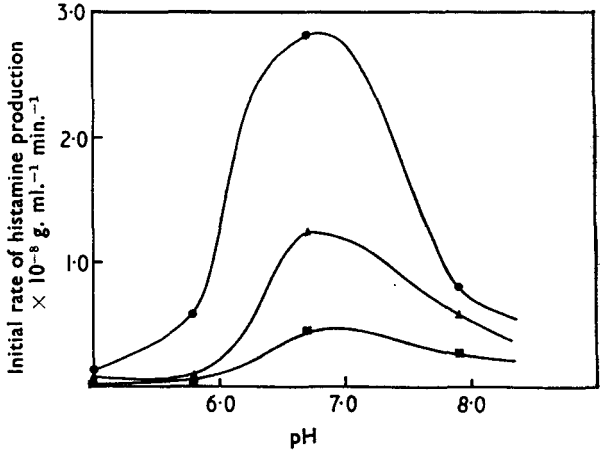

FIG. 2. The initial rate of production of histamine by the histidine decarboxylase of rat hepatoma as a function of pH and substrate concentration. Incubations at 37°. L-Histidine concentrations—● 0·25 mg./ml. ▲ 0·05 mg./ml. ■ 0·01 mg./ml.

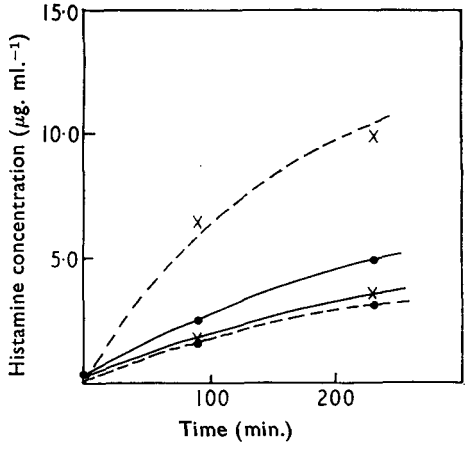

FIG. 3. The effects of benzene on the rates of production of histamine by the histidine decarboxylases of guinea-pig kidney and rat hepatoma, at 37°. Unbroken lines—experiments without benzene. Broken lines—experiments with benzene. x—guinea-pig kidney extract. ●—rat hepatoma extract.

activity of the two extracts is illustrated in Fig. 3, from which it will be seen that while the rate of histamine production by guinea-pig kidney extract is greatly increased in the presence of benzene, that by rat hepatoma extract is actually reduced. It is clear from these results that the enzymes present in the two tissues are quite different.

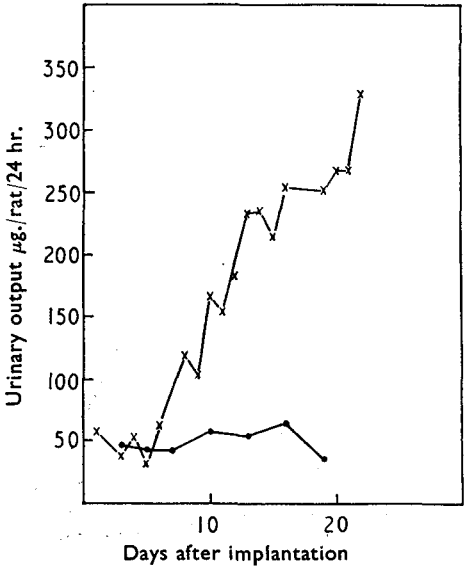

FIG. 4. Urinary histamine and 5-HIAA output of female August rats implanted with the transplantable hepatoma, F-HEP. x—histamine output per rat per day (μg.). ●—5-HIAA output per rat per day (μg.).

If the Michaelis-Menten treatment is assumed to apply to these systems, in spite of the crude nature of the enzyme-containing tissue extracts, then the Michaelis constant (K_m) and the maximum rate of histamine production (V), under the various experimental conditions,

TABLE I

EFFECT OF pH ON THE MAXIMUM RATE OF HISTAMINE PRODUCTION (V), AND ON THE VALUE OF THE MICHAELIS CONSTANT (K_m) OF HISTIDINE DECARBOXYLASES AT 37°

