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INHIBITION OF THE EFFECTS OF THYROXINE BY 5,5-DIPHENYL-2-THIOHYDANTOIN*

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Abstract—5,5-Diphenyl-2-thiohydantoin (DPTH), the sulfur analog of 5,5-diphenylhydantoin (dilantin), inhibited the induction of rat tissue mitochondrial α-glycerophosphate dehydrogenase (GPD) and the stimulation of whole-body metabolic rate by exogenous thyroxine (T_4). It did not affect the tissue uptake, the biliary excretion, or the enterohepatic recirculation of T_4. Like propylthiouracil, it increased the serum protein-bound iodine (and free T_4) when exogenous T_4 was given, and decreased the deiodination *in vivo* of the hormone. In thyroidectomized rats, DPTH did not inhibit the stimulation of general protein synthesis produced by exogenous T_4, and it had only a little or no effect on the induction of glucose 6-phosphatase, succinic dehydrogenase, and malic enzyme by T_4, or on the induction of tyrosine transaminase by hydrocortisone. The inhibition of GPD induction by DPTH could not be explained by its effects on the general distribution or metabolism of T_4.

SOME thioamides, such as thiouracil derivatives, decrease the effectiveness of administered thyroxine (T_4). More hormone must be given to restore the metabolic rate in rats fed 2-thiouracil (TU)[1] or 6-methyl-2-thiouracil (MTU)[2] than in surgically thyroidectomized rats, and the daily T_4 requirement to maintain a euthyroid state of rats receiving 6-propylthiouracil (PTU), TU, or MTU is doubled.[3] The mitochondrial α-glycerophosphate dehydrogenase (GPD) of some rat tissues increases in response to T_4 administration, and this also is partially inhibited by TU and PTU.[4,5]

In a study of the peripheral inhibition of thyroxine by thiohydantoins derived from amino acids, several 3-phenyl-2-thiohydantoins with nonpolar substituents at the No. 5 position (including the leucine derivative, PTH-leucine) were found to have some effect.[6] In the process of surveying the effects of phenyl thiohydantoins and other thioamides on the induction of GPD by T_4, 5,5-diphenyl-2-thiohydantoin (DPTH), the sulfur analog of dilantin, was found to inhibit effectively the increases in GPD and overall metabolic rate normally observed after T_4 administration. However, all effects of T_4 were not inhibited equally, and this report describes the effect of DPTH on the metabolism and actions of T_4. The purpose was to define the extent of and the mechanism by which DPTH inhibited the peripheral effect of T_4.

METHODS AND MATERIALS

Male rats (Holtzman or Blue Spruce) weighing 200–240 g at sacrifice were fed *ad lib.* a basal diet containing 30% casein, 54% sucrose, 10% Wesson or Mazola oil,

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4% low-iodine Phillips and Hart salt mixture (KI = 1·48 g/50 lbs of salt), plus the vitamin mixture previously described.[7] The compounds studied were added to this basal diet in the concentrations shown with the results. The thyroid hormones were injected subcutaneously in alkaline 0·9% NaCl; for very low concentrations (0·4 μg T_4/0·2 ml), a more concentrated stock solution was diluted with 1% gelatin immediately before use. Alpha-glycerophosphate dehydrogenase (GPD, EC 1.1.99.5) activity was determined manometrically as previously described,[8] except that $MgSO_4$ was omitted from the flask; GPD activity has been expressed as microliters of oxygen consumed per 10 min per 150 mg of wet tissue at 30°. Malic enzyme (EC 1.1.1.40) activity was assayed in the supernatant of a liver homogenate (12,000 *g* for 20 min) according to the method of Ochoa,[9] as modified by Tepperman and Tepperman,[10] and expressed as micromoles of NADPH formed per minute per gram of wet liver at 30°. Tyrosine transaminase (EC 2.6.1.5) activity was determined on the supernatant (31,000 *g* for 30 min) from a 1:5 liver homogenate (w/v) in 0·15 M KCl by the method of Diamondstone,[11] and is expressed as micromoles of *p*-hydroxyphenylpyruvate formed per 10 min per gram of wet liver at 37°. All results have been recorded as mean ± S.E. of the mean for groups of six to nine rats.

For studies of T_4 and 3,5,3′-triiodo-L-thyronine (T_3) distribution and metabolism, the ^{131}I compounds (sp. act. 30–50 μc/mg) were dissolved in alkaline 0·9% NaCl at a concentration of 5·0 μc/ml and injected once subcutaneously (1·0 μc of radioactive hormone per 100 g of body weight). Tail cups collected the feces and prevented coprophagy. Tissue, feces, serum, and urine samples and washed trichloroacetic acid (TCA) precipitates of serum [^{131}protein-bound iodine (PBI) values] were counted. Serum nonradioactive PBI was determined chemically by Bioscience Laboratories, Van Nuys, Calif.

