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UNUSUAL CHARACTERISTICS OF THE DOSE-DEPENDENT UPTAKE OF PROPYLTHIOURACIL BY THYROID GLAND *IN VIVO*

EFFECTS OF THYROTROPIN, IODIDE OR PHENOBARBITAL PRETREATMENT

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Abstract—Dose dependency of propylthiouracil (PTU) uptake by the thyroid gland was investigated in intact, adult male rats after a single intraperitoneal dose of PTU. PTU pharmacokinetics in rat serum, liver and lung were independent of the size of this single dose of PTU. However, in the thyroid gland, PTU concentrations corrected for dose were disproportionately high at low doses. At low PTU doses, a transport mechanism contributes substantially to thyroidal PTU uptake, but at high PTU doses this transport system becomes saturated. Thus, at serum PTU concentrations of 5 μg/ml or above, thyroid to serum (T/S) PTU ratios are independent of PTU dose and serum concentration. Alteration in the functional status of the thyroid changed the relationship between serum and thyroidal PTU concentrations. Thyroxine administration prior to an intraperitoneal PTU dose of 5 mg/kg reduced ratios of T/S PTU concentrations. However, thyrotropic hormone (TSH) preadministration had little effect on early thyroidal PTU concentration, but appeared to exert an effect at later times. Chronic administration of either potassium iodide or phenobarbital prior to PTU increased T/S PTU ratios, again suggesting an effect of TSH on PTU metabolism in the thyroid.

Thyroidal accumulation and subsequent elimination of the antithyroid drugs propylthiouracil (PTU) [1, 2] and methimazole [3], as well as of the thiourea [4, 5] and thiouracil [5, 6], have been investigated in rats and humans. These studies relied heavily on ratios of thyroid to serum drug concentration because such ratios depend primarily on the passage of drug from serum into thyroid gland, whereas absolute concentrations in serum or thyroid tissue can be altered by numerous environmental factors. Previously reported ratios greater than unity for thyroid to serum concentrations of antithyroid compounds [1–6] could have arisen from thyroidal binding of PTU, carrier-mediated PTU uptake, or a combination of these and other factors. Pharmakiotis and Alexander [7] observed that, although PTU metabolites in rat thyroid were bound to protein, PTU itself was not covalently bound. Binding of PTU to thyroid, however, could occur through other types of chemical bonds; if such binding does not take place to an appreciable extent, the work of Pharmakiotis and Alexander [7] raises the possibility that PTU accumulates in the thyroid by active transport. Previously published work has not dealt with relationships between thyroidal uptake of antithyroid drugs and serum drug concentrations, nor with the potential influence on thyroidal drug accumulation of endogenous or exogenous substances. Accordingly, the present study evaluates thyroidal PTU uptake as a function of serum PTU concentration, dose, and prior administration of thyrotropic hormone (TSH), thyroxine or potassium iodide (KI).

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MATERIALS AND METHODS

Materials. Adult, male Sprague–Dawley rats (Charles River Breeding Laboratories, Inc., Wilmington, MA), weighing 200–300 g and maintained on Purina Formulab Chow No. 5008 (iodine content 1.7 ppm) and tap water *ad lib.* were used. [^{35}S]PTU (Amersham, Arlington Heights, IL) had a specific activity at the time of injection of either 127 mCi/m-mole or was combined with unlabeled PTU to a specific activity of 0.34 mCi/m-mole. The radiochemical purity was 93 per cent, as determined by thin-layer chromatography.

Methods. For the dose dependency experiment, 96 rats, each to receive a single intraperitoneal dose of PTU, were divided into four groups of 24 each. Each group was given a different dose of PTU. Group A received only [^{35}S]PTU at a dose of 1 μCi/100 g body weight, equivalent to a PTU dose of 0.01 mg/kg. Groups B, C and D received unlabeled carrier PTU in addition to the [^{35}S]PTU tracer. Group B received a 5 mg/kg dose of PTU with 1 μCi/100 g tracer. Group C received a 12.5 mg/kg PTU dose with 2.5 μCi/100 g tracer. Group D received a 25 mg/kg PTU dose with 5 μCi/100 g tracer. All injections were in 0.9% NaCl, pH 8.8.

