Inhibitors of Hydroxyindole-*O*-methyltransferase: Indolealkylpiperazines

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Abstract □ Two antipsychotic agents, oxypertine and haloperidol, were both found to be *in vitro* inhibitors of bovine pineal hydroxyindole-*O*-methyltransferase. A series of indolealkylpiperazines structurally related to oxypertine was evaluated for inhibitory activity with the enzyme. The most potent inhibitor of this study, 1-(2-chlorophenyl)-4-[2-(5,6-dimethoxy-3-indolyl)ethyl]piperazine, exhibited a mixed-type inhibition. The possible mode of binding of these inhibitors to the enzyme was discussed.

Keyphrases □ Hydroxyindole-*O*-methyltransferase—indolealkylpiperazines as inhibitors, *in vitro* □ Indolealkylpiperazines—tested *in vitro* as inhibitors of hydroxyindole-*O*-methyltransferase, mode of binding □ Haloperidol—*in vitro* inhibition of hydroxyindole-*O*-methyltransferase, mode of binding □ Oxypertine—*in vitro* inhibition of hydroxyindole-*O*-methyltransferase, mode of binding

Oxypertine, 1-[2-(5,6-dimethoxy-2-methyl-3-indolyl)-ethyl]-4-phenylpiperazine (I), a compound that has been reported to be effective in the treatment of schizophrenia (1–4), was found in our laboratories to be a potent *in vitro* inhibitor of hydroxyindole-*O*-methyltransferase. A widely accepted hypothesis proposes the formation of psychotomimetic compounds from endogenous biogenic amines, such as serotonin (5-hydroxytryptamine), as an etiological factor in schizophrenia. The formation of a compound such as *O*,*N*,*N*-trimethylserotonin (*O*-methylbufotenine) in the body can be prevented by an inhibitor of hydroxyindole-*O*-methyltransferase, and this might explain the mechanism of action of antipsychotic agents such as oxypertine. In the present study, a series of indolealkylpiperazines structurally related to oxypertine was evaluated for their inhibitory activities with hydroxyindole-*O*-methyltransferase.

EXPERIMENTAL[1]

Compounds—The compounds (Table I) were dissolved in glacial acetic acid and then diluted with water to a concentration of 0.2% acetic acid.

Assay—Hydroxyindole-*O*-methyltransferase was isolated from beef pineal gland and purified according to the method of Axelrod and Weissbach (5). Incubation was carried out with *N*-acetylserotonin and *S*-adenosyl-L-methionine-methyl-^{14}C according to the procedure previously described (6).

RESULTS AND DISCUSSION

The inhibitory activity of oxypertine (I) increased with the substitution of H for CH_3 in the 2-position. The 1.5-fold increase in activity of VIII over I could indicate a lack of tolerance of the enzyme for the 2-CH_3 group. The undesirable effect of the methyl group at the 2-position in I could be overcome with a 2-ethyl group; VII was slightly more active than VIII and was twice as active as I. The extended carbon chain might have resulted in an involvement of the terminal CH_3 of CH_2CH_3 group to a hydrophobic region of the enzyme.

The inhibitory activity of I did not increase to a large extent with the substitution of a fluorine atom on the *para*-position of the phenyl ring to give II. The introduction of a hydrophobic group or atom such as CH_3 ($\pi = +0.56$) or Cl ($\pi = +0.71$) (7), however, increased the inhibition of the enzyme. Thus, compounds with *o*-CH_3 (IV) and *m*-CH_3 (VI) groups were both better inhibitors (1.5-fold) than I. Similarly, the *p*-Cl of III doubled the inhibitory activity. No increase in inhibition was observed with substitution of an *o*-OCH_3 group in V; this could be attributed to the hydrophilic nature of the OCH_3 group ($\pi = -0.02$), which differs from the other two hydrophobic CH_3 and Cl groups.

In the 2-H series, no increase in activity of VIII was observed by the *p*-CH_3 group of IX; the substitution of an *o*-Cl group in X, however, increased inhibition by more than fivefold.

Extension of the side chain of VIII by inserting CH_2 between the piperazinyl and phenyl groups resulted in about a 1.5-fold loss of activity, indicating the decrease in the tolerance of the enzyme for the $CH_2C_6H_5$ group of XI.

There was a requirement of at least two methylene (CH_2) groups to bridge the indole nucleus with the piperazinyl moiety of VIII, since shortening of the chain to one CH_2, as in XII, reduced the activity 22-fold.

The binding of the two piperazinyl nitrogen atoms remained to be evaluated. In compounds of the present study, the two nitrogens, both being tertiary amines and one of the two also an anilino nitrogen, are in fact weakly basic. No correlation can be found between the inhibitory activities and their pKa values (Table II).

Haloperidol, another antipsychotic drug, was also found to inhibit hydroxyindole-*O*-methyltransferase *in vitro* (Table I); its activity was about one-sixth of the oxypertine (I).

