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ALTERATIONS IN HISTIDINE DECARBOXYLASE ACTIVITY DURING ANAPHYLACTIC SHOCK IN THE RAT

BY

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The similarity between the signs of anaphylactic shock and the pharmacological action of histamine on different organs of the dog and guinea-pig has been well documented (Dale, 1929). However, the similarity does not extend to all species, and other endogenous substances such as 5-hydroxytryptamine (Sanyal & West, 1958a), and bradykinin (Brocklehurst & Lahiri, 1962; Dawson, Starr & West, 1966) have been implicated. Whereas Sanyal & West (1958b) showed that depletion of the histamine stores in the rat by polymyxin B or by *x*-ray irradiation did not change the severity of the anaphylactic reaction in rats, Mota (1957) claimed that pretreatment of rats with compound 48/80 (a histamine liberator) inhibited the shock. Both Mota (1957, 1958) and Code, Cody, Hurn, Kennedy & Strickland (1961) found that the plasma histamine level of rats was raised during anaphylactic shock, although they did not identify the source of the released histamine. Later, Kahlson, Rosengren & Thunberg (1964, 1966), using a radioactive technique, reported that the formation of histamine in some tissues of the rat (particularly in the lung and gut) was increased in anaphylaxis.

At least two enzymes capable of forming histamine in mammals have been described in recent years; one is specific for L-histidine, and the other is non-specific as it decarboxylates aromatic aminoacids other than histidine (Lovenberg, Weissbach & Udenfriend, 1962). Using carboxyl-labelled histidine, it has recently been shown that the enzyme in the thin fundic portion of rat stomach possesses some properties like those of the specific enzyme whereas that in the less active pyloric portion resembles more the non-specific enzyme (Radwan & West, 1967).

The present work was planned (a) to investigate the location and nature of the enzyme responsible for the increased formation of histamine during anaphylactic shock in the rat, and (b) to determine the role this increased histamine formation plays in the pathogenesis of the syndrome.

METHODS

Groups of four or more male Sprague-Dawley rats (obtained from Fisons Pharmaceutics Ltd., Holmes Chapel), weighing 120 to 150 g were used in most experiments. Female animals of similar

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weight were only used for estimating total body histamine formation. All rats were fed on a cube diet (No. 41B, Associated London Flour Millers Ltd.), allowed unrestricted drinking water, and housed at $21 \pm 0.5°$ C.

Histidine decarboxylase activity of rat tissues

The isotopic dilution technique, devised by Schayer, Rothschild & Bizony (1959) and modified by Kahlson, Rosengren & Thunberg (1963), was used. In brief, ring-labelled 2-^{14}C-L-histidine was incubated with 400 mg minced tissue in phosphate buffer of optimum pH value in the presence of aminoguanidine (an inhibitor of histaminase). Incubation proceeded at 37° C for 3 hr, and then trichloroacetic acid was added to precipitate the proteins. The formed ^{14}C-histamine was isolated by ion-exchange chromatography, and then converted to the dibenzenesulphonylhistamine derivative (BSH) which was purified by repeated crystallization until constant activity of the product was obtained. The radioactivity of the derivative was counted in a Packard Tricarb liquid scintillation counter with an efficiency of about 55%, as determined by the channel ratio method of Baillie (1960). Histidine decarboxylase activity has been expressed as d.p.m./g tissue or μg histamine formed/g tissue/3 hr; 31,000 d.p.m. are equivalent to about 1 μg ^{14}C-histamine. An inhibitor of the specific enzyme (α-methylhistidine) and one of the non-specific enzyme inhibitors (α-methyldopa) were each tested in amounts ranging from 100 to 1,000 μg/4 ml. incubation mixture.

