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Histamine Methyltransferase: Inhibition and Potentiation by Antihistamines

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SUMMARY

The activities of histamine methyltransferase preparations partially purified from several mammalian sources are activated by low concentrations of histamine (K_m = 6–9 μM) and are markedly inhibited by histamine concentrations in excess of 10 μM. A variety of antihistaminic drugs are potent competitive inhibitors of histamine methyltransferase in the presence of histamine concentrations below 10 μM, but enhance enzyme activity at higher histamine concentrations. These effects of antihistamines correlate partially with their antihistaminic activity.

INTRODUCTION

Methylation of histamine by histamine methyltransferase (EC 2.1.1.8) is the predominant mode of inactivation of this amine in most animal species (1–3). Histamine methyltransferase occurs ubiquitously in animal tissues and exhibits a high degree of substrate specificity, acting only upon histamine and a few histamine analogues, but not on most imidazoles (4).

Several drugs, including chlorpromazine, antimalarials, and 5-hydroxytryptamine, are potent inhibitors of partially purified preparations of histamine methyltransferase (5, 6). Netter and Bodenschatz (7) reported that some antihistamines weakly inhibited crude preparations of the enzyme. Recently, in attempts to elevate the normally low levels of histamine in the mammalian brain (8, 9), we evaluated the effects of several known inhibitors of histamine methyltransferase. Although well-known potent inhibitors, such as quinacrine, failed to alter brain levels of histamine, the antihistamine dexbrompheniramine was one of the few drugs capable of significantly elevating levels of histamine in the mouse brain (10). Accordingly, in the present study we have examined the interactions of a variety of antihistaminic agents with partially purified histamine methyl transferase preparations from mouse brain.

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MATERIALS AND METHODS

Animals used were male albino mice (30–40 g) of the CF-1 strain from Carworth Farms, New City, N. Y.; male albino rats (200–250 g) from Huntingdon Farms, West Conshohocken, Pa.; and male albino guinea pigs (350–500 g) from the Bar F Rabbitry, Perry Hall, Md.

S-Adenosyl-L-[*methyl*-^{14}C]methionine (51.2 mCi/mmole) was obtained from New Eng-

land Nuclear Corporation. Histamine dihydrochloride and (−)-*S*-adenosyl-L-methionine iodide were obtained from Calbiochem. Quinacrine hydrochloride and L-histidine hydrochloride were purchased from Nutritional Biochemicals Corporation. Sources of donated drugs are recorded under ACKNOWLEDGMENTS at the end of the text.

Partial purification of mouse brain histamine methyltransferase. Histamine methyltransferase was partially purified from the brains of male albino mice (5). Whole brains of 20 mice were homogenized in 4 volumes of 0.25 M sucrose prepared in 5 mM sodium phosphate buffer, pH 7.4 containing 0.1% Triton X-100 (11), and centrifuged at 78,000 × *g* for 30 min. Ammonium sulfate was added to the supernatant fluid to achieve 50% saturation, followed by centrifugation at 10,000 × *g* for 20 min. To the decanted supernatant fraction, more ammonium sulfate was added until 75% saturation was reached. The final precipitate, obtained by centrifugation at 10,000 × *g* for 10 min, was dissolved in 10 ml of 10 mM sodium phosphate buffer, pH 7.4, and dialyzed overnight against 2 liters of 1 mM sodium phosphate buffer, pH 7.4. The specific activity of the final preparation was 6 times greater than that of the original supernatant fraction of the brain homogenate. The partially purified enzyme lost no appreciable activity when stored for 2 months at −15°.

Method of assay. Histamine methyltransferase activity was assayed by a modification of the procedure of Snyder and Axelrod (12). The incubation and extraction were performed in 15-ml, glass-stoppered centrifuge tubes. The reaction mixture consisted of 200 µl 0.05 M sodium phsophate buffer (pH 7.4), 10–50 µl of partially purified histamine methyltransferase (0.01–0.05 mg of protein), various amounts of histamine, 13.3 nmoles of *S*-adenosyl-L-methionine, and 1.7 nmoles of *S*-adenosyl-L-[*methyl*-^{14}C]methionine. The final reaction volume was 300 µl. The final concentration of *S*-adenosyl-L-methionine in the reaction mixture was 50 µM.

After 15 min of incubation at 37°, the reaction was stopped by the addition of 0.5 ml of 0.05 M borate buffer, pH 10. Six milliliters of a toluene and isoamyl alcohol (1:1) were added, and the tube was shaken for 15 min. After centrifugation at 1500 × *g* for 10 min, 4 ml of the organic phase were transferred to a scintillation vial containing 2 ml of ethanol and 10 ml of toluene phosphor (0.4% 2,5-diphenyloxazole and 0.1% *p*-bis[2-(5-phenyloxazolyl)]benzene). ^{14}C was measured in a Packard model 3375 liquid scintillation spectrometer, with an efficiency of 64%. Drugs were initially incubated for 5 min with the enzyme and histamine, although they had a similar effect on histamine methyltransferase activity when this preliminary incubation step was omitted. After prior incubation, labeled and unlabeled *S*-adenosyl-L-methionine were added to the incubation mixture, and the activity of the enzyme was measured as above.

