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# Prolactin and Growth Hormone Synthesis: Effects of Perphenazine, α-Methyltyrosine and Estrogen in Different Thyroid States[1] (39076)

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There are many indications that thyroid hormones exert some influence over the secretion of prolactin (PRL) by the rat anterior pituitary gland. It is well established, for example, that thyroid ablation leads to a reduction in pituitary PRL content (1–3), and Meites and coworkers (2, 4) have suggested that thyroid hormones promote PRL secretion via a direct action on the gland. On the other hand, tumor and plasma PRL levels have been reported to increase when rats bearing a PRL-secreting tumor (MtTW15) are rendered hypothyroid by administration of propylthiouracil (3). It also has been demonstrated (5) that thyroid hormones suppress the spontaneous release of PRL by pituitary fragments or dispersed cell preparations incubated *in vitro*, as well as the release induced by ˋρGlu–His–Prl–$NH_2$ (TRH).

Drugs such as perphenazine (6) and α-methyltyrosine (7), which depress catecholamine activity in the hypothalamus, and estrogen (6) previously have been shown to enhance the rate of PRL synthesis in normal rats. We have examined the effects of thyroid deficiency and thyroxine on the ability of these agents to promote PRL synthesis in an attempt to clarify the relationship between thyroid hormones and the functional status of pituitary lactotrophs.

Amesbury *et al.* (8) have reported that estrogen also prevents the severe depletion of pituitary growth hormone (GH) content normally induced by thyroid deficiency in the rat. In view of this finding and the fact that brain monoamines have been implicated in the regulation of GH secretion (9, 10), GH synthesis was monitored throughout these experiments.

*Materials and methods.* Male Sprague–Dawley rats thyroparathyroidectomized at 34–36 days of age (THX rats) and sham-operated controls were obtained from Hormone Assay Labs., Chicago, Ill. The THX rats were provided with 0.2% $CaCl_2$ in their drinking water, while sham-operated rats received tap water. All animals had free access to a commercial rat chow (Charles River) and were housed four to five per cage in a temperature-controlled room (23 ± 1°C) under artificial illumination (0600–1800).

All animals were weighed 2–4 weeks after surgery, and any THX rats with body weights comparable to the sham-operated animals were discarded (11). In the first series of experiments, intact and THX rats were then injected with one of the following: polyestradiol phosphate (Estradurin, Ayerst), perphenazine (Trilafon, Schering), dl-α-methyl-ρ-tyrosine methyl ester HCl (Sigma) or 0.9% NaCl, which was used as a diluent for these agents. The estrogen was administered as a single dose (200 μg) 5 days prior to sacrifice, while perphenazine (5 mg/kg) and α-methyltyrosine (250 mg/kg) were injected daily for 4 days. In other experiments, some treated and untreated THX rats also received a single injection of l-thyroxine ($T_4$, 200 μg/kg) 48 hr before sacrifice. The $T_4$, a generous gift from Travenol Laboratories, was dissolved in 1 ml of 0.05 *N* NaOH and then diluted to 50 ml with 0.9% NaCl. All solutions for injection were freshly prepared and were administered sc.

The rats were killed by decapitation. Each anterior pituitary gland was incubated in a separate flask containing 1 ml of tissue culture Medium 199 (Microbiological Associates, Inc.) and 10 μCi of leucine-4,5-$H^3$ (30 Ci/mmole, International Chemical and Nuclear). The incubations were conducted at 37°C in a Dubnoff shaker, under an atmosphere of 95% $O_2$–5% $CO_2$. After 6 hr

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of incubation, the medium was aspirated and saved for electrophoresis and radioimmunoassay. The glands were rinsed twice with ice-cold saline, weighed and individually homogenized in 0.75 ml of distilled water. Homogenates and medium samples were immediately frozen over dry ice and stored at −20°C for later analysis.

