Biochemical Pharmacology, Vol. 20, pp. 183–191. Pergamon Press. 1971. Printed in Great Britain

4,4,6-TRIMETHYL-3,4-DIHYDROPYRIMIDINE-2-THIOL, AN EFFECTIVE INHIBITOR OF DOPAMINE-β-HYDROXYLATION *IN VIVO*

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(*Received* 13 *February* 1970; *accepted* 1 *May* 1970)

Abstract—4,4,6-Trimethyl-3,4-dihydropyrimidine-2-thiol, although considerably less potent (1/500) than disulfiram as an inhibitor of dopamine-β-hydroxylation *in vitro*, is 10–20 times as potent as disulfiram, both administered intraperitoneally, *in vivo*. The pyrimidinethiol decreases the concentration of norepinephrine in the brain (mice and rats) but does not decrease the concentration of dopamine in that organ; it inhibits the depleting action of methyldopa and α-methyl-*m*-tyrosine on brain and heart norepinephrine, and of α-methyl-*m*-tyramine and α-methyl-dopamine on heart norepinephrine, and its effects upon tissue catecholamines are additive to those of DOPA and of a monoamine oxidase inhibitor; it inhibits restoration of the tyramine-pressor response in reserpine-treated rats by α-methyldopamine. The pyrimidinethiol is perhaps twice as potent as disulfiram as an inhibitor of alcohol metabolism in the rat, and in this function it, like disulfiram, acts at the aldehyde dehydrogenase step of oxidation. The pyrimidinethiol, like other thiourea derivatives, blocks the uptake of iodine by the rat thyroid gland *in vivo*. In this function it is about 1/150 as potent as propylthiouracil and six times as potent as disulfiram. The pyrimidinethiol is well absorbed after oral administration; for this reason, and because of its potency advantage over disulfiram, the compound should be superior to dilsulfiram in a number of situations.

A NUMBER of compounds are known to inhibit the enzyme, dopamine β-hydroxylase. Included among the inhibitors are chelating agents,[1–3] sulfhydryl compounds,[4] and benzyloxyamine and its derivatives;[5] however, the compound most widely used for studies *in vivo* is tetraethylthiuramdisulfide (disulfiram) which, although not a chelating agent itself, is reduced *in vitro* and *in vivo* to the compound, diethyldithiocarbamic acid,[6] a strong copper chelator.[7]† The sodium salt of the carbamic acid has been used *in vivo* as a β-hydroxylase inhibitor,[11] and a related substance, sodium phenylethyldithiocarbamate, has been proposed for the same purpose.[12] A derivative of disulfiram[13] and other *N,N*-disubstituted dithiocarbamic acids[14] are more potent than the parent compounds. Certain aromatic and aliphatic thiourea derivatives have been shown to inhibit dopamine β-hydroxylase *in vitro* and to lower the concentration of norepinephrine in the brains of rats and mice *in vivo*.[15]

In the course of examining compounds to find a novel or more potent inhibitor of dopamine β-hydroxylase *in vivo*, we observed also that a number of thioureas, and indeed some thioamides, were effective in lowering norepinephrine concentrations in

* With the technical assistance of James A. Totaro, David C. Titus and Elizabeth R. Wynosky.

† For discussions of dopamine β-hydroxylase and its inhibition by disulfiram, recent reviews [8–10] may be consulted.

the mouse brain, while either not affecting or in fact elevating brain dopamine concentrations. The most potent compound encountered was 4,4,6-trimethyl-3,4-dihydropyrimidine-2-thiol;* the properties of this substance as a dopamine β-hydroxylase inhibitor are described in this communication. Certain other activities of the pyrimidinethiol, properties which it shares with other thiourea derivatives and with disulfiram, are reported also.

METHODS

Norepinephrine and dopamine concentrations in tissues were determined by a variation of the trihydroxyindole method,[16] described previously.[17] Female mice of the Carworth CF1 strain were used, except where specified otherwise.† Compounds were administered as solutions or micronized suspensions in 5 per cent carboxymethylcellulose, and doses are stated as milligrams base weight per kilogram of body weight.

