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STUDY OF THE RELATIONSHIP OF HISTAMINASE AND DIAMINE OXIDASE ACTIVITIES IN VARIOUS RAT TISSUES AND PLASMA BY SENSITIVE ISOTOPIC ASSAY PROCEDURES

MICHAEL A. BEAVEN and RICHARD E. SHAFF

Pulmonary Branch, National Heart and Lung Institute, National Institutes of Health, Bethesda, Md. 20014, U.S.A.

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Abstract—In this laboratory, histaminase activity has been assayed by measurement of the release of tritium from side-chain labeled β-^{3}H-histamine, and this assay has proved useful in a variety of animal and clinical studies. In the present study, the assay was compared with that of diamine oxidase activity which uses ^{14}C-labeled putrescine as substrate. Both activities are believed to be due to the enzyme, diamine oxidase, which has been shown to catalyze the deamination of diamines as well as histamine. The data showed that β-^{3}H-histamine and ^{14}C-putrescine were deaminated by the same enzyme in a variety of rat tissues and that the assays were interchangeable. The labeled amines competed for deamination, and their deamination was inhibited to the same extent by aminoguanidine which is a specific inhibitor of diamine oxidase. There was also a close correlation in the values obtained by the two assays in all tissues. The deamination of each amine was affected differently by changes in pH and substrate concentration, as has been shown previously for purified preparations of diamine oxidase. Substrate inhibition was, for example, observed with histamine, but not with putrescine. An unexpected finding was that histamine, in the concentrations usually encountered in tissues, markedly inhibited diamine oxidase activity. Concentrations of 10^{-5} M histamine (1·1 μg/g) inhibited putrescine deamination by 55 per cent and 10^{-4} M (11·1 μg/g) by more than 90 per cent.

A number of tissues, which include placenta, plasma from pregnant women and kidney, have the ability to oxidatively deaminate histamine. This activity, usually referred to as histaminase activity,* has been attributed to the enzyme, diamine oxidase (diamine, O_2 oxidoreductase [deaminating], EC 1.4.3.6.), which deaminates histamine and aliphatic diamines such as putrescine [1]. Several years ago, a sensitive tritium-release assay of histaminase activity was developed in this laboratory to replace the more time consuming biological and radiochemical assay procedures [2]. The assay is based upon the use of side-chain labeled β-^{3}H-histamine, which undergoes deamination with the quantitative release of the tritium label. Tritium is not released by monoamine oxidases [2]. The assay has been used in a variety of clinical [3–7] and animal [8, 9] studies, and high histaminase activity was found in kidney and intestine [5] in humans and intestine, thymus and adrenals in rat [2]. Abnormally high enzyme activity was also found in the tumor, medullary carcinoma of the thyroid [3–6], and elevated histaminase activity in plasma or tissue is a specific test for this tumor in man [6].

A simple and elegant procedure devised by Kobayashi *et al.* [10, 11] is available for the assay of diamine oxidase activity. In this assay, ^{14}C-labeled putrescine is used as substrate. The deaminated product, ^{14}C-γ-aminobutyraldehyde, spontaneously cyclizes to form ^{14}C-Δ^1-pyrroline which can be extracted directly from the incubation mixture into a toluene-based scintillation mixture and assayed for ^{14}C. The procedure has been adopted widely to follow the rise in plasma diamine oxidase during pregnancy (see, for example, Tryding and Willert [12], Hansson *et al.* [13] and Southren *et al.* [14–18], where it has been shown to parallel the rise of histaminase activity [19–20]. The assay has been used to measure diamine oxidase activity in tissues, although comparisons with tissue histaminase activities have not been made.

The purpose of the present work was to determine if the assay of histaminase activity with β-^{3}H-histamine as substrate was interchangeable with the assay of diamine oxidase activity using ^{14}C-putrescine as substrate. The effect of pH, substrate concentration and aminoguanidine, a specific inhibitor of diamine oxidase [21], on the deamination of the two amines was compared in human plasma and various rat tissues. Evidence of competition for deamination between histamine and putrescine and a correlation between histaminase and diamine oxidase activities in these tissues was also sought.

