INHIBITION OF IODOTHYRONINE 5'-DEIODINASE BY THIOUREYLENES; STRUCTURE–ACTIVITY RELATIONSHIP

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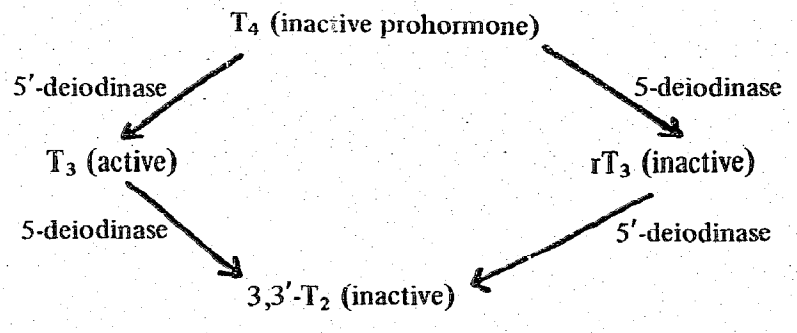
1. Introduction

Thiourea derived compounds are known for their goitrogenic activity [1]. These substances block the biosynthesis of thyroxine (T_4) by inhibiting thyroid peroxidase [2]. Among them are the 2-thiouracil (TU) derivatives, which have an additional inhibitory effect on the deiodinactive metabolism of thyroid hormone in peripheral tissues [3,4]. T_4 is considered as a prohormone, which is converted into the biologically active form of thyroid hormone, 3,3',5-triiodothyronine (T_3), by the enzyme iodothyronine 5'-deiodinase [5]. Deiodination of T_4 may also yield the inactive metabolite 3,3',5'-triiodothyronine (rT_3). This latter reaction is probably mediated by a second enzyme, iodothyronine 5-deiodinase [5]. The main pathway of rT_3 degradation is by 5'-deiodination into 3,3'-diiodothyronine [5]. The latter may also be produced by 5-deiodination of T_3 [5]:

These enzymes contain essential sulfhydryl groups and thiol cofactors are required for activity [5]. Deiodination follows a ping–pong mechanism which involves the oxidation of an enzyme-sulfhydryl group [6–8]. This is substantiated by the findings of an uncompetitive inhibition by TU of the 5'-deiodination of T_4 [6,9] and of 3,3',5'-triiodothyronine (rT_3) [7,10]. This inhibition is competitively obviated by thiols such as dithiothreitol (DTT) and by 1-methyl-2-mercaptoimidazole (MMI) or thiourea [6–8]. A similar mode of inhibition, although not competitive with MMI or thiourea, has been observed with sulfite and thiosulfate [8]. In consideration of the high reactivity of TU towards sulfenyl iodides (–SI) as compared with ordinary disulfides [11], we suggested that an E–SI complex is formed during deiodination [7].

The low activity of MMI in the deiodination of iodothyronines in vivo [2] as well as in vitro [6,8]



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compared with its potency similar to that of TU in inhibiting thyroid peroxidase activity is intriguing. Nevertheless, also in the iodination of thyroglobulin by thyroid peroxidase the formation of an intermediate E–SI complex is implied [11–13]. Furthermore, it has been shown that MMI is even more reactive than TU towards β-lactoglobulin sulfenyl iodide [11]. The aim of the present study was to elucidate the features in structure of MMI compared with TU rendering it virtually without effect on the deiodination process. To this end various analogues of TU and MMI were tested. It was found, among other things, that methylation of N_1 (as in MMI) of thioureylenes greatly reduces 5′-deiodinase inhibitory activity.

2. Materials and methods

2.1. *Compounds*

2-Thiouracil (TU), 2-mercaptopyrimidine, 2-thiocytosine, 6-methyl-TU, 2-thiobarbituric acid, 5-carboxy-TU and 2-thiohydantoin were obtained from Sigma, St. Louis, MO. 2-Mercaptobenzimidazole, 2-mercaptobenzoxazole, 2-mercaptobenzothiazole and 3-mercapto-1,2,4-triazole were purchased from Fluka AG, Buchs and 1-methyl-2-mercaptoimidazole (MMI) from Nogepha, Alkmaar. 5-Propyl-TU and 6-propyl-TU were kindly supplied by Dr P. J. H. Eggels, University Hospital 'Dijkzigt', Rotterdam. 1-Methyl-6-propyl-TU was donated by Dr R. H. Lindsay, Veterans Administration Hospital, Birmingham, AL and 2-mercaptoimidazole by Dr E. Bäuerlein, Max-Planck-Institut, Heidelberg.

