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THE SELENIUM ANALOG OF 6-PROPYLTHIOURACIL

MEASUREMENT OF ITS INHIBITORY EFFECT ON TYPE I IODOTHYRONINE DEIODINASE AND OF ITS ANTITHYROID ACTIVITY

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Abstract—6-Propylthiouracil (PTU), a widely used antithyroid drug for the treatment of Graves' disease, is also a potent inhibitor of Type I iodothyronine deiodinase (ID-1). Inhibition of ID-1 was attributed initially to the formation of a mixed disulfide between PTU and a putative cysteine residue at the active site. It has been demonstrated recently that ID-1 is a selenium-containing enzyme, with selenocysteine, rather than cysteine, at the active site. It seemed possible, therefore, that the selenium analog of PTU (PSeU) might be a more potent inhibitor of ID-1 than PTU. To test this possibility, we developed a procedure for the synthesis of PSeU, and we compared PSeU and PTU as inhibitors of ID-1 in a test system containing ^{125}I-rT_3, rat liver microsomes, and dithiothreitol. Deiodinase activity was measured by the increase in ^{125}I-iodide. PTU and PSeU were tested at 0.1, 0.3, 1 and 3 μM. Based on results of four separate experiments, the drugs were essentially equipotent as inhibitors of ID-1, although statistical analysis suggested that PSeU may be slightly more potent than PTU. PTU and PSeU were also compared for antithyroid activity *in vivo* and *in vitro*. As inhibitors of the catalytic activity of thyroid peroxidase (TPO), the two drugs were essentially equipotent in iodination and guaiacol assays involving measurements made shortly after the addition of H_2O_2. However, in *in vivo* experiments with rats, PSeU showed no appreciable inhibition of organic iodine formation in the thyroid, whereas PTU, as expected, was a potent inhibitor. The lack of inhibition of organic iodine formation *in vivo* by PSeU suggests that, unlike PTU, it is not concentrated by the thyroid gland. In an iodination system in which H_2O_2 was generated by glucose-glucose oxidase, both PTU and PSeU, when present at 10 μM, acted as reversible inhibitors of iodination. However, when the drug concentration was raised to 50 μM, TPO was inactivated and iodination was irreversibly inhibited. These results suggest that PTU and PSeU inhibit TPO-catalyzed iodination by similar mechanisms. Under the same conditions, the selenium analog of methimazole (another widely used antithyroid drug) does not inactivate TPO. It acts primarily as a reversible inhibitor of TPO-catalyzed iodination.

Key words: thyroid; propylthiouracil; selenium; Type I iodothyronine deiodinase; antithyroid drugs

PTU§ is an antithyroid drug, widely used in the treatment of Graves' disease (hyperthyroidism). Its antithyroid effect depends primarily on its ability to block TPO-catalyzed iodination of tyrosyl residues in thyroglobulin, thereby reducing the biosynthesis of thyroxine in the thyroid gland [1].

PTU is also a potent inhibitor of ID-1, an enzyme present in liver, kidney, thyroid, and other tissues, which converts T_4 to the biologically active thyroid hormone T_3. A mechanism for the inhibition of ID-1 by PTU was proposed by Leonard and Visser [2]. They obtained evidence for the formation of a mixed disulfide between PTU and a putative cysteine residue at the active site, leading to irreversible inactivation of the enzyme. However, it was subsequently demonstrated by Berry *et al.* [3] that ID-1 contains selenocysteine rather than cysteine at the active site. They proposed a modified mechanism of inhibition of ID-1 by PTU, based on the scheme originally proposed by Leonard and Visser, but involving formation of an enzyme-Se-S-PTU adduct rather than the previously postulated mixed disulfide [4]. This modified scheme raised the possibility that the selenium analog of PTU (PSeU) might be a more potent inhibitor of ID-1 than PTU itself, as formation of the Se-Se bond would be expected to occur more readily than formation of the Se-S bond. Seleno compounds are usually more reactive (nucleophilic) than their sulfur analogs.

In the present study, we developed a procedure for synthesizing PSeU, and we compared PSeU with PTU for inhibition of ID-1 in rat liver microsomes. PSeU and PTU were also compared for their ability

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§ Abbreviations: PTU, 6-propyl-2-thiouracil; PSeU, 6-propyl-2-selenouracil; MMI, 1-methyl-2-mercaptoimidazole; MSeI, 1-methyl-2-selenoimidazole; TPO, thyroid peroxidase; ID-1, Type I iodothyronine deiodinase; T_4, thyroxine; T_3, 3′,3,5-triiodothyronine; and rT_3, 3′,5′,3-triiodothyronine.

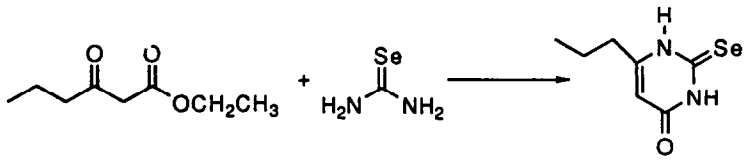

Fig. 1. Synthetic pathway for preparation of the selenium analog of PTU.

to inhibit organic iodine formation in rat thyroids *in vivo*, and for inhibition of TPO catalytic activity *in vitro*. We previously reported [5] results of a similar study with another widely used antithyroid drug, methimazole (MMI) and its selenium analog.