In each experiment two types of blank determinations were obtained, both of which gave identical values. One consisted of omitting histamine methyltransferase from the reaction, while in the other histamine was omitted. Concentrations of substrates and drugs are expressed as the final concentration in the reaction mixture. Preliminary experiments gave the following results. (a) The addition of aminoguanidine, nialamide, tranylcypromine, or Mg^{++}, all at 0.1 mM, did not affect histamine methyltransferase activity. (b) Histamine methyltransferase activity was linear with time for 30 min when the concentration of histamine was varied from 1 mM to 0.1 µM, and was linear with enzyme concentration over the range studied. (c) Addition of drugs at the end of the incubation period failed to affect the content of ^{14}C in the organic phase. Therefore it was concluded that none of the drugs studied affected the extractibility of methylhistamine into toluene–isoamyl alcohol mixture.

RESULTS

Substrate inhibition of histamine methyltransferase, and effect of diphenhydramine. In initial experiments histamine methyltransferase activity was assayed at a variety of histamine concentrations in the presence of different concentrations of *S*-adenosyl-L-methionine (Fig. 1). Increasing the histamine concentration from 0.1 to 10 µM enhanced the enzyme activity at the three *S*-adenosyl-L-methionine concentrations examined. The

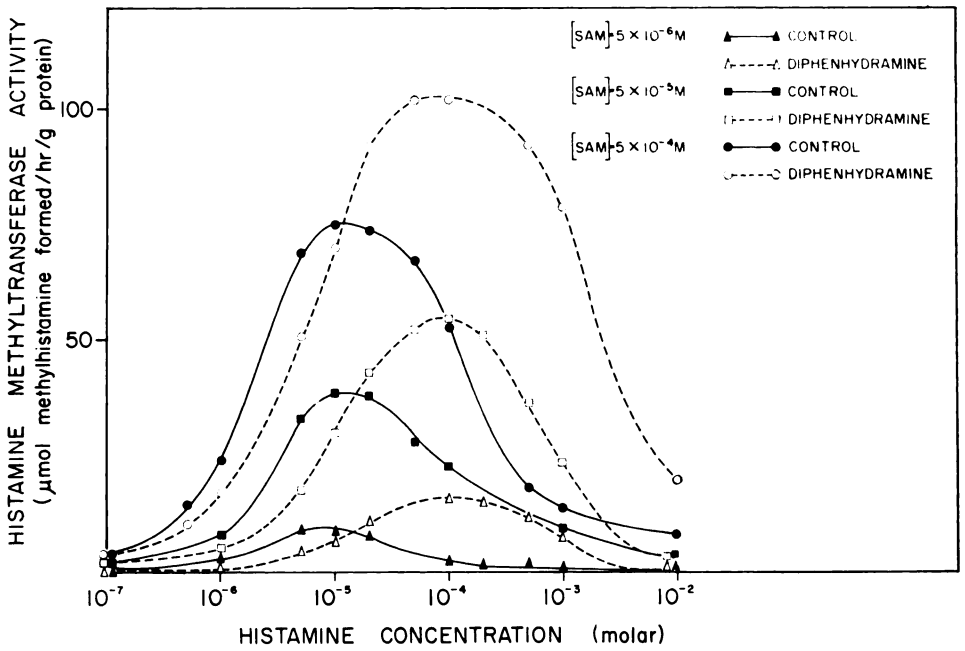

FIG. 1. *Substrate inhibition of histamine methyltransferase and effect of diphenhydramine at various S-adenosyl-L-methionine (SAM) concentrations*

The enzyme activity was estimated with increasing concentrations of histamine (0.1 μM–10 mM) at three concentrations of *S*-adenosyl-L-methionine. Histamine methyltransferase activity in the presence of diphenhydramine (0.1 mM) was also measured at each concentration of histamine and *S*-adenosyl-L-methionine used. Each point is the mean of three determinations.

K_m value of histamine for histamine methyltransferase estimated from these data was about 6–9 × μM, somewhat lower than the K_m reported by Brown *et al.* (5) for guinea pig kidney and by Gustafsson and Forshell (4) for pig liver. The K_m value for histamine was the same at all three *S*-adenosyl-L-methionine concentrations. The K_m for *S*-adenosyl-L-methionine was 30 μM at histamine concentrations of 5, 10, and 100 μM. Histamine methyltransferase activity decreased markedly at histamine concentrations in excess of 10 μM. At 100–200 μM, the enzyme activity was reduced to about 50% of the maximal activity obtained at 10 μM. The relative decrease in activity with increasing histamine concentrations was similar at all three concentrations of *S*-adenosyl L-methionine.

