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PRELIMINARY COMMUNICATIONS

INHIBITION OF HISTAMINE-N-METHYLTRANSFERASE AND HISTAMINASE (DIAMINE OXIDASE) BY A NEW HISTAMINE H_2-RECEPTOR AGONIST, DIMAPRIT

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Dimaprit, S-[3-(N,N-dimethylamino)propyl]isothiourea, was introduced recently as a selective agonist for histamine H_2 receptors (1). The compound was reported to possess less than 0.001% of the activity of histamine on H_1 receptors (guinea pig ileum) and to be highly active toward tissues with H_2 receptors (rat uterus, guinea pig right atrium and gastric mucosa). Dimaprit stimulated gastric secretion in several species and in cat was 4 to 5 times more potent than histamine in stimulating secretion (1). Since the enzyme, histamine-N-methyltransferase (EC 2.1.1.8) (HNMT), is inhibited *competitively* by antagonists of both H_1 (2) and H_2 receptors (3), we have investigated the possible effect of Dimaprit on HNMT and on other enzymes involved in histamine metabolism. In this communication, we report that Dimaprit inhibits *noncompetitively* HNMT and, in higher concentrations, diamine oxidase (DAO) (EC 1.4.3.6). The possibility that Dimaprit acts on a second inhibitory site on these enzymes is discussed.

Materials and Methods

The sources of enzymes were as follows. For histidine decarboxylase, a soluble extract of rat gastric mucosa was used. A rat was given pentagastrin, 1 mg/kg s.c., to induce histidine decarboxylase activity (4). Two hours later, the glandular portion of the stomach was removed, washed carefully to remove bacteria (5), homogenized in 9 vol of 0.1 M, pH 6.8, sodium phosphate buffer. The homogenate was centrifuged at 18,000 g to remove particulate matter. A soluble extract was prepared likewise from rat ileum as the source of DAO (6). A partially purified preparation of HNMT was prepared from frozen guinea pig brain as described elsewhere (7). The concentration of these preparations in the final incubation mixtures was 1 mg stomach/20 µl, 1 mg intestine/200 µl and 50 µg protein/40 µl respectively.

Dimaprit was a gift from Dr. W. A. M. Duncan, Smith Kline and French Laboratories,

Welwyn Garden City, England. The dihydrochloride salts of histamine and 1-methyl-4-(β-aminoethyl)imidazole (1-methylhistamine) were purchased from Sigma Chemical Co., St. Louis, Mo. and Calbiochem, San Diego, Ca., respectively. Radiochemicals were obtained from Amersham Searle Corp., Arlington Heights, Ill., and New England Nuclear Corp., Boston, Mass. Side chain labeled β-^{3}H-histamine was prepared from side chain labeled β-^{3}H-L-histidine as previously described (9).

Histidine decarboxylase activity was assayed by measurement of the $^{14}CO_2$ release from L-histidine-(^{14}C-carboxyl carbon)*, DAO activity by measurement of tritium release from β-^{3}H-histamine (8) and HNMT by the formation of ^{14}C-methylhistamine from histamine in the presence of S-adenosyl-L-methionine (^{14}C-methyl) (9): unless otherwise stated, the final concentration of substrate in these assays was L-histidine 2.5×10^{-4}, histamine 7.5×10^{-8}, and histamine 5×10^{-7} M respectively. The concentration of substrate was varied by the addition of unlabeled substrate. All values reported are the average of duplicate experiments.

Results and Comments

In our initial survey, Dimaprit was found to inhibit HNMT and, in higher concentrations, DAO. The compound had no effect on histidine decarboxylase activity (Table 1). The inhibition of both HNMT and DAO was noncompetitive (Fig. 1A and B). In these and 2 other experiments, the apparent K_i values as determined by the Dixon plot (10) ranged from $7\text{-}9 \times 10^{-6}$ M for HNMT and $2.5\text{-}3.0 \times 10^{-4}$ M for DAO. In comparison with other published K_i values, Dimaprit was more effective an inhibitor of HNMT than the majority of the histamine H_1 antagonists tested by Taylor and Snyder (2), more effective than the H_2 antagonist burimamide (K_i 2.8×10^{-4} M (3) but less effective than the antimalarial drug Quinacrine (K_i 1×10^{-7}) (2).

Table 1. Percent inhibition of histamine-metabolizing enzymes by Dimaprit in vitro

Dimaprit conc. (M)	Histidine decarboxylase (rat gastric mucosa)	Diamine oxidase (rat ileum)	Histamine-N-methyltransferase (guinea pig brain)
10^{-6}	0	1	7
10^{-5}	0	3	56
10^{-4}	0	14	89
10^{-3}	2	70	90
2.5×10^{-3}	1	81	95

Substrate concentrations are given in the text.