Cell fractionation. For studies of the distribution of radioactivity in subcellular fractions, 3·6 ml of a 1:3 liver homogenate (w/v) in cold buffer (0·25 M sucrose, 0·025 M KCl, 0·005 M $MgCl_2$, 0·05 M Tris, pH 7·5) was diluted with 8·4 ml of additional buffer and centrifuged at 12,000 *g* for 15 min. Eight ml of the supernatant was recentrifuged at 105,000 *g* for 60 min to separate the microsomes from the supernatant.

Three ml of the original homogenate was mixed with 6 ml of cold 2·4 M sucrose containing 1 mM $MgCl_2$, and 6 ml of this mixture was layered over 2 ml of the same sucrose. After centrifugation at 105,000 *g* for 60 min, the nuclei collected as a clean gelatinous pellet at the bottom of the tube while the debris floated to the surface.[12]

^{14}C-orotic acid and ^{3}H-leucine incorporation. Weanling male rats were thyroidectomized (Hormone Assay Laboratories), maintained on 1% calcium lactate, and used 5–6 weeks later when their body weights had plateaued at approximately 160 g. ^{14}C-orotic acid (sp. act., 60·8 mc/m-mole) in 0·9% NaCl (10 μc/ml) was injected once i.p. (4 μc/100 g body wt.) 30 min before sacrifice. ^{3}H-leucine (sp. act., 6000 μc/mole) in 0·9% NaCl (6·25 μc/ml) was also injected once i.p. (25 μc/100 g body wt.) 30 min before sacrifice. The ^{14}C and ^{3}H were counted in a liquid scintillation system as described below.

In the orotic acid experiments, the liver cellular fractions were separated as described previously and the RNA from each fraction was extracted and hydrolyzed by the method of Schmidt and Thannhauser.[13] The acidified extract containing hydrolyzed RNA was neutralized with NaOH and added to Bray's scintillant fluid

for counting.[14] The samples were counted in a Beckman LS-150 liquid scintillation system with external standardization. The supernatant containing the hydrolyzed RNA was also analyzed for ribose by the orcinol color test.[15]

For studies of leucine incorporation, a 1:10 liver homogenate (w/v) in water was prepared and analyzed for protein by the biuret reaction[16] or by the method of Lowry *et al.*[17] Five ml of 10% TCA (containing 0·1% nonradioactive leucine) was added to 1 ml of the homogenate and centrifuged to collect the protein precipitate. The latter was washed with ethanol–ether (3:1) and ether, and resuspended in 2·0 ml of 1·0 M Hyamine hydroxide in methanol. The precipitate was dissolved by incubating at 75–77° and 0·1 ml of the solution was mixed with 15 ml of toluene scintillant fluid [4 g 2,5-diphenyloxazole, 0·2 g 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene per liter of toluene] and counted 48 hr later in a Beckman LS-150 liquid scintillation system.

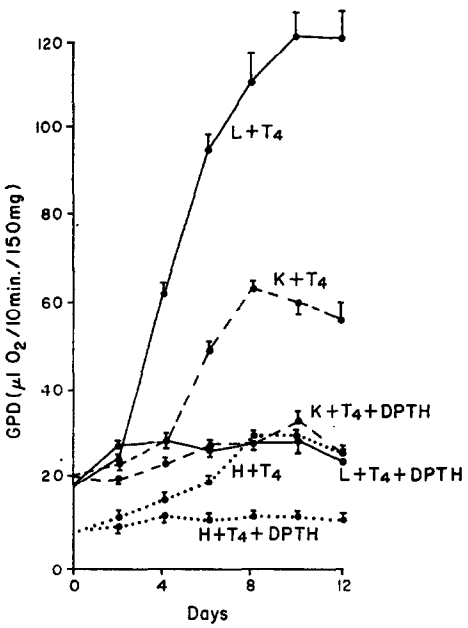

FIG. 1. Effect of DPTH on the T_4-induced increase in liver (L), kidney (K), and heart (H) glycerophosphate dehydrogenase (GPD). Groups of six to eight male rats were fed the basal diet ± 0·06% DPTH for the number of days shown, and were injected subcutaneously with 15 μg T_4/100 g body wt./day.