Diphenhydramine (0.1 mM) had a biphasic effect on histamine methyltransferase activity. At histamine concentrations below 10 μM, diphenydramine inhibited the enzyme activity. The relative degree of inhibition varied, depending on the histamine concentration. Kinetic analysis of this inhibition by the method of Dixon (13) indicated that diphenhydramine was a competitive inhibitor, with a K_i value of 40 μM (Table 1). At histamine concentrations in excess of 10 μM, diphenhydramine markedly augmented enzyme activity. At 0.1 mM histamine, diphenhydramine maximally enhanced histamine methyltransferase activity, about 2-fold. At histamine concentrations above 0.1 mM, enzyme activity in the presence of diphenhydramine diminished, but less precipitously than in the absence of diphenhydramine. Accordingly, at 1 mM histamine, enzyme activity was augmented 4-fold by diphenhydramine. The pattern of interaction of diphenhydramine and histamine methyltransferase was similar at all three concentrations of *S*-adenosyl-L-methionine (Fig. 1).

Effects of various concentrations of antihistaminic drugs on enzyme activity. Because of the striking effects of diphenhydramine on histamine methyltransferase activity, a variety of

TABLE 1

K_i values for inhibition of mouse brain histamine methyltransferase by antihistamines and related drugs

The values presented are the means of two independent determinations of K_i values by the method of Dixon (13). Inhibition by all drugs was competitive (Fig. 4). Each determination was carred out in triplicate with five concentrations of inhibitor at histamine concentrations of 1 and 5 μM (Fig. 4). These histamine concentrations were used because, with more than 10 μM histamine, the drugs increased histamine methyltransferase activity. In independent determinations none of the values shown differed by more than 20%.

Drug	K_i	Drug	K_i
	$\times 10^7$		$\times 10^7$
Quinacrine	1	Dimethindene	140
d-Chlorpheniramine	7	Fenazoxine	150
d-Brompheniramine	9	Tripelennamine	160
Chlorpheniramine	15	Carbinoxamine	230
Brompheniramine	19	Diphenylpyraline	240
Promethazine	90	Antazoline	310
l-Chlorpheniramine	90	Diphenhydramine	400
l-Brompheniramine	100	Triprolidine	450
Bromdiphenhydramine	100	Chlorcyclizine	470
Pyrathiazine	110	Haloperidol	500
Thioridazine	120	Cyclizine	1200
Chlorpromazine	140	Diazepam	2000

antihistaminic and related agents were examined for their effects on this enzyme. Initial experiments utilized a fixed concentration of histamine (0.1 mM) with drug concentrations ranging from 0.1 μM to 10 mM. At lower drug concentrations, almost all the agents examined enhanced enzyme activity, while at higher drug concentrations they were inhibitory (Fig. 2). Dexbrompheniramine and dexchlorpheniramine were the most potent agents in augmenting histamine methyltransferase activity, eliciting a maximal increase of approximately 2-fold at 2 μM. At concentrations greater than 10 μM, these two drugs progressively inhibited the enzyme. Interestingly, *l*-isomers of brompheniramine and chlorpheniramine, which are much weaker antihistamines than the *d*-isomers, were about 10% as potent in augmenting histamine methyltransferase activity.

Of the drugs that enhanced histamine methyltransferase activity, diazepam was the least potent, requiring a 1 mM concentration to elicit a maximal enhancement of activity. Most of the drugs at lower concentrations showed a similar pattern of potentiation of enzyme activity, followed by inhibition at higher concentrations. However, quinacrine, the most potent inhibitor studied *in vitro* (Table 1), failed to enhance enzyme activity significantly at any drug concentration.

In addition to the agents which are clinically employed as antihistamines, the phenothiazines chlorpromazine and thioridazine, the tricyclic antidepressant desmethylimipramine, and the butyrophenone haloperidol also showed the biphasic effects on histamine methyltransferase manifested by the antihistaminic drugs. Chlordiazepoxide and sodium pentobarbital failed to alter the enzyme activity at any concentration, nor did histidine and α-hydrazinohistidine (not depicted) have any effect.