HNMT is also inhibited by the reaction product 1-methylhistamine (11). In our hands, the inhibition by methylhistamine (apparent K_i $2\text{-}4 \times 10^{-4}$ M) appeared to be competitive (Fig. 1C) but at higher substrate concentrations (5×10^{-6} M) noncompetitive. At high substrate

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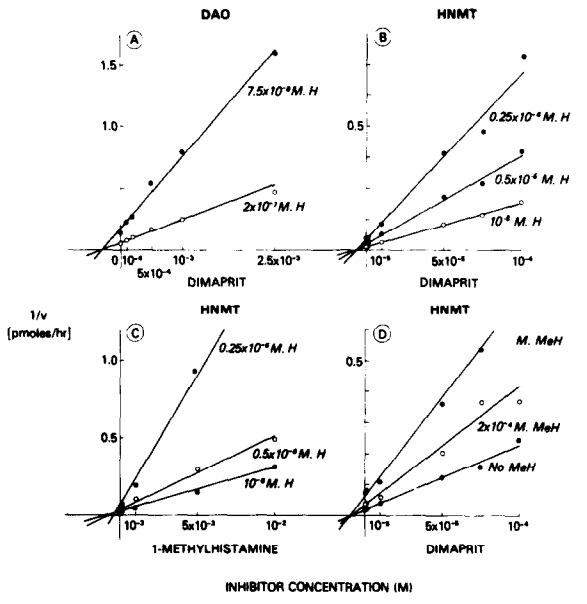

Fig. 1. Inhibition of DAO by Dimaprit (A), and HNMT by Dimaprit (B), 1-methylhistamine (C) and by both inhibitors (D), as plotted according to the method of Dixon (10). The molar concentration of histamine (H) and methylhistamine (MeH) was as indicated and that of histamine in D was 0.5×10^{-6} M. The inhibition of DAO and HNMT by Dimaprit was noncompetitive (A and B) and the inhibition of HNMT by MeH competitive (see text) (C). The K_i for HNMT by Dimaprit was unaltered by MeH (D).

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concentrations, the inhibition of HNMT by Diamprit and methylhistamine was enhanced. When Dimaprit and 1-methylhistamine were tested in combination, the data indicated that the K_i value for Dimaprit was unaltered by the presence of 1-methylhistamine (Fig. 1D) and vice versa. Because of the crude nature of the HNMT preparation, more detailed analysis of the enzyme kinetics was not undertaken.

Discussion

The present data indicate that Dimaprit is a weak inhibitor of DAO and a stronger inhibitor of HNMT. Since methylation is a major route of inactivation in most species and is the only route of inactivation in stomach of some species (12), there is a question as to the possible contribution of HNMT inhibition to the pharmacological activity of this drug. No information is available as to the metabolism or distribution of the drug in the body. It is a longer acting drug than histamine (W. A. M. Duncan, personal communication) and is presumably metabolized and excreted less rapidly than histamine. A maximum rate of gastric secre-

tion was reported to be elicited when Dimaprit was infused at the rate of 1.25×10^{-6} moles/kg/min in rat, 3×10^{-5} moles/kg/hr in dog and 4×10^{-8} moles/kg/min in cat (1). It is conceivable that in rat the levels of Dimaprit may become sufficient to inhibit, at least partially, HNMT in stomach. It is clear, however, that gastric secretion is elicited in cat with levels of Dimaprit well below those required to inhibit HNMT and that the drug acts directly on H_2 receptors in the gastric mucosa. This is apparent from the fact that the action of Dimaprit is blocked competitively by the H_2 antagonists (1).

The noncompetitive inhibition of HNMT and DAO by Dimaprit could indicate that the drug acts at a site other than the active site on these enzymes. HNMT (2) and DAO (6,13), are inhibited by high concentrations of substrate. In the case of DAO, it has been proposed that histamine acts on a second inhibitory site which has an affinity for "delocalized π electrons" of the imidazole ring (14). Inhibition of the catalytic activity is readily studied with DAO, because other substrates of this enzyme, e.g. putrescine, are not inhibitory (6). Such studies are difficult with HNMT as histamine is the sole substrate and the enzyme has yet to be purified to a high degree. When purer preparations of HNMT become available, Dimaprit and related substances (15) may be useful compounds to test for the presence and characteristics of inhibitory site(s).

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