Biochemical Pharmacology, Vol. 16, pp. 1627–1631. Pergamon Press Ltd. 1967. Printed in Great Britain

INHIBITION OF HISTAMINE-*N*-METHYLATION BY SOME ANTIHISTAMINES

K. J. NETTER* and K. BODENSCHATZ

Department of Pharmacology, University of Hamburg, Germany

(*Received* 24 *November* 1966; *accepted* 16 *February* 1967)

Abstract—*N*-methylation of histamine by imidazole-*N*-methyl transferase can be inhibited *in vitro* by antihistamines of the benzimidazole and ethylene diamine types. Clemizole and tripelennamine as well as some of their derivatives were used in this study. Rat kidney homogenate served as enzyme source. In final concentrations of $2{\cdot}5 \times 10^{-4}$ M both groups of antihistamines inhibit by about 50 per cent. From a Lineweaver–Burk plot the inhibition appears to be competitive. In accordance with observations on other series of drugs the inhibitory activity is higher when the hydrogen in para-position of a benzyl group is substituted by halogens, activity rising in that order: H, Cl, Br. The effect of these antihistamines does not seem to be mediated through their interaction with functional sulfhydryl groups. *In vivo* blocking of histamine *N*-methylation cannot be achieved because of the toxicity of the antihistamines at the required dosage. Attempts to deplete the animals of activated methyl groups by large doses of nicotinamide and to produce an 'amethylosis' were unsuccessful.

THE METABOLIC inactivation of histamine in the rat proceeds through two main pathways. They are oxidation to imidazole acetic acid and *N*-methylation plus oxidation to *N*-methyl-imidazole acetic acid.[1] The first reaction, which is catalysed by diamine oxidase (DAO), can be almost selectively inhibited by aminoguanidine, both *in vivo* and *in vitro*.[2] Simultaneous blocking of the second reaction, which is catalysed by the imidazole-*N*-methyl transferase (IMT), would yield valuable information as to the physiologic role of histamine in the organism. It also would contribute to a better understanding of the sex difference in histamine catabolism in rats.[3, 4] Recent experiments have shown that some antimalarial drugs inhibit histamine methylation both *in vitro*[5] and *in vivo*.[6] Besides antimalarials a weak antihistamine, chlorpromazine, has been found to inhibit IMT.[7, 8] Therefore it seemed of interest, whether other antihistamines exert the same effect.

We examined a series of antihistamines of the benzimidazole and ethylene diamine types and, for comparison, promazine and chlorpromazine. The observation that these substances block IMT competitively suggests a structural similarity between the binding site of IMT and the tissue receptors for histamine. Furthermore an increase of inhibitory activity was observed when the para-hydrogen of the benzyl group of the antihistamines was substituted by halogens. Nicotinamide is methylated in the organism by a nicotinamide-*N*-methyl transferase with the aid of S-adenosyl-methionine (SAM)[9, 10] in a similar way as is histamine by IMT. The possibility was examined

* Present address: Department of Pharmacology, University of Mainz, 65 Mainz, Germany.

that overloading with nicotinamide might deprive the animals of metabolically available active methyl groups and thus slow down histamine methylation.

METHODS

Experiments were carried out with male Wistar rats, which were allowed a standard laboratory diet and water *ad libitum*. Histamine was determined fluorimetrically by reacting the extracted histamine with *o*-phthaldialdehyde,[11] which was obtained from "Calbiochem", Los Angeles, California. The fluorophore was measured at 450 mμ in a filter photometer ('Eppendorf') after activation at 366 mμ. The activity of IMT was determined in rat kidney homogenates. Kidneys were removed after decapitation and bleeding of the animals and homogenized immediately at 3° in 20 vol. of ice-cold sucrose solution (0·25 M) in a glass homogenizer. The supernate obtained after 10 min of centrifugation at 1200 *g* served as the enzyme. The incubation mixture (4 ml) contained 1 ml of the supernatant (=50 mg of fresh tissue weight), 200 μmole of phosphate buffer (pH 7·2), 0·4 μmole of aminoguanidine sulfate (Th. Schuchardt AG, Munich) for inhibition of DAO, and 20 μmole of $MgCl_2$. Activated methyl groups were added as S-adenosyl-methionine ("Calbiochem") (0·5 μmole). Five minutes after the addition of the inhibitor, histamine dihydrochloride was added in

