HISTAMINE AS AN IMPURITY IN SAMPLES OF HISTIDINE

By N. G. Waton

From the National Institute for Medical Research, Mill Hill, London, N.W.7

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Commercial samples of histidine are shown to contain histamine, generally in small quantities, but occasionally between 100 and 260 μg./g. When this fact is taken into account in incubation experiments of tissue extracts no evidence is found for the presence of histidine decarboxylase in extracts of cat liver or kidney. This is in contrast to the original claim by Holtz, Heise and Spreyer.

The first and often quoted evidence for the presence of histidine decarboxylase in cat tissue is the finding by Holtz and Heise (1937) and by Holtz, Heise and Spreyer (1937-1938) of the formation of histamine by cat liver brei incubated with histidine. This finding has not been confirmed (Waton, 1956) and the present experiments provide a possible explanation for this discrepancy since it was found that commercial samples of histidine may contain high amounts of histamine, the presence of which can easily simulate formation of histamine in incubation experiments with animal tissue if this factor is not excluded by control experiments. Hitherto the presence of histamine as impurity in commercial samples of histidine has been mentioned only by MacKay and Shepherd (1960) and by White (1960).

Methods

Nineteen samples of histidine were assayed for histamine. Of fifteen samples, commercially obtained, twelve were the L-monohydrochloride, one the DL-dihydrochloride, one the free DL-amino-acid and one the free D-amino-acid. In addition, four samples of the free DL-amino-acid prepared several years ago in this laboratory, were examined.

The samples were made 1/200 either in water if the salt, or in dilute hydrochloric acid (0·02N) if the free amino-acid was used, and assayed on the atropinised guinea-pig ileum preparation suspended in 5 ml. oxygenated Tyrode's solution. The contractions obtained were abolished by doses of mepyramine maleate and recovered in parallel with histamine, which according to Reuse (1948) is good evidence for the identification of histamine.

Preparation and incubation of tissue extracts was carried out according to the method of Holtz, Heise and Spreyer (1937–1938). Fresh cat liver or kidney tissue was minced and ground with silver sand and 0·05M disodium phosphate buffer (1 g. tissue/5 ml. buffer), centrifuged and the supernatant shaken with kaolin (1 g./20 ml.) for 15 min. to remove histaminase from the extracts; after further centrifugation, the supernatants were removed and used for incubation.

Samples of 5 ml. of liver extracts or of 3 ml. of kidney extracts were mixed with an equal volume of histidine (4 mg./ml.) with or without 1 ml. or 0·6 ml. of toluene respectively. Immediately after mixing nitrogen was

blown into each flask for 5 min. and the flasks were stoppered and incubated for 18 hr. at 37°. Their histamine content was assayed on the arterial blood pressure of a cat anaesthetised with pentobarbitone sodium.

RESULTS

Histamine Content in Samples of Histidine

In Table I are shown the histamine contents obtained for 19 samples of histidine. Three samples contained either no histamine or less than 2 μg./g., six samples contained 4–8 μg./g., seven samples 10–20 μg./g. but three samples contained as much as 106, 160 and 240 μg./g.

TABLE I

HISTAMINE CONTENT (μg./g.) OF 19 SAMPLES OF HISTIDINE

	Histidine samples	Histamine μg./g.
1.	Commercial L- monohydrochloride ..	6
2.	" " " ..	6
3.	" " " ..	8
4.	" " " ..	8
5.	" " " ..	10
6.	" " " ..	14
7.	" " " ..	16
8.	" " " ..	16
9.	" " " ..	20
10.	" " " ..	106
11.	" " " ..	160
12.	" " " ..	240
13.	" DL- dihydrochloride	<2
14.	" free DL- amino-acid	<2
15.	" free D- amino-acid	<2
16.	Old laboratory free DL- amino-acid ..	4
17.	" " " " " " ..	6
18.	" " " " " " ..	14
19.	" " " " " " ..	14

Incubation of Extracts with histidine

Two samples of histidine, No. 9 and No. 10 of Table I containing 20 and 106 μg./g. histamine respectively were chosen for the incubation experiments.

Liver Extracts

Samples of liver extracts incubated for 18 hr. with histidine contained detectable amounts of histamine only if the histidine sample contained a high amount of histamine. The histamine assayed was fully accounted for by the histamine impurity of the histidine sample.

A typical experiment is illustrated in Fig. 1 which gives an assay, on the arterial blood pressure of the cat, of samples of 1 ml. of liver extract incubated with histidine either samples 10 or 9. If no histamine had been formed or destroyed, the 1 ml. liver extract would have contained 212 ng. of histamine, if histidine sample 10, and 40 ng., if histidine sample 9 had been used for incubation. The 1 ml. of the liver extract incubated with histidine sample 10 either with or without toluene, gave a depressor action (at E and B) smaller or equal to that of 200 ng. histamine (at A and L) and slightly less than that of the liver extract which had not been

incubated but to which the same amount of histidine sample 10 had been added (at H). The effect of 2 mg. histidine sample 10 which is equivalent to the amount of the added histidine in 1 ml. of liver extract is shown for comparison (at K).

The 40 ng. histamine present in 2 mg. histidine sample 9 was insufficient to lower the blood pressure (at J) and the 1 ml. of liver extract to which 2 mg. of this sample of histidine had been added remained ineffective whether the sample had been incubated with (at F) or without toluene (at C). The figure shows further that the liver extract produced no fall in arterial blood pressure either when incubated without histidine in the absence or presence of toluene (at D and G) or when not incubated but containing the histidine (at I).

From these results it is not only evident that there is no formation of histamine by liver brei on incubation with histidine but also that by the use of a sample of histidine with a high histamine impurity, an apparent formation of histamine is simulated if this impurity is not taken into consideration.

