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Stimulation of mouse liver glutathione *S*-transferase activity in propylthiouracil-treated mice *in vivo* by tri-iodothyronine

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Female C57B1/6J mice were given drinking water containing 0.05% propylthiouracil to induce a hypothyroid condition. Mitochondrial glycerol-3-phosphate dehydrogenase activity, used as an index of hypothyroidism, was 57.1 ± 4.5 and 29.4 ± 3.8 nmol/min per mg of protein for control and propylthiouracil-treated animals respectively. Administration of tri-iodothyronine resulted in an approx. 4.5-fold increase in dehydrogenase activity in propylthiouracil-treated animals. A dose-dependent increase in hepatic GSH *S*-transferase activity in propylthiouracil-treated animals was observed at tri-iodothyronine concentrations ranging from 2 to 200 μg/100 g body wt. This increase in transferase activity was seen only when 1,2-epoxy-3-(*p*-nitrophenoxy)propane was used as substrate for the transferase. Transferase activity with 1-chloro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene as substrate was decreased by tri-iodothyronine. Administration of actinomycin D (75 μg/100 g body wt.) inhibited the tri-iodothyronine induction of transferase activity. Results of these studies strongly suggest that tri-iodothyronine administration markedly affected the activities of GSH *S*-transferase by inducing a specific isoenzyme of GSH *S*-transferase and suppressing other isoenzymic activities.

INTRODUCTION

GSH *S*-transferase (EC 2.5.1.18) represents a family of enzymes or binding proteins that have been identified in a variety of species and tissues. GSH *S*-transferase has been extensively studied as a major detoxification system during the metabolism of drugs, xenobiotics and carcinogens (Garry *et al.*, 1977; Benson *et al.*, 1978; Jakoby, 1978; Chasseaud, 1979). Transferases from rat and human liver have been characterized biochemically and immunologically (Habig *et al.*, 1974). At least four immunologically distinct forms of transferases have been identified in rats. These have been identified as E, A (or C), B and AA (Habig *et al.*, 1976). More recently multiple forms of rat liver GSH *S*-transferase have been shown to be homodimers or heterodimers composed of subunits of distinct M_r values (Hayes *et al.*, 1980; Mannervik & Jenson, 1982).

Three major forms of cytosolic GSH *S*-transferase, designated F1, F2 and F3, have been purified from mouse liver; a minor form, F4, was also characterized (Lee *et al.*, 1981). These isoenzymes exhibited a moderate degree of substrate specificity and distinct kinetic parameters towards different substrates. F1 and F2 transferases showed complete immunological identity. However, no cross-reactivity was observed between antisera to F1 or F2 transferase and to F3 transferase.

Hepatic GSH *S*-transferases are inducible by microsomal-drug-metabolizing-enzyme inducers such as phenobarbital and polycyclic aromatic hydrocarbons (Mukhtar & Bresnick, 1976; Kulkarni *et al.*, 1978). Sparnins *et al.* (1982) showed that several dietary constitutents increased the transferase activity in female ICR/HA mice. In rats hepatic transferase B concentration increased by 30% over the basal level in hypophysectomized or thyroidectomized rats (Arias *et al.*, 1976). T_4 restored the transferase B activity to normal values.

Extrathyroidal metabolism of T_4 is the major source of T_3 in man and experimental animals (Braverman *et al.*, 1970; Schwartz *et al.*, 1971). The compound PTU has been shown to be a very potent inhibitor of the conversion of T_4 into T_3, and has been used to induce hypothyroidism in experimental animals (Oppenheimer *et al.*, 1972; Chopra, 1977; Visser, 1979). Mitochondrial glycerol-3-phosphate dehydrogenase acivity has been used as an excellent index of thyroid-hormone action in rodents (Oppenheimer, 1979).

In the studies presented below, dietary administration of PTU (0.05% in the drinking water) was used to induce hypothroidism in mice. The activity of mitochondrial glycerol-3-phosphate dehydrogenase was used as an index of the hypothroid state. We investigated the effects of T_3 on hepatic GSH *S*-transferase activity in female C57B1/6J mice.

MATERIALS AND METHODS

Chemicals

1,2-Dichloro-4-nitrobenzene and 1-chloro-2,4-dinitrobenzene were obtained from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.). These compounds were recrystallized from ethanol/water before use. 1,2-Epoxy-3-(*p*-nitrophenoxy)propane, GSH, L-T_3 and actinomycin D were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Animals

C57B1/6J female mice, 4–6 weeks old, were obtained from Jackson Laboratory (Bar Harbor, ME, U.S.A.).

