action of MMIQ would seem to be limited to the respiratory chain. However, if the data in Figs. 2 and 3 are compared to the results shown in Fig. 4, it may be observed that MMIQ inhibits succinate oxidation in mitochondria but not in submitochondrial particles. Therefore, the possibility that MMIQ affects the influx of succinate into the mitochondria cannot be fully discarded.

In comparison to other inhibitors of electron transport which preferentially act on the NADH-CoQ segment of the respiratory chain, MMIQ possesses some unique properties. It differs from rotenone and piericidin [6, 7] in that these two inhibitors are more potent than MMIQ [8], and in addition, MMIQ inhibits the oxidation of succinate by intact mitochondria. In this respect, it is more like octylguanidine [2, 3], which at low concentrations (30 μM) inhibits the coupled oxidation of NAD-dependent substrates and at much higher concentrations (300 μM) inhibits the coupled oxidation of succinate. Nevertheless, the inhibiting action of octylguanidine is reversed by uncouplers [9], while MMIQ inhibits uncoupler-stimulated respiration. Since MMIQ, octylguanidine and ethidium bromide possess a positive charge at a pH around 7, these three compounds most probably act on the same site or through a similar mechanism; however, it is probable that the binding of MMIQ to the membrane would be more stable owing to stronger hydrophobic interactions in addition to charge interactions. Thus, MMIQ would not be released from the membrane by the uncoupler-induced collapse of the electrochemical potential, as is the case for octylguanidine and ethidium bromide [2, 4].

Another important characteristic of MMIQ is that it inhibits electron transport at concentrations 50 per cent lower than those that inhibit phosphodiesterase activity [1]. This may indicate that MMIQ affects the contraction–relaxation cycle of muscle (see Ref. 1) by interfering with the mitochondrial energy-transducing system, particularly if it is considered that mitochondria, by regulating cytoplasmic Ca^{2+} levels, may affect muscle contraction [10]. This is a possibility that will be explored.

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Biochemical Pharmacology, Vol. 27, pp. 1507–1509.

0006-2952/78/0515-1507/$02.00/0

Elevation of brain histamine levels by diaminopyrimidine inhibitors of histamine *N*-methyl transferase

(*Received* 29 *July* 1977; *accepted* 28 *September* 1977)

In the central nervous system (CNS) of mammalian species, histamine is metabolized primarily by methylation of the imidazole ring [1]. This reaction requires *S*-adenosyl methionine (SAM) as the methyl donor and is catalyzed by the enzyme histamine *N*-methyl transferase (HMT). In rats, however, tissues other than brain contain histaminase and thus have an alternative pathway for the metabolism of histamine. Among several antimalarial drugs that inhibit the methylation of histamine *in vitro*, pyrimethamine, a 2,4-diaminopyrimidine, has been reported to be a potent inhibitor of this enzyme [2]. The initial clinical studies of a pyrimethamine analog, metoprine, related to its evaluation as an anticancer agent, indicated that this compound produced CNS, cutaneous, and gastrointestinal toxicities [3–5], possibly involving inhibition of histamine catabolism. Similar CNS and cutaneous toxicities occurred during clinical studies of the anticancer agent triazinate, a diamino-*s*-triazine [6]. This report describes the effects of these anticancer agents on the activity of HMT *in vitro* and *in vivo*.

[^{14}C]methyl-SAM (sp. act. 57.8 mCi/m-mole) and [^{3}H]methyl-SAM (sp. act. 10.5 Ci/m-mole) were purchased from New England Nuclear; histamine, quinacrine and chloroquine were secured from Sigma Chemical Co., St. Louis, MO. Triazinate was obtained from the Drug Development Branch of the National Cancer Institute, Bethesda, MO. Pyrimethamine [2,4-diamino-5-(4′-chloro phenyl)-6-ethyl pyrimidine; BW 63U], metoprine [2,4-diamino-5-(3′,4′-dichlorophenyl)-6-methyl pyrimidine; DDMP; BW 197U], etoprine, the corresponding 6-ethyl analog of metoprine (DDEP; BW 276U) and triazinate [α-2-chloro 4-[4,6-diamino-2,2-dimethyl-*s*-triazine-1(2H)-yl]phenoxyl]-*N*,*N*-dimethyl-*m*-toluamide ethanesulfonic acid; TZT; NSC 139105] were dissolved in 0.01 N HCl prior to use.

