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ENZYMATIC HISTAMINE DEGRADATION BY HUMAN SKIN

D. FRANCIS, M.W. GREAVES & S. YAMAMOTO[1]
Institute of Dermatology, Homerton Grove, London, E9 6BX

1 Degradation of histamine by homogenized human skin *in vitro*, in the presence of the cofactor S-adenosyl methionine, indicates the presence of the histamine metabolizing enzyme histamine-*N*-methyl transferase in human skin. Under the experimental conditions described, no significant histamine degradation by diamine oxidase was observed.

2 The enzyme activity is temperature-sensitive with an optimum at 37°C. The enzyme is stable in intact excised skin at −20°C, but unstable in homogenized skin at this temperature.

3 Little or no enzyme activity is present in mid- or deep dermis, but the distribution of the enzyme between superficial papillary dermis and epidermis is uncertain.

4 The presence of a potent histamine degrading mechanism raises the possibility that histamine-*N*-methyl transferase activity may be an important modulating factor in histamine-mediated skin disorders

Introduction

The presence of the histamine-degrading enzyme diamine oxidase in guinea-pig skin was recently described (Yamamoto, Francis & Greaves, 1976). We have proposed a role for this enzyme in the regulation of histamine-mediated skin inflammation, since administration of the diamine oxidase inhibitor, aminoguanidine, caused amplification of the passive cutaneous anaphylaxis reaction in this species. Histamine is a mediator of a variety of common inflammatory dermatoses in man. The presence of a histamine catabolizing mechanism in human skin might, therefore, be an important modulating factor in these skin disorders. In this paper we report the presence and preliminary characterization of histamine degrading enzyme activity in human skin.

Methods

Human skin

Clinically healthy skin removed from the breast, arm, face or abdomen during radical mastectomy or plastic surgery was used. Unless otherwise stated, the skin was stored intact at −20°C, until used.

Skin homogenate

After the subcutaneous fat was removed by surgical scissors, the skin was cut into square pieces, of about 3 mm, with a blade. The pieces were homogenized with 0.01 M phosphate buffered saline (PBS) at pH 7.3, in an ice bath, with a homogenizer (I.L.A. X-1020). The homogenate was filtered through cotton gauze. The filtrate was used as a source of histamine-degrading enzyme, the fibrous residue containing little or no activity.

Assay of histamine degrading enzyme activity

The homogenates obtained from human skin contained endogenous histamine, in concentrations ranging from 600 to 3600 ng/ml. The endogenous histamine in the homogenate was used as a substrate for the study of histamine degrading enzyme activity in skin. Aliquots of the homogenate were incubated in stoppered test tubes. The reaction volume was 0.6–0.7 ml and the incubation time 60 min at 37°C unless otherwise stated. All determinations were carried out in duplicate. After incubation, the reaction was terminated by cooling on ice and the reaction mixture was diluted to 5 ml with ice-cold Tyrode solution and boiled for 10 min, followed by bioassay of the histamine content of the sample. The activity of the histamine degrading enzyme was expressed as the absolute amount of histamine degraded in the reaction mixture after incubation. In each experiment, control samples were kept on ice during the period of incubation and were used to determine the initial histamine content of the homogenate. Histamine degradation at 0°C was negligible (see Results section). In experiments designed to show the presence of enzymatic histamine degradation due to histamine-*N*-methyl transferase, the methyl group donor

[1] Present address: Hiroshima University School of Medicine, Hiroshima, Japan.

S-adenosyl methionine (S-AM), was included in the incubation mixtures (72 nmol per sample, approximately 0.1 mM) (Brown, Tomchick & Axelrod, 1959).

Assay of histamine activity

Histamine was measured by bioassay, on the guinea-pig isolated ileum preparation in the presence of atropine 1 μM. Each bioassay was carried out in duplicate. In experiments in which aminoguanidine was included in the incubation mixtures, the concentration was equalized in all samples before bioassay, as well as in the histamine standard solutions and Tyrode wash solution.

Thin-layer chromatography (t.l.c.)

