Relative Antithyroid Effects of 2-Thiouracil, 2-Thiouridine and 2-Thio-UMP (37388)

MEI-YING WONG YU,[1] JOZEF SEDLAK,[2] AND RAYMOND H. LINDSAY[3]
(Introduced by R. S. Teague)

Departments of Pharmacology and Medicine, University of Alabama in Birmingham; Pharmacology Research Unit, VA Hospital, Birmingham, Alabama 35233

The structural similarities between thioracil and uracil suggest that antithyroidal effects of thiouracil may be related to its involvement in nucleic acid metabolism. This hypothesis is supported by the report of Sadhu (1) which indicated that the goitrogenic effect of thiouracil in rats could be overcome by equimolar concentrations of uracil. These results could not be confirmed by other investigators (2–4) using uracil to thiouracil ratios as high as 300:1 and thiouracil inhibition of iodination in sheep thyroid slices was not affected by 8:1 ratios of uracil (3). Complicating these observations is the report by Van Middlesworth (5) that many commercial uracil preparations contain a thiouracil-like contaminant which may itself be goitrogenic.

More recently, reports have demonstrated that thiouracil may be converted to thiouridine by uridine phosphorylase (6) or thymidine phosphorylase (7, 8). Further metabolism to thio-UMP is catalyzed by uridine kinase (6). In addition, a one-step conversion of thiouracil to thio-UMP is accomplished by UMP pyrophosphorylase (9). Metabolism of thiouracil to its nucleoside and nucleotides by thyroid tissue has received little attention; however, rat thyroid readily utilizes uracil and orotic acid, incorporating them into nucleotides and RNA (10), and contains high levels of uridine phosphorylase and uridine kinase. Furthermore, thyroid tissue has been shown to concentrate thiouracil (11). Thus conditions in the thyroid appear favorable for thiouracil conversions to nucleosides and nucleotides.

The objectives of this study were to determine whether thyroid tissue from various species possess the enzymes capable of forming the thiouracil nucleoside and nucleotide and whether these metabolites are involved in antithyroidal effects attributed to thiouracil.

Materials and Methods. Tissue extracts were prepared and assayed for uridine phosphorylase (12) and uridine kinase (12) according to methods previously described. Thymidine phosphorylase was assayed by a slight modification of the spectrophotometric method described by Friedkin and Roberts (14). The enzyme extract was prepared by homogenizing tissue in 4 vol of 0.05 *M* Tris buffer (pH 6.0) and centrifuging at 105,000 *g* for 30 min. The supernatant was used as the source of the enzyme. For UMP pyrophosphorylase assays, tissue was homogenized in 5 vol 0.05 *M* potassium phosphate buffer (pH 7.5) and centrifuged at 600*g*. The supernatant was then centrifuged at 15,000*g* for 30 min and the resulting supernatant was used as a source of the enzyme. Assays were carried out with the isotopic method previously described in detail (9).

Porcine thyroid peroxidase was prepared by a modification of methods described by Coval and Taurog (15) and Hosoya and Morrison (16). Fresh hog thyroid glands were freed of connective tissue and fat, then cut into small pieces. Tissue weighing 88.7 g was washed several times with a large volume of cold 0.9% NaCl (approx 500 ml) and homogenized in a buffer–ice mixture in

[1] Present address: Box 236, Department of Zoology, University of Texas at Austin, Austin, Texas 78712.

[2] Present address: SAV, Endokrinologicky Ustav, Kramare, Limbova cesta, Bratislava, Czechoslovakia.

