Biochemical Pharmacology, Vol. 26, pp. 1092–1094. Pergamon Press, 1977. Printed in Great Britain.

Inhibition of dopamine β-hydroxylase by anti-thyroid agents, methimazole and propylthiouracil

(*Received* 2 *September* 1975; *accepted* 7 *December* 1976)

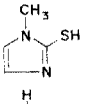
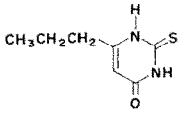
1-Methyl-2-mercaptoimidazole (methimazole) and 6-propyl-2-thiouracil (propylthiouracil) are potent inhibitors of thyroid iodide peroxidase which catalyzes the initial step of thyroid hormone biosynthesis [1, 2]. On the other hand, various sulfhydryl compounds such as mercaptoethanol, cysteine, glutathione and coenzyme A are known to be inhibitors of dopamine β-hydroxylase [3,4-dihydroxyphenylethylamine, ascorbate:oxygen oxidoreductase (hydroxylating), EC 1.14.2.1] (DBH) which catalyzes the biosynthesis of norepinephrine (NE) from dopamine (DA) [3]. Since both methimazole and propylthiouracil are thought to be sulfhydryl compounds, studies on the effects of these two drugs and their analogues on DBH have been carried out. This communication described an inhibitory mechanism of methimazole, propylthiouracil and their analogues on DBH *in vitro*.

N-ethylmaleimide and catalase were obtained from Sigma Co. Methimazole, 1-methylimidazole, 2-mercaptoimidazole, propyl-thiouracil and 2-thiouracil were gifts from Chugai Pharmaceutical Co., Tokyo, Japan. All other chemicals were reagent grade or commercially available.

Dopamine β-hydroxylase of bovine adrenal gland was highly purified according to the method of Friedman and Kaufman [4]. A spectrophotometric assay using tyramine as the substrate was employed to determine enzyme activity [5]. The incubation mixture (1 ml) contained the following components: potassium phosphate buffer (pH 5.5), 100 mM; ascorbic acid, 10 mM; fumaric acid, 12 mM; tyramine hydrochloride, 10 mM; enough crystalline catalase to give maximal stimulation of the reaction rate; and 20–40 μg of the purified enzyme. Concentrations of inhibitors were decreased over the range of 0.5 to 10 mM until the inhibition fell below 50 per cent. The inhibitor concentration producing 45–55 per cent inhibition (I_{50}) was determined graphically [6].

Purified bovine adrenal DBH was inhibited by methimazole and 2-mercaptoimidazole but not by 1-methylimidazole and imidazole. The inhibitory effects of these drugs are summarized in Table 1. DBH was also inhibited by propylthiouracil and 2-thiouracil (Table 1). Methimazole, 2-mercaptoimidazole, propylthiouracil and 2-thiouracil, which contain sulfhydryl or thiol residues in their molecule, inhibited DBH, but 1-methylimidazole and imidazole, which do not contain sulfhydryl residues, failed to inhibit DBH up to a concentration of 50 mM. It has been reported that various sulfhydryl compounds such as cysteine, gluthathione and coenzyme A inhibit purified DBH from bovine adrenal gland [3]. It is likely that sulfhydryl residues or thiol residues in these compounds are essential for inhibition of DBH. Unlike the other sulfhydryl compounds reported previously [3], the inhibition of DBH by methimazole and propylthiouracil was not reversed by the addition of *N*-ethylmaleimide, but the inhibition by 2-mercaptoimidazole and 2-thiouracil was partially reversed by

Table 1. Effect of various compounds on purified bovine adrenal DBH

Compounds		I_{50}* (mM)
	Methimazole	3.7
	2-Mercaptoimidazole	1.4
	1-Methylimidazole	No effect†
	Imidazole	No effect†
	Propylthiouracil	1.5
	2-Thiouracil	4.6

* Concentration of drug producing 50 per cent of DBH activity.
† Fifty mM of the compounds has no effect on DBH activity.