Tissue extract	pH	K_m mole. litre^{-1}	V mole. litre^{-1} min.$^{-1}$
Guinea-pig kidney	9·2	$4{\cdot}5 \times 10^{-2}$	$3{\cdot}8 \times 10^{-7}$
	7·8	$7{\cdot}2 \times 10^{-2}$	$3{\cdot}3 \times 10^{-7}$
	6·7	—	—
Rat hepatoma	7·8	$1{\cdot}4 \times 10^{-4}$	$0{\cdot}77 \times 10^{-7}$
	6·7	$6{\cdot}8 \times 10^{-4}$	$3{\cdot}6 \times 10^{-7}$
	5·8	68×10^{-4}	$2{\cdot}0 \times 10^{-7}$

can be determined from a plot of the reciprocal of the initial reaction rate against the reciprocal of the substrate concentration (Lineweaver and Burk, 1934). The results obtained are given in Tables I and II. The value of K_m for the enzyme of guinea-pig kidney is approximately 5×10^{-2} mole litre^{-1} as compared with a value of 7×10^{-4} mole litre^{-1}

for that of rat hepatoma, showing that the enzyme in the tumour has the greater affinity for L-histidine. From Table II it will be seen that for both enzymes the values of K_m appear to be raised in the presence of benzene. However benzene greatly increases V in the case of guinea-pig kidney, but has little effect in the case of rat hepatoma. This suggests that benzene exerts its main influence on guinea-pig kidney extract either by increasing the rate of breakdown of the enzyme-substrate complex or by increasing the concentration of enzyme molecules available (Waton, 1956).

We have confirmed the finding that guinea-pig kidney extracts have high DOPA and 5-HTP decarboxylase activities when studied manometrically (Blaschko, 1942; Smith, 1960). However, using the same method we

TABLE II

EFFECT OF BENZENE ON THE MAXIMUM RATE OF HISTAMINE PRODUCTION (V), AND ON THE VALUE OF THE MICHAELIS CONSTANT (K_m) OF HISTIDINE DECARBOXYLASES AT 37°

Tissue extract	pH	Km mole litre^{-1}		V mole litre^{-1} min.$^{-1}$	
		without benzene	with benzene	without benzene	with benzene
Guinea-pig kidney	9·2	$3{\cdot}3 \times 10^{-2}$	$5{\cdot}8 \times 10^{-2}$	$3{\cdot}3 \times 10^{-7}$	$25{\cdot}0 \times 10^{-7}$
Rat hepatoma	6·7	$8{\cdot}7 \times 10^{-4}$	$17{\cdot}4 \times 10^{-4}$	$5{\cdot}5 \times 10^{-7}$	$5{\cdot}9 \times 10^{-7}$

have been unable to detect these two enzymes in extracts of rat hepatoma at pH values of 5·0, 5·8 or 6·7. The apparent absence of 5-HTP decarboxylase from rat hepatoma is consistent with the unchanged urinary output of 5-HIAA from female August rats implanted with F-HEP, as shown in Fig. 4. On the other hand, in keeping with the high histidine decarboxylase activity of the tumours, the urinary histamine rose dramatically as previously reported (Mackay, Marshall and Riley, 1960). The histidine decarboxylase activity of rat hepatoma, unlike that of guinea-pig kidney, does not therefore appear to be associated with DOPA and 5-HTP decarboxylase activities. The above results indicate that the histidine decarboxylase present in rat hepatoma is a completely different enzyme from that which occurs in guinea-pig kidney.

Acknowledgement. The authors are grateful to Dr. K. S. Kirby and Professor A. Haddow for supplying the August rats and hepatoma transplant. One of the authors (D. M.) also wishes to acknowledge the financial support of Smith, Kline and French Laboratories Ltd.

REFERENCES

Blaschko, H. (1942). *J. Physiol.*, **101**, 337–349.
Kahlson, G. (1960). *Lancet*, **1**, 67–71.
Lineweaver, H., and Burk, D. (1934). *J. Amer. chem. Soc.*, **56**, 658–666.
Macfarlane, P. S., Dalgliesh, C. E., Dutton, R. W., Lennox, B., Nyhus, L. M., and Smith, A. N. (1956). *Scot. med. J.*, **1**, 148–155.
Mackay, D., Marshall, P. B., and Riley, J. F. (1960). *J. Physiol.*, **153**, 31P.
Mackay, D., and Shepherd, D. M. (1960). *Brit. J. Pharmacol.*, **15**, 552–556.
Smith, S. E. (1960). *Ibid.*, **15**, 319–327.
Telford, J. M., and West, G. B. (1961). *J. Physiol.*, in the press.
Waton, N. G. (1956). *Biochem. J.*, **64**, 318–322.