Total body histamine formation. Changes in the rate of endogenous histamine formation were followed by determining the urinary free-histamine in female rats housed in separate metabolic cages (Gustafsson, Kahlson & Rosengren, 1957). Each rat was given 5 ml. distilled water orally to increase the urinary flow, after injecting aminoguanidine (50 mg/kg) intraperitoneally to inactivate the histaminase (Schayer, Wu & Smiley, 1954). Urine was collected for periods of 24 hr in small beakers containing 0.1 ml. N-HCl. The total volume was measured and its content of free histamine was assayed on the isolated atropinized guinea-pig ileum. Specificity of the response was checked with mepyramine maleate. All values of histamine refer to the base. When tests were made to determine the effect of inhibitors of histidine decarboxylase on the daily output of histamine, these were injected intraperitoneally, at a dose level of 100 mg/kg, when collection commenced.

Histamine content of the tissues. The method used was that described by Parratt & West (1957). Briefly, the tissues were extracted with 10% (w/v) trichloroacetic acid (5 ml./g), the excess acid was removed by ether, and the aqueous solutions were assayed on the isolated atropinized guinea-pig ileum. Rats were killed at different times after the injection of α-methylhistidine, α-methyldopa, or 4-bromo-3-hydroxy-benzyloxyamine (NSD-1055), a powerful inhibitor of both the specific and the non-specific histidine decarboxylases (Levine, Sato & Sjoerdsma, 1965).

Anaphylaxis in rats. Groups of 10 rats were sensitized by an intraperitoneal injection of either horse serum (0.5 ml.) or fresh hen's egg-white (0.5 ml. of a 50% v/v solution in 0.9% saline), each mixed with *Bordetella pertussis* vaccine (0.25 ml. of $80{,}000 \times 10^6$ organisms/ml.). Twelve days later, they were injected intravenously, under light ether anaesthesia, either with 1 ml. or with 0.2 ml. of the corresponding antigen. With the larger challenging dose, mortality rates were determined over the next 24 hr; with the smaller dose, rats were killed 3 hr later to provide the tissues for histidine decarboxylase determinations. Other groups of sensitized rats were injected intravenously with 1 ml. 0.9% saline and then killed; the histidine decarboxylase activities of the tissues of these rats served to show whether these values had been altered by sensitization 12 days earlier. Further groups of sensitized animals were injected intraperitoneally, 3 hr before the challenge with antigen (1 ml.), with α-methylhistidine or NSD-1055 (100 mg/kg).

RESULTS

Factors affecting histidine decarboxylase activity

Effect of pH. Figure 1 shows the effect of changing the pH value of the incubation mixture on the activity of histidine decarboxylase in lung, liver and spleen of rats. The optimal pH value of the lung is about 7 while that of the liver and the spleen is about 7.4. It has been previously reported that the corresponding values for the thick

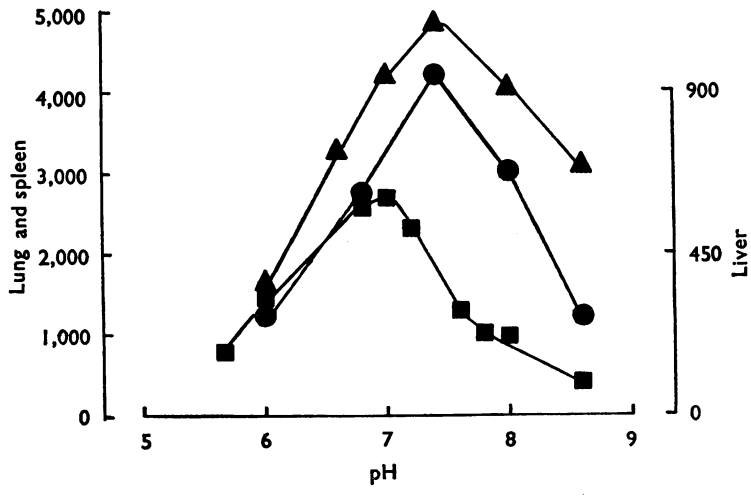

Fig. 1. Effect of pH on the histidine decarboxylase activity of liver (●——●), lung (■——■), and spleen (▲——▲) of rats, expressed as d.p.m./g tissue. Note the different scales.

pyloric part and the thin fundic part of the stomach are about 7.6 and 5.6 respectively (Radwan & West, 1967) and in all subsequent work these optimal pH values were used.

