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FORMATION OF HISTAMINE IN THE GUINEA-PIG

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Abstract—The formation of histamine from histidine has been studied in the guinea-pig using a non-isotopic method *in vitro*. Main sites of histamine formation are the kidney, liver and small intestine. Foetal tissues form little histamine. Studies of the optimal conditions for histidine decarboxylase activity suggest that the guinea-pig possesses only one enzyme capable of forming histamine. In contrast with the rat, histidine decarboxylase activity in the guinea-pig is not modified by injections of glucocorticoids or by exposure to cold.

The origin of tissue histamine in mammals is not clear. Recently, Udenfriend and his colleagues[1,2] have suggested that tissues contain at least two enzymes which form histamine from histidine; first, a non-specific enzyme which is able to decarboxylate all the naturally occurring L-amino acids,[1] and secondly, a seemingly specific L-histidine decarboxylase thought to be present in all mast cells[2] and probably identical with the non-mast cell enzyme present in high concentration in the rat foetus.[3] The relative roles of these enzymes in the control of histamine production have not, however, been established, and their distribution has not been systematically studied. Histamine formation has been studied in closest detail in the rat, and in this species Telford and West[4] have suggested that there may be at least three histamine-forming enzymes. The evidence for this hypothesis is that the optimal conditions for enzyme activity (e.g. optimal pH) vary from tissue to tissue, and that after injections of glucocorticoids or histamine liberators, or after exposure of the animals to cold, enzyme activity is reduced in one tissue (adult liver) but increased in another (adult pyloric stomach). The present communication describes a preliminary series of experiments into the origin of histamine in the guinea-pig, and the results suggest that this species differs from the rat in possessing only one histamine-forming enzyme.

METHODS

Animals. All guinea-pigs had the characteristics of the pure English variety; for breeding and maintenance purposes the procedures of Paterson[5] were followed. The animals were fed on a cube diet (Ralston Purina Co., U.S.A.), allowed drinking water *ad libitum*, given a supplement of fresh green food daily, and were housed at 25–30°.

Groups of 3 female guinea-pigs weighing 450–550 g were used in all experiments unless otherwise stated. Foetal tissues were pooled from the foetuses of groups of 3 guinea-pigs weighing 450–550 g on the day of mating; the average number of young per litter was 2·9. All animals were killed by cutting the throat after preliminary stunning.

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Histidine decarboxylase activity of tissue homogenates. Pooled tissue from freshly killed guinea-pigs was ground in a glass mortar with a small quantity of sand and 0·9% (w/v) sodium chloride solution (4 ml/g tissue). The resulting homogenate was centrifuged at 4,000 rev/min for 5 min, and the supernatant fluid removed for incubation. The composition of the incubation mixture was: tissue homogenate, 4·0 ml; phosphate buffer (M/20 KH_2PO_4), 4·8 ml.; aminoguanidine (neutralized, 10 mg/ml.), 0·1 ml.; L-histidine (neutralized, 15 mg/ml.), 1·0 ml.; pyridoxal-5-phosphoric acid (50 μg/ml.), 0·1 ml.; benzene, 20 mg. Benzene was added last to avoid evaporation. The mixture was immediately shaken, and incubated for 4 hr at 37° under constant agitation. The reaction was stopped by reducing the pH of the solution to 4·0 with N-hydrochloric acid and by cooling to 4°. The mixture was then centrifuged at 4,000 rev/min for 5 min and after neutralisation with N-sodium hydroxide the histamine content of the supernatant was determined. In each experiment, mixtures with and without the substrate (histidine) and mixtures containing boiled homogenate or no homogenate were incubated and assayed for histamine; each incubation was performed in triplicate. The final volume of all mixtures was 10 ml. The mean histamine content of mixtures incubated in the presence of histidine less the mean histamine content of mixtures incubated in the absence of histidine gave the amount of histamine formed from histidine. The amounts of histamine (μg) formed per gram of tissue and per tissue were used as indices of histidine decarboxylase activity. Each result is the mean of three experiments unless otherwise stated.

Histidine decarboxylase activity of guinea-pig ileum. An attempt was made to determine the histamine forming capacity of the ileum in conditions which are more physiological than pertains in saline homogenates. This was done by suspending segments of ileum at 37° for 4 hr in oxygenated Tyrode solution containing histidine, and by then measuring the amount of histamine formed.