RESULTS

Inhibition of GPD induction

Figure 1 shows that feeding 0·06% DPTH in the diet effectively prevented the T_4-induced increase in liver, kidney, and heart GPD. DPTH also blocked the induction of liver GPD by triac (3,5,3′-triiodothyroacetic acid), tetrac (3,5,3′,5′-tetraiodothyroacetic acid), isopropyl-T_2 (3,5-diiodo-3′-isopropyl thyronine), tetraprop (3,5,3′,5′-tetraiodothyropropionic acid), and T_3 (3,5,3′-triiodo-L-thyronine) (Table 1). PTU and the phenylthiohydantoin of leucine (PTH-leucine) blocked only the T_4-induced

TABLE 1. EFFECTS OF PTU, DPTH, AND PTH-LEUCINE* ON THE INDUCTION OF LIVER GPD BY SEVERAL THYROACTIVE COMPOUNDS

Compound	Dose	Liver GPD (μl O_2/10 min/150 mg)†			
		Basal diet	0·06% PTU	0·06% DPTH	0·2% PTH-leucine
Triac	30	132 ± 10	115 ± 5	67 ± 6	132 ± 11
Tetrac	100	122 ± 6	162 ± 10	39 ± 4	
Isopropyl-T_2	3	76 ± 8	127 ± 6	49 ± 4	106 ± 11
Tetraprop	75	54 ± 4	110 ± 13	27 ± 4	
Thyroxine	15	125 ± 9	58 ± 4	25 ± 2	50 ± 3
T_3	8	117 ± 7	126 ± 9	54 ± 2	111 ± 6

* PTH-leucine is one of several 3-phenyl-2-thiohydantoins with nonpolar substituents at the No. 5 position that inhibit the induction by T_4 of the rat liver mitochondrial GPD.[6] See text for abbreviations.

† Groups of six to eight male rats were fed the basal diet ± PTU, DPTH or the phenylthiohydantoin of leucine (PTH-leucine) for 11 days while receiving a daily subcutaneous injection of one of the compounds indicated (dose = μg/100 g body wt./day).

TABLE 2. EFFECT OF DPTH ON LIVER PROTEIN CONCENTRATION, SERUM PBI AND METABOLIC RATE*

	Basal diet	0·06% DPTH
15-Day wt. gain (g)	80 ± 4	58 ± 5
Liver GPD (units/100 mg wet wt.)	20 ± 1·8	6 ± 0·9
Total liver GPD (units/total liver)	1538 ± 145	531 ± 71
GPD (units/mg protein)	0·66 ± 0·06	0·22 ± 0·03
Liver wt. (as % body wt.)	4·68 ± 0·11	5·74 ± 0·14
Protein (μg/mg liver)	202 ± 6	186 ± 6 (NS)
Total liver protein (g)	2·33 ± 0·09	2·45 ± 0·07 (NS)
Chemical PBI (μg/100 ml)	4·8	3·0
Metabolic rate (O_2/m²/hr)†	6·5 ± 0·7	6·4 ± 0·9 (NS)
Metabolic rate (+ T_4)†	13·6 ± 0·8	8·2 ± 0·7

* Rats, 170–210 g, were fed the basal diet ± 0·06% DPTH for 14 days before the livers were analyzed for proteins[17] and GPD. Chemical PBI levels were determined on pooled sera from each group. Metabolic rates were determined[7] after 17–18 days of feeding with and without the daily injection of 40 μg T_4/100 g body wt. Mean ± S.E. (nine rats/group); NS, not significantly different.

† Six rats per group, the rats weighed about 300 g.

increase in GPD; by contrast with DPTH, they had no effect on the inductions by T_3 and triac, and even potentiated the effects of tetrac, isopropyl-T_2, and tetraprop.

Feeding DPTH for 2 weeks inhibited growth somewhat (Table 2) and gave thyroidectomy levels of liver GPD. DPTH also gave a modest increase in liver weight, but had no effect on liver protein concentration, and was therefore not a general inhibitor of protein synthesis. It did not have any effect on the control metabolic rate,[7] but severely inhibited the stimulation of the metabolic rate by exogenous T_4.

Figure 2 shows that the effect of DPTH was not due to a more rapid destruction of the enzyme. After an 11-day buildup of the liver enzyme by T_4 stimulation, new protein synthesis was halted temporarily by cycloheximide, and the rate of loss of the

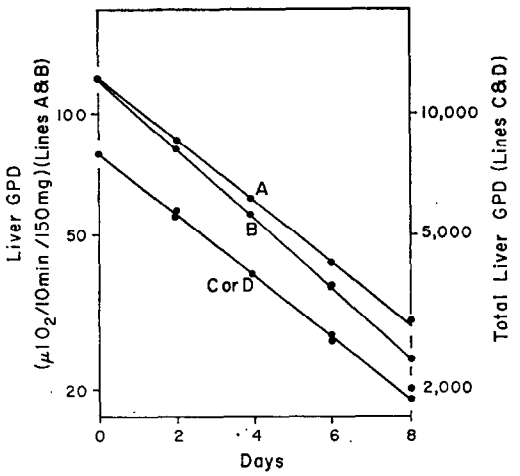

FIG. 2. Effect of DPTH on the half-life of liver GPD. All rats were fed the basal diet for 11 days and injected subcutaneously with 15 μg T_4/100 g body wt./day. On the twelfth day, T_4 administration was discontinued; all rats were given a single intraperitoneal injection of 120 μg cycloheximide/100 g body wt. and, in some groups, the basal diet was replaced by 0·06% DPTH. Liver GPD was determined on the days indicated after commencement of the DPTH diet. Liver GPD half-life for rats fed basal diet (A = 3·8 days) or DPTH (B = 3·3 days), based on unit concentration of liver GPD. The liver GPD half-life (3·7 days) for rats fed basal diet (C) or DPTH (D), based on total liver GPD.