2.2. *Methods*

Conversion of rT_3 into 3,3′-diiodothyronine ($3,3'\text{-}T_2$) by rat liver microsomal fraction in the presence of DTT was studied essentially as in [14]. In brief, 0.1 μM rT_3 was reacted with 7 μg microsomal protein and various substances to be tested in 0.25 ml 0.05 M phosphate, 3 mM EDTA and 1 mM DTT (pH 6.5). After incubation for 20 min at 37°C, the reaction was stopped by the addition of 1 ml 0.06 M barbitone buffer containing 0.1% bovine serum albumin and 0.1% SDS (pH 8.6). The amount of $3,3'\text{-}T_2$ produced was measured with a specific radioimmunnoassay in 50 μl of the extract [15]. The reaction was started by addition of microsomes. In the control experiments incubation was carried out without microsomes, which are added only following the addition of SDS buffer. Deiodinase activity was corrected for the amount of $3,3'\text{-}T_2$ produced in the controls. The non-enzymatically formed $3,3'\text{-}T_2$ was only a minor fraction of that produced in the presence of microsomes, being generally < 5%. If necessary, test substances were dissolved in 0.1 N NaOH, rapidly followed by dilution to the desired concentration with incubation buffer and adjusting of pH.

3. Results

Figure 1 shows the effect of increasing concentrations of various TU derivatives on the conversion of rT_3 into $3,3'\text{-}T_2$. The relative activities of these compounds are given in table 1. Uracil has been shown not to affect deiodinase activity, pointing to the essential nature of the 2-mercapto group [7]. Similarly, deletion of the 4-hydroxyl group or replacement by an amino group greatly reduces inhibitory activity (fig.1, table 1). Polar substituents at C_5 and C_6 –

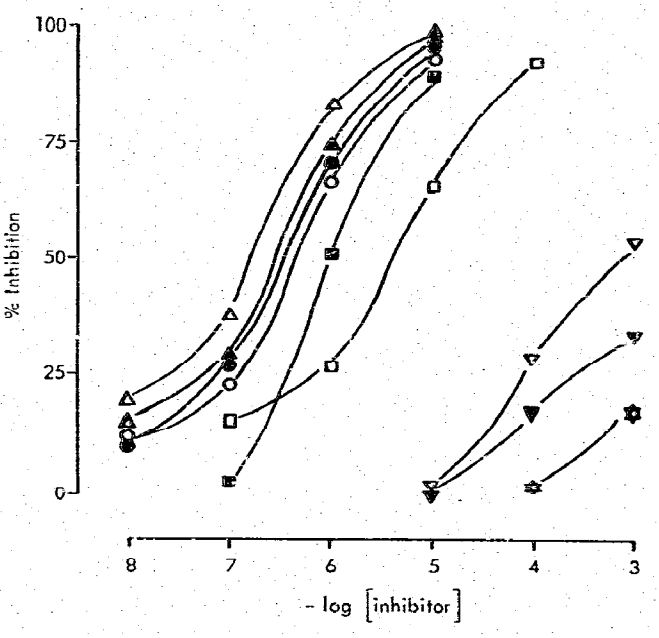
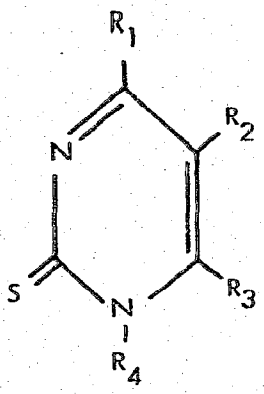


Fig.1. Inhibition of the conversion of rT_3 into $3,3'\text{-}T_2$ by increasing concentrations of TU derivatives. For details see section 2 and for explanation of symbols see table 1. Results are mean of 3 closely agreeing experiments performed in duplicate.

