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The Distribution and Properties of a Histamine-methylating Enzyme*

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A major pathway of histamine metabolism in mammals is methylation of the imidazole ring (2–4) to yield 1-methyl-(β-aminoethyl)-imidazole (methylhistamine). An enzyme, imidazole-*N*-methyl transferase, which carries out this reaction has been shown in this laboratory (1), and independently by Lindahl (5), to utilize *S*-adenosylmethionine as a methyl donor. This paper describes the purification of imidazole *N*-methyl transferase from the soluble supernatant fraction of guinea pig brain, as well as its ubiquitous distribution in various tissue and species, and some of its properties.

METHODS

Materials—Histamine dihydrochloride was obtained from Nutritional Biochemical Corporation, and histamine dihydrochloride-2-C^{14} from Nuclear-Chicago. These compounds were used without further purification. Nonradioactive and methyl-C^{14}-*S*-adenosylmethionine were synthesized enzymatically from ATP and methionine (6). Methylhistamine was prepared by the method of Rothschild and Schayer (7) and was shown by paper chromatography to be free from histamine and acetylhistamine.

Assay of Imidazole-N-methyl Transferase—An assay system which measures the enzymatic *N*-methylation of histamine has been described in a previous paper (1). In this procedure, methylhistamine is almost quantitatively separated from histamine by extraction into chloroform at an alkaline pH (7). However, some histamine breaks down to a chloroform extractable product, thus increasing the "blank" value. For more precise work, methyl-C^{14}-*S*-adenosylmethionine and nonradioactive histamine have been used, since less than 1% of the radioactive cofactor is extracted into chloroform under the conditions of the assay system.

Incubations were carried out in glass-stoppered centrifuge tubes at 37°. After the incubation period, one-third volume of 1.0 N NaOH was added, the solution was saturated with sodium sulfate, and methylhistamine extracted with 3 volumes of chloroform. After centrifugation, the aqueous layer was removed by aspiration, and an aliquot of the chloroform phase was transferred to a glass vial for liquid scintillation counting. The chloroform was evaporated to dryness in a stream of warm air, the residue dissolved in 0.5 ml of hyamine (8) and counted in a Tri-Carb liquid scintillation spectrometer after the addition of 10 ml of phosphor.

* A preliminary report of this work appeared previously (1).

RESULTS

Organ and Species Distribution of Enzyme—Table I summarizes the widespread distribution of imidazole-*N*-methyl transferase in tissues of several species. All values were obtained for the soluble supernatant fraction, except for sciatic nerve and adrenal glands, where whole homogenates were used. Values are relative to the most active organ, guinea pig brain, taken as 100, which formed about 30 mμmoles of methylhistamine per 200 mg of whole tissue per hour. Treatment of rats or guinea pigs with iproniazid, a monoamine oxidase inhibitor, did not alter the enzyme activity in any organ.

Purification of Enzyme from Guinea Pig Brain (Table II)—Because of the relatively high activity of imidazole-*N*-methyl transferase in guinea pig brain, this organ was used for purification of the enzyme. All procedures were carried out at 0–4°.

Step 1. Soluble Supernatant Fraction from Brain—Whole brains from 30 adult male guinea pigs were homogenized with 10 volumes of isotonic sucrose and centrifuged at 50,000 × *g* for 30 minutes. Enzyme activity was confined entirely to the soluble supernatant fraction.

Step 2. Precipitation with Ammonium Sulfate (45 to 70% Saturation)—To 2000 ml of the soluble supernatant fraction, 554 g of $(NH_4)_2SO_4$ were added. After centrifugation at 10,000 × *g* for 10 minutes, the precipitate was discarded and 342 g of $(NH_4)_2SO_4$ were added to the supernatant solution. This was centrifuged in the same manner and the supernatant fraction discarded. The resulting precipitate was dissolved in 300 ml of 10^{-2} M sodium phosphate buffer, pH 7.4, and dialyzed overnight at 4° against 10 liters of 10^{-3} M sodium phosphate, pH 7.4.

