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The Effect of Altered Thyroid State on Prolactin Binding to Liver and 7,12-Dimethylbenz(a)anthracene-Induced Mammary Tumors in Rats[1] (40237)

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A relationship between altered thyroid function and breast cancer in women has been suggested, but remains unexplained (1). In order to better understand the possible role of thyroid hormones in this disease several workers have studied the effects of altered thyroid state on the growth of carcinogen-induced, hormone-dependent mammary tumors in rats. Tumor incidence was enhanced (2–4), suppressed (5–8) or unchanged (9, 10) following administration of 7,12-dimethylbenzanthracene (DMBA) to hypothyroid rats. Jull and Huggins (11) found that thyroidectomy reduced the incidence of methylcholanthrene-induced mammary tumors and that large doses of thyroxine (T_4; 1 mg/day) inhibited tumor formation, whereas lower doses (0.5 mg/day) appeared to hasten tumor appearance. Thus, the role of thyroid hormones during the induction of mammary tumors by polycyclic hydrocarbons in rats remains to be defined. Further, there is a paucity of data relating the effects of altered thyroid state to the growth rates of established DMBA-induced mammary tumors. Shellabarger (12) suggested that tumor growth was inhibited in hypothyroidism, but Chen *et al.* (13) reported that average tumor diameter per rat continued to increase following thyroidectomy and at the end of 3 weeks was not different than in control rats. The effects of hyperthyroidism on growth rates of established DMBA tumors is unknown. In normal rat mammary gland thyroid hormones antagonize the mammary development promoted by endogenous prolactin (PRL) (4, 14, 15) and pharmacological doses of thyroid hormones suppress lactation (16). The mechanism of this apparent antagonism between PRL and thyroid hormones is not known, but could be explained if thyroid hormones reduced PRL binding activity in mammary tissue in a manner similar to that observed following the suppression of lactation by estradiol (17). However, Vonderhaar (18) recently reported an increased responsiveness to PRL of mouse mammary gland explants incubated in the presence of thyroid hormones, and she suggested that thyroid hormones might enhance PRL binding. Several hormonal manipulations are now known which alter growth rates and PRL binding of DMBA-induced tumors in tumor-bearing rats (17, 20–23). Our recent findings that the inhibitory effect of large doses of estrogen on the growth of hormone-dependent DMBA-induced tumors may be related to decreased PRL-binding activity (17) stimulated our interest in the possibility that thyroid hormones might also change PRL-binding activity. In this report therefore we present results of experiments designed to evaluate the effects of altered thyroid states on the growth of established DMBA-induced tumors and to examine PRL-binding activity in tumors under these conditions.

Materials and methods. Female Sprague–Dawley rats were purchased from Charles River Breeding Laboratories (Wilmington, MA), fed and watered *ad libitum* and housed under controlled conditions (23°, 14 hr light, 10 hr darkness). Mammary tumors were induced by the weekly administration (gastric incubation) of 5 mg DMBA (Sigma Chemical Co., St. Louis, MO) in sesame oil (1.0 ml) beginning at 50 days of age and continuing for 5 weeks. Tumors began to appear 4–5 weeks after the last dose of DMBA. Tumor measurements were made 3 times weekly with calipers and tumor size was expressed as the product of two perpendicular diameters. Rats bearing tumors of sufficient size (2–3 sq cm) were randomly assigned to one of the following treatments: Control, hypothyroid or hyperthyroid. Rats were made hypothyroid by the administration of propylthiouracil (PTU, Sigma, 0.1% w/v) in the drinking wa-

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ter for 21 days (24). Hyperthyroid state was induced by daily sc injections of L-thyroxine (T_4, 75 μg/100g body wt in 0.3 ml of 0.9% NaCl) for 10 days (25). Similar treatments have been shown to effectively alter thyroid state (24, 26). Control and hypothyroid rats received daily sc injections (0.3 ml) of saline. After 10–21 days of treatment, the rats were decapitated, blood was collected for plasma PRL analysis, and livers and tumors were rapidly excised, rinsed in ice-cold 0.9% NaCl and frozen in liquid N_2. The tissues were stored at −20° until assayed for prolactin binding activity.

PRL binding to microsomal membrane particles (100–300 μg protein) prepared from tumors and livers of tumor-bearing rats was determined as previously described (17) using 60,000–80,000 cpm of ^{125}I-PRL (ovine, estimated specific activity 50–80 μCi/μg) prepared by the lactoperoxidase technique (27) and purified by passage through Sephadex G-100 prior to use in the binding assay. Specific ^{125}I-PRL binding was defined as the difference observed in the cpm bound in the absence and presence of an excess (2 μg/ml) of unlabeled ovine PRL.

Plasma PRL concentrations were determined by radioimmunoassay using materials provided by the National Institute of Arthritis, Metabolism, and Digestive Diseases.

Membrane protein concentrations were determined (28) using bovine serum albumin as the standard. The specific PRL binding data were expressed as cpm/100 μg protein and are presented as the mean ± SE of the observations in each group. Statistical comparisons were made using analysis of variance and χ^2 analysis.