GPD from the liver was then determined in the presence and absence of DPTH. The slight effect of DPTH when based on unit concentration calculations was attributed to the modest enlargement of the liver with GPD-poor tissue as a result of DPTH feeding. When calculated on the basis of total liver GPD, the half-life was 3·7 days in the presence or absence of DPTH.

Distribution and metabolism of T_4

The effects of DPTH on the uptake of T_4-^{131}I by liver, kidney, heart and skeletal muscle were measured by a method similar to that of Osorio *et al.*[18] There was no indication in the results that DPTH seriously retarded the uptake of T_4 by the tissues or decreased the half-life values of T_4.

Rats were fed basal diet or a diet containing 0·06% DPTH. On days 10 and 11, all rats were injected subcutaneously with 100 μg T_4 per 100 g body wt. during the morning. During the night of day 11, each rat was injected subcutaneously with 1·0 μc T_4-^{131}I per 100 g body wt. Eight rats were sacrificed from each group at 5- or 6-hr intervals. The per cent dose of ^{131}I was measured in the tissues and serum and the uptake ratios[18] were calculated. At 10 hr, the uptake ratios for liver, kidney, heart and muscle were 0·71, 1·10, 1·11 and 1·11 respectively. At the end of 85 hr, the values were 0·74, 1·18, 1·38 and 1·02 respectively. (A ratio of less than one indicates that T_4 is taken up at a faster rate in the treated rats than in the controls.) Half-life values of the ^{131}I were found by plotting the log of per cent dose per gram of tissue (or 1 ml serum) against time. Half-life values for whole serum, PBI, liver, kidney, heart and muscle from rats fed the basal diet were 10·8, 9·0, 9·6, 9·6, 10·8 and 12·0 hr respectively; and 12·6, 11·4, 11·4, 12·0, 12·6 and 13·8 hr, respectively, for the tissues from the DPTH-treated animals.

TABLE 3. EFFECT OF DPTH ON THE SUBCELLULAR DISTRIBUTION OF T_4*

Cell fraction	Basal diet† (counts/min $\times 10^{-3}$)	Basal diet† (% counts/min in TCA ppt.)	0·06% DPTH (counts/min $\times 10^{-3}$)	0·06% DPTH (% counts/min in TCA ppt.)	P (counts/min)
Homogenate	91·6 ± 3·8	66	108·3 ± 3·4	72	0·01
Cell sap	37·2 ± 1·2		33·8 ± 0·8		NS
Nuclei (counts/min/mg protein)	0·6 ± 0·05	44	0·6 ± 0·00	42	NS
Microsomes (counts/min/mg protein)	0·8 ± 0·09	81	1·1 ± 0·05	86	0·01

* All rats were injected s.c. with 15 μg T_4/100 g body wt./day for 11 days while being fed basal diet ± DPTH. All rats were injected s.c. with 22 μc T_4-^{131}I/100 g body wt. 10 hr before sacrifice. The liver was homogenized, centrifuged, and a sample of each fraction was counted. TCA precipitates of some fractions were also counted. Protein was determined by the biuret method.[16] Counts × 10^{-3} are based on liver samples of 100 mg. Means ± S.E. are shown for groups of seven to nine rats.

† DPTH had no effect on the concentration of nuclear protein, but slightly increased (to 125% of control) the concentration of microsomal protein in the liver. Seven of the nine nuclear homogenates from the DPTH-fed rats gave cloudy biurets which were not cleared by ether. Consequently, the mean nuclear protein value for DPTH-fed rats is based on the two clear samples. Rats on the basal diet did not produce cloudy biurets (nuclear) and this mean is based on all 8 samples.

The subcellular distribution of T_4 in rats fed the basal diet ± DPTH and injected with T_4 for 11 days is given in Table 3. Slightly more counts were found in the liver homogenate from DPTH-fed rats, but the same percentage of these counts was precipitable by TCA in both groups. The radioactivity in the supernatant (105,000 *g*) and nuclear fraction was not affected by DPTH. DPTH produced a significant increase in the ^{131}I (180%) and protein concentration (125%) in the microsomal fraction of the cells, as well as an increase in radioactivity per milligram of microsomal protein. The slight increase in radioactivity in liver cells associated with DPTH administration may be largely accounted for by the increase in ^{131}I in the microsomes.