Rats were exsanguinated by cardiac puncture during light ether anesthesia 2, 4, 6, 8, 10, 12, 18 and 24 hr after the single dose of PTU. Blood was drawn into silicon-coated, evacuated, glass tubes. In some rats the left lateral lobe of the liver and the right lobe of the lung were removed, weighed and placed on ice. Thyroid glands of all rats were removed and stored on ice until homogenization in 1 ml of cold, glass-distilled water in a glass homogenizer with a ground glass pestle. Liver and lung specimens were homogenized in glass-distilled

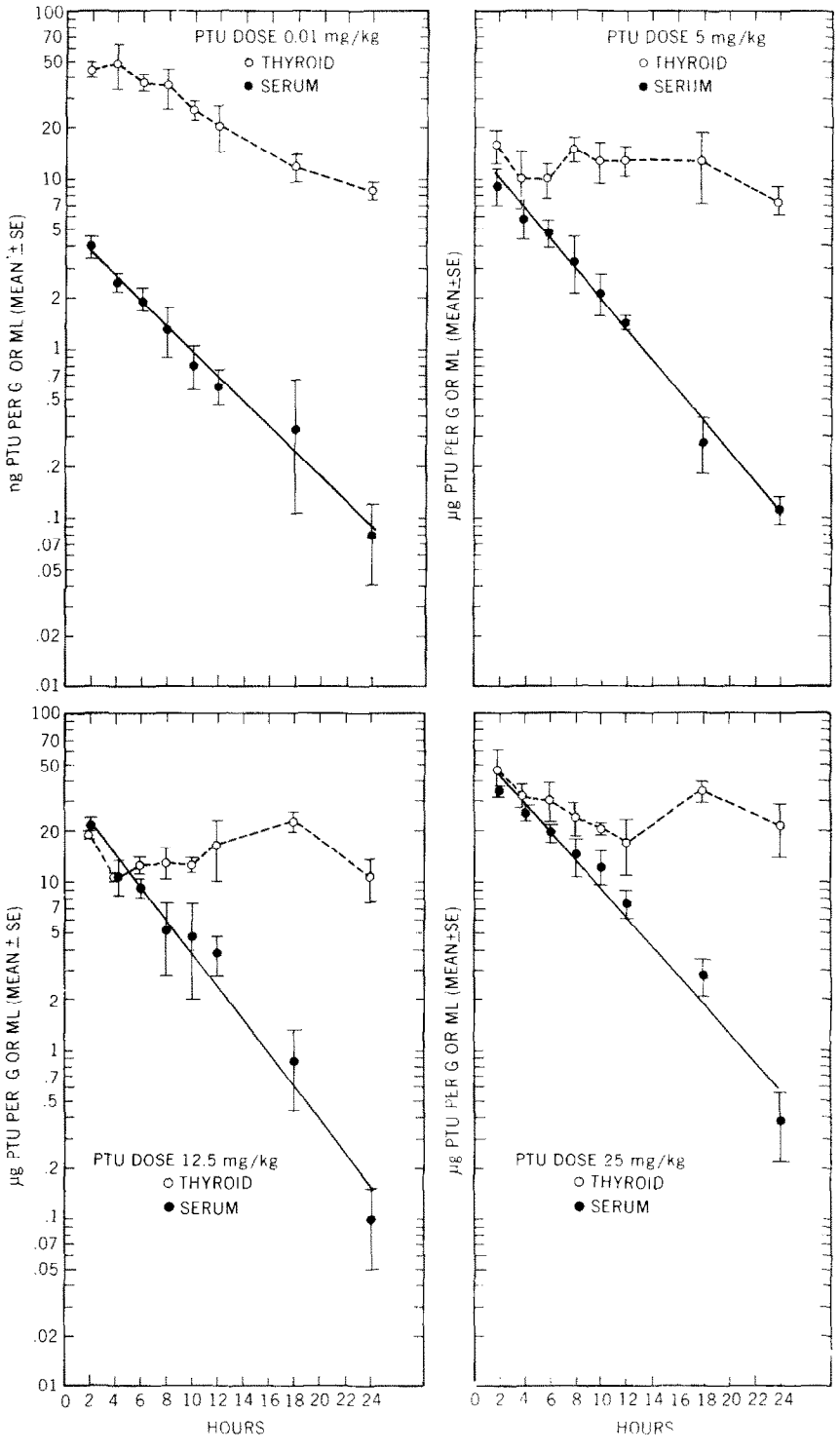

Fig. 1. PTU concentration vs time in serum (●) and thyroid (○) after a single i.p. dose of PTU of (a) 0.01 mg/kg, (b) 5 mg/kg, (c) 12.5 mg/kg and (d) 25 mg/kg. Each point represents the mean and SE of three rats.

[^{35}S]PTU concentrations were then measured in homogenates of thyroid, liver and lung.

PTU assay. A 1-ml aliquot of serum or of homogenates of thyroid, liver and lung was added to 600 mg ammonium sulfate and vortexed to dissolve the ammonium sulfate. Ten ml chloroform was added and PTU extracted by shaking for 20 min, followed by centrifugation at 1000 *g* for 10 min. An 8.5-ml aliquot of the organic phase was removed and evaporated at 35–40° on a rotary evaporator (Buchler Instruments, Fort Lee, NJ). Approximately 72 per cent of the PTU in the sample was recovered in the residue.

water using a Brinkman polytron homogenizer.

Thin-layer chromatographic analysis indicated that, for serum, thyroid, liver and lungs at 2–24 hr after administration of a 0.01 mg/kg dose of [^{35}S]PTU, less than 7 per cent of the total radioactivity in the chloroform extract failed to migrate as authentic PTU. Therefore, total radioactivity in the chloroform extract accurately reflects the amount of [^{35}S]PTU present in each of these tissues. The ascending solvent system used for analysis of the chloroform extracts [ethanol–1 M ammonium acetate (7.5:3.0) on 250 μm thickness cellulose plates] effectively separates PTU metabolites from PTU [8].