MATERIALS AND METHODS

Materials. β-^{3}H-histamine [4(5)-(2-aminoethyl-2-^{3}H)imidazole] was prepared by decarboxylation of β-^{3}H-L-histidine, 6·8 Ci/m-mole (New England Nuclear Corp.), with bacterial histidine decarboxylase [2]; β-^{3}H-methylhistamine was prepared from the

* In this paper, "diamine oxidase activity" denotes the enzyme activity assayed with ^{14}C-putrescine as substrate, and "histaminase activity" when β-^{3}H-histamine was used as substrate. The term, "diamine oxidase," is used specifically to refer to the enzyme, diamine oxidase.

β-^{3}H-histamine by methylation with *S*-adenosylmethionine and guinea-pig brain histamine-*N*-methyltransferase as described previously [2]. Labeled putrescine—1,4-^{14}C-putrescine, 11 mCi/m-mole, and 2,3-^{3}H-putrescine—was obtained from New England Nuclear Corp. 1-Methyl-4-(2-aminoethyl)-imidazole (methylhistamine) was prepared by Regis Chemical Co.; histamine dihydrochloride and putrescine dihydrochloride were obtained from Schwarz/Mann (Orangeburg, N.Y.) and aminoguanidine sulfate from Fischer Scientific Co. (Washington, D.C.).

Tissue and plasma collection. Tissues were obtained from male Sprague–Dawley rats, 180–220 g. The blood was collected from rats by cardiac puncture at various times after the intravenous injection of heparin (4000 units/kg) and from pregnant women during their third trimester of pregnancy. The blood was drawn into syringes containing heparin (5 units). Plasma was obtained by centrifugation of the blood at 2000 *g* for 20 min. Tissues and plasma were frozen on Dry Ice and stored at −20°. Tissues were homogenized immediately before assay in 9 vol. of 0·1 M sodium phosphate buffer, pH 6·8 or pH 7·4, as required. Plasma was used undiluted.

Assay procedures. Histaminase activity was determined by measurement of the release of tritium from β-^{3}H-histamine as described previously [2]. Tissue homogenate or plasma was incubated at 37° with β-^{3}H-histamine (15 pmoles, 0·1 μCi) and 0·1 M sodium phosphate buffer, pH 6·8, total volume of 0·2 ml, in the bottom of a Thunberg tube. Incubations were for 60 min unless stated otherwise. The reaction was stopped by the addition of 0·2 ml of 5 mM solution of unlabeled histamine. The reaction mixture was frozen in the Thunberg tubes and the air evacuated. Water was then collected as ice in the upper part of the Thunberg tube and assayed for tritium.* The deamination of β-^{3}H-methylhistamine was determined by measurement of tritium release by the same procedure.

The assay of diamine oxidase activity was based upon that of Okuyama and Kobayashi [10], but was modified so that the volumes were the same as those used for the histaminase assay. Tissue homogenates or plasma were incubated with ^{14}C-putrescine (2 nmoles, 0·1 μCi) or β-^{3}H-putrescine (2 nmoles, 0·16 μCi) and 0·1 M sodium phosphate buffer, pH 7·4, in a volume of 0·2 ml. At the end of the incubation, 0·2 ml of an aminoguanidine solution (2×10^{-5} M) in 0·1 M sodium phosphate buffer, pH 7·4, was added to stop the reaction. The labeled deaminated product was extracted from the incubation mixture directly into 10 ml of a PPO–POPOP† toluene scintillation mixture after the addition of sodium bicarbonate and assayed for radioactivity as described by Okuyama and Kobayashi [10]. In another experiment, the addition of sodium bicarbonate was omitted as noted in the text. The assumption was made that all of the labeled deaminated product was recovered by the two extractions. Identical results were obtained using either ^{14}C-putrescine or β-^{3}H-putrescine as substrate, although the values for the assay blanks run in the absence of enzyme were lower for ^{14}C-putrescine.