Step 3. Purification with Calcium Phosphate Gel—Calcium phosphate gel was added in a ratio of 2.5 mg of gel per mg of protein to 270 ml of the solution obtained from Step 2. After 10 minutes, the suspension was centrifuged at 10,000 × *g* for 10 minutes and the precipitate discarded.

Step 4. Alumina Gel Cγ Adsorption—Alumina gel Cγ (2 mg per mg of protein) was added to the supernatant solution obtained from Step 3. After centrifugation, the precipitate was washed once with cold distilled H_2O and then eluted with 150 ml of 0.1 M potassium phosphate, pH 7.4. The eluate was dialyzed overnight against 10^{-3} M sodium phosphate, pH 7.4, lyophilized, and dissolved in 60 ml of H_2O. This preparation, like the transferase from mouse liver (1), lost no activity after several months at −10°. This procedure accomplishes about a 30-fold purification of the enzyme.

Properties of Purified Imidazole-N-methyl Transferase—The product of the reaction (methylhistamine) is formed by transfer of the methyl group of *S*-adenosylmethionine to histamine (*cf.* (1) Table I). Addition of Mg^{++}, reduced glutathione, or Versene was without effect. The pH optimum for imidazole-*N*-methyl transferase was studied with purified preparations obtained from mouse liver (1) and guinea pig brain. In a final concentration of 0.05 M phosphate buffer, maximal activity occurred between pH 7.2 to 7.4, although there was considerable activity between pH 6.5 to 8.0.

K_m values for histamine and *S*-adenosylmethionine were 3.5×10^{-5} M and 3.8×10^{-4} M, respectively, for the purified enzyme from guinea pig brain.

Substrate Specificity—The possibility of methylation of nitrogen in the 3- as well as in the 1-position of the imidazole ring was examined. A convenient technique for distinguishing between the two ring *N*-methylated isomers depends upon the marked difference in solubilities of their dipicrate salts. Schayer *et al.* (3) have shown that 1-methylhistamine forms an insoluble dipicrate that can be separated from the 3-methyl isomer by recrystallization. The specific activity of the chloroform extractable product formed enzymatically remained constant after four recrystallizations from water as the dipicrate with carrier 1-methylhistamine, indicating that the enzyme specifically catalyzes methylation of position 1 of the imidazole ring. However, the presence of trace amounts of the 3-methyl isomer cannot be excluded.

The methylation of histidine was attempted with use of trace amounts of L-histidine-2-C^{14} (Nuclear-Chicago), excess *S*-adenosylmethionine, and the purified brain enzyme. After incubation for 3 hours at 37°, carrier 1-methyl-DL-histidine was added and crystallized from the reaction mixture as the dipicrate. No methylhistidine-C^{14} was detected after 5 recrystallizations from water.

A variety of imidazoles was examined as possible substrates or inhibitors of imidazole-*N*-methyl transferase. Reaction mixtures containing purified guinea pig brain enzyme, limiting amounts of *S*-adenosylmethionine-C^{14} (2×10^{-5} M), 1×10^{-4} M histamine, and various imidazole derivatives (1×10^{-4} M) were incubated for 30 minutes at 37° and the formation of methylhistamine-C^{14} was measured. The following compounds had no effect on this reaction: histidine, methylhistidine, imidazoleacetic acid, methylimidazoleacetic acid, imidazole, thiolhistidine, ergothioneine, urocanic acid, *N*-acetylhistidine, and imidazole-4,5-dicarboxylic acid. If methylated, none of these compounds except unsubstituted imidazole would interfere with the assay of methylhistamine. The product, methylhistamine, inhibited the reaction 40% at 1×10^{-4} M. This inhibition was largely, but not completely, reversed by excess substrate, indicating the competitive nature of this reaction.

Effect of Common Inhibitors and Drugs on Enzyme Activity—The following compounds did not affect the methylation of histamine at 10^{-3} M concentration: sodium cyanide, sodium arsenite, iodoacetamide, sodium bisulfite, or sodium azide. However, *p*-chloromercuribenzoate inhibited the reaction 91% at 10^{-5} M.