Residues of the chloroform extracts were redissolved in 50 μl chloroform and aliquots placed in scintillation vials for counting. Two ml ethanol and 10 ml of a scintillation mixture consisting of 5 g PPO* and 200 mg POPOP per litre of toluene were added to each vial. The counting efficiency of each sample was measured using an external standard. With the specific activity of the [^{35}S]PTU and the percentage recovery available, we converted the data from dis./min to ng or μg PTU per unit weight or volume.

Effect of pretreatment with TSH, KI or phenobarbital on PTU thyroidal uptake. Male Sprague–Dawley rats (200–300 g) were divided into groups of 16. Each group received a different pretreatment before a single 5 mg/kg i.p. dose of PTU with a [^{35}S]PTU tracer (1 μCi/100 g body wt). Serum and thyroid were analyzed for PTU concentrations at hourly intervals with two animals for each time point.

Thyrotropic hormone (TSH), as a sterile powder from bovine pituitary (Sigma Chemical Co., St. Louis, MO), was determined by the manufacturer to have an activity of 0.5 to 1.0 I.U./mg. Group E was pretreated with a single i.p. injection of TSH (2 I.U. in 0.9% NaCl 1 hr before injection of PTU). Group F received three i.p. doses of TSH (each TSH dose consisted of 2 I.U. 8 hr apart, with the last dose 1 hr before administration of PTU). Group G received 15 μg L-thyroxine sodium salt (Sigma) i.p. once daily for 7 consecutive days before PTU. Group H received a single dose of KI (50 mg/kg i.p. from Lyne's potassium iodide solution, N.F.) 1 hr before PTU. Group I received 5 mg KI i.p. twice daily for 3 days before PTU, with a final dose 1 hr before PTU. A dosage regimen for phenobarbital (PB) shown previously to stimulate hepatic microsomal drug-metabolizing enzymes [9] was administered to group J. This regimen consisted of PB (100 mg/kg i.p. in 0.9% NaCl) daily for 3 days prior to PTU administration.