Table 1
Inhibitory activity of TU derivatives

Inhibitory activity of thiouracil (TU) analogues



	R_1	R_2	R_3	R_4	Relative activity
▼ 2-mercaptopyrimidine	H	H	H	H	<0.001
▽ 2-thiocytosine	NH_2	H	H	H	0.001
● 2-thiouracil (TU)	OH	H	H	H	1.0
■ 5-carboxy-TU	OH	CO_2H	H	H	0.35
△ 5-propyl-TU	OH	C_3H_7	H	H	2.0
□ 6-hydroxy-TU[x]	OH	H	OH	H	0.08
○ 6-methyl-TU	OH	H	CH_3	H	0.71
▲ 6-propyl-TU (6-PTU)	OH	H	C_3H_7	H	1.25
✡ 1-methyl-6-PTU	OH	H	C_3H_7	CH_3	<0.001

[x] 2-thiobarbituric acid

Figures are based on the concentration giving 50% inhibition of deiodinase activity and are expressed relative to that of TU. Symbols refer to fig.1

especially a hydroxyl group at C_6 – give a decrease in potency. Alkyl substituents, on the other hand, do not affect activity to a great extent. The most effective substance is 5-propyl-TU, being twice as active as TU. Methylation of N_1 yields an almost inactive compound.

The effect of increasing concentrations of various MMI analogues on the 5'-deiodination of rT_3 is illustrated in fig.2. The activities of these substances relative to that of TU are given in table 2. For comparison, fig.2 shows also the effects of addition of thiourea, which appears to be practically inactive. MI has 18% of the activity of TU. In this case the hydroxyl substituent at C_4 is not obligatory, but even

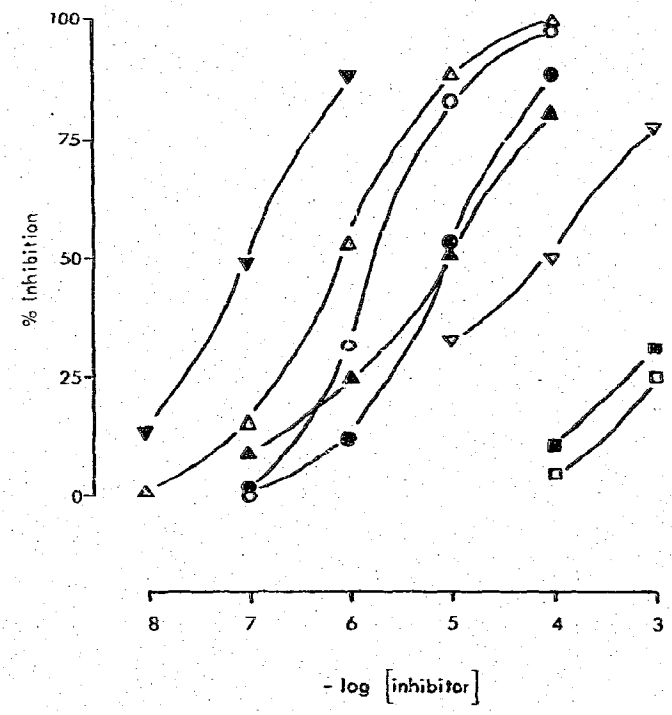
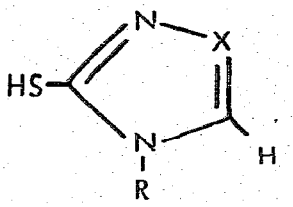
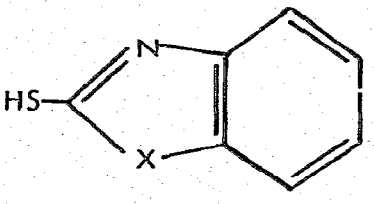


Fig.2. Inhibition of the conversion of rT_3 into 3,3'-T_2 by increasing concentrations of MMI analogues. For details see section 2 and for explanation of symbols see table 2; in addition, the effect of thiourea is shown (□). Results are means of 3 closely agreeing experiments performed in duplicate.