Disc gel electrophoresis was performed as described by Davis (12) using a separating gel containing 7% acrylamide at pH 8.9 and a Tris–glycine buffer (pH 8.3) in both electrode compartments of a Canalco electrophoresis apparatus. The anterior pituitary homogenates were adjusted to a final concentration of 1% Triton X-100 (Rohm and Haas) before electrophoresis to lyse hormone storage granules. After 10 min at room temperature, a concentrated solution of large-pore gel was added to each treated homogenate to form the sample gel. An aliquot of each incubation medium was mixed directly with the large-pore gel solution, omitting the treatment with Triton. All samples were electrophoresed in duplicate, using a current of 5 mA/column. The separated proteins were stained overnight in 7% acetic acid containing 0.5% Amido Black 10 B (EM Reagents), and excess stain was then removed electrophoretically.

Extracts of serial segments from unstained columns were subjected to radioimmunoassay to verify the position of the PRL band. The location of GH was confirmed by comparison of the sample patterns with that produced by a purified preparation of rat GH (NIAMDD-Rat GH-B-1) electrophoresed under the same conditions.

Radioactivity in polyacrylamide gels was measured by a slight modification of the method described by Zaitlin and Hariharasubramanian (13). Transverse segments containing the GH and PRL bands were cut from the stained columns and placed in scintillation vials containing 1 ml of an NCS (Amersham/Searle)–water solution (92:8, vol/vol). The vials were capped tightly, incubated overnight at 50°C, and then cooled to room temperature. Toluene-based scintillation fluid (13) was added to each vial (10 ml), and the samples were counted 24 hr later in a Beckman LS-233 liquid scintillation spectrometer. A uniform counting efficiency of 43–44% was obtained under these conditions, and quench corrections were unnecessary.

The PRL content of the incubation medium samples was measured by a double antibody radioimmunoassay using the materials and protocol kindly supplied by the NIAMDD Rat Pituitary Hormone Distribution Program. Sheep anti-rabbit gamma globulin was obtained from Dr. Ann Johanson of the University of Virginia. Each sample was assayed in triplicate at 2 or 3 dilutions, and all samples from a given experiment were measured within a single assay. Results are expressed in terms of NIAMDD-Rat PRL-RP-1. In some experiments, PRL levels in serum collected at the time of decapitation also were determined by radioimmunoassay.

All results are presented as means ± SEM. A 2 × 2 factorial analysis of variance (14) or Student's *t*-test was used for the statistical evaluation of the data, and a $P \leq 0.05$ was considered statistically significant.

*Results.* Anterior pituitary glands obtained from male rats 2–4 weeks after thyroidectomy incorporated 52–71% less $H^3$-leucine into PRL during a 6-hr *in vitro* incubation than glands provided, by sham-operated donors (Figs. 1A–C). As shown in Figs. 1D–F, a single injection of $T_4$ (200 μg/kg) administered 48 hr prior to sacrifice slightly but significantly increased the rate of PRL synthesis in THX rats.

Four daily injections of perphenazine (5 mg/kg) or α-methyltyrosine (250 mg/kg), or a single dose (200 μg) of polyestradiol phosphate injected 5 days before sacrifice, greatly stimulated PRL synthesis in both intact and THX animals (Figs. 1A–C). Thyroid deficiency had no significant influence on the response to perphenazine, but α-methyltyrosine ($P < 0.01$) and estrogen ($P < 0.05$) were relatively more effective in THX than in intact rats. Treatment with α-methyltyrosine, for example, increased PRL synthesis by 179 ± 12% in intact rats and by 375 ± 51% in THX rats. In the same manner, $T_4$ was found to exert a suppressive effect on the rise in PRL synthesis elicited by perphena-