In order to study the absorption and enterohepatic recirculation of T_4,[19] rats were fed basal diet ± DPTH, gavaged with 1·0 μc T_4-^{131}I/100 g body wt., and serum and tissue levels of ^{131}I were determined 10 hr later. DPTH only slightly decreased the serum level of ^{131}I (1·6 ± 0·03 vs. 1·9 ± 0·04% dose/ml serum), but had no significant effect on the tissue levels of this isotope. The results show that DPTH did not seriously interfere with the gastrointestinal absorption or enterohepatic recirculation of T_4.

When exogenous T_4 was administered, both DPTH and PTU increased the bound T_4 and the free T_4 concentrations in the serum to the same degree; the per cent of free T_4 was unchanged (Table 4). The concentration of protein-bound T_4 was increased by PTU or DPTH plus exogenous T_4, the latter as measured by any of three methods: T_4 by column (ion-exchange resin), chemical PBI, or Tetrasorb ^{125}PBI. When no exogenous T_4 was given, DPTH decreased the PBI, presumably because of its mildly goitrogenic properties.

Effects of DPTH and PTH-leucine on the excretion of T_4 and T_3

DPTH and PTH-leucine inhibited the deiodination *in vivo* of T_4, since they decreased the urinary excretion of ^{131}I after an injection of radioactive T_4 (Fig. 3). The fecal

TABLE 4. EFFECTS OF DPTH AND PTU ON SERUM THYROXINE*

Treatment	T_4 by column (μg/100 ml)	Free T_4 (mμg/100 ml)	Free T_4 (%)	PBI (μg/100 ml)	Tetrasorb ^{125}PBI (μg/100 ml)
Basal diet + T_4	6·6	4·9	0·073	9·1	9·2 ± 1·4
DPTH + T_4	13·4	8·5	0·064	15·0	12·6 ± 1·6
PTU + T_4	10·7	8·6	0·080		

* Rats were fed basal diet ± 0·06% DPTH or 0·06% PTU for 11 days and were injected s.c. with 15 μg T_4/100 g body wt./day. Pooled frozen sera were analyzed for T_4 by column, free T_4, and PBI by Bioscience Laboratories (single determinations). The Tetrasorb ^{125}PBI determinations were run individually on the sera of six to eight rats per group by Mr. Eugene Keran.

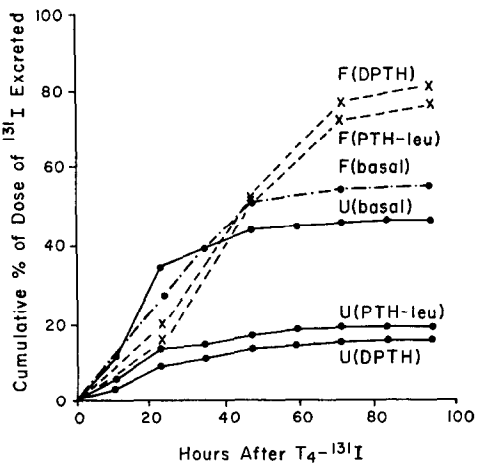

FIG. 3. Effects of DPTH and PTH-leucine on the excretion of injected T_4-^{131}I. All rats received subcutaneous injections of 15 μg T_4/100 g body wt./day for 10 days while fed a basal diet ± 0·06% DPTH or 0·2% PTH-leucine. A single subcutaneous injection of T_4-^{131}I (1·0 μc/100 g body wt.) was given on the tenth day. Tail cups prevented coprophagy. The diets and nonradioactive T_4 injections were continued throughout the experiment. The results are expressed as the cumulative per cent of the dose of injected ^{131}I excreted in the urine (U) or feces (F).

excretion of ^{131}I was increased secondarily, and the total excretion was essentially unaltered after 94 hr. The urinary ^{131}I did not bind to serum proteins and was presumed to be iodide. These results are similar to those reported by Morreale de Escobar and Escobar del Rey[3] for PTU.

Both DPTH and PTH-leucine decreased the urinary excretion and increased the fecal excretion of ^{131}I after an injection of T_3-^{131}I. Whereas on the basal diet the rats excreted 75 per cent of an 8 μg dose of T_3/100 g body wt. as ^{131}I in urine in 48 hr (19 per cent in feces), the values were 49 (36 per cent in feces) and 37 per cent (24 per cent in feces) for rats receiving DPTH or PTH-leucine respectively. Hence, both compounds interfered with the deiodination *in vivo* of T_3, but only the DPTH inhibited the GPD response to T_3 (see Table 1).