Results and discussion. The induction of hypothyroidism by PTU administration or hyperthyroidism by T_4 injection did not significantly alter plasma PRL in rats bearing DMBA-induced tumors (Table I). Likewise, neither of these treatments had a significant effect on the growth rates (Table I) or the PRL binding activities (Table II) of these hormone dependent tumors. (Note that due to the small size of some tumors, not all of those detailed in Table I could be analyzed for PRL-binding in Fig. 1 and Table II.)

In contrast to the results seen in tumors, PRL binding to liver membrane particles was very greatly reduced (85%) following either PTU- or T_4-treatment (Table II). Gelato *et al.* (19) reported a similar decrease in liver PRL binding following thyroidectomy. The latter workers also showed that daily injections of 2.5 or 10 μg T_4/100 g body wt restored PRL binding to normal. These results (Table II) showed that pharmacological doses of T_4 reduced PRL binding to liver. Scatchard

TABLE II. PRL-BINDING TO TUMORS AND LIVERS OF TUMOR-BEARING RATS.

	PRL binding (cpm/100 μg protein)[a]		
	Control	PTU	T_4
Tumors	7581 ± 648 (19)	6356 ± 399 (35)	6063 ± 480 (30)
Liver	10647 ± 1793 (7)	1719 ± 553 (8)[b]	1621 ± 304 (7)[b]

[a] Values are mean ± SE of the number of observations in parentheses.
[b] $P < 0.01$ vs. controls.

TABLE I. THE EFFECT OF PTU AND T_4 TREATMENT ON DMBA-INDUCED TUMOR GROWTH, PLASMA PRL AND BODY WEIGHT.

	Control	PTU	T_4
Number of rats	7	8	8
Number of tumors	29	43	32
Tumor growth[a]			
% Regressing	3	14	3
% Static	34	37	22
% Growing	63	49	75
Plasma PRL (ng/ml)	22 ± 7[b] (7)	38 ± 8 (8)	41 ± 14 (4)
Body wt (g)			
Pretreatment	288 ± 11[b] (7)	261 ± 5 (8)	274 ± 6 (8)
Treated 10 days	283 ± 11 (7)	273 ± 12 (8)	279 ± 12 (8)
Treated 21 days	298 ± 5 (7)	262 ± 7 (8)	—

[a] Tumor growth responses were determined during the final 10 days of treatment and were defined as follows: regressing >20% decrease in tumor size; growing >20% increase in tumor size; and static, <20% change in tumor size.
[b] Values are the mean ±SE of the number of observations shown in parentheses.

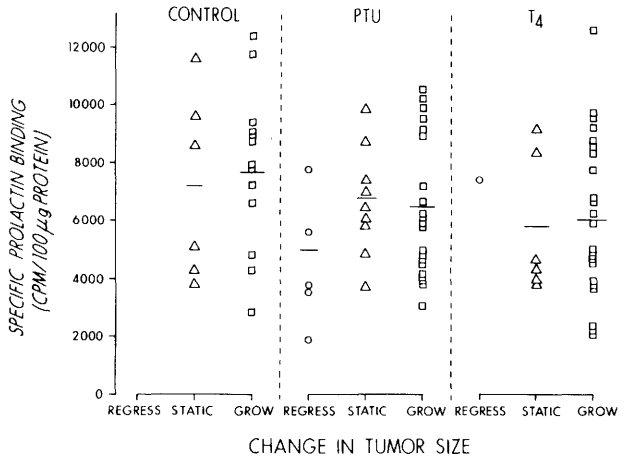

FIG. 1. Growth responses and prolactin binding activity in individual tumors following PTU or T_4 treatment. Prolactin binding activity was measured as described in the text. Horizontal lines represent the mean PRL binding activity observed in each group. Tumor growth response was determined during the final 10 days of treatment and defined as follows: regressing, >20% decrease in tumor size; growth >20% increase in tumor size, and static, <20 change in size.

analyses of PRL binding to liver membranes from control, PTU- and T_4-treated rats revealed no alterations in the apparent affinity of PRL binding, which ranged from 2.0 to 3.6 $\times 10^9 M^{-1}$ (data not shown). The differences in binding activity observed in these experiments can, therefore, be attributed to a decrease in the number of hormone binding sites per unit of membrane protein; recovery of protein in the particulate membrane fraction was not affected by PTU- or T_4 treatment (data not shown).

Our results showed, therefore, that the growth rates of established DMBA-induced tumors, PRL binding to these tumors and plasma PRL in tumor-bearing rats were not affected by hypo- or hyperthyroidism. Large, obvious changes in PRL-binding to livers occurred in the treated, tumor-bearing animals, emphasizing the lack of change in the tumors. Many endocrine factors have now been found which alter PRL-binding in liver (17, 19, 21–23, 29, 30) but the physiological role of the liver PRL receptors remains unknown.

Summary. The effects of propylthiouracil (PTU) and pharmacological doses of thyroxine (T_4) on growth and prolactin (PRL)-binding activity of DMBA-induced mammary tumors were studied. The hypo- or hyperthyroidism induced by these respective treatments did not affect the growth rate of established tumors, PRL binding activity in these tumors or plasma PRL in the tumor-bearing rats. In contrast, both PTU and T_4 treatments caused a large reduction of PRL binding activity in the liver.

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