While the present study was in progress, Visser *et al.* [6] reported that the selenium analog of PTU is about twice as potent as PTU as an inhibitor of ID-1 activity. However, as indicated below, we observed little difference between the two drugs in their inhibitory effects on ID-1.

MATERIALS AND METHODS

Synthesis of the selenium analog of PTU. PSeU was prepared by condensation of selenourea with ethyl 3-ketohexanoate (Fig. 1), analogous to the published procedure for the preparation of PTU [7]. The product was a colorless crystalline solid, m.p. 189–190°. It was homogeneous on reversed-phase HPLC, and was characterized by proton and carbon NMR, IR, and elemental analysis. PSeU appears to be slightly sensitive to light and oxygen, slowly developing a pink coloration (finely divided red elemental selenium) on standing for extended periods in diffuse light. Details of its preparation and characterization will be published separately.*

TPO. Highly purified porcine TPO was prepared as previously described [8, 9]. The value for A_{412}/A_{280} was 0.48.

Measurement of ID-1 activity. The incubation system generally contained 0.5 μM ^{125}I-rT_3, 5 mM dithiothreitol, 25 μg/mL rat liver microsomal protein, and varying concentrations of drug (0.1 to 3 μM), in 65 mM phosphate buffer, pH 7.2, containing 2 mM EDTA. The incubation period was usually 20 min, and deiodination was measured by the increase in $^{125}I^-$, corrected for a blank. Deiodinase activity was expressed as picomoles rT_3 deiodinated per minute per milligram of rat liver microsomal protein. A more detailed description of the procedure was reported previously [5].

Inhibition of TPO-catalyzed iodination by PTU and PSeU; concentration–inhibition curves. The incubation system contained 5 nM highly purified porcine TPO, 100 μM ^{125}I-iodide, 0.5 mg/mL BSA, varying concentrations of drug, and 100 μM H_2O_2, in 65 mM phosphate buffer, pH 7.0. As described previously [5], the reaction was started with H_2O_2 and stopped after 1 min by inactivating the TPO with a large excess of MMI. Organically bound ^{125}I was determined by paper chromatography, and results were expressed as percent of the control value obtained in the absence of drug.

Inhibition of TPO-catalyzed guaiacol oxidation by PTU and PSeU. The incubation system contained 10 nM highly purified porcine TPO, 2 mM guaiacol, 0.5 mg/mL BSA, varying concentrations of drug, and 314 μM H_2O_2. As described previously [5], the reaction was started in a cuvette with H_2O_2, and ΔA_{470} was measured at 15 sec. Results were expressed as percent of the control value (no drug).

Inhibition of organic iodine formation in thyroids of rats injected with PTU or PSeU. Two separate experiments were performed. In each experiment, the rats were divided into 6 groups, each containing 2 or 3 animals. The rats were injected i.p. with: (1) saline (controls), (2) PSeU, 0.1 μmol/100 g, (3) PSeU, 0.3 μmol/100 g, (4) PSeU, 1 μmol/100 g, (5) PTU, 0.1 μmol/100 g, or (6) PTU, 0.3 μmol/100 g. The procedure for measurement of organic iodine formation in the thyroid was exactly as described in our previous study [5], except that whole thyroid homogenate was used, rather than thyroid supernatant.

Time-course of inhibition of TPO-catalyzed iodination by PTU and PSeU. The incubation mixture (500 μL) contained 10 nM TPO, 100 μM $^{125}I^-$, 0.5 mg/mL BSA, 1 mg/mL glucose, 0.5 μg/mL glucose oxidase, and various concentrations of PTU or PSeU, in 67 mM phosphate buffer, pH 7.0. The reaction was started at 37° by the addition of glucose oxidase. At intervals of 5, 15, 30, and 45 min, 50 μL of incubate was added to a small plastic tube containing 5 μL of 0.1 M MMI to stop the reaction. The tubes were kept in an ice bath, and after all the samples had been collected, 25 μL was applied to a filter paper strip for chromatography in collidine-NH_4OH [10]. The fraction of the total ^{125}I on the paper that remained at the origin (organically bound ^{125}I) was determined, and these values were plotted against time of incubation.