TABLE 1. INHIBITION OF HISTAMINE-*N*-METHYLATION BY ANTIHISTAMINES

Inhibitor		% Inhibition
Control: Hist. metabolized 3·4 ± 0·2 μg/25 min	(7)	0 ± 0
Benzyl-2-pyrrolidyl-methyl-benzimidazole-HCl	(3)	24 ± 3
p-Cholro-benzyl-2-pyrrolidyl-methyl-benzimidazole-HCl (Clemizole, Allercur°)	(7)	49 ± 10
p-Bromo-benzyl-2-pyrrolidyl-methyl-benzimidazole-HCl	(5)	75 ± 4
Control: Hist. metabolized 2·2 ± 0·1 μg/15 min	(7)	0 ± 0
Promazine	(5)	29 ± 8
Chlorpromazine	(5)	40 ± 2
Control: Hist. metabolized 2·00 μg/15 min		0
Tripelennamine-HCl (Pyribenzamine°) 1·55 μg/15 min		23
p-Chloro-tripelennamine-HCl (Synpen°) 0·95 μg/15 min		52
p-Bromo-tripelennamine-HCl (Hibernon°) 0·60 μg/15 min		70

The figures represent mean values ± standard deviations except for the single experiment in the lower part. For experimental details see 'Methods'. The number of experiments is given in parentheses.

varying amounts, usually 5 or 6 μg (calculated as the free base). The incubations of Table 1 were carried out with 6 μg. The volume was adjusted to 4 ml with sucrose solution and the mixture was shaken at 37° under an atmosphere of air at about 120 opm. The reaction was stopped after 15 or 25 min by adding 1 ml of 2 N perchloric acid. Control experiments showed that the decrease of histamine concentration was linear for at least 30 min. The inhibitors did not interfere with fluorimetry.

The biological half life of histamine *in vivo* was estimated as described earlier, by determining blood histamine concentrations after injection into pentobarbital-anesthetized (45 mg/kg) male rats.[3] 5 mg/kg of histamine were injected via carotid

artery cannula after blocking DAO by 20 mg/kg of aminoguanidine sulfate and bilateral nephrectomy (to remove most of IMT activity).

RESULTS

Inhibition of IMT by antihistamines. Halogen substitution and inhibitory activity

The antihistamines chosen for study have been congeners of clemizole (Allercur°) and of tripelennamine (Pyribenzamine°). The degree of inhibition at inhibitor concentrations of $2{\cdot}5 \times 10^{-4}$ M varies between 20 and 70 per cent as is shown by the experiments of Table 1. It is known for certain antihistamine structures that halogen substitution increases their antihistaminic potency.[12, 13, 14] This applies also for the inhibition of IMT by members of the clemizole and tripelennamine families. The inhibitory activity increases from hydrogen to chlorine to bromine as substituents in the para-position of the benzyl ring, as can be seen from Table 1. The table further demonstrates that promazine and chlorpromazine behave in the same way.

Lack of inhibition by other compounds

Besides the benzimidazoles a number of other compounds with imidazole structure have been tested for their ability to inhibit IMT *in vitro* at a final concentration of 10^{-3} M. They are *N*-acetyl-histamine, di-*n*-butyl-histamine, histidyl-histidine, 3-methyl-histidine, caffeine, carnosine, murexine, pilocarpine, theophylline, and ureidoimidazole. With the exception of pilocarpine, which inhibits by about 50 per cent, these substances were found to lack inhibitory activity. Other compounds such as creatine, glycocyamine, dilantin, thiamine, 3-aminotriazole, pyrogallol, and nicotinamide likewise did not inhibit under the same conditions. Nicotinamide has already been described as being non-inhibitory.[15] These results were obtained by incubation of 25 mg of kidney tissue for 30 min as described above.