Confirmation of the identity of histamine degrading enzyme activity was obtained by thin layer chromatography of the products of [^{14}C]-histamine catabolism by skin. A skin homogenate was prepared from 3.25 g of skin in 10.8 ml PBS; 0.5 ml aliquots of the homogenate were added to 6 tubes on ice. To each tube was added 0.19 μCi of [^{14}C]-histamine dihydrochloride (equivalent to 84 ng histamine base) and the tubes divided into 3 sets of duplicates. S-AM (72 nmol) was added to one set of duplicates and all the reaction volumes were made up to 0.65 ml with PBS. One set of samples containing S-AM and one set without S-AM were incubated for 60 min at 37°C and the remaining two tubes without S-AM were kept on ice during the incubation, as controls. At the end of the incubation the samples were frozen at −20°C and freeze-dried overnight. To the residues, 300 μl of methanol was added and the tubes thoroughly shaken. Aliquots (150 μl) from each duplicate sample were applied as 3 cm bands on glass t.l.c. plates (200 × 100 × 0.25 mm silica gel G, Anderman). A sample from each duplicate was run on either side of the same plate. Approximately 10 μl aliquots of histamine dihydrochloride, methylhistamine dihydrochloride and imidazole acetic acid hydrochloride solutions (all 2 mg/ml in methanol) were applied at the origin, in the centre of the plate. The plates were then developed to 15 cm in a closed, equilibrated glass tank, using the solvent system chloroform/methanol/ammonia; 70 : 30 : 5 by volume. The plates were then removed, dried and developed a second time in the same solvent system, as this was found to give a better separation of histamine from its metabolites. Dry scrapes (1 cm) of each sample run were washed into scintillation vials, containing 8 ml of scintillant, with 5 ml of methanol and counted on an L.K.B. counter for 300 seconds. The remaining third of each t.l.c. plate was developed in an iodine tank and the R_F values of histamine, methylhistamine and imidazole acetic acid determined.

To compare the degradation of [^{14}C]-histamine observed using t.l.c. with that measured by bioassay, a further set of three duplicate samples was set up, using the same homogenate. The samples were prepared and incubated as for the t.l.c. samples, except that 84 ng of histamine base was added instead of [^{14}C]-histamine. The histamine content of the samples was then determined by bioassay.

Solvents and chemicals

All solvents were of analytical grade or redistilled before use. The chloroform was redistilled over anhydrous $CaCl_2$ and 1% (by vol.) of absolute ethanol was added to the redistillate to stabilize it. The scintillant for liquid scintillation counting was composed of: toluene/ethoxyethanol, 1500 : 900, v/v; PPO/naphthalene, 10.5 : 112.5, by weight.

[U-^{14}C]-histamine dihydrochloride (specific activity 270 mCi/mmol) was obtained from The Radiochemical Centre, Amersham. The following commercial chemicals were also used: histamine dihydrochloride (Koch-Light Laboratories Ltd.), 1-methylhistamine dihydrochloride (Calbiochem Ltd.), imidazole-4-acetic acid hydrochloride, S-adenosyl-L-methionine chloride and aminoguanidine hemisulphate (Sigma London Chemical Company Ltd.), histamine acid phosphate (BDH).

Results

Degradation of histamine by homogenates of human skin

In 3 experiments samples of separate homogenates were incubated in the absence of S-AM for periods ranging from 15–60 minutes. The results are given in Figure 1. Degradation of histamine in these samples did not exceed 10% of the total, in any experiment. Additional samples of the same homogenates were incubated in the presence of S-AM. Substantial degradation occurred in samples to which S-AM had been added (Figure 1). Prolonging the incubation from 15 min to 60 min caused a progressive increase in the amount of histamine degraded. S-AM was therefore included in the incubation mixture in all subsequent experiments.

Aminoguanidine

In order to exclude the possibility that diamine oxidase contributed to the observed degradation of histamine by the skin homogenate, the experiments were repeated in the presence of the specific inhibitor of diamine oxidase, aminoguanidine, (Blaschko, Friedman, Hawes & Nilsson, 1959) in concentrations ranging from 1 to 100 μM. The incubation period was 60 minutes. Aminoguanidine caused little or no reversal of histamine degradation in the presence of

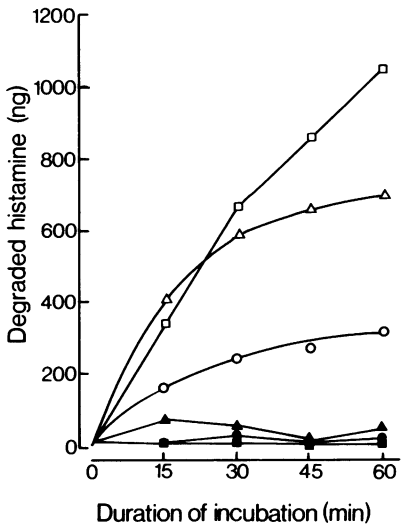

Figure 1 The effect of S-adenosyl methionine (S-AM) on the time course of the degradation of histamine in samples from 3 separate homogenates of human skin. All homogenates were produced by homogenizing skin in 4 volumes of PBS (w/v). The curves (○), (□) and (△) show the degradation of endogenous histamine in samples, containing 443 ng, 1800 ng and 742 ng of histamine respectively, in the presence of S-AM. Curves (●), (■) and (▲) show the corresponding values when samples were incubated without S-AM.