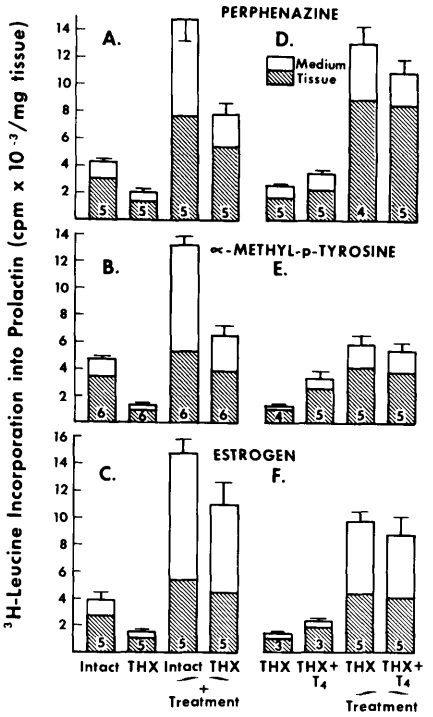


FIG. 1. Effects of perphenazine, α-methyltyrosine and estrogen on prolactin synthesis in normal, thyroidectomized and $T_4$-treated male rats. Perphenazine (5 mg/kg) and α-methyltyrosine (250 mg/kg) were injected daily (sc) for 4 days before sacrifice. A single sc injection of polyestradiol phosphate (200 μg) or $T_4$ (200 μg/kg) was administered 5 days or 48 hr prior to sacrifice, respectively. Each anterior pituitary gland was incubated for 6 hr (37°C) in 1 ml of TC medium 199 containing 10 Ci of leucine-4,5-$H^3$, and the prolactin was isolated by polyacrylamide gel electrophoresis. The shaded portion of each bar represents radioactive prolactin in the gland, while the open portion shows radioactive hormone released into the incubation medium. The number of rats receiving each treatment is indicated at the base of the bar.

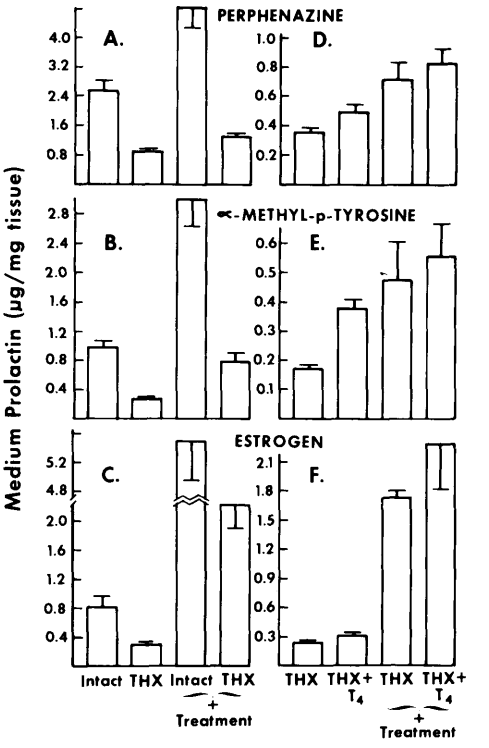


FIG. 2. Overall prolactin release *in vitro*: Effects of perphenazine, α-methyltyrosine and estrogen in normal, thyroidectomized and $T_4$-treated male rats. Prolactin levels in the incubation medium samples from the experiments described in Fig. 1 were measured by radioimmunoassay. Results are presented as mean ± SEM.

zine ($P < 0.05$), α-methyltyrosine ($P < 0.001$), and estrogen ($P < 0.01$) in THX rats (Figs. 1D–F).

Overall PRL release during incubation, as measured by radioimmunoassay, generally paralleled the level of hormone synthesis in the glands (Fig. 2). Unlike PRL synthesis, however, there was no significant relationship between the effect of perphenazine, α-methyltyrosine or estrogen on PRL release *in vitro* and the thyroid status of the recipient.