FIG. 1

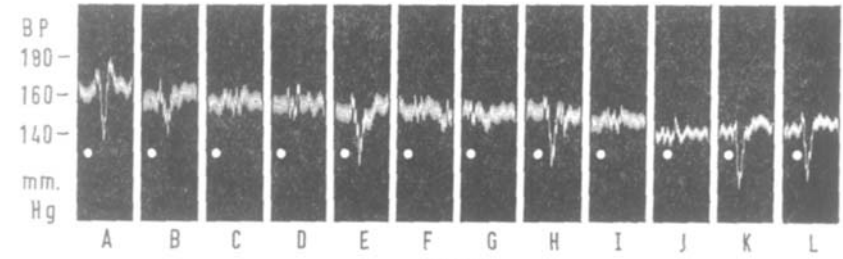

FIG. 2

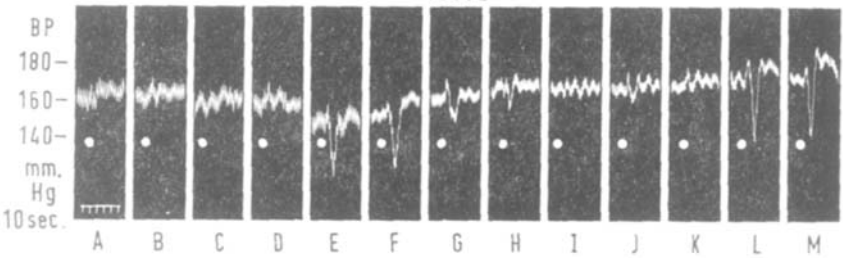

FIG. 1. Arterial blood pressure of cat anaesthetised with pentobarbitone sodium. Assay of liver extract incubated for 18 hr. Effects of 200 μg. histamine (A and L) of 2 mg. histidine sample 9 (J) and of 2 mg. histidine sample 10 (K). Effects of 1 ml. liver extract incubated with histidine sample 10 (B and E), with 2 mg. histidine sample 9 (C and F) and without histidine (D and G). B-D incubated without, and E-G with toluene. Effects of 1 ml. unincubated liver extract with 2 mg. histidine sample 10 (H) and sample 9 (I).

FIG. 2. Preparation as in Fig. 1. Assay of kidney extract incubated for 18 hr. Effects of 200 μg. of histamine (E and M), of 2 mg. histidine sample 9 (K) and of 2 mg. histidine sample 10 (L). Effects of 1 ml. of kidney extract incubated with histidine sample 9 (A and C) and with histidine sample 10 (B and D). A and B incubated without, and C and D incubated with toluene. Effect of 1 ml. unincubated kidney extract (J). Effect of 1 ml. kidney extract with 2 mg. histidine sample 10 tested after standing at 18° for 25 sec., 5, 10 and 20 min. (F-I).

Kidney Extracts

No histamine was detected in any of the 1 ml. samples of kidney extract tested after incubation for 18 hr. with histidine, with or without toluene. This result which is illustrated in Fig. 2,B was also obtained when histidine No. 10 with the high histamine impurity had been used.

The incubated kidney extract injected into the same cat, on which the liver extract had been assayed, produced no depression whether incubation was with histidine sample 9 or 10 and whether incubation was without or with toluene (Fig. 2, A–D) yet the injection of 200 ng. histamine still produced its strong depressor effect (Fig. 2, E and M). For comparison the Figure also shows the effect of unincubated kidney extract without added histidine (at J) as well as the effects of 2 mg. histidine sample 9 and 10 (at K and L) an amount equivalent to that added to 1 ml. kidney extract. The fact that the kidney extract incubated with histidine sample 10 which contained the high impurity did not produce a fall in arterial blood pressure was shown to be due to the histamine-destroying activity of the extract. This destruction occurred rapidly as was evident when kidney extract was kept with histidine No. 10 at room temperature (18°) for periods varying between 25 sec. and 20 min. (at F–I). Thus the ability of the kidney extract to destroy histamine was not abolished by the pretreatment with kaolin. Therefore the failure to detect formation of histamine by these extracts does not exclude the possibility that histamine was formed but subsequently destroyed.

Discussion

The finding that commercial samples of histidine contain histamine, generally in small amounts, but occasionally in large quantities has to be taken into account when investigating the enzymic formation of histamine by tissue extracts with histidine as substrate.

From the present results it would appear that the early report by Holtz, Heise and Spreyer (1937–1938) on the formation of histamine by cat liver is attributable to impurities of histamine in the samples of histidine used as substrate by these authors, since they did not examine the histamine content of their histidine samples, but only compared the effects on the arterial blood pressure of liver extract incubated with and without histidine. In the present experiments, it was shown that, if a histidine sample containing a high amount of histamine was used as substrate, the histamine was still present after 18 hr. incubation, but no evidence for newly formed histamine was obtained. If it had not been known that the histidine sample, used as substrate, was contaminated with a large amount of histamine, this finding might have suggested formation of histamine.

Since the kidney extracts used in the present experiments were able to destroy histamine, the kaolin treatment did not fully remove the histaminase known to abound in cat kidney (Waton, 1956) and this fact explains why kidney extracts in contrast to liver extracts, no longer contained detectable amounts of histamine after incubation for 18 hr. with histamine contaminated histidine. Holtz and Heise who detected histamine in 5

out of 30 samples of cat kidney extracts after treatment with kaolin and incubation with histidine for 18 hr. were probably more successful in removing the histaminase from at least some of their kidney extracts, but their five positive results cannot be taken as evidence for the presence of histidine decarboxylase in cat kidney.

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