In the above assays, the volume of plasma or tissue homogenate and the time of incubation were varied according to the enzyme activity in the sample. All incubations were at 37°. The final concentration of β-^{3}H-histamine was $7{\cdot}5 \times 10^{-8}$ M and β-^{3}H- or ^{14}C-putrescine, 1×10^{-5} M. In experiments where the concentration of substrate was varied or where competition between the two substrates was studied, unlabeled histamine or putrescine was added to the incubation to the required concentration. In studies of the effect of pH on deamination, 0·1 M sodium phosphate buffer was used for pH 5·8 to 8·0 and 0·1 M sodium borate buffer for pH 7·6 to 9·0. The results were calculated in terms of either per cent amine deaminated or nmoles amine deaminated/hr of incubation/g of tissue or ml of plasma after correction for control incubations run in the agsence of enzyme. Since plasma can be frozen and thawed repeatedly without loss of enzyme activity [2], plasma from pregnant women was used initially to study the kinetics of each reaction.

RESULTS

Effect of pH and substrate concentration on deamination of histamine and putrescine. With purified preparations of diamine oxidase [22, 23], changes in pH and substrate concentration are known to affect the deamination of histamine and aliphatic diamines differently. Similar differences were observed with the deamination of β-^{3}H-histamine and ^{14}C-putrescine in tissues. The rate of deamination of β-^{3}H-histamine increased with an increase in pH until pH 6·8. Above this pH, there was little further increase (Fig. 1). Control incubations run without enzyme or tissue sample gave progressively higher values once the pH was raised above 6·8. For this reason, pH 6·8 was adopted for the assay procedures. In contrast to β-^{3}H-histamine, the rate of deamination of ^{14}C-putrescine increased throughout the whole pH range studied (Fig. 1). This increase was evident when the deaminated product was extracted after adjustment of pH by the addition of sodium bicarbonate as described by Okuyama and Kobayashi [10] or when the product was extracted directly into the organic phase without adjustment of pH (Fig. 1).

With respect to substrate concentration (Fig. 2), enzyme activity was partially inhibited by histamine in concentrations above $0{\cdot}5 \times 10^{-4}$ M and was completely inhibited by concentrations above 10^{-3} M. Such inhibition was not observed with putrescine (Fig. 2). The effects of pH and histamine concentration on β-^{3}H-histamine deamination were similar for tissues. In all tissues, enzyme activity was inhibited completely by 10^{-3} M histamine.

* Tritiated water may also be measured by the addition of 0·2 ml of a suspension of Dowex-50 ("H" form) in a 2·5 mM histamine solution (1 g to 2 ml solution). After mixing the suspension and incubation mixture (5 sec on a vortex mixer), and depositing the resin by centrifugation, the supernatant fluid, 0·1 ml, is assayed for ^{3}H. Almost all, >99·2 per cent, of the labeled histamine is removed, and the tritium label remaining is tritiated water. Incubations containing 10^{-5} M aminoguanidine (assay blanks) give 150–170 cpm ^{3}H/0·1 ml of supernatant fluid compared to 65–75 cpm ^{3}H when water is separated by sublimation.

† PPO = 2,5-diphenyloxazole; POPOP = 1,4-bis-[2-(4-methyl-5-phenyloxazolyl)]benzene.