Studies *in vitro* were with beef adrenal medulla enzyme, carried through ammonium sulfate fractionation, and with the cofactors specified by Levin *et al.*[18]

For tyramine response restoration studies, male Charles River rats (300–450 g) were injected i.p. with reserpine, 5 mg/kg. Eighteen to 24 hr later, the rats were anesthetized (vinbarbital, 70–80 mg/kg, i.p.) and tracheotomies were performed. Body temperatures were maintained at 37° (rectal) by means of a heat lamp and blood pressures were taken from a carotid artery with a Sanborn transducer. Intravenous injections were via a jugular vein cannula and were flushed in with saline. The pyrimidinethiol and disulfiram were injected intraperitoneally 2 hr before the restoring agents, α-methyldopamine (30 μg/kg, i.v.) or α-methylnorepinephrine (3 μg/kg, i.v.). Injections of 100 μg tyramine were made at 10-min intervals, before and after administration of the restoring agents.

Female albino rats (about 150 g) were used for alcohol metabolism studies. They were given single i.p. doses of disulfiram or the pyrimidinethiol, and 30 min later, an oral dose of 1-^{14}C-ethanol, 4 ml/kg of a 20 per cent aqueous solution. Each rat received about 4 μc ^{14}C. The animals were housed in all glass metabolism cages, and expired CO_2 was trapped in 1 N KOH, which was changed 30, 60 and 90 min after alcohol administration and counted by liquid scintillation. Three rats were used per treatment. Inhibition caused by the compounds was calculated from average maximum rate of $^{14}CO_2$ expiration, obtained from plots of cumulative counts/min per 100 g rat versus time.

EXPERIMENTAL AND RESULTS

The pyrimidinethiol effectively lowered the concentration of norepinephrine in the brains of mice. Its intraperitoneal ED_{50} (norepinephrine measured 4 hr after administration of the compound) was 15·7 mg/kg (Table 1); thus, it is about 18 times as potent as disulfiram (ED_{50}, 275 mg/kg). The compound is active orally also, its ED_{50} by this route

* Samples of the compound were obtained from the Koppers Chemical Company and from K & K Laboratories, Inc. It is referred to in this paper as "the pyrimidinethiol".

† Concentrations of amines in the brains of these mice before drug administration were: norepinephrine, 0·48 $\pm$ 0·04 (S.D.) μg/g; dopamine, 1·04 $\pm$ 0·03 μg/g. Control heart norepinephrine was 0·89 $\pm$ 0·08 μg/g.

TABLE 1. POTENCY OF PYRIMIDINETHIOL AND DISULFIRAM IN DECREASING NOREPINEPHRINE CONCENTRATION IN THE MOUSE BRAIN*

Drug		Brain amine (fraction of normal)	
Dose (mg/kg)	Route	Norepinephrine	Dopamine†
Pyrimidinethiol			
10	i.p.	0·632	1·057
20	i.p.	0·417	1·164
40	i.p.	0·253	1·136
ED_{50} (mg/kg)		15·7	
95% Conf. limits		13·7, 18·1	
15	p.o.	0·669	1·047
30	p.o.	0·452	1·128
60	p.o.	0·357	1·183
ED_{50} (mg/kg)		29·2	
95% Conf. limits		25·6, 33·2	
Disulfiram			
100	i.p.	0·789	1·000
200	i.p.	0·576	1·060
400	i.p.	0·403	1·250
ED_{50} (mg/kg)		274·8	
95% Conf. limits		242·1, 312·6	
100	p.o.	0·912	1·063
200	p.o.	0·877	1·063
400	p.o.	0·817	1·119
800	p.o.	0·782	1·183

* Three groups of five mice/treatment. Animals were killed 4 hr after inhibitor administration.

† Correlation coefficient, brain dopamine with log dose, 0·623 ($P < 0·05$).

being 29·2 mg/kg (Table 1), while disulfiram was considerably less potent, a dose of 800 mg/kg decreasing brain norepinephrine only 25 per cent. Under the conditions of these experiments, administration of the pyrimidinethiol or disulfiram resulted in significantly increased concentrations of dopamine in the brains.