Simultaneous measurement of inhibition of iodination and of TPO guaiacol activity by PTU and PSeU. The incubation mixture was exactly the same as described in the previous section, except that the total volume was 1 mL. At intervals of 5, 15, 30, 45, and 60 min, aliquots were withdrawn for measurement of iodination and of residual guaiacol activity. The procedure for measurement of iodination was the same as that described in the previous section. For measurement of guaiacol activity, 100 μL of incubate was added to a cuvette containing 2 mL of 33 mM guaiacol and 0.05% BSA in 67 mM phosphate buffer, pH 7.0. Immediately thereafter, the reaction was started by addition of

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Table 1. Inhibition of rat liver microsomal ID-1 by PTU and PSeU

Drug concentration (μM)	Percent of control activity							
	Expt. 1		Expt. 2		Expt. 3*		Expt. 4†	
	PTU	PSeU	PTU	PSeU	PTU	PSeU	PTU	PSeU
0.1	64	58	64 ± 2	61 ± 4	85 ± 3	83 ± 3	94 ± 1	89 ± 4
0.3	35	32	34 ± 1	29 ± 1	80 ± 2	81 ± 4	76 ± 1	74 ± 3
1.0	13	13	13 ± 1	11 ± 0.2	47 ± 1	41 ± 1	47 (N = 2)	38 ± 1
3.0	3	5	5 ± 0.7	4 ± 0.4	20 ± 2	17 ± 1		

Assay conditions are given in Materials and Methods. Values in Expt. 1 are the averages of duplicate measurements; values in Expt. 2–4 are means ± SD of triplicate measurements. Control values (pmol rT_3 deiodinated/min/mg protein): Expt. 1, 430; Expt. 2, 367 ± 7.5; Expt. 3, 845 ± 56; and Expt. 4, 910 ± 17.

* 12.5 μg/mL microsomal protein.

† 11-min incubation.

10 μL of 66 mM H_2O_2 with a Calbiochem plumper, and ΔA_{470} was recorded at 1 min. A similar experiment was performed using MMI and its selenium analog, MSeI.

RESULTS

Effect of PTU and PSeU on ID-1 activity. PTU and PSeU were tested at 0.1, 0.3, 1, and 3 μM for inhibition of rat liver microsomal activity. Results of four separate experiments are shown in Table 1. The two drugs appeared to be about equipotent in their inhibitory effect. However, there was a tendency for PSeU to show slightly more inhibitory effect than PTU. Of the 15 pairs of comparisons in Table 1, the inhibitory effect of PSeU was higher in 12. The probability that this would occur by chance is <0.04 (two-tailed binomial test), if the two drugs were equipotent inhibitors.

Inhibition of TPO-catalyzed iodination by PTU and PSeU. Concentration–inhibition curves are shown in Fig. 2 for the effects of PTU and PSeU on TPO-catalyzed iodination of BSA. Based on the concentration required for 50% inhibition, PTU appeared to be very slightly more potent than PSeU.

Inhibition of TPO-catalyzed guaiacol oxidation by PTU and PSeU. These results are shown in Fig. 3. At low drug concentrations (5 and 10 μM), no difference in inhibitory potency was observed. However, when the drug concentration was raised to 25 and 50 μM, PSeU appeared to be slightly more inhibitory.

Non-enzymatic oxidation of PSeU by H_2O_2. Seleno compounds are more susceptible to oxidation than their sulfur analogs. It was of interest, therefore, to check whether non-enzymatic oxidation of PSeU by the excess H_2O_2 used in the guaiacol and iodination assays might interfere with the validity of the assay. To test this possibility, we measured the rate of disappearance of 25 μM PSeU in the presence of 100 and 300 μM H_2O_2 by following the decrease in A_{304} (λ_{max} for PSeU) in a Cary 219 spectrophotometer. With 300 μM H_2O_2 (the concentration used in the guaiacol assay), we observed a decrease of 8% in 15 sec (the time interval used for the guaiacol assay). With 100 μM H_2O_2 (the concentration used in the iodination assay), there was a decrease of 10% in 1 min (the time interval used in the iodination assay). PTU under the same conditions showed no decrease in A_{274} (λ_{max}), even after 5 min. Based on these data, the average decrease in PSeU concentration

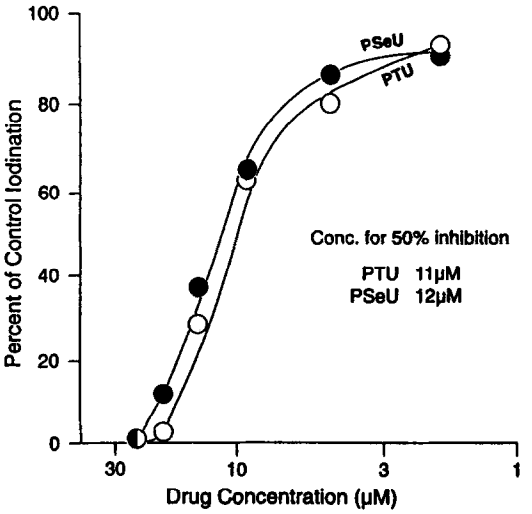

Fig. 2. Concentration–inhibition curves for TPO-catalyzed iodination of BSA by PTU and PSeU. The incubation mixture contained 5 nM TPO, 100 μM ^{125}I-iodide, 0.5 mg/mL BSA, varying concentrations of drug, and 100 μM H_2O_2, in phosphate buffer at pH 7.0 and 37°. The reaction was started with the H_2O_2 and stopped after 1 min by the addition of a large excess of MMI. In the control sample, 25 nmol I/mL was organically bound to BSA. Iodination in the presence of drug is plotted as percent of the control. All samples were incubated in duplicate, and each point represents the mean of closely agreeing duplicate determinations. A second experiment, performed under the same conditions, yielded 11 and 14 μM for the values of PTU and PSeU, respectively, required for 50% inhibition.

