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Inhibition of histamine methylation by antimalarial drugs*

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In studies designed to assess the physiologic role of biogenic amines such as serotonin and norepinephrine, one approach has been to inhibit either their biogenesis or their catabolism and note the corresponding changes in function.[1] A similar approach to the study of histamine's physiologic role should likewise be useful. Histamine is metabolized by two principal pathways: oxidation of the side chain amino group by diamine oxidase (DAO) and methylation of an imidazole nitrogen by imidazole N-methyl transferase (IMT).[2] Aminoguanidine has been known for many years to be a potent and selective inhibitor of DAO, both *in vitro* and *in vivo*.[3, 4] Although chlorpromazine has been shown to inhibit IMT both *in vitro*[5, 6] and *in vivo*,[7] it appears to be only a weakly effective inhibitor.[6, 7] The desirability for finding an inhibitor of IMT with greater effectiveness and specificity is apparent.

Attempts to inhibit catechol O-methyl transferase by quinacrine† led to the study of the effect of this compound on IMT. Quinacrine proved to be an effective inhibitor of IMT, and compounds structurally related to quinacrine were tested for similar activity. Since the most active of these compounds were antimalarial drugs, other compounds strucutrally unrelated but possessing antimalarial activity were also tested. Many of these were found to be potent inhibitors of IMT. The results of the studies are reported here.

METHODS

Imidazole N-methyl transferase activity was assayed by the procedure utilized but not described in detail by Netter *et al.*[8] The enzyme was prepared from kidney homegenates of male Sprague-Dawley rats. Kidneys were removed immediately after the rat was killed by cervical dislocation, and chilled on ice. After weighing, the kidneys were homogenized in 9 volumes of ice-cold 0·25 M sucrose and centrifuged at 30,000 *g* for 20 min. The 30,000-*g* supernatant fluid was used as the enzyme source. A representative incubation vessel contained 1 ml enzyme, 200 μmoles phosphate buffer (pH 7·2), 0·5 μmole aminoguanidine (to inhibit metabolism by DAO), 0·5 μmole S-adenosyl methionine (to serve as methyl donor), 0·09 μmole histamine, and 0·4 mμmole to 4 μmoles inhibitor in a final volume of 4 ml. Incubation vessels were preincubated in a Dubnoff metabolic shaker for 10 min at 37° in an air atmosphere to ensure temperature equilibrium and to provide a standard time period for the inhibitor to interact with the enzyme before the substrate (histamine) was added. In control experiments aliquots of the incubation mixture were removed at various times, and the rate of histamine disappearance was shown to be linear for at least 30 min. In subsequent experiments, rates were determined from aliquots removed at 0 and 30 min. In the absence of added S-adenosyl methionine, no histamine disappearance could be detected, thus indicating the specificity of the assay procedure. The enzymatic reaction was stopped by adding a 0·5 ml aliquot of the incubation mixture to an extraction tube containing 4 ml of 0·4 N $HClO_4$. The tube contents were then made alkaline by the addition of 0·5 ml of 5 N NaOH, and any fluorescent inhibitors were extracted from the aqueous phase with 5 ml chloroform. After this extraction no interference in the histamine assay was produced by any of the compounds studied. Histamine was then extracted from a portion of the alkaline

aqueous phase according to the procedure of Shore *et al.*[9] and the histamine determined fluorometrically after reaction for 3 min with 0·1% (w/v) *o*-phthaldehyde dissolved in absolute methenol.[9]

RESULTS AND DISCUSSION

Table 1 summarizes the results of experiments designed to measure the inhibition of histamine methylation. Each value represents the average of one to four determinations of the inhibition. Representatives of each of the major classes of antimalarial drugs, except for the 8-aminoquinoline, primaquine, display significant inhibitory activity. Of the 4-aminoquinolines tested, amodiaquine appears to be somwehat more active and is comparable in activity to quinacrine, an acridine derivative.

TABLE 1. PER CENT INHIBITION OF HISTAMINE METHYLATION

	10^{-6}M	10^{-5}M	10^{-4}M	10^{-3}M
Anitimalarial drugs				
Quinacrine	7	41	100	100
Chloroquine	0	39	79	100
Hydroxychloroquine	0	17	54	100
Amodiaquine		69	84	100
Primaquine		0	0	0
Quinine		0	7	53
Quinidine		12	34	97
Chlorguanide	0	0	6	63
Chlorguanide metabolite*	4	29	94	100
Pyrimethamine	8	29	91	100
Other compounds				
Amethopterin			0	0
Chlorpromazine	0	0	13	81

* 4,6-Diamino-1-*p*-chlorophenyl-1,2-dihydro-2,2-dimethyl-1,3,5-triazine (Cycloguanil).