HMT was partially purified from the cerebral cortex of bovine brain, using the ammonium sulfate fractionation and dialysis steps described by Brown *et al.* [7]. HMT incubation mixtures consisted of 10 μmoles of sodium phosphate buffer, pH 7.4, 15 nmoles histamine, 14.2 nmoles [^{14}C]-SAM, enzyme and, where appropriate, inhibitor, in a total volume of 300 μl. The reaction was initiated by the addition of [^{14}C]-SAM and incubated for 15 min at 37°. The reaction was terminated by the addition of 0.5

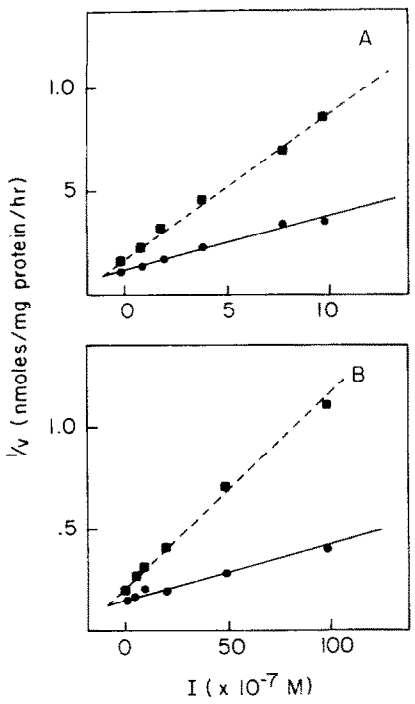

Fig. 1. Dixon plots of the effects of metoprine and triazinate on the activity of HMT. Values are the means of duplicates for each point. Panel A: metoprine (DDMP); panel B: triazinate. Key: ●——●, 5×10^{-5} M histamine; ■——■, 1×10^{-5} M histamine.

ml of 0.5 M borate buffer, pH 11, and the radioactive product was extracted with 6 ml toluene–isoamyl alcohol (1:1). Four ml of the organic extract was counted in 10 ml of an omnifluor–toluene scintillation mixture containing 2 ml of absolute ethanol. Kinetic studies were carried out at histamine concentrations ranging between 5×10^{-6} and 5×10^{-5} M, since higher concentrations of histamine have been shown to inhibit the enzyme [8]. The K_m for histamine was determined using the method of Lineweaver and Burk [9], and the various K_i values were determined by the method of Dixon [10].

To evaluate the activity *in vivo* of the diaminopyrimidines, male Sprague–Dawley rats (160–180 g) received a single oral dose of metoprine (10 mg/kg of body weight). The histamine levels in brain were determined using the enzymatic assay of Taylor and Snyder [11] as modified by Beaven *et al.* [12]. To avoid interference in the enzymatic assay, metoprine was removed from tissue extracts by chromatography on Dowex 50.

The effects of several drugs, all of which were found to be effective inhibitors of HMT isolated from bovine brain, are summarized in Table 1. Among these compounds, metoprine was the most potent inhibitor of the enzyme, producing greater than 90 per cent inhibition at a concentration of 10^{-5} M. In a series of 5-phenyl and 5-benzyl diaminopyrimidines, wide variations in potency as inhibitors of HMT were observed, and trimethoprim [13], an antibacterial agent, had relatively little activity as an inhibitor of HMT. For comparison, we include in Table 1 the effects of the antimalarial drugs chloroquine and quinacrine on the same enzyme preparation; these drugs were among the most effective inhibitors *in vitro* of HMT, as described previously [2].

The method of Dixon was used to determine the nature of the inhibition. The diaminopyrimidines and *s*-triazine were competitive inhibitors of the enzyme. This is illustrated graphically for metoprine and triazinate in Fig. 1. The kinetic data reveal that these agents are competitive with respect to histamine. Metoprine is the most potent inhibitor of the enzyme, having a K_i of 1×10^{-7} M. An important consideration concerning the likelihood of activity *in vivo* is that these compounds have K_i values at least 10-fold less than the K_m of histamine (Table 1). Studies are currently in progress to assess the effects of structurally related compounds on the metabolism of histamine and to identify the structural features necessary for these compounds to act as competitive inhibitors of HMT. In this regard, it should be noted that, in addition to diaminopyrimidines and triazines, several other classes of compounds act as inhibitors *in vitro* of HMT. We have observed that compounds containing the 4-aminoquinoline, aminoacridine, quinazoline or pyridopyrimidine nucleus are also potent inhibitors of the enzyme. Since a wide variety of compounds are capable of inhibiting this enzyme, one should be aware that unwanted side effects may be produced by some drugs via elevation *in vivo* of histamine levels.