RESULTS

Figure 1 shows the time course for the decline of PTU concentrations in serum and thyroid gland of the four groups of rats, each given a different dose of [^{35}S]PTU. Decay of PTU in serum of all four groups exhibited apparent first-order pharmacokinetics. For each group, PTU concentrations in liver and lung closely approximated those in serum. Thyroidal PTU accumulation was indicated in that PTU concentrations were higher and declined less rapidly in thyroid than in serum.

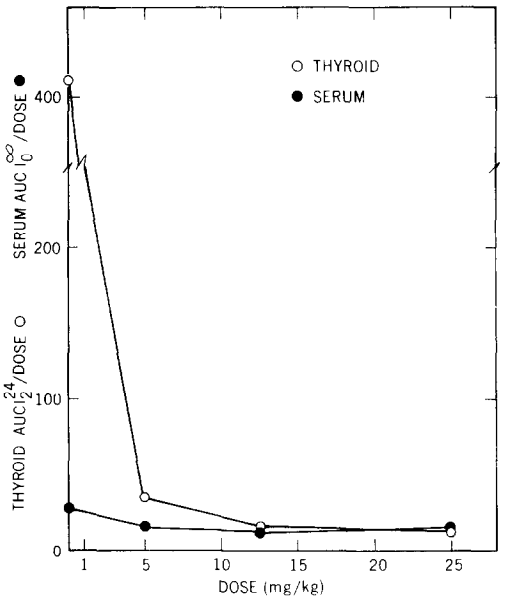

Fig. 2. Serum AUC $|_0^\infty$/dose (●) and thyroid AUC $|_2^{24}$/dose (O) vs dose. AUC means are under the curve relating plasma drug concentration to time after drug administration.

Table 1 shows pharmacokinetic parameters for PTU calculated from these data. Neither PTU elimination half-life from serum, liver or lung nor apparent volume of distribution of PTU exhibited dose dependency. In order to examine dose dependency of thyroidal PTU concentration, the area under the thyroidal PTU concentration vs time curve (AUC) from 2 to 24 hr after PTU administration was corrected for PTU dose and plotted against PTU dose (Fig. 2). The data for PTU decay in serum again showed no dose dependence, but

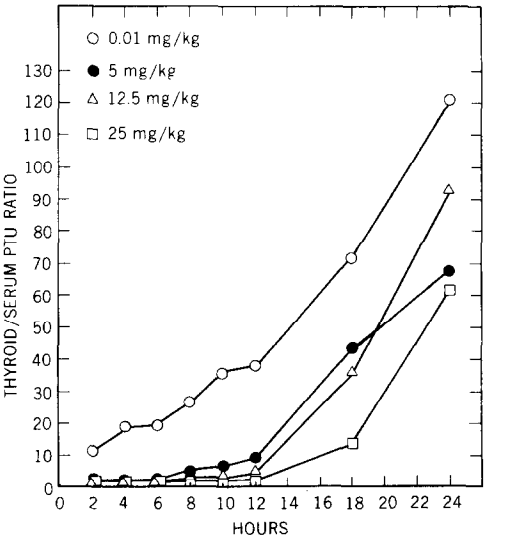

Fig. 3. Mean T/S PTU concentration ratios vs time after a single PTU dose of 0.01 mg/kg (O), 5 mg/kg (●), 12.5 mg/kg (△) and 25 mg/kg ( ). Each point represents the mean of three rats.

* PPO = 2,5-diphenyloxazole; POPOP = 1,4-bis-[2-(4-methyl-5-phenyloxazolyl)]benzene.

Table 1. Pharmacokinetic parameters for PTU in groups A–D*

	A (0.01 mg/kg)	B (5 mg/kg)	C (12.5 mg/kg)	D (25 mg/kg)
λ (hr^{-1}) from serum	0.171	0.209	0.227	0.197
$T_{\frac{1}{2}}$ (hr) from serum	4.06	3.32	3.06	3.53
Vd_{area} (l/kg)	0.437	0.315	0.370	0.330
AUC_0^{∞} ($\mu g \cdot hr/ml$) serum	28.51×10^{-3}	75.96	149.02	348.95
Thyroid $AUC\|_2^{24}$ ($\mu g \cdot hr/ml$)	410.88×10^{-3}	173.50	202.96	301.17
Body wt [9] (mean ± S. E.)	213 ± 23	260 ± 28	242 ± 10	217 ± 24