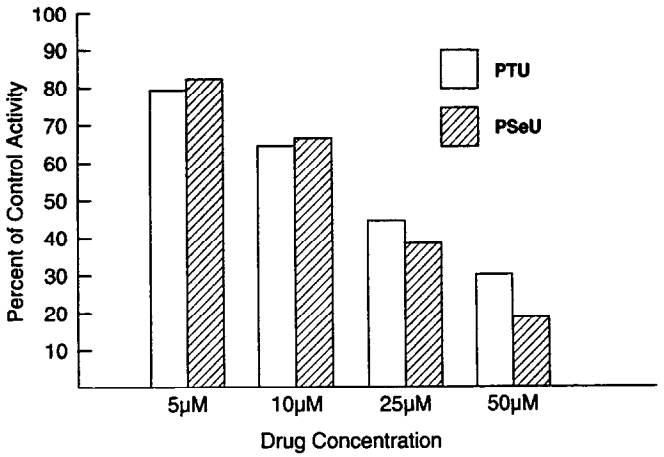

Fig. 3. Inhibition of TPO-catalyzed guaiacol activity by PTU and PSeU. The incubation mixture contained 10 nM TPO, 2 mM guaiacol, 0.5 mg/mL BSA, varying concentrations of drug, and 314 μM H_2O_2, in phosphate buffer, pH 7.0, at 24°. The reaction was started with H_2O_2 directly in a cuvette, and ΔA_{470} was measured at 15 sec. The control value (no drug) for ΔA_{470} was 0.356. Values for ΔA_{470} in the presence of drug are plotted as percent of the control. All samples were incubated in duplicate, and each result represents the average of closely agreeing duplicate determinations. Similar results were obtained in two other experiments.

attributable to direct H_2O_2 oxidation during the assay interval was 4–5%. This would be expected to have only a minor effect on the relative assay values for PTU- and PSeU-inhibited TPO activity.

Time-course of inhibition of TPO-catalyzed iodination by PTU and PSeU. These results are shown in Fig. 4. The iodination system differed from that in Fig. 2 in the use of glucose-glucose oxidase as the source of H_2O_2. Under these conditions, H_2O_2 generation is the rate-limiting step in iodination. In the presence of 10 μM PTU or PSeU, iodination was inhibited only transiently. After about 2 min there was escape from inhibition, and iodination proceeded at a rate very close to that observed in the absence of drug. However, when the drug concentration was raised to 50 μM, iodination remained completely inhibited by PSeU throughout the 45-min incubation period. With 50 μM PTU, a slight escape from inhibition was observed after 15 min.

We have reported previously [11] that inhibition of TPO-catalyzed iodination by PTU (and MMI) can be reversible or irreversible, depending on the relative concentrations of drug and iodide. Whether inhibition is reversible or irreversible depends on the relative rates of drug metabolism by the enzyme and inactivation of enzyme by the drug. With a low concentration of drug relative to I^- (10 μM PTU in Fig. 4), drug metabolism was very rapid compared with enzyme inactivation. By 2 min most of the drug had been metabolized, whereas only a small fraction of the TPO was inactivated. However, with a higher concentration of drug relative to I^- (50 μM PTU in Fig. 4), inactivation of TPO was very rapid, and only a small fraction of the drug was metabolized. The results in Fig. 4 suggest that the mechanism of inhibition of iodination by PSeU is very similar to that of PTU. The escape from inhibition observed with 10 μM PSeU suggested that, like PTU, the drug was rapidly metabolized under these conditions. This was demonstrated spectrophotometrically by following the decrease in A_{304} after the start of incubation (data not shown). The irreversible inhibition observed with 50 μM PSeU suggests that under these conditions the TPO was rapidly inactivated. More direct evidence for inactivation of TPO by 50 μM PSeU is presented in the following section.

Simultaneous measurement of effects of 50 μM PTU and PSeU on iodination and on TPO guaiacol activity. In this experiment (Fig. 5), the incubation mixture was identical to that used in Fig. 4, except that only one drug concentration (50 μM) was employed. At intervals after the start of the reaction, aliquots of the reaction mixture were withdrawn for measurement of iodination and of residual TPO activity (determined by guaiacol assay). Similar measurements were made on a control sample containing no drug. In the presence of 50 μM PSeU, TPO was rapidly inactivated. At 5 min (the earliest time interval measured), almost no guaiacol activity could be detected. As might be expected, no iodination occurred throughout the 60-min incubation period. In the sample containing 50 μM PTU, TPO was also readily inactivated. However, inactivation was apparently incomplete, and some escape from inhibition occurred after 15 min, more than was observed in Fig. 4 under similar conditions of incubation. Even in the complete absence of drug, a slower but steady inactivation of TPO was observed. Similar observations were reported previously [11]. Inactivation of TPO under these conditions was most likely due to formation of Compound III by the steadily increasing concentration of H_2O_2 generated by glucose-glucose oxidase. This reaction was probably facilitated by the steadily decreasing concentration of iodide, a known protector against TPO degradation [12].