Effect of benzene. Enzyme activities of tissues are shown in Table 1. When benzene (20 mg) was included in the incubation mixture with rat liver, the histidine decarboxylase actvity was increased three-fold; no such increase occurred in the lung, spleen, heart, stomach and small intestine. Benzene was therefore included in the incubation mixture only when assays were carried out on rat liver. Using a different radioactive technique, benzene has previously been found to potentiate the enzyme activity in the pyloric portion of the stomach by a small significant amount (about 20%).

TABLE 1

HISTIDINE DECARBOXYLASE ACTIVITY *IN VITRO* OF TISSUES OF MALE SPRAGUE–DAWLEY RATS

The results are expressed as μg histamine formed/g tissue/3 hr. Each value is the mean of at least four observations

Tissue	Histidine decarboxylase
Liver	0·02
Heart	0·03
Small intestine	0·03
Lung	0·09
Spleen	0·15
Stomach (pyloric)	1·21
Stomach (fundic)	8·67

Effect of inhibitors. When 1 mg of α-methylhistidine was included in the incubation mixture, significant inhibition (more than 40%) of the enzyme in the lung and spleen was found, but that in the liver was unaltered. However, larger amounts (up to 10 mg) of α-methyldopa inhibited the liver enzyme.

Effect of histidine decarboxylase inhibitors on histamine metabolism

Table 2 shows that a single intraperitoneal dose of NSD-1055 or of α-methylhistidine significantly decreases the histamine content in the lung and pyloric portion of the stomach by 3 hr after injection but does not change the skin histamine levels. Whereas the stomach histamine remains low for more than 24 hr, that in the lung has returned to at least 50% of the control value by this time. On the other hand, treatment with α-methyldopa failed to alter the histamine content of the tissues at either time interval. The rate of histamine formation as determined by the urinary excretion of free histamine was also significantly reduced (by more than 35%) during the first 24 hr after treatment with NSD-1055 or α-methylhistidine but returned to control levels within 48 hr. Treatment with α-methyldopa had no such effect. Thus, histamine metabolism in the rat may be reduced by treatment with inhibitors of the specific histidine decarboxylase.

TABLE 2

EFFECT OF HISTIDINE DECARBOXYLASE INHIBITORS (100 mg/kg INTRAPERITONEALLY) ON THE HISTAMINE CONTENT (μg/g) OF RAT TISSUES AT DIFFERENT TIMES AFTER INJECTION

Standard errors of the means are shown

Time after injection (hr)	NSD-1055			α-Methylhistidine		
	Skin	Pyloric stomach	Lung	Skin	Pyloric stomach	Lung
0	40·1±2·5	34·1±2·6	6·5±1·1	40·1±2·5	34·1±2·6	6·5±1·1
3	41·4±2·5	23·5±3·3	1·5±0·4	40·0±3·2	25·2±2·1	3·1±0·8
24	41·4±2·8	20·0±2·9	3·0±0·6	36·0±4·4	23·0±3·0	6·5±1·2

Changes in histidine decarboxylase during anaphylaxis

When sensitized rats were challenged with the smaller dose of the specific antigen (0.2 ml.), there were no deaths by 3 hr and significant increases in histidine decarboxylase activity were found in the lung, liver and spleen. As shown in Table 3, the enzyme activities in the heart of rats sensitized to egg-white and in the small intestine of animals sensitized to horse serum were also increased after anaphylaxis. In addition, Table 3 shows that sensitization alone does not significantly increase the levels of enzyme activity in tissues.