[3] To whom reprint requests should be addressed.

a Waring blender. This slurry was filtered through two layers of cheesecloth, passed through a fine-mesh nylon cloth then centrifuged at 30,000*g* for 1.5 hr at 0°. The pellet was suspended in 0.05 *M* Tris·HCl–1.0 × 10^{-3} *M* KI (pH 7.0) (buffer) in a final volume of 30 ml. Trypsin (3.9 mg) was added in 1.3 ml of buffer and the mixture was stirred for 2.5 hr at room temperature to solubilize thyroid peroxidase. Trypsin activity was terminated by the addition of 8.7 mg of soybean trypsin inhibitor in 1.3 ml of buffer and the mixture was centrifuged at 13,000*g* for 2 hr at 0°. The somewhat turbid and slightly colored trypsin supernatant containing most of the peroxidase activity was brought to 45% saturation with ammonium sulfate. The protein precipitate collected by centrifugation at 13,000*g* for 1 hr at 0° was redissolved in buffer (total volume 20 ml) and dialyzed 90 min each against two changes of 500 ml of fresh buffer in the cold. The dialysate (about 27 ml) contained the active hog thyroid peroxidase and was used for inhibition studies.

Peroxidase activity was assayed by a slight modification of the guaiacol test (17). The assay medium contained 117.5 μmoles of guaiacol; 58.75 μmoles of Tris·HCl (pH 7.4); and 0.6 mg of enzyme protein in a final volume of 1.2 ml. The reaction was initiated by the addition of 0.025 ml of 3.92 × 10^{-2} *M* H_2O_2. The increase in absorbance at 470 nm was measured for 12 sec during the initial linear reaction using a recording Beckman DK-2 spectrophotometer.

The antithyroidal effects of 2-thiouracil and 2-thiouridine in intact rats were determined by a modification of the method of Pitt-Rivers (18). Male Sprague–Dawley rats (100–125 g) were placed on a low iodide diet for 3 days prior to the experiments. Each animal received an intraperitoneal injection of 0.5 ml of either a blank (0.9% NaCl), thiouracil or thiouridine solution. One hour later, an injection of $Na^{125}I$ (1 μCi/0.5 ml saline, carrier free) was given intraperitoneally. Three hours after the injection of $Na^{125}I$ the animals were sacrificed and the thyroids were removed. Whole thyroid lobes were placed in ground-glass homogenizers and the total thyroidal ^{125}I uptake was obtained. The glands were then homogenized in 1 ml of the 0.05 *M* Veronal buffer at pH 8.6 containing 1.0 × 10^{-5} *M* thiouracil. One volume of 20% ice-cold trichloroacetic acid was added and the resulting precipitate was washed twice with 1 ml of 10% trichloroacetic acid. The radioactivity recovered in the final precipitate was taken as PB ^{125}I and the radioactivity in the supernatants as inorganic ^{125}I.

Results. The initial conversion of thiouracil to a nucleoside is catalyzed by uridine phosphorylase or thymidine phosphorylase with further metabolism of the nucleoside to thio-UMP being catalyzed by uridine kinase. Conversion of thiouracil to thio-UMP in a one-step reaction is accomplished by UMP pyrophosphorylase. The occurrence of these enzymes in thyroid tissue from various species was examined with the results presented in Table I. Values for rat liver are included for comparison. The highest level of activity was demonstrated for uridine phosphorylase which was consistently present and higher in all species examined than in rat liver. Thymidine phosphorylase activity was observed in rat, dog, goat and bovine thyroid but not in sheep. In all except bovine tissue, activity was only slightly less than that in rat liver preparations. Only traces of UMP pyrophosphorylase were observed in any tissue examined and were so low as to be of doubtful significance. The enzyme converting thiouridine to thio-UMP, uridine kinase, was present in all species at a level of activity less than that in rat liver. Thus thyroid tissue generally contains two enzymes, uridine phosphorylase and thymidine phosphorylase, capable of converting thiouracil to thiouridine and also the enzyme uridine kinase which catalyzes thiouridine metabolism to thio-UMP.

The acute effects of thiouracil and thiouridine on thyroid function were determined by a modification of the method described by Pitt-Rivers (18) and are presented in Table II. The effects of thio-UMP were not examined due to the general lack of cell permeability to phosphorylated nucleosides. A single dose of 0.78, 1.95, 3.9 and 7.8 μmoles of thiouracil depressed the thyroidal ^{125}I up-

TABLE I. Activity of Pyrimidine Pathway Enzymes Involved in Thiouracil Anabolism.