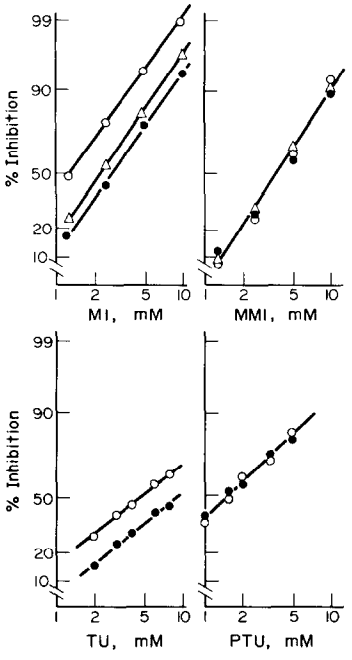


Fig. 1. Effect of methimazole (MMI), 2-mercaptoimidazole (MI), 2-thiouracil (TU) and propylthiouracil (PTU) on bovine adrenal DBH in the presence and absence of *N*-ethylmaleimide. Key: (○—○) in the absence of *N*-ethylmaleimide; (△—△) in the presence of 5 mM *N*-ethylmaleimide; and (●—●) in the presence of 10 mM *N*-ethylmaleimide.

10 mM of *N*-ethylmaleimide (Fig. 1). This would suggest that methimazole and propylthiouracil are slightly different from 2-mercaptoimidazole and 2-thiouracil as DBH inhibitors. Dixon plots show that methimazole and propylthiouracil inhibit DBH differently than do other sulfhydryl compounds (Fig. 2). This indicates that a methyl or propyl residue in methimazole or propylthiouracil would affect the chelate interaction between the sulfhydryl or thiol residue of each compound and the copper atom at the active site of the enzyme. Differences in the tautomeric frequencies among these compounds might explain the effect observed because a 1-methyl residue in the methimazole molecule and a 6-propyl residue in the propylthiouracil molecule affect the tautomeric frequencies of these compounds. When methimazole or propylthiouracil was added with the substrate and ascorbic acid (cofactor or DBH) or incubated with enzyme before adding the substrate and ascorbic acid, propylthiouracil was noncompetitive for both the substrate and ascorbic acid but methimazole was competitive for the substrate and exhibited an unknown type of inhibition for ascorbic acid. These results might suggest that inhibition of DBH by methimazole and propylthiouracil is not due to simple chelation between these compounds and copper in the enzyme. Incubation of inhibitor with enzyme before the addition of the substrate did not influence the inhibitory activity or the mode of inhibition. Both compounds were noncompetitive for fumaric acid.

Methimazole and propylthiouracil are often used in the therapy of hyperthyroidism. These drugs are thought to exhibit clinical effectiveness through suppressing thyroid hormone biosynthesis by inhibiting thyroid iodide peroxidase [1, 2]. These drugs inhibit thyroid iodide peroxidase at approximately 1×10^{-6} M *in vitro* [2] but they inhibit DBH at 10^{-3} M *in vitro*, as demonstrated in this communication. It is unlikely that methimazole or propylthiouracil alleviates hyperthyroidism by inhibiting DBH.

Acknowledgements— The authors are grateful to Dr. M. Yano, Institute for Medical Research, Banyu Pharmaceutical Co., Tokyo, Japan, for his excellent technical assistance.

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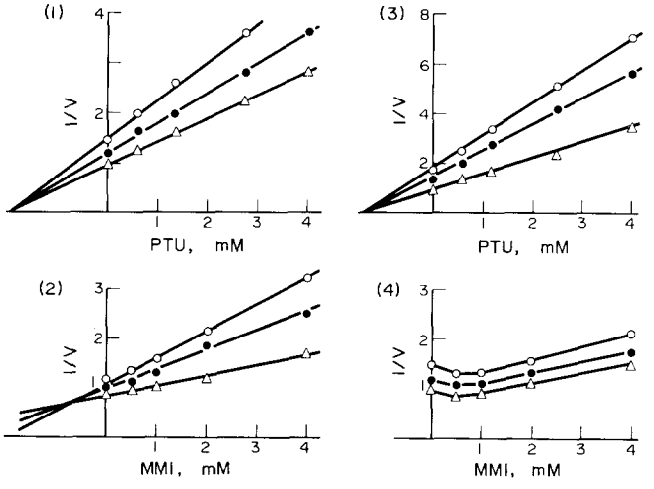

Fig. 2. Dixon plot for determining the apparent K_i of methimazole (MMI) and propylthiouracil (PTU) as inhibitors of purified bovine adrenal DBH. Panels 1 and 2: kinetics for tyramine. Key (○—○) 1.25 mM; (●—●) 2.5 mM; and (△—△) 5 mM. Panels 3 and 4: kinetics for ascorbic acid. Key: (○—○) 1.25 mM; (●—●) 2.5 mM; and (△—△) 5 mM.