Effects of drugs on enzyme activity at different histamine concentrations. Drug concentrations which had displayed maximal augmentation of histamine methyltransferase activity in initial experiments (Fig. 2) were examined for their effects on enzyme activity at histamine concentrations ranging from 0.1 μM to 10 mM (Fig. 3). Most of the drugs displayed a pattern similar to that first observed with diphenhydramine (Fig. 1), inhibiting the enzyme activity at lower histamine concentrations and potentiating it at higher levels. Only quinacrine and 5-hydroxytryptamine failed to enhance enzyme ac-

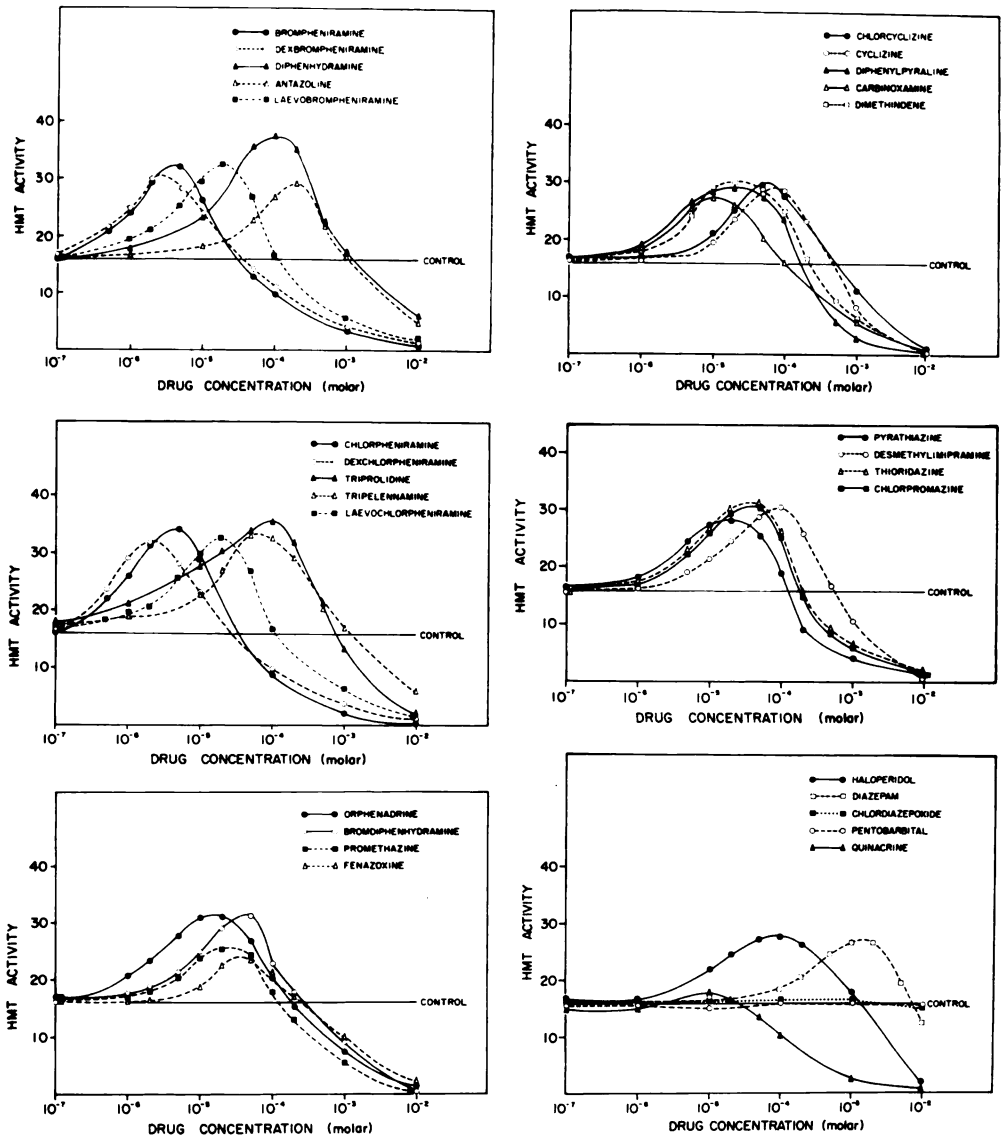

FIG. 2. *Effect of antihistamines and related drugs on activity of histamine methyltransferase (HMT)*
The enzyme activity was estimated in the presence of increasing concentrations of drugs, while the concentration of the substrate, histamine, was kept at 0.1 mM. Drugs were incubated with the enzyme for 5 min prior to the addition of *S*-adenosyl-L-methionine (50 μM). Each point is the mean of three determinations.

tivity at any concentration examined. For all other drugs, the extent of maximal augmentation of histamine methyltransferase activity was similar, and the highest enzyme activity usually occurred at histamine concentrations of about 0.1 mM. Of the drugs examined, only orphenadrine failed to inhibit the enzyme at any histamine concentration.