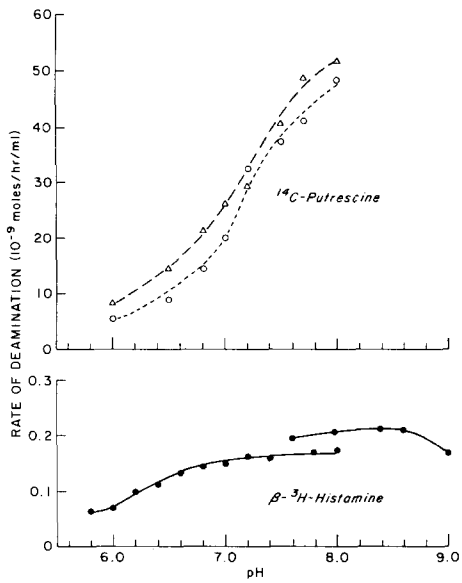

Fig. 1. Effect of pH on the rate of deamination of β-^{3}H-histamine and ^{14}C-putrescine. Enzyme source was plasma obtained from pregnant women. The deamination of β-^{3}H-histamine (●) was measured by the tritium release assay and ^{14}C-putrescine by extraction of the cyclized deaminated product, Δ^1-pyrroline. Sodium bicarbonate was added before extraction of the deaminated product in one experiment (△) and was omitted in a second experiment (○) as described under Materials and Methods. Points depict average of duplicate incubations.

Studies on the time-course of histamine deamination suggested that inhibition was due to the substrate rather than the deaminated product. At low concentrations of histamine, $7{\cdot}5 \times 10^{-8}$ M, the deamination of β-^{3}H-histamine proceeded initially at a constant rate and later decreased as substrate was utilized (Fig. 3). At higher concentrations of histamine, $2{\cdot}5 \times 10^{-5}$ M, the deamination of β-^{3}H-histamine appeared to accelerate as the reaction proceeded (Fig. 3). A decrease would be expected if

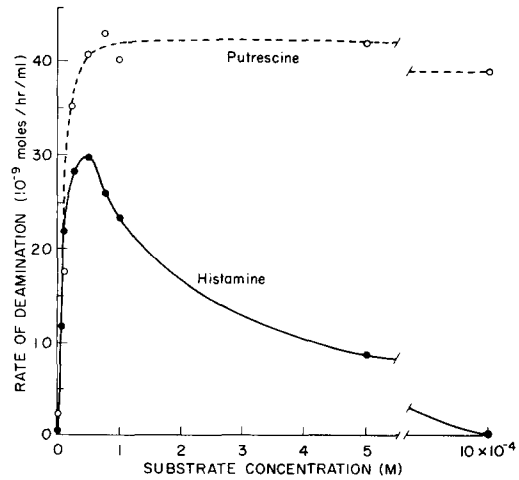

Fig. 2. Effect of substrate concentration on the rate of deamination of β-^{3}H-histamine and ^{14}C-putrescine. Incubations contained β-^{3}H-histamine or ^{14}C-putrescine and increasing amounts of unlabeled histamine or putrescine. Enzyme source was plasma obtained from pregnant women. The points depict values for individual incubations.

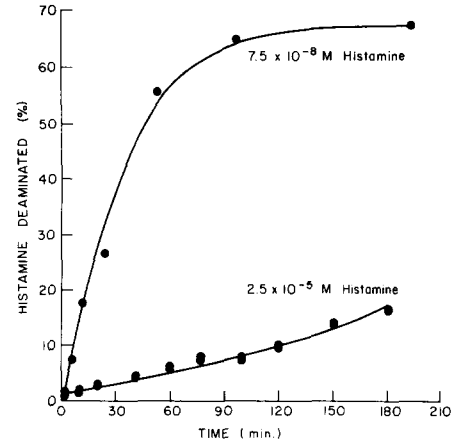

Fig. 3. Time-course of deamination of β-^{3}H-histamine, $7{\cdot}5 \times 10^{-8}$ M, alone and in the presence of $2{\cdot}5 \times 10^{-5}$ M histamine. The enzyme source was plasma obtained from pregnant women. The points depict values for individual incubations.

the deaminated product was an inhibitor of the enzyme (Fig. 3).