Table 2
Inhibitory activity of MMI analogues

Inhibitory activity of methimazole (MMI) analogues





	X	R	Relative activity
○ 2-mercapto imidazole (MI)	C-H	H	0.18
■ 1-methyl-MI (MMI)	C-H	CH_3	<0.001
● 4-hydroxy-MI[x]	C-OH	H	0.035
△ 3-mercapto-1,2,4-triazine	N	H	0.35
▼ 2-mercaptobenzimidazole	N-H		3.16
▲ 2-mercaptobenzothiazole	S		0.032
▽ 2-mercaptobenzoxazole	O		0.0035

[x] thiohydantoin

Figures are based on the concentration giving 50% inhibition of deiodinase activity and are expressed relative to that of TU. Symbols refer to fig.2

lowers inhibitory activity. As with TU, a methyl substituent at N_1 is deleterious. The triazine analogue has ~2-times the activity of MI. The most effective substance tested is 2-mercaptobenzimidazole, being ~20-times as active as MI and even 3-times as active as TU. The importance of the atom occupying position 1 is illustrated by the effects of the benzothiazole and benzoxazole derivatives. The activity of 2-mercaptobenzimidazole is decreased by 2 and 3 orders of magnitude by replacing nitrogen with sulfur and oxygen, respectively.

4. Discussion

The effect of administration of a large number of anti-thyroid compounds on the deiodination of T_4 in the rat in vivo was reported [3]. This was investigated by measuring the amount of [^{131}I]iodide excreted in the urine after the administration of [^{131}I]T_4. The main conclusions drawn [3] with regard to the structural requirements for inhibition of deiodination by thioureylene derivatives were:

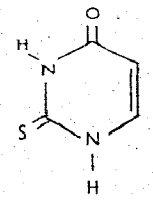
1. Substitution of N_1 of the heterocyclic ring as in MMI prevents effectiveness;
2. An oxygen meta to the thio group in the 6 membered ring is essential, but not so for the 5 membered ring;
3. Polar groups at C_6 prevent inhibition of deiodination.

Among the compounds tested 5-propyl-TU was found to be the most active. 2-Mercaptobenzimidazole was not tested.

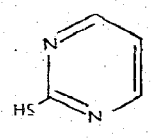
Essentially the same conclusions may be drawn from the present study. This is further strong evidence that the enzyme activity investigated in this way is at least in part responsible for the deiodination of T_4 in vivo. This would also support the hypothesis that 5'-deiodination of T_4 and other iodothyronines is mediated by this very enzyme [5,14,16]. It has been

suggested that a second enzyme mediates 5-deiodinations [5]. This 5-deiodinase also requires thiol compounds for activity and is also inhibited by TU derivatives [10,17]. Nevertheless, the main effect on thyroid hormone deiodination of 6-propyl-TU in humans is a decreased production of T_3 from T_4 and a decreased degradation of rT_3 into $3,3'\text{-}T_2$ [16]. Thus, it appears that iodothyronine 5′-deiodinase is more sensitive than the 5-deiodinase to the administration of TU. The effects observed [3], therefore, may well have been due primarily to inhibition of 5′-deiodinase activity. The close agreement between their results and ours demonstrates that inhibition of deiodination in vivo is due to a direct action on the enzyme of the compounds tested and not of their metabolites. The inactivity of MMI both in vivo and in vitro suggests that no significant *N*-demethylation occurs in the organism.

Both MMI and thiourea are potent inhibitors of thyroid peroxidase [2]. The structural requirements for thioureylenes to be active inhibitors of thyroid peroxidase are, therefore, different from those for inhibition of deiodinase activity. It has been suggested that both in iodination [11–13] and deiodination [7] the formation of an enzyme–sulfenyl iodide (E–SI) intermediate is involved. However, it is appreciated that 6-propyl TU and MMI inhibit peroxidase activity in the absence of iodide [2]. Furthermore, reaction of thiourea and MMI with β-lactoglobulin sulfenyl iodide is even faster compared with TU. The present study, therefore, has not yielded indications for the involvement of an E–SI complex in deiodination, however plausible this mechanism (see section 1).



(1)



(2)

It should be emphasized that the structures drawn in tables 1 and 2 do not reflect preferred configurations in aqueous solutions. More likely these are represented by (1) for TU and by (2) for 2-mercaptopyrimidine. The latter and those thioureylenes having N_1 blocked with a methyl group are very weak inhibitors of enzymatic deiodination. This may suggest that there is a strict requirement for a secondary amine in position 1.

Acknowledgements

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