Kinetics of inhibition of histamine methyltransferase by various drugs. Inhibition of the enzyme by a variety of drugs was analyzed by the method of Dixon (13) (Fig. 4 and Table 1). Inhibition for all drugs listed in Table 1 was competitive, as is depicted in Fig. 4 for *d*- and *l*-brompheniramine.Quinacrine was the most potent inhibitor ex-

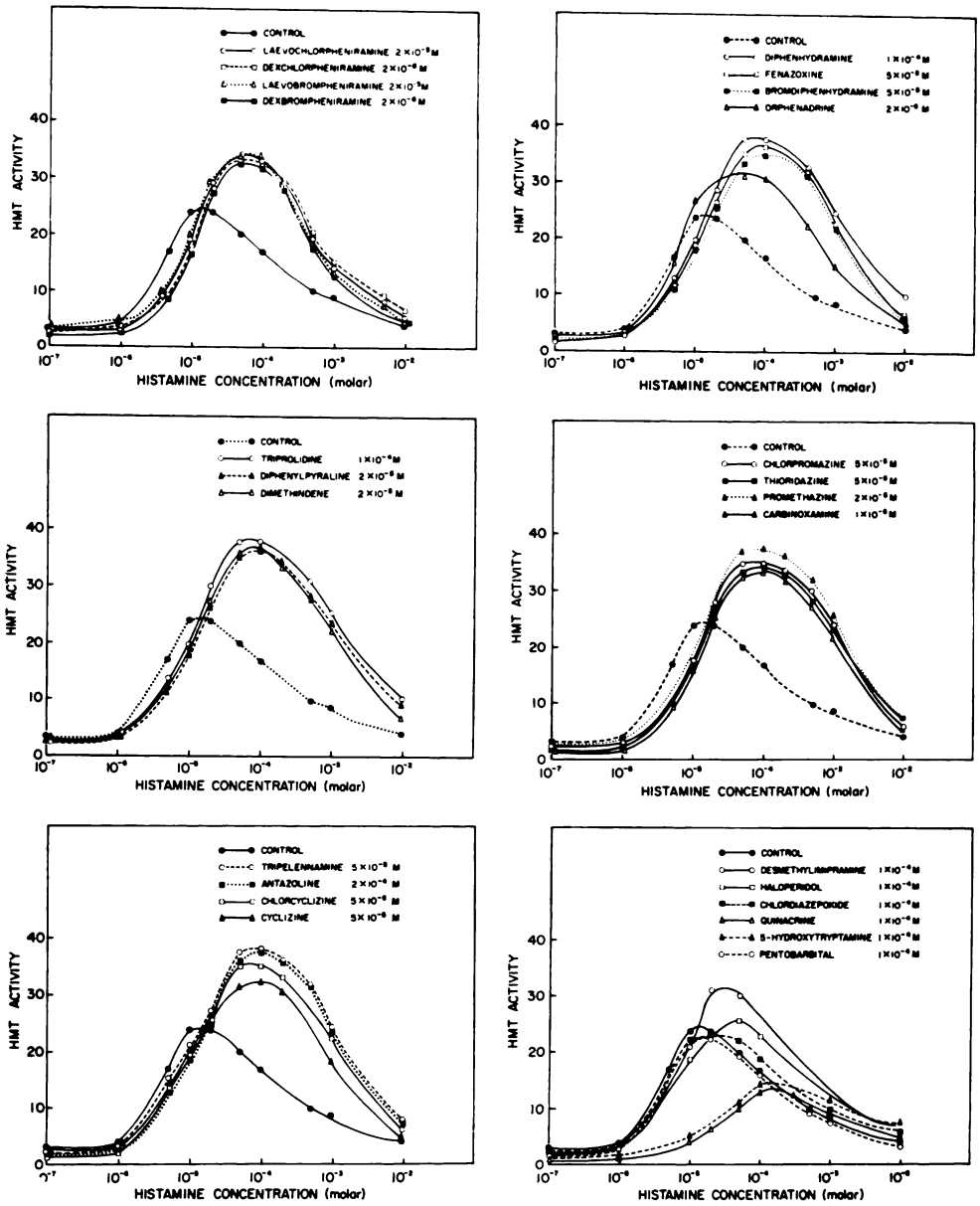

FIG. 3. *Effect of antihistamines and related drugs on activity of histamine methyltransferase (HMT) at various concentrations of histamine*

Each drug was used in a concentration that produced maximal potentiation of enzyme activity when the histamine concentration was 0.1 mM. Drugs were incubated for 5 min with the enzyme in the presence of increasing concentrations of histamine (0.1 μM–10 mM) before the addition of *S*-adenosyl-L-methionine (50 μM). Each point is the mean of three determinations.

emined, with a K_i of about 0.1 μM. The *d*-isomers of chlorpheniramine and brompheniramine were the next most active, and were about 10 times as potent as their *l*-isomers. The racemic forms of chlorpheniramine and brompheniramine were about half as active as the *d*-isomers, as would be expected because of the 10-fold greater potency of the *d*- than the *l*- forms of these drugs. The next most active group of drugs, consisting of a