Source	Sp act (μmoles/mg/hr)			
	Uridine phosphorylase	Thymidine phosphorylase	Uridine kinase	UMP pyrophosphorylase
Liver				
Rat	0.104	0.069	0.09	Trace[b]
Thyroid				
Rat	0.354	0.023	0.05	Trace[b]
Bovine	0.289	Trace[a]	0.013	Trace[b]
Guinea pig	—	—	—	Trace[b]
Dog	0.230	0.032	0.025	—
Sheep	0.261	0	0.014	0
Goat	0.125	0.022	0.011	—

[a] Less than 0.005.
[b] Less than 1.0×10^{-5}.

take 40, 76, 84 and 94%, respectively; whereas the ratio of PB ^{125}I and inorganic ^{125}I with the three higher doses was decreased to 4, 2 and 1, respectively.

A comparison of the antithyroidal effects of thiouracil and thiouridine is also shown in Table II. The administration of 3.9 and 7.8 μmoles of thiouridine inhibited iodide uptake 22 and 54%, respectvely, compared to 88% for 3.9 μmoles of thiouracil. Iodide organification was also inhibited by thiouracil but not by either amount of thiouridine. These data strongly indicate that thiouracil had a much more potent antithyroidal effect than thiouridine. The relative potencies cannot be accurately estimated but 7.8 μmoles of thiouridine produced results similar to those seen with 0.78 μmoles of thiouracil, suggesting that thiouridine is approximately 10% as effective as thiouracil.

Thiouracil has been shown to be a potent inhibitor of a highly purified thyroid peroxidase preparation (15) which apparently accounts for its antithyroidal effects. The effects of thiouridine and thio-UMP on partially purified thyroid peroxidase were examined and compared with thiouracil with the results shown in Table III. It is evident that

TABLE II. The Antithyroidal Actions of 2-Thiouracil and 2-Thiouridine in Intact Rats.

Compound	Dose (μmoles)	Thyroidal ^{125}I uptake (dpm)	Distribution of ^{125}I (%)	
			PB ^{125}I	Inorg. ^{125}I
A. Thiouracil	0	198,779 ± 12,945 (3)[a]	84.3	15.7
	0.78	118,542 ± 14,553 (3)[b]	88.1	11.9
	1.95	47,927 ± 7235 (3)[c]	80.0	20.0
	3.9	31,337 ± 1974 (3)[d]	67.8	32.2
	7.8	19,420 ± 5302 (3)[d]	49.8	50.2
B. NaCl (0.9%)		186,516 ± 22,504 (12)	88.9	11.1
Thiouracil	3.9	23,089 ± 2718 (14)[d]	71.9	28.1
Thiouridine	3.9	146,501 ± 23,281 (10)[b]	88.7	11.3
Thiouridine	7.8	87,451 ± 8889 (8)[c]	87.9	12.1

[a] The data represent the mean ± SE with the number of rats for each group indicated in the parentheses.
[b] $p > 0.05$.
[c] $p < 0.05$.
[d] $p < 0.001$.

TABLE III. Effects of 2-Thiouracil, Uracil, 2-Thiouridine, Uridine and 2-Thio-UMP on Porcine Thyroid Peroxidase.