The effect of the antihistaminic agents pyribenzamine and hydrocortisone were examined. Adult male rats and guinea pigs were treated with either 2 mg of hydrocortisone acetate or 5 mg of pyribenzamine intraperitoneally twice a day for 4 days.

TABLE I

Species and tissue distribution of imidazole-N-methyl transferase

Results are expressed as percentage of activity relative to guinea pig brain. Figures represent average values from 2 to 4 determinations on separate animals except for the cat, where a single assay was done.

Tissues were obtained from adult male animals (the cat was an adult female). They were homogenized with 4 volumes of ice cold 0.25 M sucrose (skin was ground with sea sand in a mortar) and centrifuged at 78,000 × *g* for 1 hour. Reaction mixtures containing 200 μmoles of sodium phosphate buffer, pH 7.4, 0.5 μmole of *S*-adenosylmethionine, 65 mμmoles of histamide dihydrochloride (16,000 c.p.m.), and 1 ml of soluble supernatant fraction in a final volume of 3.0 ml were incubated at 37° for 1 hour and assayed for methylhistamine-C^{14}.

	Rat	Guinea pig	Cat	Rabbit	Mouse
Liver	0	21	56	62	47
Kidney	46	63	tr.*	55	73
Ileum	29	74	tr.	13	
Lung	0	85	64	40	69
Heart	tr.	50	49	32	36
Stomach	0	92	65	60	44
Thigh muscle	tr.	46	15	25	25
Adrenal gland	0	17	52	27	
Abdominal skin	tr.	79	35	60	23
Spleen	0	78	56	29	50
Pancreas			89		
Trachea			66		
Whole brain	22	100			62
Brain stem			39	62	
Midbrain			33	42	
Cerebellum			32	69	
Cortex			72	52	
Sciatic nerve		31	40	49	

* Trace (tr) = less than 5%.

TABLE II

Purification of imidazole-N-methyl transferase from guinea pig brain

Reaction mixtures contained 540 mμmoles of histamine-C^{14} dihydrochloride (16,000 c.p.m.), 100 mμmoles of *S*-adenosylmethionine, 100 μmoles of sodium phosphate buffer, pH 7.4, and 0.5 ml of enzyme in a final volume of 2 ml.

Step	Purification stage	Total Protein	Protein*	Total units
		mg	*units/mg*	
1	Soluble supernatant fraction	13,400	0.7	9520
2	45–70% $(NH_4)_2SO_4$	1,755	4.4	7730
3	$CaPO_4$ gel, negative adsorption	188	7.7	1450
4	Alumina gel C_γ adsorption	36	25.4	920

* 1 unit = 10 mμmoles of methylhistamine formed per hour.

The soluble supernatant fractions of rat kidney and guinea pig brain, stomach, skin, and lung were assayed for their ability to form *S*-adenosylmethionine as well as methylhistamine. Enzymatic synthesis of *S*-adenosylmethionine was assayed by measuring the formation of methylhistamine after the addition

TABLE III

Inhibition of purified guinea pig brain imidazole-N-methyl transferase by chlorpromazine, bromo-lysergic acid diethylamide and serotonin

Reaction mixtures containing 54 mμmoles of histamine dihydrochloride (2.7×10^{-5} M), inhibitor, 15 mμmoles of methyl-C^{14}-*S*-adenosylmethionine (3500 c.p.m.), 130 μmoles of sodium phosphate buffer, pH 7.4, and 0.5 ml of purified enzyme (0.12 mg of protein) in a final volume of 2.0 ml, were incubated at 37°. After 30 minutes of incubation the reaction mixture was assayed for methylhistamine-C^{14}.

Compound	Inhibitor concentration*		
	5×10^{-5} M	5×10^{-6} M	5×10^{-7} M
Chlorpromazine	82	60	33
Bromo-lysergic acid diethylamide	92	56	27
Serotonin	75	33	7

* Results are expressed as percentage inhibition.

of excess ATP, $MgCl_2$, reduced glutathione, and histamine-C^{14}.[1] The treated animals were not altered either in their ability to methylate histamine or to synthesize the cofactor. Furthermore, addition of 10^{-4} M hydrocortisone acetate directly to the reaction mixture had no effect.