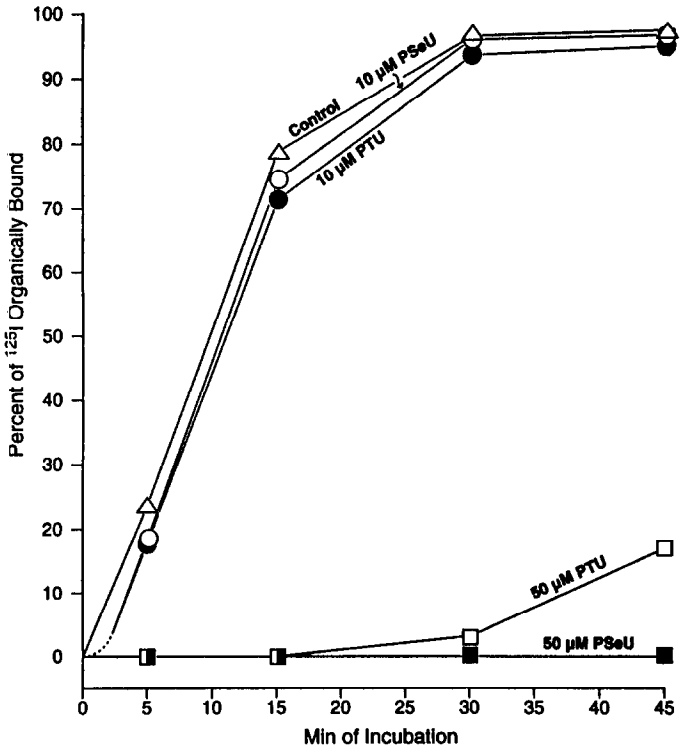

Fig. 4. Time-course of inhibition of TPO-catalyzed iodination by PTU and PSeU. The incubation mixture (500 μL) contained 10 nM TPO, 100 μM $^{125}I^-$, 0.5 mg/mL BSA, varying concentrations of drug, 1 mg/mL glucose, and 0.5 μg/mL glucose oxidase, in phosphate buffer at pH 7.0 and 37°. The reaction was started with the glucose oxidase. Aliquots of the reaction mixture were removed at the indicated intervals and treated with a large excess of MMI to stop the reaction. Values are expressed as percent of added $^{125}I^-$ organically bound to BSA. The experiment was repeated with very similar results.

The results with PTU and PSeU contrast with results obtained with MMI and its selenium analog (MSeI). We have reported previously [5], that, in the presence of H_2O_2, TPO is rapidly inactivated by MMI, but not by MSeI. It was of interest, therefore, to carry out a study with MMI and MSeI similar to that in Fig. 5. These results are shown in Fig. 6. In agreement with our previous study [5], inhibition of iodination by 50 μM MSeI was only transient, and complete escape from inhibition occurred after 15 min of incubation. Surprisingly, the guaiacol assay performed on the incubation mixture after 5 min of incubation appeared to show a very significant degree of inactivation. However, at 15 min the guaiacol activity was much higher than at 5 min and was even higher than the control value. Similar results were obtained in a second experiment. At 30 min the guaiacol activity in the presence of 50 μM MSeI was several-fold greater than the control. It is apparent, therefore, that the low value for the guaiacol activity at 5 min does not represent inactivation of the TPO. Possibly, some transient oxidation product of MSeI interfered with the guaiacol assay for TPO. Further studies are required to elucidate the mechanism of this inhibitory effect. In contrast to the results with MSeI, 50 μM MMI rapidly and irreversibly inactivated TPO. Guaiacol activity was reduced to undetectable levels, and iodination was completely inhibited throughout the 60 min incubation period.

Antithyroid effects of PTU and PSeU in vivo. Table 2 shows the results of two experiments in which PTU and PSeU were compared for their ability to inhibit thyroidal organic iodine formation after injection into rats. Similar results were obtained in both experiments. Based on the fraction of thyroidal ^{125}I that was organically bound, PSeU had no significant inhibitory effect on thyroidal organic iodine formation, whereas potent inhibition was observed with PTU. As expected [13], PTU also inhibited total thyroidal ^{125}I uptake. In the rats that received 0.3 μmol/100 g body weight, uptake was inhibited about 80%. However, PSeU also showed some inhibition of thyroidal ^{125}I uptake, despite its lack of effect on organic iodine formation. Such an effect is more characteristic of perchlorate, a drug that inhibits iodide transport but not organification of iodine. The inhibition was more pronounced in Experiment 1, but in neither experiment was the inhibitory effect dose related. Although it is possible that PSeU or one of its metabolic products has an inhibitory effect on iodide transport, such an effect would be expected to be dose related. Further studies are required to explain the observed inhibitory effect of PSeU on thyroidal ^{125}I uptake. Such inhibition was not observed with the selenium analog of MMI in our previous study [5].