In the rat, the pyrimidinethiol is about 16 times as potent as disulfiram, as judged by the effects of the compounds on brain norepinephrine concentrations (Table 2).

TABLE 2. RAT BRAIN AMINES AFTER PYRIMIDINETHIOL OR DISULFIRAM

Drug	Dose (mg/kg)	Brain amine (fraction of normal)*	
		Norepinephrine	Dopamine
Pyrimidinethiol	16†	0·567	1·079
Dilsufiram	250†	0·560	1·100

* Normal amine concentrations in rat brains were: norepinephrine, 0·45 ± 0·034 (S.D.) μg/g; dopamine, 0·68 ± 0·086 μg/g.

† Dosing 4 hr before amine assays; eight rats/treatment.

Administration of approximately equipotent doses of the two compounds to mice resulted in similar rates of depletion and repletion of norepinephrine in the brains (Table 3).

TABLE 3. MOUSE BRAIN CATECHOLAMINES AT VARIOUS TIMES AFTER ADMINISTRATION OF β-HYDROXYLATION INHIBITORS

Time (hr)	Brain amines (fraction of normal)*			
	Pyrimidenethiol (20 mg/kg, i.p.)		Disulfiram (250 mg/kg, i.p.)	
	Norepinephrine	Dopamine	Norepinephrine	Dopamine
1	0·744	1·199	0·745	1·139
2	0·541	1·183	0·560	1·152
4	0·448	1·042	0·530	1·093
6	0·561	0·979	0·648	0·992
8	0·863	1·011	0·776	1·079
16	0·897	1·052	0·866	0·963

* Three groups of five mice/treatment. P for difference between pyrimidinethiol and disulfiram at the doses given, $>0{\cdot}25$; P for drug × time intreaction, $>0{\cdot}05$.

The pyrimidinethiol inhibited norepinephrine depletion of both brains and hearts of mice after the administration of methyldopa or α-methyl-*m*-tyrosine (Table 4); it also decreased the potency of α-methyl-*m*-tyramine and of α-methyldopamine in depleting the heart of norepinephrine (Tables 5 and 6). However, the pyrimidinethiol did not

TABLE 4. EFFECT OF PYRIMIDINETHIOL ON NOREPINEPHRINE DEPLETION BY METHYLDOPA AND α-METHYL*m*-TYROSINE*

Treatment	Tissue norepinephrine (fraction of normal)	
	Brain†	Heart†
Pyrimidinethiol (50 mg/kg)	0·874	0·997
Methyldopa (85 mg/kg)	0·425	0·471
Pyrimidinethiol + methyldopa	0·748	0·659
P for interaction	$<0{\cdot}001$	$<0{\cdot}005$
Pyrimidinethiol (50 mg/kg)	0·945	1·016
α-Methyl-*m*-tyrosine (20 mg/kg)	0·486	0·506
Pyrimidinethiol + α-methyl-*m*-tyrosine	0·688	0·658
P for interaction	$<0{\cdot}001$	$<0{\cdot}001$

* Three groups of five mice/treatment. One-hour predose with pyrimidinethiol. Assays 16 hr after amino acid administration.
† No difference between tissues ($P > 0{\cdot}10$).

depress the activity of cobefrine or metaraminol in this respect (Table 5). It is interesting to note that the effect of the opticals isomers of α-methyldopamine were not affected equally by the β-hydroxylase inhibitor (Table 6). This is consistent with the fact that the L (+) form, the one inhibited most drastically, is the form which is susceptible to enzymatic β-hydroxylation.[19]

TABLE 5. INTERACTION WITH AMINES ON MOUSE HEART NOREPINEPHRINE*

	Heart norepinephrine (fraction of normal)		
Pyrimidinethiol (100 mg/kg)	0	+	P†
Amine			
None	1·000	1·098	>0·05
Cobefrine (1·4 mg/kg)	0·437	0·336	>0·05
Metaraminol (0·2 mg/kg)	0·361	0·279	<0·025
α-Methyl-*m*-tyramine (1·5 mg/kg)	0·412	0·582	<0·01

* Six groups of five mice/treatment. Pyrimidinethiol, i.p., 60 min before amine, i.p. Assays 16 hr after amine administration.