TABLE 3

EFFECT OF ANAPHYLACTIC SHOCK ON THE HISTIDINE DECARBOXYLASE ACTIVITY OF RAT TISSUES

Enzyme activities are expressed as d.p.m./g tissue. Each value is the mean of at least two observations. Rats were challenged 12 days after sensitization and killed 3 hr later

Tissue	Controls	Sensitization to egg-white	Egg-white anaphylaxis	Sensitization to horse serum	Horse serum anaphylaxis
Heart	760	858	1,315*	812	845
Small intestine	880	972	862	934	1,362*
Liver	962	1,230	6,698*	1,028	10,191*
Lung	2,640	2,980	6,800*	2,997	15,025*
Spleen	7,715	10,730	14,472*	10,852	12,835*

* Significance at the $P=0·05$ level

The increases in enzyme activity in the lung, liver and spleen at 3 hr after challenge with antigen were markedly inhibited by including α-methylhistidine in the incubation mixture (see Fig. 2). This result shows that most of the increased enzyme activity in these three tissues is of the specific type, and the negative results with α-methyldopa support this conclusion.

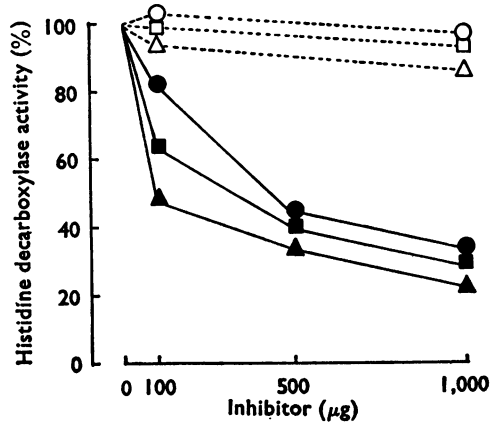

Fig. 2. Effect of including α-methyldopa (broken lines) and α-methylhistidine (continuous lines) in the incubation mixtures on the histidine decarboxylase activity of liver (●——●), lung (■——■), and spleen (▲——▲) of rats undergoing anaphylactic shock with egg-white as antigen. Enzyme activities are expressed as percentages of control incubations containing no inhibitor.

Effect of histidine decarboxylase inhibitors on anaphylaxis

When sensitized rats were challenged with the larger dose of the specific antigen (1 ml.), the mortality rate over the next 24 hr was 30%. The mortality rate was unaffected by pretreatment with α-methylhistidine. However, when NSD-1055 (100 mg/kg) was given intraperitoneally 1 hr before the challenge, the mortality rate was markedly increased (to 60%), indicating that this inhibitor of both the specific and the non-specific histidine decarboxylase does not reduce the severity of the shock but increases it. This dose of NSD-1055 was found to lower the blood pressure of the anaesthetized rat and hence the result of the antigen-antibody reaction may be aggravated by this hypotensive action. The organs where gross tissue damage and haemorrhage occurred during anaphylaxis were the small intestine and lungs when horse serum was used as antigen and the heart and lungs when egg-white was the antigen.

DISCUSSION

The results of the present work show that the histidine decarboxylase activity of some tissues of Sprague-Dawley rats is elevated in anaphylaxis. This confirms the findings of Kahlson *et al.* (1964) who used only egg-white as antigen and *B. pertussis* vaccine to aggravate the anaphylactic reaction (Malkiel & Hargis, 1952; Sanyal & West, 1958b). Whereas Kahlson *et al.* (1964) found that gross tissue damage in the small intestine during anaphylactic shock was accompanied by elevation of the histamine-forming capacity of this tissue to about 25 times the control level and that a similar increase in the decarboxylase activity occurred in the lung, much smaller increases have been found in the present study. For example, during anaphylactic shock using egg-white as antigen, increases of the order of only two- to six-fold were obtained in the lung, liver, spleen and heart; when horse serum was the antigen, somewhat larger increases (up to