*$\lambda/_{2.303}$ represents the slope of the terminal phase of the log serum PTU concentration vs time plot [10]. $AUC|_0^{\infty}$ was calculated by trapezoidal rule and Vd_{area} by the following formula:

$$Vd_{area} = \frac{F \cdot \text{dose}}{\lambda \cdot AUC_0^{\infty}}$$

where F, the bioavailability of the dose, was assumed to be 1. Thyroid $AUC|_2^{24}$ was also calculated by trapezoidal rule.

thyroid PTU concentrations were clearly dose related, a non-zero slope for this type of plot indicating non-linear pharmacokinetics. As the PTU dose decreased, a disproportionate increase occurred in the amount of PTU corrected for dose in the thyroid.

Another useful index of thyroidal accumulation of PTU was the ratio of thyroid to serum (T/S) PTU concentration. Whereas absolute PTU concentrations in thyroid and serum are affected by several variables, including location of the i.p. injection, the extent and the rate of PTU absorption from the site of administration, and distribution to extrathyroidal tissues, T/S ratios depend primarily on rates of PTU passage between thyroid and serum.

Figure 3 shows mean T/S ratios at each time point after PTU administration for groups A, B, C and D. At all times between 2 and 24 hr after PTU administration, group A which received the lowest dose of PTU had the highest T/S ratios. Increasing the PTU dose from group B to group D decreased the T/S PTU ratios, reflecting reduced PTU uptake by the thyroid gland.

Effects on PTU accumulation in the thyroid gland of pretreatment with either TSH, thyroxine, iodide or

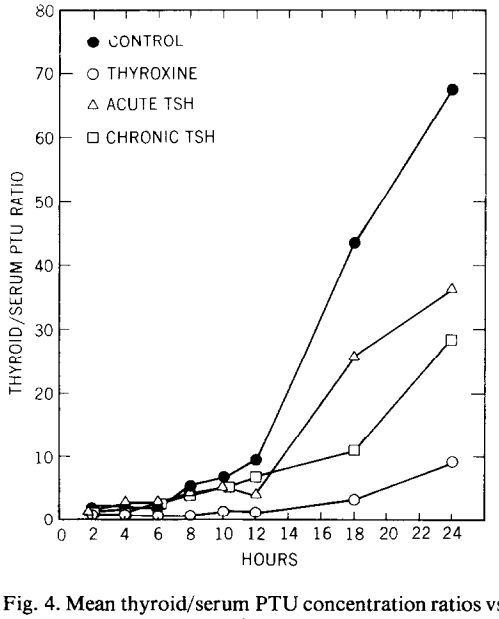

Fig. 4. Mean thyroid/serum PTU concentration ratios vs time after a PTU dose of 5 mg/kg to control (●), thyroxine (○), acute TSH (△), and chronic TSH (□) treated rats. Each point represents the mean of two rats.

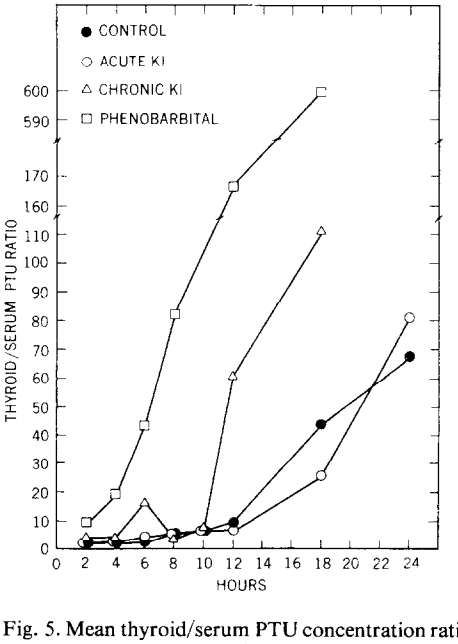

Fig. 5. Mean thyroid/serum PTU concentration ratios vs time after a PTU dose of 5 mg/kg to control (●), acute KI (○), chronic KI (△), and phenobarbital (□) treated rats. Each point represents the mean of two rats.

phenobarbital were examined using a PTU dose of 5 mg/kg. For each group, mean T/S PTU ratios were measured at each time point.