† P for effect of pyrimidinethiol.

TABLE 6. PYRIMIDINETHIOL EFFECT UPON HEART NOREPINEPHRINE DEPLETION BY OPTICAL ISOMERS OF α-METHYLDOPAMINE*

Treatment	Heart norepinephrine (fraction of normal)	Inhibition† (%)
Pyrimidinethiol (100 mg/kg)	0·988	
D(−)-α-Methyldopamine (4 mg/kg)	0·510	
Pyrimidinethiol + D(−)-α-methyldopamine	0·601	19
L(+)-α-Methyldopamine (17 mg/kg)	0·487	
Pyrimidinethiol + L(+)-α-methyldopamine	0·808	63

* Three groups of 5 mice/treatment.

† The L(+) form was inhibited more than the D(−) form ($P < 0·005$).

After the administration of DOPA to mice, the expected increases were observed in brain norepinephrine and dopamine. The pyrimidinethiol increased the latter amine also, in the same proportion, whether or not DOPA was given (Table 7). However, the combination of compounds depressed brain norepinephrine more than the pyrimidinethiol alone.

TABLE 7. PYRIMIDINETHIOL EFFECT ON BRAIN NOREPINEPHRINE AND DOPAMINE AFTER ADMINISTRATION OF DOPA*

	Tissue amine (fraction of normal)	
Treatment	Norepinephrine†	Dopamine‡
Control	1·000	1·000
DOPA (100 mg/kg)	1·167	1·157
Pyrimidinethiol (100 mg/kg)	0·323	1·220
DOPA + pyrimidinethiol	0·240	1·420

* Three groups of 5 mice/treatment. Pyrimidinethiol, i.p., 60 min before DOPA; assays 90 min after DOPA administration.

† P for DOPA and pyrimidinethiol effects, <0·05 and <0·001 respectively; P for interaction, <0·001.

‡ P for DOPA and pyrimidinethiol effects, <0·001; P for interaction, >0·25.

TABLE 8. INTERACTION OF MAO INHIBITOR (CATRON, JB-516) WITH PYRIMIDINETHIOL IN MICE*

Treatment	Tissue amine (fraction of normal)		
	Brain norepinephrine†	Brain dopamine‡	Heart norepinephrine§
JB-516 (10 mg/kg)	1·338	1·097	1·203
Pyrimidinethiol (100 mg/kg)	0·279	1·133	1·014
JB-516 + pyrimidinethiol	0·826	1·352	1·254

* Three groups of 5 mice/treatment. JB-516 given 16 hr before pyrimidinethiol; animals killed 4 hr after pyrimidinethiol.

† P for JB-516 and pyrimidinethiol effects, $<0·001$; P for interaction, $<0·10$.

‡ P for JB-516 and pyrimidinethiol effects, $<0·025$ and $>0·01$ respectively; P for interaction, $>0·25$.

§ P for JB-516 effect $<0·05$; P for pyrimidinethiol effect and interaction, $>0·25$.

TABLE 9. EFFECT OF β-HYROXYLATION INHIBITORS ON RESTORATION OF THE PRESSOR RESPONSE TO TYRAMINE IN RESERPINE-PRETREATED RATS

Pretreatment*	β-Hydroxylation inhibitor	Restoring agent	N†	Pressor response to tyramine (100 μg) (mm Hg, mean ± S.D.)
None	None	Saline	10	75 ± 16
Reserpine	None	Saline	8	12 ± 4
Reserpine		α-Methyldopamine (30 μg)	18	62 ± 17
Reserpine	Pyrimidinethiol (mg/kg)	α-Methyldopamine (30 μg)‡		
	1·56		3	70 ± 8
	6·25		3	50 ± 14
	25·00		4	28 ± 0§
	100·00		4	15 ± 5§
Reserpine	Disulfiram (mg/kg)	α-Methyldopamine (30 μg)‡		
	1·56		3	60 ± 12
	6·25		3	53 ± 12
	25·00		4	46 ± 6§
	100·00		3	35 ± 6§
	400·00		4	28 ± 7§
Reserpine		α-Methylnorepinephrine (3 μg)	14	74 ± 13
Reserpine	Pyrimidinethiol (mg/kg)	α-Methylnorepinephrine (3 μg)		
	12·5		4	65 ± 13
	50·0		4	63 ± 16
	100·0		4	58 ± 5§
Reserpine	Disulfiram (mg/kg)	α-Methylnorepinephrine (3 μg)		
	100·0		5	76 ± 13
	400·0		4	55 ± 13§