Inhibition by aminoguanidine. The deamination of β-^{3}H-histamine and ^{14}C-putrescine was inhibited to the same extent by aminoguanidine (Table 1). The enzyme was inhibited approximately 50 per cent by $2{\cdot}5 \times 10^{-8}$ M aminoguanidine and 99 per cent by $2{\cdot}5 \times 10^{-6}$ M aminoguanidine.

Competition for deamination by histamine and putrescine. In tissues (Table 2) and plasma (Fig. 4), putrescine inhibited the deamination of histamine, and conversely histamine the deamination of putrescine (Fig. 4). As can be seen from the data in Table 2, the inhibition was similar in all tissues. Histamine was a stronger inhibitor. Concentrations of histamine as low as $0{\cdot}3 \times 10^{-5}$ M (0·3 μg/ml) and $0{\cdot}75 \times 10^{-5}$ M (0·8 μg/ml) inhibited putrescine deamination by 25 and 55 per cent, respectively, whereas the same concentrations of putrescine inhibited histamine deamination by less than 10 per cent (Fig. 4). The inhibition by either amine could be reversed by addition of excess substrate. Analysis of the data

Table 1. Inhibition of β-^{3}H-histamine and ^{14}C-putrescine-deaminating activity of human plasma by aminoguanidine*

Aminoguanidine concn† (moles/incubation)	Per cent inhibition in deamination of: β-^{3}H-histamine (histaminase activity)	^{14}C-putrescine (diamine oxidase activity)
5×10^{-14}	3	
5×10^{-13}	17	19
$2{\cdot}5 \times 10^{-12}$	29	28
5×10^{-12}	58	50
$2{\cdot}5 \times 10^{-11}$	83	69
5×10^{-11}	95	91
5×10^{-10}	99·4	99
5×10^{-9}	100	99·6
5×10^{-8}	100	100

* Deamination of β-^{3}H-histamine and ^{14}C-putrescine was measured in human plasma, obtained from pregnant women, in the absence and presence of aminoguanidine as described in Materials and Methods. The values are the average of three experiments.

† Amount added to incubation mixture (volume 200 μl).

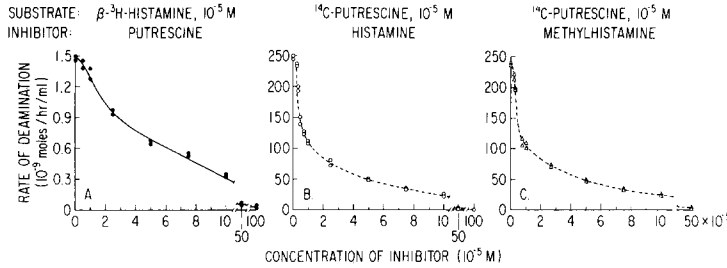

Fig. 4. Competition of various substrates for deamination by plasma diamine oxidase. The three graphs show the inhibition of deamination of (A) β-^{3}H-histamine by putrescine, (B) ^{14}C-putrescine by histamine, and (C) ^{14}C-putrescine by 1,4-methylhistamine. The incubations contained β-^{3}H-histamine diluted with unlabeled histamine, 10^{-5} M, or ^{14}C-putrescine, 10^{-5} M, and increasing concentrations of unlabeled putrescine, histamine or methylhistamine. Enzyme source was plasma obtained from pregnant women. Points depict values for individual incubations.

Table 2. Inhibition of histaminase activity in rat tissues by putrescine*

Tissue	Per cent activity remaining in presence of putrescine (mM) 0	0·02	0·04	0·1	0·5	1·0
Ileum	100 (9470)†	85	64	53	23	15
Thymus	100 (913)	78	67	50	19	
Plasma, after heparin‡	100 (298)	78	47	47	19	12

* Histaminase activity was assayed with β-^{3}H-histamine (7.5×10^{-8} M) as substrate in the presence of putrescine as indicated.

† Values in parentheses indicate the histaminase activity (nmoles/hr/g) in the absence of putescine.