While DPTH administration increased the fecal excretion of T_4 over a period of

several days, it did not have any effect on the acute biliary excretion of injected T_4 ^{131}I. Rats were injected i.p. with 5 μc T_4-^{131}I/100 g body wt. about 90 min prior to ether anesthesia; the bile duct was cannulated via the duodenum (using Clay-Adams No. PE-10 polyethylene tubing) and bile was collected for 4–5 hr under anesthesia. DPTH (fed in the diet for 11 days) did not alter the bile flow rate (0·25 $\pm$ 0·03 ml bile/half hour vs. 0·21 $\pm$ 0·02 ml for control), nor the concentration of ^{131}I excreted into the bile (0·19% of dose/half hour for each group).

Similarly, DPTH did not increase the unit concentration of UDP-glucuronyltransferase (EC 2.4.1.17) in the liver. A 1:10 liver homogenate (w/v) in 0·15 M KCl was centrifuged for 15 min at 1500 *g*, and the supernatant was assayed for this enzyme[20] with *o*-aminophenol substrate. Enzyme units from control and DPTH-treated rat livers were 84 $\pm$ 5 and 71 $\pm$ 7/100 mg of liver respectively. It has been suggested that a single enzyme uses T_4, *o*-aminophenol and bilirubin as substrates for glucuronidation.[21,22]

Orotic acid incorporation into liver RNA

Tata and Widnell[23] have shown that a single injection of T_3 or T_4 given to thyroidectomized rats will stimulate the incorporation of orotic acid into liver RNA. In similar studies (Table 5), we have found that thyroidectomy decreased the total

TABLE 5. EFFECT OF DPTH ON THE STIMULATION OF OROTIC ACID INCORPORATION INTO LIVER RNA BY T_4*

	Homogenate	Nuclei	Cell sap	Microsomes	No. of rats
TX, BD	5·1 $\pm$ 0·20	62·5 $\pm$ 6·7	2·4 $\pm$ 0·14	0·99 $\pm$ 0·12	9
TX, BD + T_4	8·70 $\pm$ 0·59	81·2 $\pm$ 5·3	4·5 $\pm$ 0·16	1·5 $\pm$ 0·06	10
TX, DPTH	3·30 $\pm$ 0·50	42·3 $\pm$ 8·2	1·4 $\pm$ 0·12	0·36 $\pm$ 0·04	10
TX, DPTH + T_4	5·2 $\pm$ 0·32	73·1 $\pm$ 9·2	2·9 $\pm$ 0·45	0·72 $\pm$ 0·69	9
Intact BD	10·0 $\pm$ 0·36				6

* Thyroidectomized (TX) rats were fed basal diet (BD) $\pm$ 0·06% DPTH for three nights before T_4 was injected s.c. (60 μgT_4/100 g body wt. 44 hr before sacrifice). All rats were injected i.p. with 4 μc orotic acid-^{14}C/100 g body wt. 30 min before sacrifice. Specific activity of orotic acid = 60·8 mc/mole (no carrier added). The liver cells were fractionated, the RNA was extracted, and samples were counted and subjected to an orcinol color test for ribose. This table reports the specific activity of RNA-^{14}C as counts per minute ($\times$ 10^{-3}) per micromole RNA ribose. Diets were continued until sacrifice.

incorporation of orotic acid into liver RNA (to 50% of the intact level), and that this decrease was partially restored by a single injection of T_4 in all fractions of the cell examined. DPTH decreased the incorporation of orotic acid into RNA *per se*, but did not prevent the stimulation by exogenous T_4. While DPTH had its own effect on RNA synthesis, it did not prevent the stimulation of transcription by T_4.

Leucine-^{3}H incorporation into liver protein

Thyroidectomy lowered the rate of incorporation of tritiated leucine into liver protein, and this decreased incorporation was fully restored by one injection of T_4 (Table 6). DPTH had no effect on the unstimulated rate of leucine incorporation into

TABLE 6. EFFECTS OF DPTH ON LEUCINE-^{3}H INCORPORATION INTO LIVER PROTEIN*

	BD	BD + T_4	DPTH	DPTH + T_4
A. Thyroidectomized†	1·83 ± 0·11	2·95 ± 0·15	2·02 ± 0·10	3·11 ± 0·20
B. Intact‡	2·61 ± 0·15	3·66 ± 0·13	2·80 ± 0·12	3·47 ± 0·11
C. Mitochondria§	0·29 ± 0·04	0·68 ± 0·12	0·36 ± 0·05	0·65 ± 0·10

* The data presented are mean specific activities of labeled protein (counts/min × 10^{-3}/mg protein) isolated from liver homogenates (A, B) or mitochondria (C). Each group contained six rats, except A, where 15–16 rats per group were used.

† Thyroidectomized rats were fed basal diet (BD) ± 0·06% DPTH for 3 nights prior to a single subcutaneous injection of T_4 (60 μg/100 g body wt.) and were sacrificed 44 hr later. Exactly 30 min before sacrifice, each rat was injected intraperitoneally with 25 μc leucine-^{3}H/100 g body wt. Diets were continued until sacrifice.