ten-fold) were detected in the small intestine, liver and lung. The heart and lungs were the chief target organs affected during egg-white anaphylaxis, whereas the small intestine and lungs suffered most damage in horse serum anaphylaxis, tissue in which major changes occurred in the histidine decarboxylase activity. The reasons for these contrasting results in location and magnitude are probably not to be found in the methods and techniques used, for these were similar except for one procedure used by Kahlson *et al.* (1964); this was the use of a histamine antagonist, mepyramine, and a 5-hydroxytryptamine antagonist, methysergide, both of which were administered to the rats 15 min before the second challenging dose of antigen, presumably to reduce mortality by 3 hr after the last injection. Furthermore, Levine (1966) reported that rats of the Sprague-Dawley strain but from different sources not only often behave differently to drugs (and he used, among others, an inhibitor of histidine decarboxylase) but they also have significantly different histamine levels in their tissues. In the present work, Sprague-Dawley rats were secured from only one source and the histamine concentrations in the skin, pyloric stomach and lung of these animals were consistent when tested at different times of the year.

The increase in histidine decarboxylase activity of some tissues induced by anaphylaxis is mostly of the specific type as it is inhibited by α-methylhistidine but not by α-methyldopa and has an optimal pH value about 7. This does not imply that these tissues do not contain the non-specific enzyme which may be present in small amounts and may be masked by the high level of the specific enzyme. The liver enzyme, for example, appears to be of the non-specific type as its activity is potentiated by benzene (Waton, 1956) and not inhibited by α-methylhistidine and yet its increased activity after anaphylactic shock can be markedly inhibited by α-methylhistidine, an inhibitor of the specific enzyme.

The histamine levels in the pyloric stomach and lung were greatly reduced by treatment with inhibitors of the specific histidine decarboxylase but not with α-methyldopa. These two tissues are lacking in mast cells, the chief storage sites of histamine (Riley & West, 1953), and hence the reduction in histamine content is probably mediated through inhibition of the enzyme and not through a greater histamine release. This conclusion is supported by the observation that inhibitors of the specific enzyme also reduced the urinary excretion of free histamine. Tissues such as the skin which have a high mast cell population were not depleted of histamine by such treatment. The action of NSD-1055 was more marked and more prolonged than that of α-methylhistidine, and this may be due to the fact that NSD-1055 is an inhibitor of both the specific and non-specific enzymes.

In the present work, the increased histidine decarboxylase activities of some tissues of Sprague-Dawley rats undergoing anaphylactic shock were markedly inhibited by including α-methylhistidine, an inhibitor of the specific enzyme, in the incubation mixtures. However, such an inhibitor failed to reduce the mortality rate in anaphylaxis, and it is possible, therefore, that histamine is not one of the major mediators in rat anaphylaxis, a finding previously reported by Sanyal & West (1958b). Recently, bradykinin was found in the blood of rats undergoing anaphylactic shock (Brocklehurst & Lahiri, 1962) and this may play a more important role in this syndrome than does histamine (Dawson & West, 1965). It is a very potent dilator of small blood vessels in most mammals.

SUMMARY

1. Using an isotopic method which allows the isolation, crystallization and counting of ^{14}C-histamine formed from ^{14}C-L-histidine, estimations have been made of the histidine decarboxylase activities *in vitro* of different tissues of Sprague-Dawley rats. In descending order of magnitude, these are stomach, spleen, lung, small intestine, heart and liver. The enzyme is of the specific type except in the case of the liver.

2. Potent inhibitors of the specific histidine decarboxylase decrease the histamine levels in the stomach and lung and reduce the urinary excretion of free histamine.

3. The specific histidine decarboxylase activities of the liver, lung and spleen are significantly increased during anaphylactic shock. Inhibitors of the specific enzyme markedly reduce the increased activity *in vitro*. However, injections of these inhibitors do not modify the mortality rates of rats undergoing anaphylactic shock.

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