Compound concn ($\times 10^{-4}$ M)	Sp act[a] (OD_{470}/min/mg protein)	Inhibition (%)
Control	1.33	None
Thiouracil		
0.2	1.09	18.0
0.5	0.807	39.3
1.0	0.596	55.2
Uracil		
10	1.17	12.0
50	0.761	42.8
100	0.519	61.0
Thiouridine		
10	1.33	None
50	1.08	18.8
100	0.814	38.8
Uridine		
10	1.33	None
50	1.33	None
100	1.33	None
Thio-UMP		
1.1	1.33	None
5.7	1.18	11.3
11.0	0.945	28.9

[a] The specific activity represents the total change in absorbance at 470 nm/mg of protein.

thiouridine was much less potent than thiouracil since 1×10^{-2} M thiouridine was required to produce essentially the same degree of inhibition as 5×10^{-5} M thiouracil. Thio-UMP exerted some inhibition and appeared to be more potent than thiouridine since thio-UMP at 1.1×10^{-3} M inhibited 29% of the peroxidase activity. However, the purity of this thio-UMP preparation was reexamined and thiouracil was found to account for approximately 5% of the sample. This amount of thiouracil could account for the inhibition observed with thio-UMP. The effects of uracil and uridine on peroxidase activity were also studied. Inhibition was produced by uracil although it was much less potent than thiouracil; however, the effect was greater than that produced by thiouridine. Uracil at a 1×10^{-2} M concentration inhibited the enzyme activity approximately as much as thiouracil at 1×10^{-4} M. Uridine did not produce any inhibition even at a concentration of 1×10^{-2} M.

Discussion. The presence of thyroidal enzymes capable of converting thiouracil to thiouridine and thio-UMP was demonstrated in Table I. Uridine phosphorylase, the most active enzyme observed, does utilize thiouracil but it is a poor substrate and little conversion occurs (6). The activity of thymidine phosphorylase, which readily utilizes thiouracil, was low but comparable to that in rat liver or not found. UMP-pyrophosphorylase activity was either absent or insignificantly low and would be of little importance in thiouracil metabolism in the gland. Consequently, the thyroid may have difficulty in carrying out the initial conversion of thiouracil to thiouridine or thio-UMP.

Once thiouridine is formed, metabolism to thio-UMP and other nucleotides probably occurs as with rat liver. Preliminary experiments (data not shown) in which thiouridine-2-^{14}C was incubated with enlarged rat thyroids demonstrated the formation of radioactive peaks after column chromatography identical to peaks identified as thio-UMP nucleotides in the acid-soluble fraction of rat liver slices (19). Incorporation into RNA also appeared to occur. The failure of Van Erkelens (20) Maloof and Soodak (3) and Lindsay, Nakagawa and Cohen (4) to find any evidence for the incorporation of thiouracil into nucleotides or RNA in the thyroid was probably due to the difficulty in converting thiouracil to thiouridine. This problem is also encountered with rat liver.

The results obtained with both intact rats and partially purified thyroid peroxidase clearly demonstrate that thiouracil was many times more potent as an antithyroid substance than thiouridine or thio-UMP. If activation of thiouracil had occurred as a result of metabolic conversion to these compounds, the contrary would have been expected. Consequently, it is highly unlikely that conversion to a nucleoside or nucleotide is essential for thiouracil to exert its antithyroidal action and thiouracil effects on RNA synthesis by this mechanism are probably not involved. In fact, it is unlikely that metabolic activation by any mechanism is involved in the thioura-

cil effect since Maloof and Soodak (21) found that the metabolism of thiouracil-^{35}S was greatly reduced in the hypophysectomized rat, yet thiouracil was as effective in inhibiting the formation of PB ^{131}I in the thyroid of this animal as in the thyroid of the normal rat.

Thiouracil in intact rats was a much more potent inhibitor of iodide uptake and organification of iodide than thiouridine. However, some inhibition was observed with thiouridine. This effect may not be due to the intact thiouridine molecule since it had little effect on peroxidase activity. Conversion to thiouracil can be readily accomplished by uridine phosphorylase (6). Degradation of thiouridine to thiouracil by this enzyme could have resulted in the thyroidal inhibition observed.

The inhibitory effect of uracil on thyroid peroxidase is surprising but is probably explained by Van Middlesworth's report (5) of the presence of a thiouracil-like contaminant in many commercial uracil preparations. The high concentrations of uracil tested could have provided enough of this contaminant to produce the inhibition observed.

