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IMPROMIDINE, A POTENT INHIBITOR OF HISTAMINE METHYLTRANSFERASE (HMT) AND DIAMINE OXIDASE (DAO)

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Impromidine is a specific histamine H_2 receptor agonist and is reported to be 9-100 times more potent than histamine in several test preparations [1-3]. Because its structure (I) (Fig. 1) includes imidazole and guanidine groups and the fact that HMT is inhibited by a wide variety of histamine receptor agonists and antagonists [4-9,11], we have tested its ability to inhibit HMT (EC 2.1.1.8), DAO (EC 1.4.3.6) and histidine decarboxylase (EC 4.1.1.22) (HdD) and compared its actions to those of SKF 91488, aminoguanidine (II) (inhibitors of HMT [4,10] and DAO [11], respectively) and the recently developed H_2-receptor antagonist ICI 125,211 (Tiotidine) (III) [12].

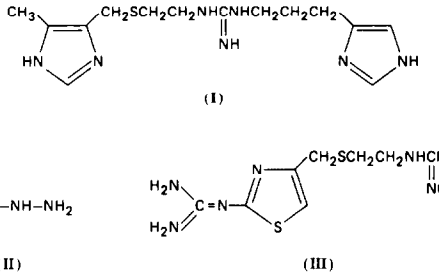

Fig. 1

METHODS

Enzyme preparations were from the following sources. HdD and DAO activity were soluble extracts of gastric mucosa and ileum [10]. HMT activity was obtained from rat kidney and was partially purified by ammonium sulfate fractionation [13] and further purified by the procedures described by Sellinger and associates [14].

Histidine decarboxylase activity was assayed by measurement of $^{14}CO_2$ release from L-histidine-1-[carboxyl-^{14}C] [15], DAO activity by the measurement of tritium release from [β-^{3}H]-histamine [16] and HMT activity by measurement of the rate of conversion of histamine to [^{14}C]-methylhistamine in the presence of S-adenosyl-L-methione [^{14}C-methyl] [17]. Concentrations of labeled substrates were L-histidine, 2.5×10^{-4} M; histamine, 10^{-7} M (for DAO) and 5×10^{-7} M (for HMT); S-adenosylmethionine, 5×10^{-6} M, and were diluted with unlabeled substrates for kinetic studies. Reagents were from sources described previously [17]. All values were the mean of duplicate determinations and graphs were plotted by the method of least squares by computer analysis.

RESULTS

The effects of the drugs are summarized in Table 1. Impromidine and SKF 91488 inhibited both HMT and DAO; aminoguanidine inhibited DAO and, at very high concentrations, HMT; and Tiotidine was a weak inhibitor of both enzymes. With Impromidine, the inhibition

Table 1. Per Cent Inhibition of histidine decarboxylase (HdD), histamine methyltransferase (HMT) and diamine oxidase (DAO)*

Drug concentration (M)	Impromidine			SKF 91488			Aminoguanidine			Tiotidine		
	HdD	HMT	DAO	HdD	HMT	DAO	HdD	HMT	DAO	HdD	HMT	DAO
5×10^{-9}	--	--	43	--	--	--	--	--	9	--	--	--
10^{-8}	--	--	52	--	--	--	--	--	17	--	--	--
2.5×10^{-8}	--	--	78	--	--	--	--	--	30	--	--	--
5×10^{-8}	--	0	87	--	--	--	--	--	49	--	--	--
10^{-7}	1	19	93	--	9	--	--	--	67	--	--	--
5×10^{-7}	--	21	--	--	31	--	--	--	--	--	--	--
10^{-6}	0	46	--	--	49	3	--	--	--	--	0	8
2.5×10^{-6}	--	80	--	--	--	--	--	--	--	--	--	--
5×10^{-6}	--	90	--	--	82	10	--	--	--	--	--	--
10^{-5}	3	--	--	--	90	22	--	7	--	--	12	39
10^{-4}	13	99	--	0	99	67	--	4	--	--	56	89
10^{-3}	--	--	--	12	--	95	13	38	--	--	93	100

*Substrate concentrations: histidine, 2.5×10^{-4} M; histamine, 5×10^{-7} M. Values for inhibition of DAO by SKF 91488 were reported previously [5].

of HMT (K_i, 5×10^{-7} M) and DAO (K_i, 7×10^{-9} M) was competitive, whereas the inhibition of DAO (K_i, 5×10^{-8} M) by aminoguanidine was noncompetitive (Fig. 2). The inhibition of Impromidine was reversible; dilution of mixtures containing enzyme and partially inhibitory concentrations of Impromidine restored activity to values that were predicted from the kinetic curves. The inhibition of HMT by SKF 91488, as reported previously [4], appeared to be noncompetitive in the presence of low concentrations of histamine ($< 5 \times 10^{-7}$ M). With higher substrate concentrations ($> 10^{-6}$ M), this did not appear to be the case (Fig. 2), but inhibition of HMT was also apparent with these concentrations of histamine.