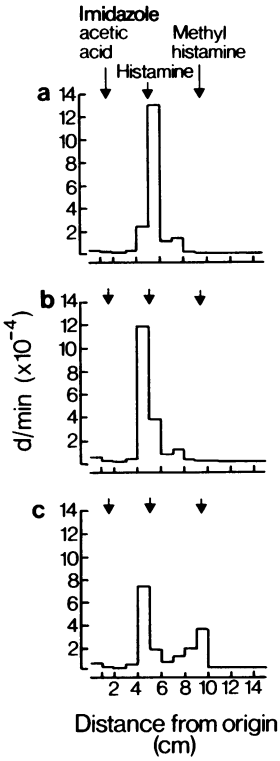

Figure 2 Thin-layer chromatographic separation of histamine catabolites produced by incubation of [^{14}C]-histamine with human skin homogenate. All reaction mixtures contained homogenate and [^{14}C]-histamine, but only in (c) was S-adenosyl methionine (S-AM) added. The reaction mixtures in (b) and (c) were incubated for 60 min at 37°C, (a) being kept on ice during the incubation to serve as control. Each histogram shows the average profile of duplicate samples run on the same t.l.c. plate. The mean distances run by authentic histamine and its catabolites, methylhistamine and imidazole acetic acid, are indicated by arrows.

S-AM. There was no significant inhibition by aminoguanidine of the slow degradation of histamine in the absence of S-AM.

Thin-layer chromatography

Figure 2 shows that the two major catabolites of histamine, methylhistamine and imidazole acetic acid can be separated on t.l.c. using the solvent system described in the Methods section. The mean R_F values, for histamine, methylhistamine and imidazole acetic acid were 0.34, 0.63 and 0.11 respectively. The percentage of the total recovered radioactivity which ran with histamine (between 4 and 6 cm) plus methylhistamine (between 8 and 10 cm) on the t.l.c. plates was between 77.8% and 78.8% for the 3 pairs of samples. The corresponding value obtained, where [^{14}C]-histamine alone was run on the same system, was 77.1%. In the samples incubated with S-AM, [^{14}C]-methylhistamine represented 29.2% and [^{14}C]-histamine 48.6% of the total radioactivity recovered. From these figures, 37.5% of the [^{14}C]-histamine was converted to [^{14}C]-methylhistamine, which compares to a total degradation measured by bioassay of 45%, (representing 139 ng loss of histamine activity). In the absence of S-AM, the bioassay measured a 14.7% loss of histamine activity. On t.l.c., no radioactivity co-chromatographed with imidazole acetic acid or methylhistamine in samples incubated at 37°C without S-AM, giving an almost identical profile to samples kept on ice during the incubation. These results confirm that the observed enzymatic degradation of histamine is attributable to histamine-*N*-methyl transferase.

Effect of temperature on histamine-N-methyl transferase

In two experiments, samples of skin homogenates from different donors were incubated for 60 min at

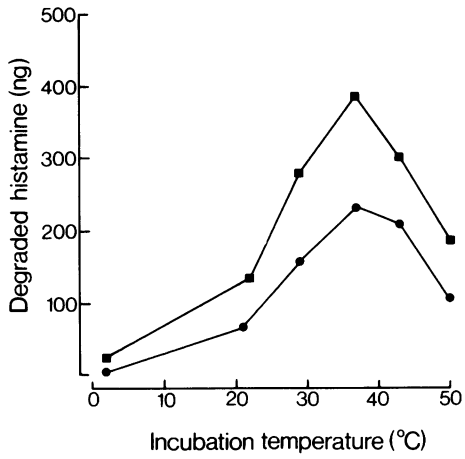

Figure 3 The effect of temperature on histamine *N*-methyl transferase (HNMT) activity. The upper (■) and lower (●) curves show the degradation of histamine in samples, containing 754 ng and 709 ng of endogenous histamine respectively, derived from 2 homogenates of human skin. The skin was homogenized in 3.2 volumes of PBS (w/v) in both cases and all incubations contained S-adenosyl methionine.

temperatures ranging from 2°C to 50°C. The results are shown in Figure 3. Maximum histamine degradation occurred at about 37°C. Increase of the temperature to 43°C caused a drop in activity. Cooling to 29°C caused reduction of histamine degradation and at 2°C degradation was almost completely inhibited.

*Stability of histamine-*N*-methyl transferase*

There was no detectable loss of enzyme activity in intact skin samples stored at −20°C for up to 27 days. A single sample stored at −20°C for 18 h after homogenization showed a 62.5% decrease in histamine degrading enzyme activity after 60 min incubation with S-AM, with respect to a sample of the same homogenate assayed immediately after homogenization. Therefore, skin was either used fresh or stored intact at −20°C.