Chlorguanide has little if any antimalarial activity *in vitro*, and is thought to be cyclized *in vivo* to its active dihydrotriazine metabolite (4,6-diamino-1-*p*-chlorophenyl-1,2-dihydro-2,2-dimethyl-1,3,5-triazine, Cycloguanil).[10] Similarly, in these studies chlorguanide itself has minimal activity in inhibiting histamine methylation, but its triazine metabolite is one of the more active compounds tested. Pyrimethamine, related structurally to the chlorguanide metabolite, has a similar inhibitory pattern. Quinine inhibits IMT only slightly; its isomer, quinidine, is somewhat more active. Primaquine, the only 8-aminoquinoline derivative tested, does not inhibit methylation even at a concentration of 10^{-3}M.

Pyrimethamine and the triazine metabolite of chlorguanide are thought to exert their antimalarial activity by inhibiting folic reductase.[11] Amethopterin, another inhibitor of folic reductase,[12] was tested and found to be inactive in inhibiting histamine methylation.

Chlorpromazine was assayed in this system as a basis for comparison. The degree of inhibition produced by chlorpromazine in this system is comparable to that observed when purified IMT was used.[6] The data indicate that in this *in-vitro* system all of the 4-aminoquinoline derivatives, quinacrine, pyrimethamine, and the chlorguanide metabolite are more active than chlorpromazine in inhibiting IMT. The antimalarial drugs are thus the most effective inhibitors of IMT *in vitro* so far reported. As such, they should serve as useful tools in the study of histamine metabolism. These studies *in vitro* are being extended to ascertain the effectiveness of the compounds in inhibiting the methylation of histamine *in vivo*.

Since the ability to inhibit IMT is shared by all the clinically useful antimalarials (except primaquine), the significance of this inhibition in contributing to either the pharmacologic or toxic effects of the compounds is of interest. This inhibition is believed to be the only biochemical effect shared by all of these antimalarial drugs.[13] Studies are currently in progress to determine the mechanism of this inhibition and to ascertain the effect of these compounds on other methylation reactions utilizing S-adenosyl methionine as the methyl donor.

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Department of Pharmacology,
The George Washington University,
School of Medicine,
Washington, D.C., U.S.A.

VICTOR H. COHN

† S. Spector, personal communication.

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Monoamines in isolated nerve ending particles*

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STUDIES on the localization and distribution of catecholamines (CA) in the adrenergic nervous system and terminals have been greatly facilitated by the specific histochemical fluorescence method developed by Hillarp and co-workers (for references, see Dahlström and Fuxe[1]). The monoaminergic nerve terminals have abundant, brightly fluorescent enlargements (varicosities).[2–4] In view especially of the studies relating endogenous CA and 5-HT to the fraction containing the isolated nerve-ending particles (NEPS) or synaptosomes,[5–7] it was of importance to determine whether the amine-containing varicosities observed in the fluorescence microscope are related to the synaptosomes.

MATERIALS AND METHODS

The brain stem of Sprague–Dawley rats was homogenized in cold 0·32 M sucrose and the P_2 residue of Gray and Whittaker[8] was obtained by differential centrifugation. This pellet was resuspended in isotonic sucrose and subfractionated by density gradient centrifugation.[6] The three well-defined subfractions of P_2 (A, B and C) were isolated and smears were prepared on micoscope slides. These slides were treated with formaldehyde gas at 80° for 1 hr. With this treatment CA and serotonin (5-HT) are converted to intensely fluorescent compounds, the former being green or yellow-green and the latter yellow. The specificity and chemical basis of this reaction have been discussed recently.[1]

RESULTS

The three subfractions (P_2–A, P_2–B and P_2–C) were examined in the fluorescence microscope. From normal rats, the P_2–B smear was covered with a myriad of intensely green to yellow-green (and possibly a few yellow) fluorescent spots of about the same size as varicosities (Fig. 1). The P_2–A and P_2–C smears had relatively much fewer numbers of fluorescent spots. The P_2–B slides showed no

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