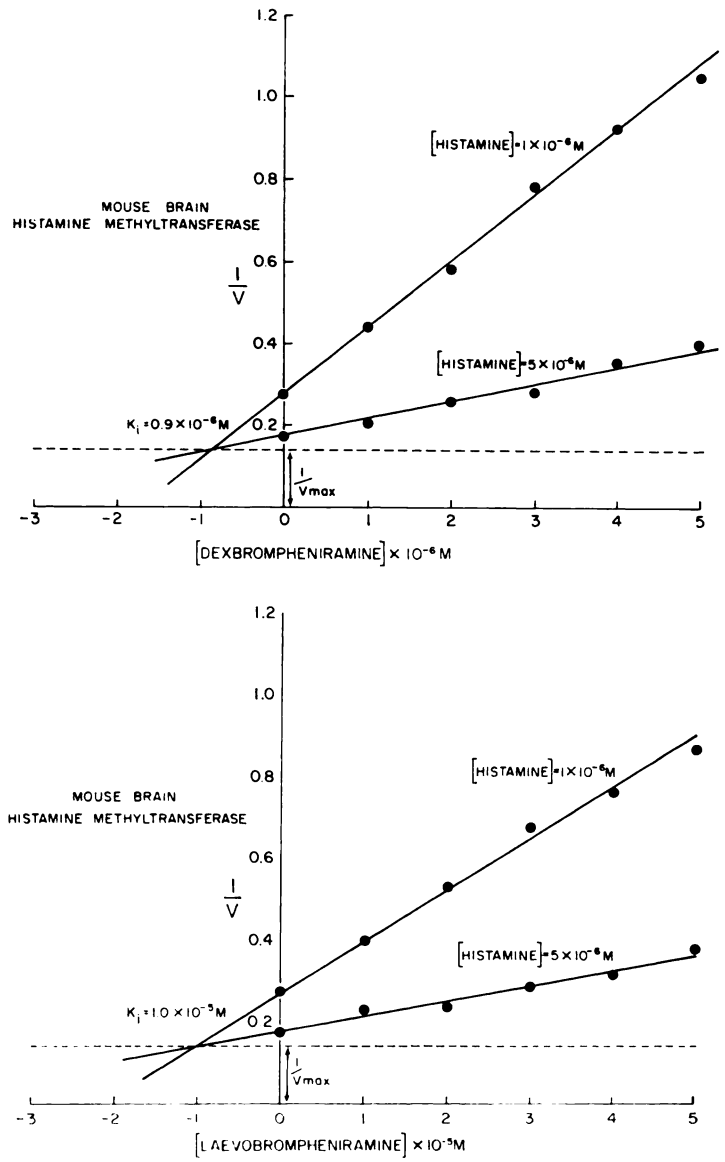

FIG. 4. *Kinetic analysis of inhibition of histamine methyltransferase by d- and l-brompheniramine*
The enzyme was incubated with increasing concentrations of drugs in the presence of histamine, 1 or 5 μM, before the addition of *S*-adenosyl-L-methionine. The method of graphical analysis was that of Dixon (13). K_i values for all drugs determined in this manner are listed in Table 1.

variety of agents with K_i values between 10 and 20 μM, were about one-fifth as potent as the chlor- and brompheniramines. These agents include *l*-brompheniramine, *l*-chlorpheniramine, promethazine, bromdiphenhydramine, pyrathiazine, thioridazine, chlorpromazine, dimethindene, fenazoxine, and tripelennamine. Drugs with K_i values between 20 and 50 μM included carbinoxamine, diphenylpyraline, antazoline, diphenhydramine, triprolidine, chlorcyclizine, and haloperidol. Cyclizine had a K_i value of 0.12 mM.

With the striking exception of quinacrine, there was a fairly close correlation between the relative potencies of various drugs in inhibiting and in elevating histamine methyltransferase activity (Table 1 and Fig. 2).

Inhibition of neurotransmitter uptake sys-

TABLE 2

Inhibition of uptake of catecholamines, 5-hydroxytryptamine, and γ-aminobutyric acid into striatal and hypothalamic synaptosome-containing homogenates by d- and l-isomers of chlorpheniramine and brompheniramine

Homogenates were prepared from the corpus striatum or the hypothalamus of rats (17, 18) and were incubated with drugs ranging in concentration from 10^{-4} to 10^{-8} M, along with 0.1 μM concentration of [^{3}H]norepinephrine, [^{3}H]dopamine, or [^{3}H]5-hydroxytryptamine or with 1 μM [^{14}C]γ-aminobutyric acid. At a concentration of 0.1 μM, 5-hydroxytryptamine does not enter catecholamine synaptosomes (19). ID_{50} values were determined from log probit plots of percentage inhibition at four concentrations of inhibitor in triplicate after subtracting 0° uptakes as blanks (18). Data presented are the mean of three independent determinations, for which the standard errors of the means were not greater than 10% of the ID_{50} values.