Two guinea-pigs of the same sex and weight (400–450g), which had been starved for 12 hr, were used in each experiment. The small intestines were removed from the freshly killed animals, and segments measuring approximately 70 cm unstretched length were suspended at zero tension in 120-ml baths of oxygenated Tyrode solution. After the segments had been suspended for 5 min, the loose intestinal contents were removed by emptying the baths and refilling with fresh Tyrode solution; removal of intestinal matter was facilitated by previously making 2 or 3 small slits along the ileal wall. Aminoguanidine (4 mg) and pyridoxal-5-phosphoric acid (200 μg) were then added to each bath; L-histidine (60 mg) was added to one bath. After incubation for 4 hr, the two solutions were run off and immediately assayed for histamine. The histamine content of the solution containing histidine less the histamine content of the solution not containing histidine gave the amount of histamine formed.

Assay of histamine. Bioassays were performed on the isolated ileum of the guinea-pig, using a 15 ml. bath of oxygenated atropinized Tyrode solution at 32°. On occasion, extracts were also assayed on the blood pressure of an anaesthetized dog. The specificity of the responses was checked with mepyramine maleate. All values of histamine refer to the base.

RESULTS

Optimal conditions for histamine formation. Highest rates of histamine formation were obtained using 1 g tissue and 15 mg L-histidine in 10 ml incubation mixtures

(Fig. 1). The presence of benzene (20 mg) increased enzyme activity 10–20 times in all tissues, and in contrast with the rat,[6] the addition of pyridoxal (5 μg) further increased activity by 20–50 per cent (Table 1). In each tissue the optimal pH for enzyme activity was 8·0–8·5, and as shown in Fig 2, the pH curves were similar over the range 4·0–10·5.

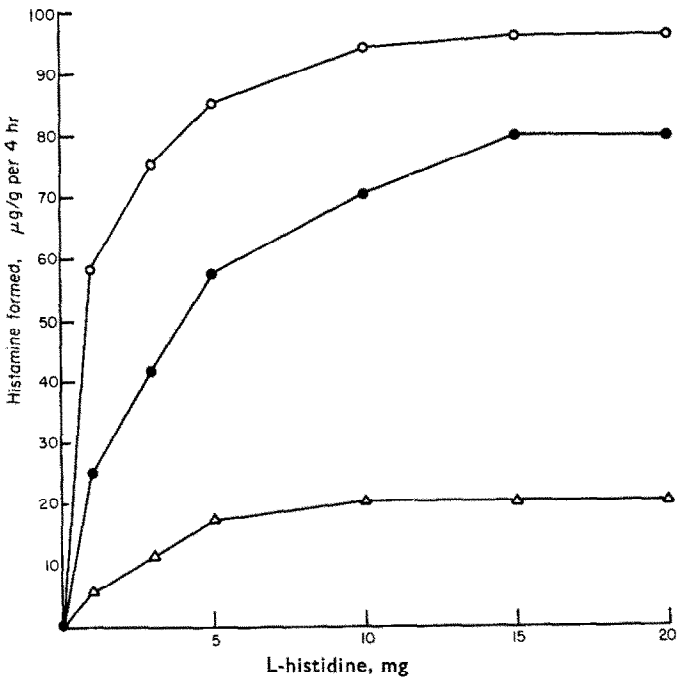

FIG. 1. Effect of substrate concentration on the histidine decarboxylase activity of guinea-pig kidney (○—○), ileum (●—●) and liver (△—△) expressed as μg histamine formed/g tissue per 4 hr.

The incubation times required for maximal formation of histamine varied from 3 to 5 hr, although maximal formation usually occurred within 4 hr. The progress curves of the reaction were similar to those previously recorded for tissues of the rat,[7] and were of the general form typical of most enzymatic reactions in which a reduction in velocity occurs with time. The unknown factors which contribute to the fall in

TABLE 1. EFFECT OF BENZENE (20 mg/g) AND PYRIDOXAL-5-PHOSPHORIC ACID (50 μg/g) ON THE HISTIDINE DECARBOXYLASE ACTIVITY OF GUINEA-PIG TISSUES EXPRESSED AS μg HISTAMINE FORMED/g PER 4 HR

Tissue	Histamine formed (μg/g per 4 hr)		
	Benzene absent Pyridoxal absent	Benzene present Pyridoxal absent	Benzene present Pyridoxal present
Liver	1·5	17·5	23·6
Kidney	9·2	96·4	122·8
Ileum	3·3	54·1	76·2
Stomach	0·4	5·4	7·6
Lung	0·2	2·1	3·1

velocity with time result in the curves not fitting the standard equations of homogeneous chemical reactions, and hence it is only during the early stages of the incubation period that the amount of histamine formed gives a true measure of the rate of histamine production. In all tissues, the rate of histamine formation is greatest during the first 30 min, up to 60 per cent of the total histamine production being formed within this time. During this early stage of the incubation period the relationship between histamine formed and time is linear, and it is thus possible to determine for each tissue the initial velocity of the reaction (μg histamine formed/g per min) by drawing a tangent to the origin of the curve.