* Reserpine (5·0 mg/kg, i.p.), 18–22 hr pretreatment; inhibitor, 2 hr i.p. pretreatment.

† N = number of animals tested.

‡ Slope of dose response for pyrimidinethiol, 30·9; for disulfiram, 13·1; P for difference, $<0·001$.

§ Significant difference from corresponding response in animals without β-hydroxylation inhibitor.

In vitro, at a concentration equal to that of substrate (dopamine, 3×10^{-4} M), the pyrimidinethiol inhibited the β-hydroxylation of dopamine 72 per cent. Inhibition was minimal (5 per cent) at a concentration of 3×10^{-5} M; thus, the I_{50} of the pyrimidinethiol is in the order of 2×10^{-4} M. The I_{50} values for sodium diethyldithiocarbamate and for disulfiram under these conditions were estimated to be 2×10^{-7} and 4×10^{-7} respectively. Therefore, the pyrimidinethiol is about 1/1000 as potent as sodium diethyldithiocarbamate, and 1/500 as potent as disulfiram as an inhibitor of dopamine β-hydroxylation *in vitro*.

Administered in addition to a monoamine oxidase inhibitor (Catron), the pyrimidinethiol had about the same proportionate effect upon brain catecholamines as when administered alone (Table 8); i.e. there was no evidence for interaction between the two enzyme inhibitors.

Both disulfiram and the pyrimidinethiol prevented restoration by α-methyldopamine of the tyramine-pressor response in reserpine-treated rats (Table 9). It is estimated that about 25 mg/kg of the pyrimidinethiol and 250 mg/kg of disulfiram would be required to produce a 50 per cent reduction in the restoring action of α-methyldopamine under the conditions of these experiments.

Prior to the administration of a restoring agent, responses to tyramine in the reserpine-treated rats were small, and no further reduction in response was produced by either β-hydroxylation inhibitor. However, disulfiram at 400 mg/kg, but not at 100, and the pyrimidinethiol at 100 mg/kg, but not at 50, significantly reduced the effectiveness of α-methylnorepinephrine as a tyramine response restoring agent.

The pyrimidinethiol, like other thiourea derivatives, inhibited the uptake of I_2 in the thyroid of the rat (Table 10). In this respect, the compound was about 6 times as potent as disulfiram, and 1/150 as potent as propylthiouracil.

TABLE 10. EFFECT OF PYRIMIDINETHIOL AND DISULFIRAM ON IODINE UPTAKE IN THE RAT THYROID*

Compound	ED_{50} (mg/kg)	95% Confidence limits	Slope†
Propylthiouracil	0·094	0·048, 0·183	31·73
Pyrimidinethiol	14·13	9·18, 21·73	90·90
Disulfiram	82·79	55·46, 123·60	51·35

* Three dose levels/compound; five rats/dose level.
† Slope of regression line, per cent inhibition on log dose.

TABLE 11. EFFECT OF DISULFIRAM AND PYRIMIDINETHIOL ON ETHANOL METABOLISM IN THE RAT

Dose (mg/kg, i.p.)	Inhibition of $^{14}CO_2$ expiration	
	Dilsulfiram (%)	Pyrimidinethiol (%)
3·125		7·0
6·25		43·6
12·5		53·4
25·0	58·5	84·7
50·0	75·3	78·9

The pyrimidinethiol, like disulfiram, interfered with the metabolism *in vivo* of ethanol. At a dose of 25 mg/kg, disulfiram inhibited the expiration of $^{14}CO_2$ by 58·5 per cent (Table 11), while the pyrimidinethiol at the same dose inhibited 84·7 per cent. It is estimated that the pyrimidinethiol is some 1·5 to 2 times as potent as disulfiram in this respect.