Substance	ID_{50} values			
	Chlorpheniramine		Brompheniramine	
	d	*l*	*d*	*l*
	M	*M*	*M*	*M*
Hypothalamus				
[^{3}H]Norepinephrine	3.3×10^{-6}	4.8×10^{-6}	4.8×10^{-6}	5.8×10^{-6}
[^{3}H]5-Hydroxytryptamine	4.1×10^{-5}	4.8×10^{-5}	5.0×10^{-5}	4.9×10^{-5}
[^{14}C]γ-Aminobutyric acid	3.8×10^{-4}	4.8×10^{-4}	4.1×10^{-4}	3.3×10^{-4}
Corpus striatum				
[^{3}H]Dopamine	3.1×10^{-6}	4.2×10^{-6}	3.8×10^{-6}	4.3×10^{-6}

tems and amine oxidase by isomers of brompheniramine and chlorpheniramine. Of the large number of clinically employed antihistamines examined in this study, the most potent antihistaminic agents are brompheniramine and chlorpheniramine. These were also the most potent inhibitors of histamine methyltransferase among the the antihistaminic drugs studied. Moreover, it is well known that the *d*-isomers of these agents are considerably more active as antihistamines than the *l*- forms, both *in vitro* and *in vivo* (14). The *d*-isomers of these drugs were about 10 times as potent as inhibitors and as augmenters of enzyme activity than the *l*-isomers. Chlorpheniramine and brompheniramine potentiate catecholamines *in vivo* (15) and inhibit their uptake by brain synaptosomes (16). To ascertain whether biological effects such as neurotransmitter reuptake and other amine-related enzyme activities might be affected differently by the *d*- and *l*-isomers of these drugs, the following experiments were performed.

Synaptosome containing homogenate preparations from the hypothalamus or corpus striatum of the rat (17) were incubated with low concentrations of radiolabeled norepinephrine, dopamine, serotonin, or γ-aminobutyric acid, with or without *d*- or *l*-isomers of chlorpheniramine and brompheniramine. As had been observed previously, these drugs were potent inhibitors of dopamine uptake into striatal synaptosomes (Table 2). However, there was no difference in the potencies of the *d*- and *l*-isomers, both displaying ID_{50} values of about 3–4 μM. These drugs had similar potencies in inhibiting norepinephrine uptake into hypothalamic synaptosomes, but again there was no difference between the *d*- and *l*-forms of the drug. At 0.3–0.4 mM, the *d*- and *l*-forms of chlorpheniramine and brompheniramine were able to inhibit γ-aminobutyric acid accumulation by hypothalamic synaptosomes with no apparent difference between the isomeric forms of the drugs.

To determine whether these drugs would affect amine-oxidizing enzymes, we examined their effects upon the monoamine oxidase activity of rat liver homogenates and diamine oxidase activity of the rat ileum (18) (Table 3). Both *d*- and *l*-chlorpheniramine at 0.1 mM inhibited monoamine oxidase activity of rat liver homogenates about 50% when tyramine was used as substrate, but

TABLE 3

Effects of isomers of chlorpheniramine and brompheniramine on monoamine oxidase activity

Rat liver monoamine oxidase activity was assayed with methylhistamine (0.1 mM) or tyramine (0.1 mM as substrate. Enzyme activity was assayed by the amine oxidase fluorescence assay of Snyder and Hendley (20). Each value is the mean of four determinations, using a drug concentration of 0.1 mM. No differences between the means of values for each isomer were significant ($p < 0.05$).

Drug	Inhibition	
	Methylhistamine as substrate	Tyramine as substrate
	%	%
d-Chlorpheniramine	14.3	46.3
l-Chlorpheniramine	10.8	52.8
d-Brompheniramine	11.9	58.9
l-Brompheniramine	12.2	61.2

only 11–14% with methylhistamine as substrate. In both cases there was no difference between the potencies of the *d*- and *l*-isomers of the drug. Neither isomeric form of chlorpheniramine or brompheniramine altered diamine oxidase activity of the rat ileum when assayed at 0.1 mM.

DISCUSSION

In the present study the kinetics of histamine methyltransferase and its interaction with a variety of drugs was examined, using a 6-fold purified enzyme preparation from mouse brain. With this preparation the K_m value (5.4 μM) for histamine was lower than that reported for guinea pig brain (5) and pig liver (4). In separate experiments, histamine methyltransferase was purified about 6-fold through the ammonium sulfate step (5) from mouse stomach, liver, and kidney and rat brain, liver, and kidney. In all these tissues the K_m for histamine was in the range of 6.0–9.0 μM, and the enzyme activity declined at histamine concentrations greater than 10 μM. As had been found with mouse brain histamine methyltransferase, diphenhydramine (0.1 mM) inhibited histamine methyltransferase prepared from these tissues when the histamine concentration was below 0.1 mM, but enhanced enzyme activity at histamine concentrations greater than 0.1 mM.