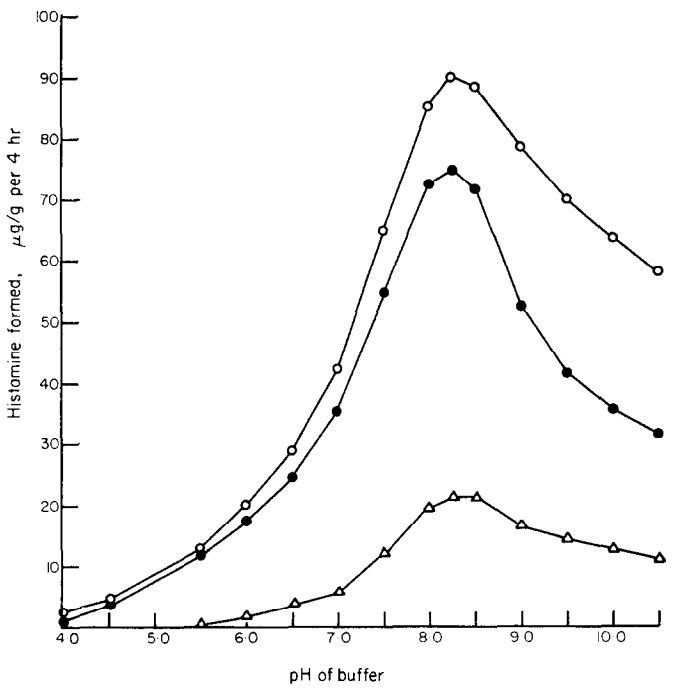

FIG. 2. Effect of pH on the histidine decarboxylase activity of guinea-pig kidney (○—○), ileum (●—●) and liver (△—△) expressed as μg histamine formed/g tissue per 4 hr.

Histamine formation in adult guinea-pigs. The richest sources of histidine decarboxylase in the adult guinea-pig are the kidney, small intestine and liver (Table 1). The stomach, lung and colon are able to form smaller amounts of histamine, while the skin, brain, heart, spleen, caecum and rectum appear to possess no ability to form histamine. In the kidney, histamine formation takes place mainly in the cortex, an enzyme activity of 205 μg/g per 4 hr being found in cortex as opposed to 32 μg/g per 4 hr in medulla.

When calculated from the initial velocity of the reaction, histamine forming capacity is considerably higher than when calculated from the total amount of histamine formed during prolonged incubation (Table 1). For example, the kidney forms 0·92 μg histamine/g per min. This represents 221 μg/g in 4 hr (5·6 mg/tissue per day), as compared with 102 μg/g in 4 hr (2·6 mg/tissue per day) when estimated as total histamine production.

The initial velocity of the enzyme reaction is similar in all tissues when the amount of enzyme used for incubation is the same. Thus, when the amount of enzyme used for incubation is such as to give a maximal histamine formation of 10 μg/g per 4 hr (range 5–15 μg/g per 4 hr), the initial velocity of the reaction is 0·15 μg/g per min (range 0·12–0·20 μg/g per min) in all tissues. Since the optimal conditions for enzyme activity are also similar in all tissues, it seems likely that the adult guinea-pig possesses only one enzyme capable of forming histamine from histidine.

TABLE 2. HISTAMINE-FORMING CAPACITIES OF GUINEA-PIG TISSUES WHEN ESTIMATED AS THE INITIAL VELOCITY OF THE ENZYME REACTION (μg HISTAMINE FORMED/g PER MIN) AND WHEN ESTIMATED AS TOTAL HISTAMINE PRODUCTION (μg HISTAMINE FORMED/g PER 4 HR)

Tissue	Histamine-forming capacity				
	Estimated as initial velocity			Estimated as total production	
	μg/g per min	μg/g per 4 hr	μg/tissue per day	μg/g per 4 hr*	μg/tissue per day
Liver	0·18	43·2	5935·8	21·3 (15·5–32·5)	2926·8
Kidney	0·92	220·8	5564·4	101·7 (72·6–153·0)	2562·6
Ileum	0·73	175·2	4730·4	75·1 (52·4–93·0)	2028·0
Duodenum	0·70	168·0	806·4	61·5 (55·0–68·4)	295·2
Stomach	0·11	26·4	507·0	6·6 (4·0–10·8)	126·6
Colon	0·03	7·2	125·3	2·7 (1·0–3·9)	47·0
Lung	0·03	7·2	181·2	2·5 (1·5–4·8)	63·0

* Figures in brackets represent the range of values obtained from seven or more experiments, and are included to show the wide variations that occur in the histidine decarboxylase activity of control animals.