Mechanism of inhibition

IMT contains sulfhydryl groups, that are necessary for its catalytic function.[15] It is conceivable, therefore, that the inhibition by the tested antihistamines may be caused by interaction with the essential SH-groups of the enzyme. In this case protection of the sulfhydryl groups by D,L-cysteine should abolish the inhibition. Respective experiments, however, revealed no change in the degree of inhibition, when $2{\cdot}5 \times 10^{-3}$ M of D,L-cysteine were added. On the other hand, in control experiments, IMT was completely inactive in the presence of $1{\cdot}5 \times 10^{-4}$ M of *p*-chloromercuribenzoate (*p*CMB) and could be almost fully reactivated by cysteine ($2{\cdot}5 \times 10^{-3}$ M). Thus the action of the antihistamines studied does not seem to be caused by an interference with the SH-groups of the enzyme.

Kinetic experiments showed the inhibition of IMT to be of the competitive type according to double reciprocal velocity versus substrate concentration diagrams. The substrate constant and its standard deviation is $2{\cdot}33 \pm 0{\cdot}86 \times 10^{-5}$ ($n = 7$). It increases with the addition of the antihistamines. In contrast to the antihistamines *p*CMB causes a non-competitive inhibition of IMT. Competitive inhibition is also observed with promazine and chlorpromazine, as has been described for nicotinamide-*N*-methyl transferase.[10] Provided that *p*CMB reacts specifically with sulfhydryl groups, which has been shown to be the case for microsomal hydroxylations,[16] this observation points to the difference in mode of action between the antihistamines and *p*CMB.

In vivo *experiments*

Interruption of both principal pathways for histamine inactivation *in vivo* by a combination of aminoguanidine and the antihistamines cannot be achieved because of the relatively high toxicity of the latter. No prolongation of histamine half life could be observed after intravenous infusion of 25–50 mg/kg of clemizole. This drug dose causes hematuria, convulsions, and pulmonary edema, although the i.p. LD_{50} in mice is given as 400 mg/kg.[17] Similarly, pretreatment of rats with 40 mg of tripelennamine per animal over a period of 4 days produces no inhibition of histamine-*N*-methylation *in vitro*,[7] although the dilution of the material involved in the preparatory procedure does not exclude a reversible inhibition.

It was attempted to decrease the rate of histamine-*N*-methylation by application of large doses of nicotinamide or dimethyl-amino-ethanol, which are known to become methylated. Overloading with these compounds could eventually deplete the animal of activated methyl groups and thus minimize the intracellular availability of SAM for the IMT. After i.p. application of 500 mg/kg of nicotinamide twice daily for 3 days as well as after feeding a diet containing 2 per cent of nicotinamide for 12 days the biological half life of histamine remains unchanged. The same is true, when dimethyl-aminoethanol is given in doses of 500 mg/kg prior to and 150 mg/kg during the experiment in which the animals were pretreated with 20 mg/kg of aminoguanidine sulfate and nephrectomized. Thus competition for SAM by a fully substrate-saturated nicotinamide-*N*-methyl transferase does not seem to reduce IMT activity *in vivo*, even when most of IMT is removed by nephrectomy.

DISCUSSION

From a metabolic aspect the suppression of allergic manifestations by antihistamines should be caused by a decrease of histamine concentration at the specific receptor sites. Therefore, impairment of histamine inactivation by these compounds would be expected to lead to the opposite effect. This seeming paradox will find its explanation in the fact that both, tissue receptor and inactivating enzyme, bind histamine as well as antihistamines, especially when the antihistamines contain the imidazole configuration. However, the affinity of the enzyme is much lower, as is evidenced by the high concentrations of antihistamines required for inhibition of IMT. Nevertheless a certain structural similarity of both binding sites is suggested.