Groups E and F received TSH as a single dose of either 2 I.U. or as three doses of 2 I.U., each 8 hr apart, respectively. Figure 4 shows the mean T/S PTU concentration ratios. Both TSH treatments exerted only slight effects on PTU uptake, producing small decreases in T/S PTU ratios at times after 6 hr. Effects of TSH or thyroxine on thyroidal PTU accumulation were better illustrated by blocking endogenous TSH production and release through thyroxine pretreatment. As shown in Fig. 4, a 7-day course of thyroxine reduced T/S PTU concentration ratios drastically at all times examined.

Figure 5 shows how acute and chronic iodide pretreatment affected thyroidal PTU concentrations. A single KI dose of 50 mg i.p. given 1 hr before PTU failed to alter thyroidal PTU intake. However, chronic KI pretreatment altered thyroidal PTU concentrations. Marked increases in T/S PTU ratios occurred after a 3-day course of KI pretreatment.

Phenobarbital administered daily for 3 days stimulated thyroidal PTU uptake (Fig. 5).

DISCUSSION

These experiments performed in intact, adult male rats were designed to investigate effects of PTU dose and of pretreatment with KI, thyroxine and phenobarbital on PTU accumulation by the thyroid gland. The results disclosed that the PTU accumulated by the rat thyroid gland decreased as the dose of PTU increased. The large T/S concentration gradient suggests accumulation of PTU by carrier mediated uptake or extensive tissue binding. PTU also enters the thyroid by passive diffusion and is metabolized to some extent by the thyroid [1, 2, 11]. Our results provide evidence that thyroidal uptake of PTU is mediated by both saturable and unsaturable processes.

To identify the precise PTU serum concentration at which PTU accumulation becomes saturated, we plotted mean T/S PTU ratios for groups B (5 μg/kg), C (12.5 mg/kg) and D (25 mg/kg) against serum PTU concentration (Fig. 6). At low serum PTU concentrations (less than 5 μg/ml), T/S PTU ratios were greater than 2. At serum PTU concentrations below 5 μg/ml, the T/S ratio rose, regardless of the initial dose of PTU, suggesting that at low PTU doses active transport of PTU may occur by a carrier mechanism. Probably at low serum PTU concentrations, the saturable process contributes mainly to thyroidal PTU concentration. As serum PTU concentrations increase, passive diffusion of PTU into the thyroid makes increasing contributions, while the contribution of the saturable process becomes constant.

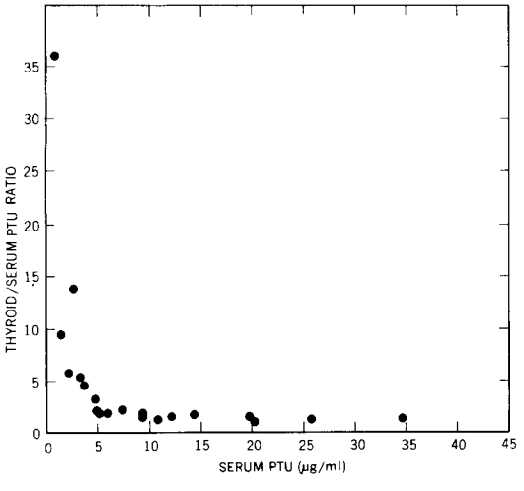

Fig. 6. T/S PTU concentration ratio vs serum PTU concentration for individual rats from groups B, C and D. T/S values greater than 35 are not included.

These studies reveal that elimination rates of PTU from serum are dose-independent, confirming a similar observation in humans after i.v. infusion of PTU [12]. Our results on liver and lung suggest that these tissues do not accumulate PTU against a concentration gradient.

The relationship between serum PTU concentration and the T/S PTU ratio changes with the functional status of the thyroid gland. TSH regulates the metabolism of many compounds within the thyroid gland. Therefore, we were very surprised that our regimen of TSH pretreatment exerted little effect on thyroidal PTU concentrations. However, T/S PTU ratios were lowered at late time points. Several explanations may be offered for our failure to observe an early stimulatory effect of TSH on PTU uptake.