Abbreviations used: T_3, tri-iodothyronine; T_4, thyroxine; PTU, propylthiouracil.

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Animals were fed *ad libitum* until the time they were used. In studies involving stimulation of transferase activity by T_3, animals were given a single or multiple intraperitoneal injections (0.2 ml) of T_3 dissolved in 0.9% NaCl. Control animals were injected with saline only.

In experiments involving actinomycin D, the test substance was dissolved in 10% (v/v) ethanol. Test animals were given intraperitoneal injections of actinomycin D (75 μg/100 g body wt.) 1 h before and 24 h after injection of T_3. Control animals received intraperitoneal injections of 10% ethanol only (Beil *et al.*, 1980).

To induce hypothyroidism, animals were given drinking water containing 0.05% PTU for 4–6 weeks. Control animals received normal drinking water. Animals were killed, and hepatic mitochondria were isolated and assayed for glycerol-3-phosphate dehydrogenase activity by the procedure of Lee & Lardy (1965).

Preparation of 105000 *g* supernatant fraction

Mice were killed by cervical dislocation. Livers were immediately removed and placed in ice-cold 0.25 M-sucrose. Livers were then minced with scissors and homogenized in a Dounce homogenizer at a 1:4 (v/v) ratio of minced liver to 0.25 M-sucrose. The homogenate was centrifuged at 9000 *g* for 20 min. The supernatant was then centrifuged at 105000 *g* for 1 h. The supernatant fluid was used for the assay of GSH *S*-transferase activity.

Determination of GSH *S*-transferase activities

GSH *S*-transferase activities with 1-chloro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene as substrates were determined spectrophotometrically by the procedure of Habig *et al.* (1974). Assay of GSH *S*-epoxidetransferase activity was performed by the procedure of Fjellstedt *et al.* (1973). The assay mixture contained 0.1 M-potassium phosphate buffer, pH 6.5, 10 mM-GSH, 0.5 mM-1,2-epoxy-3-(*p*-nitrophenoxy)propane and various amounts of supernatant fraction in a total volume of 1 ml.

Protein was determined by the method of Lowry *et al.* (1951), with crystalline bovine serum albumin as the standard.

Analysis of results

Values are expressed as means ±S.D. The data were analysed by using Student's *t* test; *P* values greater than 0.05 were not considered significant.

RESULTS

Induction of hypothyrodism by PTU

Table 1 shows the effects of PTU on mitochondrial glycerol-3-phosphate dehydrogenase activity. Animals were maintained on PTU for 4–6 weeks. PTU treatment resulted in a marked suppression of glycerol-3-phosphate dehydrogenase activity. When PTU-treated animals were given a single intraperitoneal injection of T_3 (200 μg/100 g body wt.) the glycerol-3-phosphate dehydrogenase activity was stimulated above the activity in the euthyroid controls animals. A dose–response investigation of T_3 treatment on glycerol-3-phosphate dehydrogenase activity was also carried out (results not shown). At physiological doses of T_3 (2 μg/100 g body wt.), glycerol-3-phosphate dehydrogenase activity in PTU-treated animals was similar to that in the euthyroid control.

Table 1. Effects of PTU and T_3 on hepatic mitochondrial glycerol-3-phosphate dehydrogenase activity

Animals were rendered hypothyroid by the procedures described in the Materials and methods section. Animals were given two consecutive daily intraperitoneal injections of T_3 (200 μg/100 g body wt.). Values represent the means ±S.D. for four or more independent experiments. Livers from two animals were pooled and assayed for each independent experiment. Significance of difference from control: $*P < 0.05$; $**P < 0.001$.

Treatment	Glycerol-3-phosphate dehydrogenase activity (nmol/min per mg of protein)	(% of control)
None	57.1±4.5	(100)
PTU	29.4±3.8*	51
PTU+T_3	125.7±5.3**	220

Table 2. Effect of PTU treatment on hepatic GSH *S*-transferase activity

Cytosolic GSH *S*-transferase activities were determined by the procedures described in the Materials and methods section. Animals were maintained on water containing 0.05% PTU for 4 weeks. Values represent the means ±S.D. for five separate experiments using two animals for each experimental manipulation. Significance of difference from respective control: $*P < 0.05$.