Apparently the presence of the imidazole ring in the inhibitor molecule does not necessarily produce inhibitory activity, since a number of compounds containing this configuration have no such effect. The histamine releasing polymeric compound 48/80 also does not inhibit IMT,[7] although it should be expected to combine with histamine binding sites at least in the mast cell, because histamine release does not seem to require cellular energy as judged from the lack of increase in oxygen uptake.[18]

Halogenation of the studied antihistamines in the para position of the benzyl moiety increases their fixation to the histamine binding site of IMT. This is in agreement with earlier observations that halogenated congeners are pharmacologically more active. But pharmacological activity is not always increased by halogenation. Thus the suppression of histamine asthma in guinea pigs by chloro-tripelennamine requires eight times the dose of tripelennamine itself.[17]

The lack of inhibition of histamine methylation *in vivo* shows that nicotinamide in large doses fails to produce a sufficiently severe 'amethylosis', at least at the site of

the intracellular localization of IMT. So far, however, even the direct proof of a decrease of intracellular concentration of SAM is lacking. Separate effects on different pools of SAM do not seem to provide an explanation, since both enzymes, IMT and nicotinamide-*N*-methyl transferase, are localized in the cytoplasm.[10, 15] This points further to the problem whether both enzymes are identical, a question which would suggest purification and specificity studies.

Acknowledgements—For the generous supply of the various antihistamines used in this study we are indebted to Ciba AG, Wehr/Baden, Diwag GmbH, Berlin, Farbenfabriken Bayer AG, Leverkusen, J. R. Geigy AG—Thomae, Biberach/Riss, and especially to Schering AG, Berlin.

We thank Dr. Parkhurst A. Shore, University of Texas, for his advice during the early stages of the work and for his help with the correction of the manuscript.

REFERENCES

1. R. W. Schayer, *Physiol. Rev.* **39**, 116 (1959).
2. W. Schuler, *Experientia* **8**, 230 (1952).
3. K. J. Netter, V. H. Cohn and P. A. Shore, *Am. J. Physiol.* **201**, 224 (1961).
4. S. H. Snyder and J. Axelrod, *Biochim. biophys. Acta* (Amst.) **111**, 416 (1965).
5. V. H. Cohn, *Biochem. Pharmac.* **14**, 1686 (1965).
6. V. H. Cohn, K. S. Kim and E. Richens, *Fedn Proc.* **25**, 691 (1966).
7. D. D. Brown, R. Tomchick and J. Axelrod, *J. biol. Chem.* **234**, 2948 (1959).
8. T. White, *Fedn Proc.* **23**, 1103 (1964).
9. G. L. Cantoni, *J. biol. Chem.* **189**, 203 (1951).
10. R. A. Salvador, and R. M. Burton, *Biochem. Pharmac.* **14**, 1185 (1965).
11. P. A. Shore, A. Burkhalter and V. H. Cohn, *J. Pharmac. exp. Ther.* **127**, 182 (1959).
12. C. R. Ensor, D. Russell and G. Chen, *J. Pharmac. exp. Ther.* **112**, 318 (1954).
13. A. Labelle and R. Tislow, *J. Pharmac. exp. Ther.* **113**, 72 (1955).
14. Z. Votava, J. Metysova und Z. Horakova, *Arch. exp. Path. Pharmak.* **236**, 25 (1959).
15. K. M. Lindahl, *Acta physiol. scand.* **49**, 114 (1960).
16. K. J. Netter und S. Jenner, *Arch. exp. Path. Pharmak.* **255**, 120 (1966).
17. W. Schulemann und H. Friebel, *Dt. med. Wschr.* **78**, 540 (1953).
18. G.F. Kahl und K. J. Netter, *Naunyn-Schmiedebergs, Archs. exp. Path. Pharmak.* **256**, 55 (1967).