‡ Same as A, except all rats were intact and weighed about 100 g each at sacrifice.

§ Liver mitochondria were isolated from liver homogenates from the rats used in A.

protein, did not prevent the increased incorporation due to T_4 in either thyroidectomized or intact rats, and did not inhibit the stimulation of leucine-^{3}H incorporation into mitochondrial protein by T_4 (Table 6). Since DPTH had no effect on the unit concentration of total protein, the changes in total counts were due to alterations in the rate of incorporation of tritiated leucine into protein. DPTH did not prevent the stimulation of general protein synthesis by T_4 even while it was specifically blocking the induction of GPD in the mitochondria.

Other enzyme inductions

The stimulation of liver tyrosine transaminase (TT) by hydrocortisone-21-acetate (HCA) represented an enzyme induction by a hormone other than T_4. DPTH had no effect upon the basal level of TT, nor upon the induction of TT by HCA and was, therefore, not a general inhibitor of the induction process.

Male rats were adrenalectomized (Hormone Assay Laboratories), maintained on 1% NaCl for 2–3 weeks, and fed the basal diet ± 0·06% DPTH for the last 3 days; the DPTH plus HCA rats also received an alkaline saline solution containing 2 mg DPTH/100 g body wt., i.p., 1 hr before and 2 hr after the HCA. HCA was given i.p. (2·5 mg in 0·2 ml propylene glycol/100 g body wt.) 5 hr before sacrifice. TT values for rats on basal diet, basal diet + DPTH, basal diet + HCA and basal diet + DPTH + HCA were 36 ± 2, 29 ± 3, 202 ± 7 and 227 ± 16 units respectively.

The malic enzyme of rat liver can be induced by fasting–refeeding as well as by T_4 administration.[10] DPTH decreased the basal level of malic enzyme in weanling rats from 4·1 ± 0·3 to 1·5 ± 0·4 units. It also partially inhibited the induction of the enzyme when T_4 was the inducer (8·1 ± 0·4 with DPTH vs. 12·2 ± 0·7 for control), when GPD was inhibited 78 per cent (18 vs. 80) by the same dose of T_4 (3 μg T_4/100 g body wt./day for 11 days). However, when the rats were starved for 48 hr and refed for 48 hr, the enzyme levels in the untreated vs. DPTH-treated rats were 6·8 ±0·3 and 7·0 ± 0·3 units respectively. In the latter experiments, all rats were fed the basal diet *ad lib.* for 7 days, starved for the next 48 hr, and refed basal diet for the next 48 hr. One group was injected subcutaneously with 2·0 mg DPTH/100 g body wt./day during the period of starvation and refeeding. This group was refed *ad libitum*, and the

control rats were individually pair-refed with the DPTH-treated rats. Hence, one induction process for the malic enzyme operated normally in the presence of DPTH, but its stimulation by T_4 was partially blocked.

Two other enzymes which can be induced to a limited extent by exogenous T_4 are the mitochondrial succinic dehydrogenase (SDH; EC 1.3.99.1)[24] and the microsomal glucose 6-phosphatase (G6Pase; EC 3.1.3.9).[25] The administration of a high dose of T_4 (40 μg/100 g/day) to thyroidectomized rats for 6 days (subcutaneously) significantly stimulated the activity of both SDH[26] and G6Pase[27] in the liver, and 0·06% DPTH in the diet did not alter either the basal or the stimulated levels of these enzymes. The G6Pase values for rats receiving basal diet, basal diet + T_4, basal diet + DPTH or basal diet + DPTH + T_4 were 7·1 $\pm$ 0·20, 14·9 $\pm$ 0·48, 6·1 $\pm$ 0·47 and 13·4 $\pm$ 0·75 μmoles phosphate per min per g of fresh liver at 37° respectively. The SDH activities (μl O_2/20 min/10 mg liver at 30°) were 78 $\pm$ 2·3, 116 $\pm$ 5·1, 79 $\pm$ 4·6 and 125 $\pm$ 4·4 respectively.

The growth of thyroidectomized rats was increased from 0 to 3·5 g/day by the daily subcutaneous injections of 0·4 or 15 μg T_4 per 100 g body wt. DPTH alone did not cause any weight loss, but DPTH completely blocked the growth effects of 0·4 μg T_4 and reduced the growth response to 2 g/day when the rats were given 15 μg T_4.