Some factors affecting histamine formation in adult guinea-pigs. Histidine decarboxylase activity of guinea-pig kidney is inhibited by DL-α-methyldopa, L-dopa, semicarbazide and catechol.[8] The inhibitory effect of these drugs on histamine formation was tested in other tissues, and it was found that when added to incubation mixtures their relative potencies are the same whatever the tissue. This further suggests that the histamine-forming capacity of guinea-pig tissues is due to the activity of a single enzyme. As found for kidney by Mackay and Shepherd,[8] the most potent inhibitor is DL-α-methyldopa, 100 μg/g tissue completely preventing formation of histamine. L-dopa, semicarbazide and catechol are somewhat less potent, 50 and 100 per cent inhibition being obtained in each tissue by approximately 50 μg/g and 500 μg/g respectively of each drug.

In rats, histidine decarboxylase activity is reduced in the liver and increased in the stomach after injections of glucocorticoids or after exposure of the animals to cold.[4] In the guinea-pig, on the other hand, no changes were found in the histidine decarboxylase activity of the liver, kidney and ileum after injections of adrenocorticotrophic hormone (40 I.U./kg daily for 4 days), cortisone (25 mg/kg daily for 4 days) or

prednisolone (20 mg/kg daily for 4 days), or after exposure of the animals to cold (8–12°) for up to 48 hr. The addition of glucocorticoids to incubation mixtures likewise did not modify the formation of histamine except in very large doses (1–5 mg/g).

In a further series of experiments, groups of guinea-pigs were starved for periods of 12–140 hr. No significant changes in the rate of histamine formation by the liver and kidney were detected. In the ileum the response was variable and in 2 groups of animals starved for 130–140 hr histidine decarboxylase activity was reduced by more than 60 per cent; activity was maintained at control values, however, in the presence of larger amounts of pyridoxal (100–500 μg/g). It is of interest that guinea-pig dopa decarboxylase is also decreased on starvation, activity likewise being restored by pyridoxal.[9] In rats, histidine decarboxylase activity is reduced during semistarvation.[10]

Foetal and young guinea-pigs. Histidine decarboxylase activity was not detected in the foetal guinea-pig before the 20th day of gestation. On the 40th day of gestation, the foetal liver formed 6·0 μg histamine/g per 4 hr, the foetal kidney 23·8 μg/g per 4 hr, and the foetal small intestine 6·6 μg/g per 4 hr. In the liver and kidney, enzyme activity increased thereafter as pregnancy progressed, and adult values were reached by term. In the small intestine, on the other hand, enzyme activity had increased to only 30·0 μg/g per 4 hr at term, and adult values were not reached until shortly before weaning; in 7 day old animals the small intestine formed 33·7 μg histamine/g per 4 hr; in 14 day old animals, 40·3 μg/g per 4 hr; and in 28 day old animals, 70·4 μg/g per 4 hr.

The optimal conditions for enzyme activity in foetal liver, kidney and small intestine are similar to those required for adult tissues. Enzyme activity was not detected in other foetal tissues or in the placenta.

Histamine formation by ileal segments. Segments of ileum suspended in oxygenated Tyrode solution for 4 hr at 37° form large quantities of histamine from added histidine. The values of histamine obtained, the means of 6 experiments, were 18·3 μg in the absence of histidine and 98·5 μg in the presence of histidine (60 mg), giving a total histamine production of 80·2 μg/4 hr or 17·9 μg histamine/g tissue per 4 hr. The ability of ileal segments to form histamine was unaffected by the addition to the medium of up to 200 mg of benzene: this is in contrast to the gross potentiation of histamine formation produced by benzene when ileal homogenates are used (Table 1). It is possible, however, that some or all of the histamine synthesized by the ileal segments was due to the activity of an enzyme different from that being measured in the homogenate experiments, for example a bacterial enzyme.