Kinetic studies were carried out with the four indolealkylpiperazines (I, VIII–X) having low I_{50} values. Lineweaver-Burk

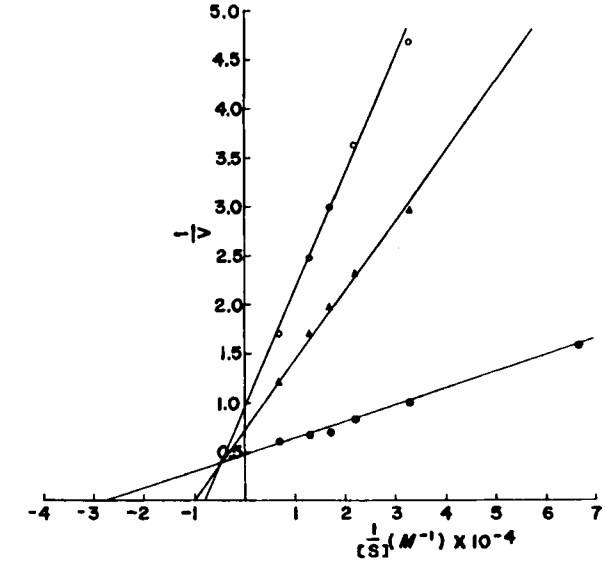
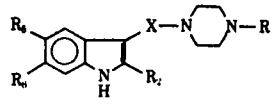


Figure 1—*Lineweaver-Burk plot of inhibition of bovine pineal hydroxyindole-O-methyltransferase by X. Key:* ●, *no inhibitor (0.2% acetic acid);* △, 5×10^{-6} M; *and* ○, 9×10^{-6} M.

[1] All indolealkylpiperazines (Table I) were supplied by Sterling-Winthrop Research Institute, Rensselaer, N. Y., and haloperidol (Haldol) was supplied by McNeil Laboratories, Inc., Fort Washington, Pa.

Table I—Inhibition of Hydroxyindole-*O*-methyltransferase by Indolealkylpiperazines



Compound	R_5	R_6	R_2	X	R	I_{50}[a], m*M*
I[b]	OCH_3	OCH_3	CH_3	CH_2CH_2	C_6H_5	0.078
II[b]	OCH_3	OCH_3	CH_3	CH_2CH_2	C_6H_4F-*p*	0.069
III	OCH_3	OCH_3	CH_3	CH_2CH_2	C_6H_4Cl-*p*	0.038
IV[b]	OCH_3	OCH_3	CH_3	CH_2CH_2	$C_6H_4CH_3$-*o*	0.045
V[c]	OCH_3	OCH_3	CH_3	CH_2CH_2	$C_6H_4OCH_3$-*o*	0.084
VI[b]	OCH_3	OCH_3	CH_3	CH_2CH_2	$C_6H_4CH_3$-*m*	0.047
VII[b]	OCH_3	OCH_3	C_2H_5	CH_2CH_2	C_6H_5	0.039
VIII	OCH_3	OCH_3	H	CH_2CH_2	C_6H_5	0.049
IX	OCH_3	OCH_3	H	CH_2CH_2	$C_6H_4CH_3$-*p*	0.047
X	OCH_3	OCH_3	H	CH_2CH_2	C_6H_4Cl-*o*	0.0089
XI	OCH_3	OCH_3	H	CH_2CH_2	$CH_2C_6H_5$	0.072
XII	OCH_3	OCH_3	H	CH_2	C_6H_5	1.09
XIII	H	H	H	CH_2CH_2	C_6H_5	>0.3
Haloperidol						0.46

[a] Concentration of an inhibitor giving 50% inhibition of the enzyme. [b] Hydrochloride salt. [c] Tartrate.

Table II—K_i and pKa values for Indolealkylpiperazines

Compound	K_i, μM[a] Competitive	K_i, μM[a] Noncompetitive	pKa[b]
I	13.2	61.4	6.6
VIII	8.1	53.6	6.8
IX	8.7	53.2	6.9
X	1.5	8.5	7.0
XII	—[c]	—[c]	7.1

[a] Substrate: *N*-acetylserotonin; $K_m = 3.75 \times 10^{-5}$ *M*. [b] A sample of 0.01 mmole of each compound was dissolved in 15 ml. ethanol and made up to 25 ml. with water. To the solution was added 1 ml. of 0.025 *N* HCl, and it was back-titrated with 0.0227 *N* NaOH. The pKa values were calculated by the method of Albert and Serjeant (9). [c] Not determined.

plots, using the method of least squares, showed that all four compounds exhibited a mixed-type inhibition with the bovine pineal hydroxyindole-*O*-methyltransferase. The plot of the best inhibitor, X, is illustrated in Fig. 1. By using the method of Krupka (8), the K_i values were then calculated (Table II). The inhibition of *O*-methylation of *N*-acetylserotonin by these compounds was more competitive than noncompetitive in nature. The K_i values of all compounds, especially the competitive component, are the same order of magnitude as the I_{50} values of the corresponding compounds listed in Table I.

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