*Localization of histamine-*N*-methyl transferase*

Epidermis was gently scraped from fresh stretched skin, at room temperature, with a blunt scalpel blade. Histological examination of the dermal and putative epidermal fractions revealed that the dermal fraction was devoid of epidermis. The remainder contained the entire epidermis but was also contaminated with elements of the upper papillary dermis. The histamine degrading enzyme activity of these fractions, as assayed in 2 experiments, is given in Table 1. In one experiment there was little or no degradation of histamine by the dermis, although enzyme activity in the other fraction, which contained epidermis as well as elements of upper dermis, was considerable. In the second experiment the results were similar although a small amount of enzyme activity was present in the dermal fraction.

Discussion

No significant degradation of histamine takes place if fresh homogenized human skin is incubated in the presence of histamine. However, if the methyl donor S-adenosyl methionine (S-AM) is introduced into the reaction mixture, temperature-dependent, progressive histamine degradation occurs, indicating the presence of histamine degrading enzyme activity. Dependence of the reaction on S-AM suggested that the observed degradation was due to histamine-*N*-methyl transferase (Brown *et al.*, 1959). This was confirmed

Table 1 Values for the degradation of histamine in incubates containing epidermis (plus papillary dermis) and dermis alone, in the presence and absence of S-adenosyl methionine (S-AM).

	Experiment 1		*Experiment 2*	
	Epidermis	*Dermis*	*Epidermis*	*Dermis*
Total histamine in test samples (ng)	302	219	1455*	868
Histamine degraded in incubate without S-AM	70	19	0	73
Histamine degraded in incubate containing S-AM	302	2	1090	160

The skin samples in the two experiments shown were from different individuals. Both epidermis and dermis were homogenized in 5 volumes of PBS (w/v). * The total histamine value for samples of epidermis in experiment 2 includes 500 ng of added histamine base. The incubation time for experiment 2 was 90 min and for experiment 1, 60 minutes.

by showing that [^{14}C]-methylhistamine was formed from [^{14}C]-histamine, when incubated with a human skin homogenate in the presence of S-AM, using thin-layer chromatography.

Two major pathways for histamine catabolism are recognized (Schayer, 1959), catalysed by histamine-*N*-methyl transferase and diamine oxidase respectively. Schayer & Cooper (1956) found that histamine-*N*-methyl transferase was the principal histamine metabolizing pathway in man. We were unable to detect evidence of diamine oxidase activity in human skin, either by t.l.c. or bioassay. T.l.c. of the reaction products of [^{14}C]-histamine incubated with a human skin homogenate did not demonstrate the production of any imidazole acetic acid, the final product of the oxidative deamination of histamine. Also, histamine degradation was not inhibited by the specific diamine oxidase inhibitor, aminoguanidine.

Although skin is a major target organ for histamine-mediated allergic reactions, histamine degradation in human skin does not appear to have been studied before. Abdominal skin of the rabbit, mouse and guinea-pig contains significant amounts of histamine-*N*-methyl transferase (Brown *et al.*, 1959) and our own studies (Yamamoto *et al.*, 1976) have shown that guinea-pig skin contains diamine oxidase, as well as histamine-*N*-methyl transferase. It is of interest that no significant metabolism of histamine could be detected in the absence of S-AM. S-AM is a major naturally occurring methyl donor (Baldessarini & Kopin, 1963) formed intra-cellularly from adenosine triphosphate (ATP) and methionine and is used as a methyl group donor by a number of enzymes. The explanation of sub-threshold amounts of this cofactor in the skin homogenates of the present experiments is speculative, but could be explained by failure of *de novo* synthesis after homogenization, together with rapid destruction or use by the several other methylating enzymes which require this cofactor.

The localization of histamine-*N*-methyl transferase in skin requires further study. We were unable to obtain clear separation of epidermis from dermis under conditions that would preserve the activity of the enzyme and at the same time yield sufficient epidermis for study. The method used yielded epidermal samples contaminated with papillary dermis. These samples contained all or almost all enzyme activity. This activity could have derived from epidermis, upper papillary dermis or both. However, there was little or no activity in mid or lower dermis. The closely related enzyme, catechol-*O*-methyl transferase, is present in both epidermis and dermis, although the concentration is higher in epidermis (Bamshad, 1969).

In vivo blockade of enzymatic histamine degradation brings about marked enhancement of the passive cutaneous anaphylaxis reaction in the guinea-pig (Yamamoto *et al.*, 1976). Since methylhistamine, the product of the metabolism of histamine by histamine-*N*-methyl transferase, has very little histamine-like biological activity (Lee & Jones, 1949), studies of enzymatic histamine degradation in the skin of patients with urticaria and other histamine-mediated skin disorders, would be of great interest.

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