Treatment	Substrate	Activity (nmol/min per mg of protein)
None	1-Chloro-2,4-dinitrobenzene	3642±220
PTU	1-Chloro-2,4-dinitrobenzene	5390±380*
None	1.2-Dichloro-4-nitrobenzene	82±6
PTU	1,2-Dichloro-4-nitrobenzene	215±19*
None	1,2-Epoxy-3-(*p*-nitrophenoxy)-propane	139±10
PTU	1,2-Epoxy-3-(*p*-nitrophenoxy)-propane	91±10

Effects of PTU treatment on hepatic GSH *S*-transferase activities

Data in Table 2 show the effects of PTU treatment on GSH *S*-transferase activities with several different substrates. PTU treatment resulted in a significant ($P < 0.05$) increase in transferase activity when 1-chloro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene were used as substrates. When 1,2-epoxy-3-(*p*-nitrophenoxy)-propane was used as substrate PTU treatment resulted in a significant decrease in transferase activity. These data suggested that either PTU concentration or T_3 concentration exerted different effects on the various enzymic activities of GSH *S*-transferases.

To ascertain whether PTU or T_3 was the direct cause of the differential effects on transferase activity, the following experiments were done. PTU-treated animals were given daily intraperitoneal injections of T_3 for 3 days. Animals were killed 24 h after the last T_3 injection,

and transferase activities were measured. As shown in Table 3, T_3 treatment of PTU-treated animals resulted in a significant decrease in transferase activity when 1-chloro-2,4-dinitrobenzene or 1,2-dichloro-4-nitrobenzene was used as substrate. However, when 1,2-epoxy-3-(*p*-nitrophenoxy)propane was used as substrate, a significant ($P < 0.01$) increase in transferase activity was observed. These data suggested that T_3 and not PTU was the direct affector of the various transferase activities.

Effects of increasing doses of T_3 on GSH *S*-transferase activity with 2-epoxy-3-(*p*-nitrophenoxy)propane as substrate

Data in Table 4 show the effects of increased concentrations of T_3 on transferase activity in hepatic tissue. Treatment of PTU-induced hypothyroid mice with T_3 resulted in a dose-dependent increase in transferase activity. This activity was significantly ($P < 0.05$) stimulated at a dose of T_3 as low as 2 μg/100 g body wt. Maximum stimulation of transferase activity was seen at a dose of 20–200 μg/100 g body wt. The addition of T_3 to the enzyme assay mixture did not result in an increase in transferase activity.

Time course of T_3 stimulation of GSH *S*-transferase activity

Increase in transferase activity (Table 5) with 1,2-epoxy-3-(*p*-nitrophenoxy)propane as substrate was observed as early as 24 h after T_3 injection. This activity was stimulated approx. 2-fold at 48 h and was maximally stimulated at 72 h. When transferase activity was measured at 96 h after T_3 treatment, it was markedly lower than that at 72 h.

Effect of actinomycin D on T_3-stimulated GSH *S*-transferase activity

Data in Table 6 show that T_3 treatment resulted in an approx. 3-fold increase in hepatic transferase activity in PTU-treated mice. This increase in activity was abolished in the actinomycin D-treated animals. Transferase activity in mice treated wth PTU only was not significantly affected by the actinomycin D treatment. In

Table 3. Effects of PTU and T_3 treatment on hepatic GSH *S*-transferase activity

Animals were treated with PTU as described in Table 2. Animals were given two consecutive daily injections of T_3 (200 μg/100 g body wt.) and were killed 24 h after the last T_3 injection. Values represent the means ± S.D. for four separate experiments using two animals for each experimental manipulation. Significance of difference from respective control: $^*P < 0.05$.

Treatment	Substrate	Activity (nmol/min per mg of protein)
PTU	1-Chloro-2,4-dinitrobenzene	5220 ± 290
PTU + T_3	1-Chloro-2,4-dinitrobenzene	3992 ± 270*
PTU	1,2-Dichloro-4-nitrobenzene	180 ± 10
PTU + T_3	1,2-Dichloro-4-nitrobenzene	110 ± 10*
PTU	1,2-Epoxy-3-(*p*-nitrophenoxy)-propane	96 ± 9
PTU + T_3	1,2-Epoxy-3-(*p*-nitrophenoxy)-propane	226 ± 11*

Table 4. Effect of increasing doses of T_3 on GSH *S*-transferase activity with 1,2-epoxy-3-(*p*-nitrophenoxy)propane as substrate

Animals were maintained on PTU for 4 weeks before T_3 injections. Mice were given intraperitoneal injections of the indicated doses of T_3 for 2 consecutive days. Animals were killed 24 h after the last T_3 injection, and hepatic cytosol was assayed for transferase activity. Results represent the means ± S.D. for four separate experiments using eight animals for each experimental manipulation. Significance of difference from control: $^{**}P < 0.001$.