DISCUSSION

The origin of tissue histamine in mammals may differ in different species, but there are at least two possible major sources. First, it may be derived from bacterial decarboxylation of histidine in the alimentary tract, and secondly, it may be formed locally from histidine in the tissues. In the rat and in the dog, sterilization of the intestinal tract reduces the urinary excretion of free histamine [11, 12] and it is possible that in these species histidine is decarboxylated in the intestinal lumen, and that some of the formed histamine is absorbed by the tissues. In the dog, tissue histidine decarboxylase has not been detected,[13] and bacterial formation of histamine in the

intestine may therefore be the major or only source of the tissue amine; in the rat, on the other hand, some tissues possess a high histidine decarboxylase activity,[3, 6] and in this species the tissue histamine may be derived both locally and from bacterial decarboxylation. In the guinea-pig, it has recently been reported by Waton[14] that oral administration of histidine raises the urinary excretion of free histamine, and that this raised urinary excretion is not modified by sterilization of the intestinal lumen. The conclusion was drawn that guinea-pigs, in contrast to dogs and rats, probably do not obtain tissue histamine by bacterial decarboxylation of histidine in the intestine, but that they derive it by the decarboxylation of histidine locally in the tissues. The results of the present experiments, which show that several guinea-pig tissues possess a very high histidine decarboxylase activity, lend support to this view.

Summation of the amounts of histamine capable of being formed by the guinea-pig liver, kidney, lung and alimentary tract gives a total histamine production *in vitro* of 10–20 mg a day. It cannot, of course, be assumed that such enormous quantities of histamine are formed and metabolized under physiological conditions *in vivo*, and the results of the present determinations can be interpreted no further than to say that in certain conditions some tissues are capable of forming much histamine, while others appear to be capable of forming little or no histamine. Thus, it is reasonable to suppose that the presence of exceedingly high enzyme activities *in vitro* in guinea-pig liver, kidney and small intestine indicates that these tissues are capable of forming very considerable quantities of histamine *in vivo*. It is also possible that some histamine is formed in tissues in which no histidine decarboxylase activity can be detected using the present somewhat insensitive method, perhaps by another enzyme. Nevertheless, in the guinea-pig as in the rat, it is clear that there is no parallelism between histidine decarboxylase activity and histamine content, and the possibility exists that histamine is formed in some tissues and taken up, stored or utilized in others.

Available evidence suggests that the processes of histamine formation and uptake are sufficiently rapid to allow very large quantities of histamine to be formed in one tissue and transported in the blood stream to another without any gross changes taking place in the histamine content of the blood itself. Several investigators have demonstrated that there exists a powerful mechanism for the uptake of histamine into tissues from circulating blood: Halpern, Neveu and Wilson, for example, have shown that histamine intravenously injected into rats is removed from the blood at a rapid rate, and is distributed in the tissues where it is then metabolized.[15] Again, Furano and Green[16] have shown that mast cells from the peritoneal fluid of the rat take up histamine and retain it for at least 24 hr, both *in vivo* and *in vitro*. In this species, the histidine decarboxylase activity of the liver far exceeds that found in other tissues, and it has been concluded that the rat liver manufactures histamine for uptake and utilisation by other parts of the body.[4] In the guinea-pig, it is possible that tissue histamine is derived mainly through the activity of histidine decarboxylase in the liver, kidney and small intestine, and that these tissues between them maintain a sufficient level of the amine in the blood stream for uptake by other parts of the body as and when required. Experiments which have indicated that injected (extracellular) histamine is metabolized by tissues rather than stored by them do not invalidate such a hypothesis, since such experiments have not been performed in conditions where the tissue histamine is in an active process of physiological release and hence in need of replenishment. Unfortunately, the use of histamine releasing agents may be of little

value in this context since the nature of the biochemical changes they produce at the binding sites is not known.

The results of the present experiments suggest that the guinea-pig possesses only one enzyme capable of forming histamine from histidine. Thus, the optimal conditions for histidine decarboxylase activity are similar in all tissues, and inhibitors of decarboxylase reactions produce a similar reduction in enzyme activity in all tissues studied. The rat, on the other hand, appears to possess three histamine-forming enzymes; in this species the optimal conditions for the formation of histamine vary from tissue to tissue, and the effects of drugs on amine formation are likewise variable [3, 4, 6]. It is only in rat liver that the optimal conditions are similar to those required by the tissues of the guinea-pig. No species has been studied in detail, but at the present time it seems that the rat is exceptional in apparently possessing three enzymes capable of forming histamine.

The specificity of histidine decarboxylases requires closer study. The guinea-pig enzyme studied in the present work may well be non-specific, as the tissues which contain it often decarboxylate other amino acids, for example, 5-hydroxytryptophan[17]. However, it appears to be the only enzyme capable of forming histamine in this species, the existence in the various tissues of an enzyme whose function is clearly confined to the formation of histamine remaining to be shown.

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