The following compounds did not alter the *N*-methylation *in vitro* of histamine at a concentration of 10^{-4} M: lysergic acid diethylamide, 48/80,[2] mescaline, γ-aminobutyric acid, morphine, meprobamate, cocaine, atropine, sodium hexobarbital, strychnine, epinephrine, norepinephrine, tryptophan, and 5-hydroxytryptophan. Tubocurare and bufotenine inhibited the reaction about 50% at 10^{-4} M. When the concentration of these compounds was reduced to 3×10^{-5} M there was no effect.

Table III summarizes the striking inhibition of the purified imidazole transferase by chlorpromazine, 2-bromo-lysergic acid diethylamide, and serotonin. When a cruder enzyme preparation (purified through Step 2) was used, the inhibition was less marked. At 10^{-4} M, chlorpromazine and serotonin inhibited the reaction 50%; bromo-lysergic acid diethylamide had no effect. Furthermore, the addition of excess *S*-adenosylmethionine or histamine did not reverse the inhibition significantly.

DISCUSSION

The studies of Schayer and Cooper (2, 9) have demonstrated that ring *N*-methylation is the principal pathway of histamine metabolism in man and several other species *in vivo*. Since the product of this reaction, methylhistamine, has negligible histamine-like action (10), imidazole-*N*-methyl transferase plays a key role in the inactivation of histamine.

Tissues on which histamine exerts its effects, *i.e.* skin, lung, ileum, and stomach, are relatively high in *N*-methyl transferase activity, indicating that this enzyme can inactivate the liberated amine locally. There appears to be no obvious association between histamine levels in tissues and enzyme activity. Particularly noteworthy is the high *N*-methyl transferase activity in brain. Although histamine has been reported to be present in brain (11), no action on the central nervous system has been found to date.

Imidazole-*N*-methyl transferase activity is highest in the guinea pig, which is most sensitive to histamine, and is lowest in the relatively insensitive rat. These findings rule out the possibility that histamine sensitivity is correlated with the inability of certain species or tissues to detoxify it by *N*-methylation. On the contrary, it suggests that the enzyme represents a defense against histamine for highly sensitive animals, and may be of some survival value to them.

Thus far, histamine is the only substrate found to be methylated by this enzyme. It is of interest to note that in the case of histidine, both the 1- and 3-*N*-methyl isomers are formed *in vivo* (12, 13). Whether two separate enzymes catalyze these methylations is not known. The methylation of histidine has been shown to utilize the methyl group of methionine (14), presumably via the active form *S*-adenosylmethionine.

Little can be said at this time about the inhibition of histamine methylation by chlorpromazine, serotonin and bromo-lysergic acid diethylamide. Studies are required to determine whether these compounds have any effect on histamine metabolism in the intact animal.

SUMMARY

An enzyme, imidazole-*N*-methyl transferase, which catalyzes the transfer of the methyl group of *S*-adenosylmethionine to the nitrogen in the 1-position of the imidazole ring, has been purified 30-fold from guinea pig brain. No other imidazoles studied thus far serve as substrates for this reaction. The enzyme is widely distributed in most tissues of a number of species.

Properties of the enzyme have been examined, including pH optimum, K_m values, stability, and inhibitors. Chlorpromazine, serotonin, and 2-bromo-lysergic acid diethylamide inhibit histamine methylation by the purified enzyme.

The physiological role of this enzyme in histamine metabolism has been discussed.

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[1] All of these tissues were capable of methylating histamine with ATP and methionine, indicating that they possess the methionine-activating enzyme. The rate of formation of methylhistamine ranged from 10 to 60% of the values obtained when preformed *S*-adenosylmethionine was used.

[2] This histamine-releasing agent is a mixture of low polymers of *p*-methoxy-*N*-methylphenylethylamine (Burroughs Wellcome and Company).

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