First, exogenous TSH has a very short duration of action in the rat [13] and our TSH treatments may not have elevated serum TSH levels sufficiently to produce the effects we anticipated from our results with thyroxine and KI pretreatment.

Second, TSH may control thyroidal PTU concentrations in a biphasic fashion and may have accelerated both entry and egress of PTU to the thyroid gland (see Fig. 4). The effect of TSH on iodide transport has been shown previously to be biphasic. During the first few hours after injection, TSH decreases iodide trapping by the thyroid *in vivo* [14, 15], apparently due to stimulation of the iodide exit rate through increased cell permeability [16].

Third, TSH may stimulate thyroidal metabolism of PTU to an extent equal to or greater than that of PTU uptake. Our study only examined PTU pharmacokinetics; rates of formation and elimination of PTU metabolites were not investigated.

Effects of TSH on thyroidal PTU uptake are more apparent when synthesis and release of endogenous TSH are inhibited by thyroxine administration. Thyroxine altered thyroidal PTU uptake and reduced the T/S PTU ratio at all times studied, completely eliminating the T/S PTU concentration gradient up to 12 hr after PTU administration.

Effects of excess iodide on thyroid function vary depending on iodine dose and duration of administration [17, 18]. Excessive iodide exerts an immediate depressing effect on the organification of iodine by the rat thyroid [19, 20]. This depression, known as the Wolff–Chaikoff effect, is only temporary, an escape occurring after 3–4 days. During the Wolff–Chaikoff effect, iodination of tyrosine and the subsequent coupling reaction to form thyroxine are inhibited.

PTU accumulation increased markedly in rats receiving KI for 3 days. This enhanced PTU uptake by

the thyroid after chronic iodide administration may be mediated through TSH stimulation related to the Wolff–Chaikoff effect. However, elevated serum TSH concentrations also occur after chronic administration of KI, unrelated to the initial Wolff–Chaikoff effect [21].

Administration of 50 mg of KI 1 hr before PTU had no effect on the mean T/S PTU ratios. This dose of iodide was shown previously to inhibit competitively thyroidal radioiodide uptake in rats, reducing the T/S ratio below 1 [22]. We observed no competitive effect of KI on thyroidal PTU uptake.

Chronic PB administration induces hepatic microsomal enzymes that metabolize many drugs and some endogenous compounds. Thyroxine turnover is acceler ated by PB, leading to a compensatory increase in thyroid function [23]. PB dramatically stimulated thyroidal uptake of PTU, as shown in Fig. 5. Increased PTU uptake following PB treatment and decreased PTU uptake following thyroxine administration are consistent with the hypothesis that the saturable component of thyroidal accumulation of PTU is controlled by TSH, thyroxine or both.

In conclusion, knowledge of the factors controlling thyroidal concentration of PTU could be useful in improving regimens of these drugs. Serum concentrations of many drugs with low therapeutic index are often monitored to help select appropriate dosage, to reduce toxicity and to enhance therapeutic efficacy. However, with PTU, uptake of the drug by a saturable process at the target organ itself must also be considered. That is, PTU concentrations at action sites are not in simple equilibrium with serum PTU concentrations; rather the PTU concentration gradient between serum and thyroid changes with changing serum PTU concentrations. In euthyroid rats, the saturable component of thyroidal uptake of PTU contributes little to thyroidal PTU concentrations when serum PTU concentrations exceed 5 μg/ml. However, the contribution of that saturable component is also subject to effects of variable iodine content of the diet and changing serum concentrations of thyroxine or TSH, as described in this study. Previous work suggested that in man thyroid status influences PTU metabolism [24]. The present studies indicate that the relationship between thyroid function and serum TSH concentration may also affect PTU disposition in patients.

Since completion of our studies, a paper on the *in vitro* uptake of [^{35}S]methimazole by sheep thyroid slices appeared and reached conclusions similar to ours concerning saturation of a carrier-mediated transport system for methimazole in the thyroid gland [25].

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