In studies of a homogeneous preparation of pig liver histamine methyltransferase, no cofactor requirements were observed (4). Accordingly, it is unlikely that the substrate inhibition and the inhibitory and enhancing effects of drugs observed in this study were due to interactions with such cofactors. Moreover, the substrate inhibition of histamine methyltransferase and the effects of antihistamines were similar at several *S*-adenosyl-L-methionine concentrations. Thus it sould seem that these effects reflect an interaction of histamine and of the drugs with histamine methyltransferase itself, although only studies with a homogeneous preparation of the enzyme could completely rule out interactions of the drugs with other proteins as accounting for our findings.

The existence of substrate inhibition may or may not reflect allosteric properties of an enzyme. For enzymes that are bireactant with respect to substrate and product, investigation of allosteric effects is more difficult than in the case of enzymes with single substrates. Because of the crude nature of our enzyme preparation, detailed analysis of possible allosteric effects was not undertaken. However, preliminary investigation failed to reveal the presence of sigmoid kinetics. With respect to the order of substrate binding, the fact that the K_m for histamine methyltransferase was the same at various concentrations of *S*-adenosyl-L-methionine suggests that a ping-pong mechanism was not involved, but would be consistent with a sequential addition of substrates.

The enhanced histamine methyltransferase activity in the presence of antihistamines could reflect a reversal of substrate inhibition or a true activation of the enzyme. If the drugs activated the enzyme, one would anticipate an increase in its V_{max}. Estimates of V_{max} values obtained by extrapolation of double-reciprocal plots to intersection with the ordinate indicated that the V_{max} of histamine methyltransferase was not altered by promethazine (Fig. 5). Similar results were obtained with all the other antihistamines used in this study, suggesting that enhancement of the enzyme activity by these drugs

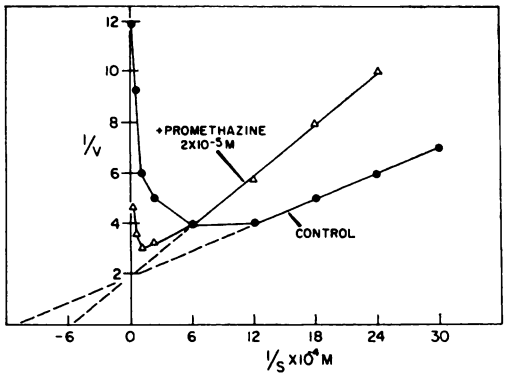

FIG. 5. *Double-reciprocal analysis of histamine methyltransferase activity at various histamine concentrations, and influence of promethazine*

Promethazine (20 μM) was incubated with the enzyme in the presence of various concentrations of histamine before the addition of *S*-adenosyl-L-methionine (50 μM). Histamine methyltransferase activity (v) is expressed as micromoles of methylhistamine ($\times 10^{-2}$) formed per hour per gram of protein.

primarily represents a reversal of substrate inhibition and not an "activation".

The ability of antihistaminic drugs to inhibit and enhance histamine methyltransferase activity showed some correlation with their antihistaminic potency. This was most evident with brompheniramine and chlorpheniramine. Thus these drugs are the most active antihistamines of those examined in the study, and were also the most potent in their interactions with the enzyme. Moreover, the *d*- isomers were 10 times as active as the *l*- isomers. In addition, other biological effects of these drugs, such as inhibition of catecholamine uptake into brain synaptosomes and inhibition of monoamine oxidase activity, failed to show stereoselectivity. It should be noted, however, that assays of the antihistaminic activity of these drugs *in vitro* show a 100-fold difference between *d*- and *l*-isomers, which is considerably greater than the differential effects we have observed on histamine methyltransferase (14).

Our findings might be construed as suggesting a parallelism between the active site of histamine methyltransferase and the histamine "receptor." Arguing against this possibility are the findings that a number of agents which are weak antihistaminic drugs, such as chlorpromazine and desmethylimipramine, were approximately as potent in their interactions with the enzyme as were drugs of similar chemical structure, such as promethazine, which are more active antihistamines. Nonetheless, the partial correlation of histamine methyltransferase inhibition and antihistaminic activity, as well as the stereoselective effects on this enzyme, but not on monoamine oxidase or transmitter reuptake, suggests that detailed investigation of the structure and kinetics of histamine methyltransferase may be heuristic in understanding histamine receptor mechanisms.

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