Both disulfiram (100 mg/kg, i.p.) and the pyrimidinethiol (50 mg/kg, i.p.) strongly depressed the expiration of $^{14}CO_2$ by rats which were given acetaldehyde (^{14}C-paraldehyde): disulfiram, 80·1 per cent; the pyrimidinethiol, 76·4 per cent. Thus, the primary target for both compounds in the oxidation of ethanol seems to be aldehyde dehydrogenase.

DISCUSSION

While the pyrimidinethiol is a poor inhibitor of the enzymatic conversion of dopamine to norepinephrine *in vitro*, it is considerably more potent than disulfiram *in vivo*. Differences in tissue distribution, metabolism or excretion of the two compounds might account for this potency reversal, but these possibilities have not been examined experimentally as yet. It seems unlikely that the pyrimidinethiol exerts its effect *in vivo* by a mechanism other than β-hydroxylase inhibition. If the compound were an inhibitor of tyrosine hydroxylase, its administration would result in depression of tissue dopamine concentrations as well as of tissue norepinephrine concentrations. Also, it is unlikely that the pyrimidinethiol functions as a catecholamine releasing agent, since it has little if any effect upon the concentration of norepinephrine in a peripheral tissue such as the heart.

The fact that the pyrimidinethiol is fully one-half as effective orally as intraperitoneally gives it further advantage over disulfiram as a β-hydroxylation inhibitor *in vivo*. Aside from potency considerations, the pyrimidinethiol and disulfiram act identically in a number of situations. They both depress the concentration of norepinephrine while producing some elevation of dopamine in the central nervous system (cf. Goldstein and Nakajima[20]). They produce little change in peripheral tissue (e.g. heart) norepinephrine, but they interfere with both central and peripheral β-hydroxylation of administered compounds such as methyldopa and α-methyldopamine, as shown by biochemical and pharmacological measures.

The mechanism underlying the minimal but significant reduction of effectiveness of α-methylnorepinephrine as a tyramine response restoring agent by the β-hydroxylation inhibitors is not known. A similar effect has been reported[21] for tyramine response restoration by norepinephrine as influenced by disulfiram.

The pyrimidinethiol also shares with disulfiram the ability to interfere with ethanol metabolism, presumably by inhibiting aldehyde dehydrogenase. Administered intraperitoneally, the pyrimidinethiol is significantly more potent than disulfiram, and this advantage should be magnified by oral administration, since the pyrimidinethiol is well absorbed from the gastrointestinal tract.

Since many thiourea derivatives inhibit the uptake of iodine by the rat thyroid, it is not surprising that the pyrimidinethiol should function in this capacity. Although the pyrimidinethiol is considerably less potent (about 1/140) than propylthiouracil, its ED_{50} for iodine uptake inhibition is of the same order as its β-hydroxylation inhibition

ED_{50} *in vivo*. However, it should be noted that disulfiram is more potent as an inhibitor of iodine uptake than it is as a β-hydroxylation inhibitor *in vivo*, by a factor of about 3.

The ability of disulfiram to inhibit tyramine response restoration in the reserpine-treated animal by DOPA, methyldopa and the related amines is well documented.[21–23] Disulfiram usually is employed in quite high doses (400 mg/kg, one or more times, i.p.), but the compound has definite effects at considerably smaller doses. For example, 50 mg/kg of disulfiram has been shown to inhibit amphetamine-induced motor hyperactivity in mice;[24] and in the present work, disulfiram, 25–100 mg/kg definitely influenced tyramine response restoration by α-methyldopamine. Since the pyrimidinethiol was some 10 times as potent as disulfiram in this respect and 15 times as potent in its effect upon brain catecholamines, it should be considerably more potent in other situations also. Presumably, the pyrimidinethiol would inhibit amphetamine-induced hyperactivity at a dose of perhaps 5 mg/kg.

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