DISCUSSION

These results indicate that Impromidine is a remarkably potent inhibitor of both HMT and DAO and is 10 times more potent an inhibitor of DAO than aminoguanidine. The drug is less potent an inhibitor of HMT than amodiaquin (K_i, 10^{-8} M) [11] but as potent as d-chlorpheniramine (K_i, 7×10^{-7} M), and more potent than SKF 91488 (K_i, 10^{-6} M) or the H_2-receptor agonist, Dimaprit (K_i, 8×10^{-6} M) [4]. Aminoguanidine and SKF 91488 remain useful experimental drugs as neither drug has intrinsic pharmacological activity when administered in large doses (see, for example, ref. 10). The inhibitory activity of Impromidine may be significant after therapeutic doses of drug in that in concentrations that produce 50 per cent of maximal stimulation of H_2 receptors (ED_{50} for guinea pig atrium, rat uterus and rat gastric secretion were 2.5×10^{-8} M, 1.8×10^{-7} M and 3×10^{-7} M, respectively) [2,3] histamine-inactivating enzymes would be partially or totally inhibited. Since the actions of exogenous [18,19] and endogenous histamine (pentagastrin-induced gastric secretion) [20] are markedly potentiated after the inhibition of these enzymes, blockade of histamine metabolism should be considered as part of the spectrum of activity of Impromidine.

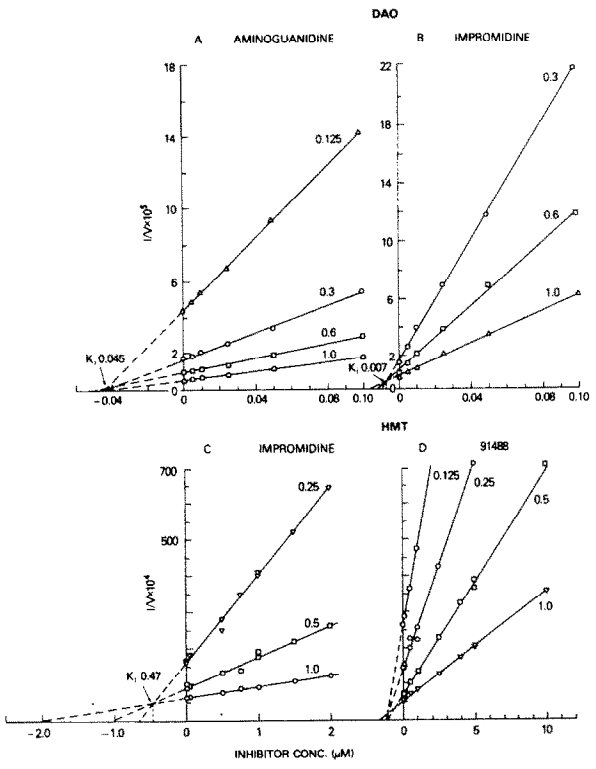

Fig. 2. Inhibition of DAO by aminoguanidine (A) and Impromidine (B) and HMT by Impromidine (C) and SKF 91488 (D) in the presence of different micromolar concentrations of histamine as indicated. The inhibition of DAO by aminoguanidine and Impromidine appeared to be non-competitive and competitive, respectively, and HMT by Impromidine competitive. Substrate inhibition (see text), however, may complicate the picture.

The presence of inhibitory sites with an affinity for groups, such as imidazole, thiourea, or guanidine, has been postulated for HMT and DAO [4] to account for substrate inhibition, reversal of this inhibition by H_1 receptor antagonists and other methylated amines [4,14], and noncompetitive inhibition of the enzymes by some inhibitors. Kinetic studies of HMT are complex, however, because the enzyme is subject to both substrate and product inhibition and there is uncertainty as to its mechanism of action (see 4 and references cited therein). Dr. I. Smith (of Smith Kline and French Research Laboratories, England) has reported to us that, at higher concentrations of histamine than were used in our earlier studies [4], inhibition of HMT by SKF 91488 was competitive. In the present studies the characteristics of inhibition by 91488 appear to alter in the presence of inhibitory concentrations of histamine. These questions will require re-examination once HMT (or its isoenzymes) has been purified and better characterized. The properties of Impromidine may prove useful in the purification of the enzyme. Our preliminary data indicate that gels prepared from Impromidine and Sepharose have a high affinity for HMT activity in the crude [13] and partially purified [14] preparations of the enzyme.

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