In the last two columns of Table 2, organic iodine formation is calculated as percent of the injected

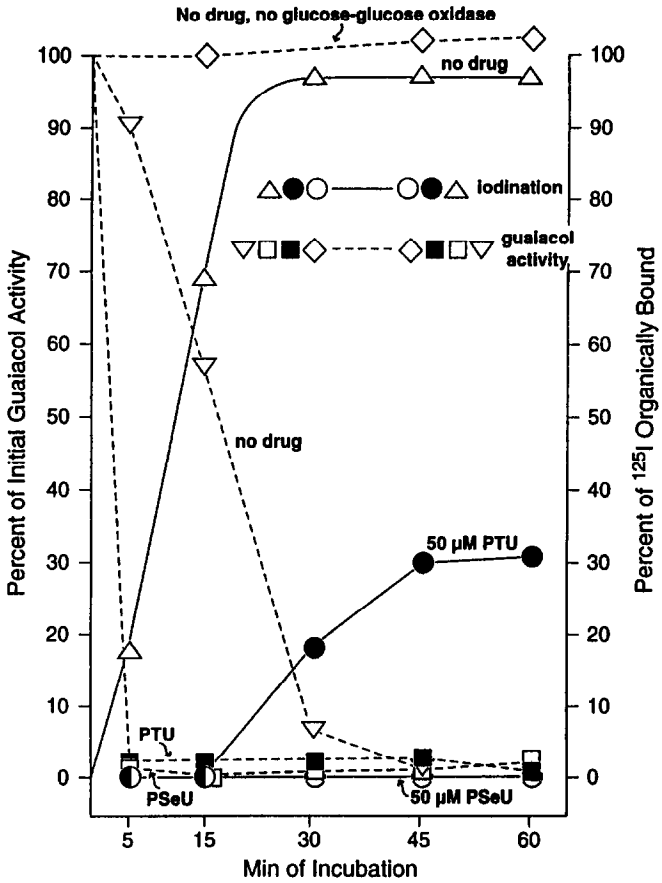

Fig. 5. Simultaneous measurement of effect of 50 μM PTU and PSeU on iodination and on TPO guaiacol activity. The incubation mixture (1 mL) contained 10 nM TPO, 100 μM $^{125}I^-$, 0.5 mg/mL BSA, 50 μM PSeU or PTU, 1 mg/mL glucose, and 0.5 μg/mL glucose oxidase in phosphate buffer, pH 7.0, at 37°. The reaction was started with the glucose oxidase. At 5, 15, 30, 45 and 60 min, aliquots were removed for measurement of iodination and residual guaiacol activity. For measurement of iodination, 50 μL of incubate was added to 5 μL of 0.1 M MMI to stop the reaction, and organically bound ^{125}I was determined by paper chromatography. Guaiacol activity was determined on a 100-μL aliquot of the incubate. The initial value for the guaiacol assay (ΔA_{470} in 1 min) was 0.232. Similar results were obtained in a second experiment performed under the same conditions. Key: (△) control iodination (no drug); (●) iodination in the presence of 50 μM PTU; (○) iodination in the presence of 50 μM PSeU; (▽) control guaiacol activity (no drug); (■) guaiacol activity in the presence of 50 μM PTU; (□), guaiacol activity in the presence of 50 μM PSeU; and (◇) guaiacol activity in the absence of drug and of glucose-glucose oxidase. Iodination is indicated by the solid lines, guaiacol activity by the dashed lines.

dose of ^{125}I present as organic ^{125}I per 10 mg of thyroid tissue. If the lower thyroidal ^{125}I uptake in the PSeU-injected rats is indeed due to an effect on iodide transport, this method of comparing PTU and PSeU for inhibition of thyroid organic iodine formation is invalid, and more weight should be given to the measurement based simply on the fraction of thyroidal ^{125}I in organic form. In any case, it is clear that, *in vivo*, PTU was a much more potent inhibitor of organic iodine formation than PSeU, in contrast to the nearly equal potencies of the drugs as inhibitors of TPO-catalyzed iodination, shown in Fig. 2. This same discrepancy between *in vivo* and *in vitro* potency was observed previously in our study comparing MMI and MSeI [5].

DISCUSSION

The discovery that ID-1 contains selenocysteine rather than cysteine at the active site [3] raised the possibility that the seleno analogs of MMI and PTU might be better inhibitors of ID-1 than their respective parent compounds. In a previous study [5], we investigated this possibility with MMI and MSeI, and we observed that replacement of the sulfur in MMI with selenium only marginally increased its inhibitory effect on ID-1. MMI itself was essentially inactive as an inhibitor of ID-1, in agreement with earlier reports [14].