Metoprine and etoprine are quite lipid soluble and readily enter the CNS. In rats, both drugs attained concentrations in the brain of approximately 2×10^{-5} M within 5 hr after oral administration of 10 mg/kg of body weight [14]. Studies on tissue distribution and elimination of metoprine in the rat have indicated a half-life of 17 hr in the brain [15]. In patients treated with metoprine for advanced malignancies, the plasma level was 10^{-5} to 10^{-6} M within a few hours after oral administration [5], and in some patients, headache and other CNS effects that may be histamine related persisted during the period of peak drug levels in the blood. Thus, these compounds are capable of reaching concentrations *in vivo* that result in significant inhibition of HMT activity *in vitro*.

The results illustrated in Fig. 2 show that metoprine, the most effective inhibitor *in vitro* of HMT, is capable of producing *in vivo* a marked elevation of brain histamine

Table 1. Inhibition of histamine methylation by several anticancer agents and antimalarial drugs*

	Per cent inhibition				K_i
	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M	$\times 10^{7}$ M
Pyrimethamine	8	18	69	94	9.0
Metoprine	30	69	93	98	1.0
Etoprine	14	52	84	96	7.6
Triazinate	9	46	82	97	6.0
Quinacrine	0	25	79	97	
Chloroquine	0	9	55	89	

* For comparison, the K_m for histamine was 9.5×10^{-6} M under the same experimental conditions. The concentration of histamine used was 5×10^{-5} M.

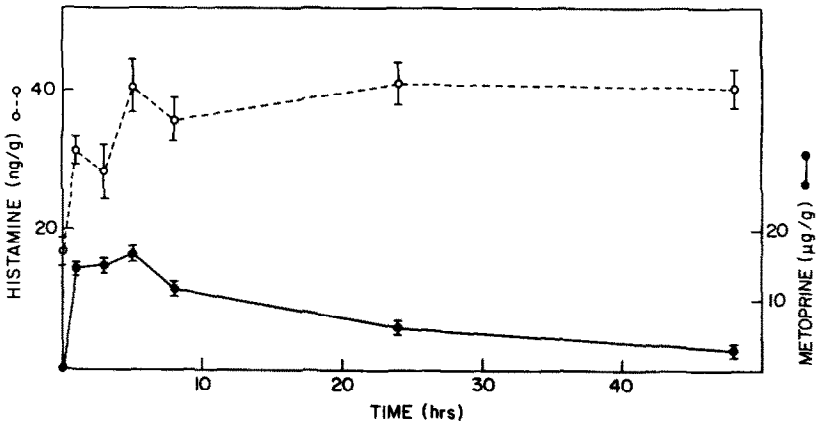

Fig. 2. Effect of metoprine on the levels of histamine in rat brain. Metoprine, 10 mg/kg, was administered orally. Each point represents the mean ± S.E.M. from five animals.

levels. Histamine levels are highest 5 hr after administration of an oral dose of metoprine and correlate with peak drug levels in the brain. Histamine levels are still elevated more than 2-fold 48 hr after administration of the drug. Metoprine levels at this time are approximately 3 μg/ml. Assuming a uniform distribution of the drug in brain, this corresponds to a concentration of 1×10^{-5} M, sufficient to produce greater than 90 per cent inhibition *in vitro* of the enzyme. This is in contrast to the effects observed with other inhibitors of HMT. Chlorpromazine and quinacrine, drugs which inhibit HMT *in vitro*, have little or no effect on the levels *in vivo* of histamine[16]. Metoprine had no effect on histamine levels in rat stomach and heart, two tissues which have been shown to lack HMT[7]. Metoprine also had no effect on the activity *in vivo* or *in vitro* of histidine decarboxylase. Thus, these diaminopyrimidines provide a means of altering histamine levels in brain and may be tools for exploring the role of histamine in CNS metabolism and neurotransmission. In addition, a biochemical test can now be applied to identify as potential chemotherapeutic agents those inhibitors of dihydrofolate reductase which lack any effect on histamine metabolism, thereby avoiding this undesirable side effect.

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