‡ Collected 30 min after the administration of heparin, 4000 units/kg, i.v.

by standard procedures (Dixon and Lineweaver–Burk plots) indicated two components in the inhibition by histamine; in low concentrations ($<10^{-5}$ M), histamine inhibited putrescine deamination non-competitively (K_i, 2.3×10^{-6}) and, in higher concentrations ($>10^{-5}$ M), it appeared to inhibit putrescine deamination competitively (K_i, 8×10^{-5}). Putrescine inhibited histamine deamination competitively (K_i, 7.5×10^{-5}) at all concentrations.

As observed in our earlier studies [2], tritium was released from β-^{3}H-methylhistamine, but not β-^{3}H-putrescine, upon incubation with homogenates of intestine, thymus, placenta or plasma from pregnant women. The release of tritium from β-^{3}H-methylhistamine was quantitative, and was blocked by aminoguanidine. β-^{3}H-methylhistamine was deaminated almost as rapidly (75–80 per cent) as β-^{3}H-histamine, and it inhibited putrescine deamination to the same extent as histamine (Fig. 4).

Comparison of histaminase and diamine oxidase activities in various rat tissues. When the two assay procedures were compared, a significant correlation ($r = 0.99$, $P < 0.001$) between histaminase and diamine oxidase activities was observed in human plasma and in various rat tissues (Fig. 5). These tissues included intestine (ileum), thymus, adrenals and plasma obtained at different times after the injection of heparin.

DISCUSSION

The term histaminase was first used by Best and McHenry [24] to describe an oxidative histamine-destroying activity in dog kidney, and it was adopted by other workers to describe the same activity in hog kidney [25], human placenta [26] and plasma from pregnant women [19, 20]. Subsequently, Zeller *et al.* (see reviews by Zeller [9, 27, 28]) showed that aliphatic diamines were also oxidatively deaminated by these tissues and competed with histamine for deamination. Zeller [27] proposed that one enzyme, diamine oxidase, was responsible for the deamination of both histamine and diamines in tissues. This concept was not accepted by Kapeller-Adler and MacFarlane [29, 30] who concluded from their studies that histaminase was a separate enzyme, since they were unable to demonstrate deamination of diamines with purified enzyme preparations. This apparent inability of the enzyme to deaminate diamines was attributed by Zeller to an artifact in assay procedures [9]. Subsequent studies have shown that purified diamine oxidase from pig kidney [23, 31–33] and human placenta [34] is capable of catalyzing the deamination of both aliphatic diamines and histamine. The in-

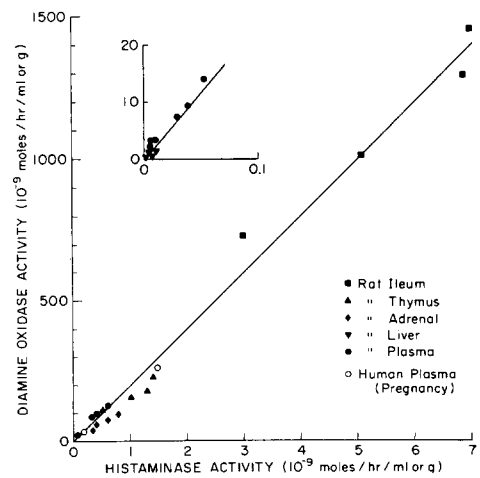

Fig. 5. Comparison of histaminase and diamine oxidase activities in individual tissues: rat intestine, thymus, adrenals, plasma and liver, and human plasma obtained from pregnant women. Rat plasma samples were obtained at various times after an intravenous injection of 4000 units/kg of heparin. Histaminase activity was assayed with β-^{3}H-histamine as substrate and diamine oxidase activity with ^{14}C-putrescine as substrate as described in Materials and Methods. The correlation between histaminase activity and diamine oxidase activity was highly significant ($r = 0.99$, $P < 0.001$).

crease in both plasma histaminase and diamine oxidase activity during pregnancy [12] or after the injection of heparin [35] also indicates that the two enzyme activities are associated. Other enzymes, however, are capable of deaminating histamine, for example, pig plasma benzylamine oxidase [22] and human serum monoamine oxidase [36], and they could contribute to the histaminase activity in certain tissues.