In the present study, we developed a procedure for the synthesis of the selenium analog of PTU,

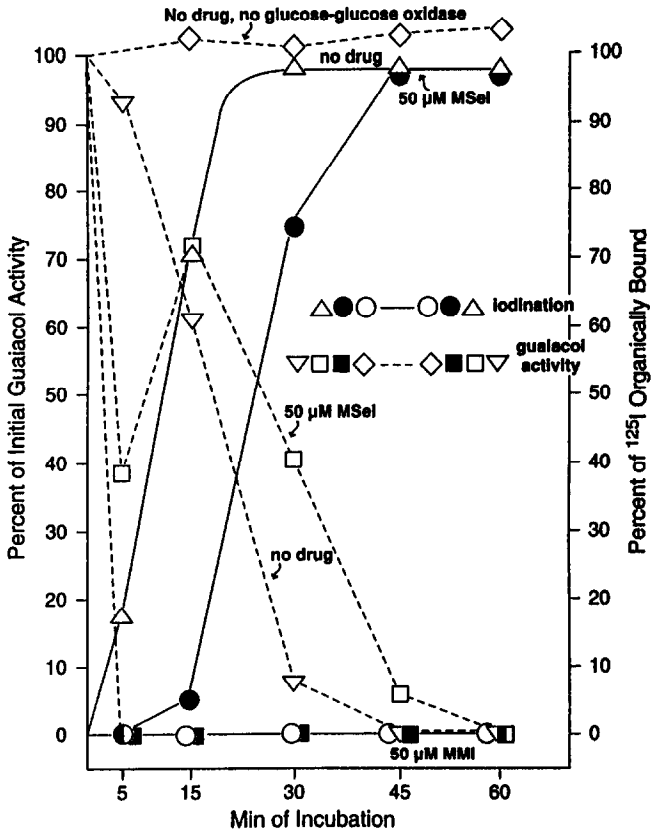

Fig. 6. Simultaneous measurement of the effect of 50 μM MMI and 50 μM MSeI on iodination and on TPO guaiacol activity. This experiment was performed in exactly the same manner as described in the legend of Fig. 5, except for the use of MMI and MSeI instead of PTU and PSeU. The initial value for the guaiacol assay (ΔA_{470} in 1 min) was 0.256. Similar results were obtained in a second experiment performed under the same conditions. Key: (△) control iodination (no drug); (●) iodination in the presence of 50 μM MSeI; (○) iodination in the presence of 50 μM MMI; (▽) control guaiacol activity (no drug); (□) guaiacol activity in the presence of 50 μM MSeI; (■) guaiacol activity in the presence of 50 μM MMI; and (◇) guaiacol activity in the absence of drug and of glucose-glucose oxidase. Iodination is indicated by the solid lines, guaiacol activity by the dashed lines.

Table 2. Inhibition of organic iodine formation in thyroids of rats injected with PSeU or PTU

Drug	Injected dose (μmol/100 g body wt)	Thyroidal ^{125}I uptake (% injected dose/10 mg tissue)		Percent of thyroidal ^{125}I organically bound		Organic ^{125}I in thyroid: Percent of injected dose/10 mg tissue		Organic ^{125}I in thyroid: Percent of control	
		Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2
None	Saline injected	1.66 ± 0.30	1.65 (2)	92.2 ± 1.1	91.9	1.53	1.52		
PSeU	0.1	1.13 ± 0.35	1.40 (2)	92.4 ± 1.1	90.7	1.04	1.27	68	84
PSeU	0.3	1.21 ± 0.23	1.36 ± 0.2 (3)	90.6 ± 2.5	92.1 ± 0.35	1.10	1.25	72	82
PSeU	1.0	1.01 ± 0.12	1.31 (2)	90.6 ± 2.3	90.0	0.92	1.17	60	77
PTU	0.1	0.57 ± 0.13	0.49 ± 0.2 (3)	65.6 ± 19	64.8 ± 5.9	0.37	0.32	24	21
PTU	0.3	0.31 ± 0.05	0.32 ± 0.04 (3)	29.6 ± 9.1	30.2 ± 12	0.092	0.097	6.0	6.4

In Expt. 1, values are means ± SD, N = three rats per group. In Expt. 2, values are presented either as averages of two rats per group or as means ± SD of three rats per group. See Materials and Methods for the procedure used.

and we compared PTU and PSeU for their inhibitory action on rat liver ID-1. The two drugs were also compared for their ability to inhibit organic iodine formation in rat thyroids *in vivo*, and for inhibition of TPO catalytic activity *in vitro*.

PTU, unlike MMI, is a potent inhibitor of ID-1. Visser *et al.* [14] called attention to the importance of the methyl substitutent at N_1 to explain the great difference between PTU and MMI as inhibitors of ID-1. They observed that 2-mercaptoimidazole (MMI lacking the N-methyl substitutent) has substantial ID-1 inhibitory activity, about 10% that of PTU. Even more striking was the observation that insertion of a methyl group at N_1 of PTU (1-methyl PTU) completely abolished ID-1 inhibitory activity. These findings suggest that formation of a hydrogen bond between the enzyme and the H atom on N_1 in PTU may be involved in the inhibitory action of the drug.