Although the metabolism of thiouracil in pyrimidine pathways leading to RNA synthesis does not appear to be involved in the antithyroid action of thiouracil, it may be related to the toxic symptoms produced by thiouracil or to thiouracil inhibition of thyroxine deiodination (5). Leukopenia, caused by the depression of marrow cell maturation after the administration of high doses of thiouracil as well as propylthiouracil (22), may be related to effects on nucleic acid synthesis.

Summary. The presence of uridine phosphorylase and thymidine phosphorylase, enzymes capable of converting thiouracil to thiouridine, was demonstrated in thyroid tissue from several species. Uridine kinase which catalyzes further metabolism of thiouridine to thio-UMP was also present. The activity of UMP pyrophosphorylase which converts thiouracil to thio-UMP in a one-step reaction was insignificant.

Comparisons of antithyroidal activity in intact rats and with partially purified porcine thyroid peroxidase demonstrate that thiouridine was approximately 10% as potent as thiouracil *in vivo* but less than 1% as active as thiouracil as an inhibitor of thyroid peroxidase. Thio-UMP was about 5% as active as thiouracil on thyroid peroxidase.

These results indicate that it is highly unlikely that thiouracil conversion to a nucleoside or nucleotide is involved in its antithyroidal action.

1. Sadhu, D. P., Amer. J. Physiol. **152**, 150 (1948).
2. Paschkis, K. E., Cantarow, A., and Stasney, J., Science **114**, 264 (1951).
3. Maloof, K., and Soodak, M., Endocrinology **68**, 831 (1961).
4. Lindsay, R. H., Nakagawa, H., and Cohen, P. P., Endocrinology **76**, 728 (1965).
5. Van Middlesworth, L., Endocrinology **77**, 946 (1965).
6. Yu, M. W., Sedlak, J., and Lindsay, R. H., Proc. Soc. Exp. Biol. Med. **139**, 1292 (1972).
7. Strominger, D. B., and Freidkin, M. J., J. Biol. Chem. **208**, 663 (1954).
8. Razzell, W. E., and Khorana, H. G., Biochim. Biophys. Acta **28**, 562 (1958).
9. Lindsay, R. H., Tillery, C. R., and Yu, M. W., Arch. Biochem. Biophys. **148**, 466 (1972).
10. Lindsay, R. H., and Cohen, P. P., Endocrinology **76**, 737 (1965).
11. Quinones, J. D., Boyd, C. M., Beierwaltes, W. H., and Poissant, G. F., J. Nucl. Med. **13**, 148 (1972).
12. Lindsay, R. H., Romine, C. J., and Wong, M. Y., Arch. Biochem. Biophys. **126**, 812 (1968).
13. Akamatsu, N., Lindsay, R. H., and Cohen, P. P., J. Biol. Chem. **239**, 2246 (1964).
14. Friedkin, M., and Roberts, D., J. Biol. Chem. **207**, 245 (1954).
15. Coval, M. L., and Taurog, A., J. Biol. Chem. **242**, 5510 (1967).
16. Hosoya, T., and Morrison, M., J. Biol. Chem. **242**, 2828 (1967).
17. Ljunggren, J. G., and Akeson, A., Arch. Biochem. Biophys. **127**, 346 (1968).
18. Pitt-Rivers, R., Ann. N. Y. Acad. Sci. **86**, 362 (1960).
19. Yu, M. W., Sedlak, J., and Lindsay, R. H., Arch. Biochem. Biophys. **155**, 111 (1973).
20. Van Erkelens, P. C., Acta Endocrinol. **18**, 229 (1955).
21. Maloof, F., and Soodak, M., Endocrinology **61**, 555 (1957).
22. Reed, P. W., and Tepperman, J., Amer. J. Physiol. **216**, 231 (1969).

Received Feb. 21, 1973. P.S.E.B.M., 1973, Vol. 143.