Our previous studies with the tritium release assay indicated that, in humans, histaminase activity is high in kidney and intestine and low in other tissues [5]. Abnormally high activity is found in tumor and serum of patients with medullary carcinoma of the thyroid [3–6]. Since diamine oxidase activity is also high, diamine oxidase is probably responsible for the high histaminase activity in the tumor and serum of these patients [6]. In rats, the high histaminase activity was found in intestine, thymus and adrenals [2, 8]. As seen in the present studies, the histaminase activity parallels diamine oxidase activity in these tissues. The competition between putrescine and histamine is another indication that the same enzyme is responsible for the deamination of both amines.

Although histamine and putrescine appear to be deaminated by the same enzyme, there are distinguishing features. Differences in the effect of pH and substrate concentration on the deamination of histamine and putrescine have been noted with purified preparations of diamine oxidase from pig kidney [22] and human placenta [23], and similar differences were noted in the present study. Blaschko *et al.* [22] have pointed out that the imidazole group of histamine (pk_a 6·8) is ionized only at acid pH, whereas the simple aliphatic diamines (pK_a 9·8) remain ionized until relatively high pH [22]. They suggested that, if only the dicationic form of the substrate interacted with diamine oxidase, the deamination of histamine would not continue to increase once the pH was above neutral pH. Although it appears that the two basic groups play an important role in the binding to the enzyme, it has not been established if ionization of both groups is necessary [37].

Other authors have shown that purified preparations of diamine oxidase are inhibited with high concentrations of histamine ($> 10^{-3}$ M) [22, 23]. The present studies indicate that inhibition may occur with relatively low concentrations (5×10^{-5} M and above) of histamine. Two explanations have been proposed for the inhibition by histamine. The first attributes the inhibition to the overlapping of two histamine molecules on the active site [37, 38]; the second has attributed the inhibition to the presence of a second inhibitory site which has a selective affinity for groups with delocalized π-electrons, such as the imidazole ring of histamine [39]. Interaction with this site is presumed to inhibit enzyme activity non-competitively [39]. The inhibition of putrescine deamination by low concentrations of histamine, as observed in our studies, could suggest the existence of such a site, and it could account for the substrate inhibition observed with histamine but not with putrescine.

The sensitivity of the enzyme to histamine is remarkable, since inhibition occurred with histamine in concentrations as low as 0·3 μg/ml. The possibility that histamine regulates the activity of diamine oxidase might be considered because histamine levels in most tissues exceed 0·3 μg/ml.

The present studies indicate that histamine and putrescine are deaminated by the same enzyme but that tritium is released from β-^{3}H-histamine but not from β-^{3}H-putrescine. In our original paper [2], we suggested that the release of tritium from β-^{3}H-histamine was consistent with the idea that the hydrogen on the beta carbon participated in the enzyme reaction as had been proposed by Werle and Pechmann [40]. However, this mechanism does not operate with β-^{3}H-putrescine, since tritium is not released from this compound. Also, as pointed out by Zeller [37], it does not operate with benzylamine derivatives which lack a beta carbon and which are good substrates for diamine oxidase. An alternative explanation for the release of tritium from β-^{3}H-histamine is that tritium is released from the deaminated product of histamine, imidazole acetaldehyde. This possibility has been discussed by Zeller [37]. If this is correct, the beta tritium of β-^{3}H-histamine must be sufficiently activated by the aldehyde carbonyl group to permit quantitative release of tritium. The tritium label of β-^{3}H-putrescine, on the other hand, may be stabilized by the tendency of the deaminated product to cyclize ^{3}H-Δ^1-pyrroline [10].

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