DISCUSSION

Since DPTH did not interfere with the determination of GPD or its rate of degradation, it presumably blocked the induction of this enzyme. It was effective against all T_4 analogs tested, whereas 2-thiouracil,[5] PTU and PTH-leucine inhibited the induction by T_4 only. Interference by DPTH with the uptake of T_4 cannot be responsible for this effect, since the amount found in liver or its subcellular fractions was not diminished. The significance of the increased T_4 concentration in liver microsomes is unknown, but the data at least show that the drug did not inhibit GPD induction by preventing the T_4 from getting to the nucleus or the microsomes of the liver cells, and it did not interfere with the absorption of T_4 from the gastrointestinal tract or with the enterohepatic recirculation.

Feeding DPTH for 2 weeks without giving exogenous T_4 gave thyroidectomy levels of liver GPD either by blocking T_4 biosynthesis or by inhibiting the peripheral effect of the endogenous T_4, or by both. Since the PBI was decreased, DPTH presumably prevented the synthesis of thyroxine in addition to its peripheral effect. With exogenous T_4, DPTH actually increased the PBI as well as the free T_4. The hyperthyroidism which could be anticipated from an increased free T_4 was blocked by the DPTH, and the inhibition of GPD was not due to a lack of free T_4 in the serum; the equilibrium between the PBI and free T_4 was not altered by DPTH. If DPTH blocks the normal utilization of T_4 in the tissues, the increased PBI could be interpreted as a "back-up" phenomenon.

It is unlikely that the effect on deiodination is responsible for the DPTH effect, as suggested by Morreale de Escobar and Escobar del Rey[3] for the PTU effect. Both DPTH and PTH-leucine inhibit the deiodination *in vivo* of T_4 and T_3, but only DPTH inhibits GPD induction by T_3. The thiouracils also inhibit the deiodination *in vivo* of T_3, D-T_4, and T_3P, but do not always inhibit their biological actions, and at least two thioamides (5,5-diallyl-2-thio-barbituric acid and 5-ethyl-5-isopropyl-2-thiobarbituric

acid) do not inhibit deiodination,[3] but do inhibit GPD induction by T_4 (unpublished observations).

From these studies, it was concluded that DPTH did not inhibit the activity of T_4 by virtue of any effects on the distribution, deiodination, excretion or detoxification of the hormone. The additional studies were, therefore, concerned with the induction process itself, and they showed that DPTH specifically blocked inductions by thyroid hormone, since it had no effect on the increased enzyme activities produced by HCA (tyrosine transaminase) or by fasting-refeeding (malic enzyme). Furthermore, all of the responses to T_4 were not affected equally in that DPTH was more effective in blocking the metabolic rate and GPD responses to T_4 than that of other T_4-sensitive enzymes (malic enzyme, G6Pase and SDH).

DPTH had no effect on general protein synthesis *per se*, or upon the T_4 stimulation of this synthesis. Since T_4 induction of GPD was blocked by the DPTH, it follows that GPD is only a small part of the total protein synthesis which is stimulated by T_4 and is not representative of the total effect of T_4 on protein synthesis.

There is a correlation between the metabolic rate and the tissue GPD in rats, as both are modified by T_4;[8] in birds, neither is affected by T_4.[28] In this study, DPTH blocked both the metabolic rate and GPD simultaneously, even though other parameters (e.g. malic enzyme, protein synthesis) were inhibited only slightly or not at all. Lee and Lardy[29] suggested that GPD may regulate the transport of electrons into the mitochondria through the glycerophosphate–dihydroxyacetone–phosphate (GP-DHAP) shuttle.[30,31] The activity of this enzyme is then a reflection of the total amount of oxygen consumed by the electron transport chain in oxidizing the hydrogens delivered into the mitochondria by the GP-DHAP shuttle. While the T_4-stimulated increase in tissue GPD may contribute importantly to the increase in whole body oxygen consumption, the latter is larger than can be accounted for by the changes in GPD alone. Sokoloff *et al.*[32–34] have suggested that the increased oxygen consumption in hyperthyroid rats is a consequence of the T_4-stimulated protein synthesis, since puromycin blocks both. However, puromycin also blocks the induction of GPD by T_4.[35] In the present studies, DPTH dramatically separated the effects of T_4 on GPD induction and general protein synthesis. Both the metabolic rate and GPD induction were inhibited, while general protein synthesis and T_4 stimulation of that synthesis were unaffected by DPTH. The increased protein synthesis in hyperthyroidism does not seem to account for the increased oxygen consumption.

The exact mechanism by which DPTH blocks the T_4 induction of GPD has not been resolved. Oppenheimer and Tavernetti[36] have reported that dilantin is structurally similar to T_4 inasmuch as the hydantoin ring provides the same bond angles between the two phenyl groups of dilantin as the ether linkage does for the benzene rings of T_4. While the sulfur atom in DPTH is essential for antithyroid activity, it would not be expected to alter the conformation of the molecule in any major way. Hence, the DPTH molecule might be able to compete with T_4 for the active induction site; why it affects GPD synthesis differently from other protein synthesis cannot be answered at the present time.

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