PTU and its selenium analog (PSeU) were tested at 0.1 to 3 μM for inhibition of ID-1 in four separate experiments. There was little difference in the inhibitory effect of the two drugs, but statistical analysis suggested a higher potency for PSeU ($P < 0.04$). However, the magnitude of this difference was small, averaging less than 15%. This is considerably less than the 2-fold greater potency for PSeU reported by Visser *et al.* [6].*

There was considerable variation among the control values in the experiments of Table 1. Higher values were obtained when the incubation time was reduced from 20 to 11 min, and also when the concentration of microsomal protein was reduced from 25 to 12.5 μg/mL. The former result suggests that the rate of deiodination was not linear with time during a 20-min incubation interval. This was confirmed in other experiments (data not shown). However, the 20-min time interval was generally used because this permitted more samples to be included in the same experiment, allowing us to test four different drug concentrations in triplicate. The effect of reducing the microsomal protein concentration, shown in Table 1, was much less marked in other experiments (data not shown). The degree of inhibition by both PTU and PSeU was lower in the experiments with the higher control values. However, the *relative* degree of inhibition by the two drugs was largely independent of the control value for deiodinase activity. This lends support to the conclusion that the two drugs inhibit the deiodinase by a similar mechanism.

We also compared PTU and PSeU as inhibitors of TPO catalytic activity. Concentration–inhibition curves were prepared for TPO-catalyzed iodination of BSA (Fig. 2). Based on the concentration required for 50% inhibition, PTU appeared to be slightly more potent than PSeU. This was also observed in experiments in which the incubation time was reduced from 1 min to 30 or 15 sec (data not shown). These observations render it unlikely that non-enzymatic oxidation of PSeU affected the relative potencies of PTU and PSeU shown in Fig. 2.

PTU and PSeU were also nearly equipotent as inhibitors of TPO-catalyzed guaiacol oxidation (Fig. 3). Our results with PTU and PSeU-inhibition of TPO guaiacol activity are in marked disagreement with those of Aboul-Enein *et al.* [15], who recently reported that PSeU is 5-fold more potent than PTU in this assay. The reason for this marked discrepancy is not clear. It may be noted, however, that Aboul-Enein *et al.* used a very crude preparation of TPO in their study. Their assay mixture contained 333 μg enzyme protein/mL, compared with 1.3 μg/mL in our procedure. It is also of interest that in our previous study [5] comparing MMI and MSeI, we observed that MSeI was only about 25% as potent as MMI as an inhibitor of TPO guaiacol activity.

Studies of the time-course of iodination in the presence of PTU and PSeU (Fig. 4) gave essentially similar results for the two drugs. When the drug concentration was 10 μM, inhibition of iodination was observed only during the first few minutes. Thereafter, there was escape from inhibition, and iodination proceeded almost at the control rate. When the PSeU concentration was raised to 50 μM, however, iodination remained completely inhibited throughout the course of the incubation. With PTU partial escape from inhibition occurred after 15 min.

The results obtained with PSeU contrast greatly with those observed with MSeI in our previous study [5]. MSeI, even when present at 50 μM, inhibited iodination only during the first 15 min of incubation. Thereafter, there was almost complete escape from inhibition, and iodination proceeded at a rate very close to that in the control. It was shown that MSeI, unlike MMI, does not inactivate oxidized TPO. In the present study, we observed that 50 μM PSeU completely inactivated TPO and irreversibly inhibited TPO-catalyzed iodination. Under the same conditions, PTU was somewhat less effective than PSeU. Inactivation of TPO appeared to be not as complete, and partial escape from inhibition of iodination was observed with PTU.

As suggested previously [12, 16], rapid inactivation of TPO by MMI and PTU very likely involves a suicide reaction between an oxidized form of the drug and the heme group of the enzyme. Presumably, therefore, based on the results of the present study, an analogous reaction occurs between PSeU and oxidized TPO. MSeI, on the other hand, as shown previously [5], and as confirmed in the present study, does not display properties of a suicide inhibitor.

The antithyroid action of PTU and PSeU *in vivo* was also investigated in the present study. Rats were injected with varying doses of the drugs, and inhibition of organic iodine formation in the thyroid was measured. In the rats injected with 1 μmol PSeU/100 g body weight, the fraction of the ^{125}I in the gland present in organic form was not significantly different from that in saline-injected controls, whereas in rats injected with 0.3 μmol PTU/100 g body weight, only 30% of the ^{125}I in the thyroid was organically bound. The much greater inhibitory effect of PTU *in vivo* contrasts greatly with the *in vitro* results (Fig. 2), in which PTU and PSeU were essentially equipotent as inhibitors of TPO-catalyzed

* Because of this discrepancy, we sent a sample of our PSeU preparation to Professor Visser for testing with his deiodinase procedure. It is of interest that with our PSeU preparation he observed only a slightly higher potency than with PTU.

iodination. A similar discrepancy between *in vivo* and *in vitro* effects was observed previously with MMI and MSeI [5]. As no significant difference was observed in the rate of peripheral metabolism of these two drugs, we suggested that MSeI is less well concentrated by thyroid than is MMI. A similar explanation may be offered for the discrepancy between the *in vivo* and the *in vitro* effects of PTU and PSeU. We have reported previously [17] that concentration of PTU by the thyroid is essential for its antithyroid activity.

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