Treatment	Activity (nmol/min per mg of protein)
PTU	100 ± 18
PTU + T_3 (2 μg/100 g body wt.)	225 ± 75**
PTU + T_3 (20 μg/100 g body wt.)	342 ± 25**
PTU + T_3 (100 μg/100 g body wt.)	350 ± 20**
PTU + T_3 (200 μg/100 g body wt.)	400 ± 70**

Table 5. Time course of T_3 stimulation of GSH *S*-transferase activity with 1,2-epoxy-3-(*p*-nitrophenoxy)propane as substrate

All animals were treated with PTU as described in Table 4. Animals were given a single dose of T_3 (200 μg/100 g body wt.) and killed at the indicated times. Hepatic cytosol was then assayed for transferase activity. Values represent the means ± S.D. for four separate experiments using eight animals for each experimental manipulation. Significance of difference from control: $^*P < 0.05$; $^{**}P < 0.001$.

Time of T_3 treatment (h)	Activity (nmol/min per mg of protein)
0	96 ± 10
24	145 ± 18*
48	210 ± 8**
72	150 ± 11**
96	135 ± 18*

Table 6. Effect of actinomycin D on hepatic GSH *S*-transferase activity with 1,2-epoxy-3-(*p*-nitrophenoxy)propane as substrate

Actinomycin D was administered to animals as described in the Materials and methods section. Animals were maintained on PTU for 4 weeks before T_3 and/or actinomycin D treatment. Values represent the means ± S.D. for three independent determinations using two animals for each experimental manipulation. Significance of difference from control: $^*P < 0.05$.

Treatment	Activity (nmol/min per mg of protein)
PTU	102 ± 7
PTU + actinomycin D	124 ± 23
PTU + T_3	290 ± 20*
PTU + T_3 + actinomycin D	136 ± 18

view of the known effects of actinomycin D on RNA synthesis (Goldberg & Friedman, 1971), these results suggest that the T_3-mediated increase in transferase activity involves synthesis of RNA.

DISCUSSION

We have examined the effects of T_3 on GSH *S*-transferase activity in livers from hypothyroid mice. Our studies showed that T_3 treatment resulted in a 3–3.5-fold increase in GSH *S*-transferase activity when 1,2-epoxy-3-(*p*-nitrophenoxy)propane was used as substrate. This increased activity was dose- and time-dependent (Tables 4 and 5). When other substrates such as 1-chloro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene were used, transferase activity in T_3-treated animals was suppressed. Previously reported studies in rats showed that hypophysectomy or thyroidectomy resulted in an increase in hepatic transferase activity. When these animals were treated with T_4 GSH *S*-transferase activities returned to control values. Hypophysectomy or thyroidectomy did not change the turnover of GSH *S*-transferase; however, there was some enhanced biosynthesis (Arias *et al.*, 1976). Although species variations with regard to the multiple forms and substrate specificity of GSH *S*-transferase have been reported (Lee *et al.*, 1981), some of our results in studies on mice are consistent with those reported in rats. The major difference is the effects of T_3 on the transferase activity when 1,2-epoxy-3-(*p*-nitrophenoxy)propane is used as substrate. Our results suggest that T_3 enhances this isoenzymic activity in the mouse. Moreover, this enhanced activity is inhibited by treatment of T_3-induced animals with actinomycin D. Since the mechanism of T_3 action is thought to involve a stimulation or an attenuation of gene expression (Oppenheimer, 1979; Seelig *et al.*, 1981), data in Table 6 suggest that T_3 treatment resulted in increased synthesis of RNA that codes for a specific isoenzyme of GSH *S*-transferase.

Although the GSH *S*-transferases have broad specificities for electrophilic substrates, their specificity for the nucleophilic thiol has been regarded as being very narrow (Habig *et al.*, 1974). Since PTU was used in these studies to induce a hypothyroid condition in the mice, one might consider the possibility that PTU acted as a substrate for the GSH *S*-transferases. However, Habig *et al.* (1984) clearly showed that PTU was not a substrate for GSH *S*-transferase. Thus the observed effects of T_3 on transferase activity may be attributed to the action of T_3 and not to that of PTU. We have also carried out studies on the effects of T_3 in euthyroid mice (results not shown). Results of these experiments were similar to those obtained in the hypothyroid animals. However, much higher concentrations of T_3 were required to stimulate transferase activity.

A report by Lee *et al.* (1981) described the purification and characterization of four isoenzymes of GSH *S*-transferase from mouse liver. These isoenzymes were designated F1–F4. Interestingly, the F4 isoenzyme was most active when 1,2-epoxy-3-(*p*-nitrophenoxy)propane was used as substrate. Conceivably, T_3 administration might result in a specific stimulation of this particular isoenzyme.

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