The effects of some treatments on serum PRL in normal, THX and $T_4$-injected rats also were investigated. Serum PRL levels in intact and THX rats injected 5 days earlier with estrogen (Expt C), for example, were 74 ± 15 ng/ml and 203 ± 31 ng/ml, respectively ($P < 0.01$). In the companion experiment (Expt F), $T_4$ suppressed serum PRL in estrogen-treated THX rats from 170 ± 4 to 35 ± 2 ng/ml ($P < 0.001$). A similar $T_4$-induced decline in serum PRL (61 ± 7 to 24 ± 4 ng/ml, $P < 0.01$) was noted in THX rats treated with perphenazine (Expt D). These findings provide some evidence for an inverse relationship between thyroid function and PRL secretion. Moreover, they may account for the fact that no treatment appeared to be more effective in THX rats than in intact or $T_4$-injected animals when PRL

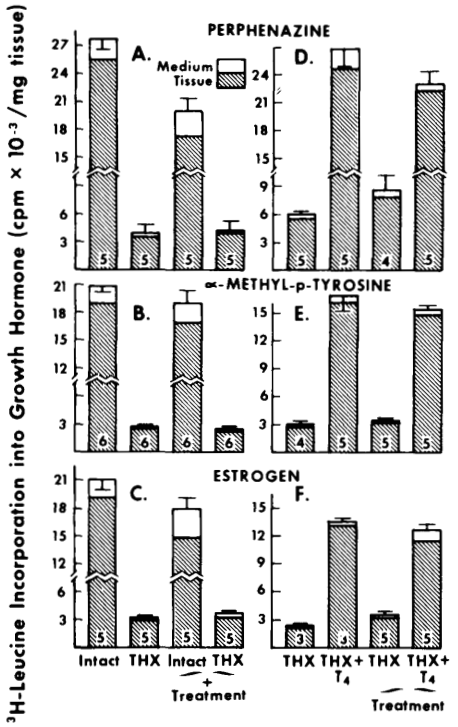


FIG. 3. Effects of perphenazine, α-methyltyrosine and estrogen on growth hormone synthesis in normal, thyroidectomized and $T_4$-treated male rats. Conditions were as described under Fig. 1.

release *in vitro* was used as an index of pituitary function, since a higher rate of PRL secretion in THX rats could lead to a reduction in pituitary hormone content (15) and thereby diminish PRL release by these glands *in vitro* (16).

Synthesis of GH declined by 84–87% as a result of thyroidectomy (Figs. 3A–C). Neither α-methyltyrosine (Figs. 3B, E) nor estrogen (Figs. 3C, F) significantly altered the rate of GH synthesis in sham-operated or THX animals. Perphenazine was also without effect in THX rats, although this tranquilizer did reduce ($P < 0.01$) GH synthesis in intact rats (Fig. 3A). The inhibitory influence of perphenazine in the male rat has been reported previously (6).

The rate of GH synthesis increased by 340–450% within 48 hr after the administration of $T_4$ to THX rats (Figs. 3D–F). In this respect, $T_4$ was equally effective in THX rats receiving perphenazine, α-methyltyrosine or estrogen.

*Discussion.* We reported several years ago that thyroid hormones are essential for the growth of a mammo-somatotropic pituitary tumor (MtTW5) as well as for some tumor-induced changes in glucose and acetate metabolism of the liver and adipose tissue (17). In a more recent study, Peake *et al.* (3) observed a marked decline in plasma and tumor (MtTW15) GH levels when rats were rendered hypothyroid six weeks after inoculation of the tumor. Since transplantable pituitary tumors are considered to be independent of hypothalamic regulation, these findings can be interpreted as an indication that thyroid hormones directly influence the rate of GH production by tumor cells.

The present study suggests that somatotrophs in the rat anterior pituitary gland may also respond directly to fluctuations in thyroid hormone levels, even though these cells appear to be subject to some form of adrenergic control (9, 10). Perphenazine and α-methyltyrosine, administered in doses which greatly suppress catecholamine activity in the hypothalamus, had no significant effect on the decline in the rate of GH synthesis which results from thyroidectomy or on the restoration of GH synthesis induced by administration of $T_4$ to THX rats. It is unlikely, therefore, that changes in brain catecholamine activity are responsible for the effects of thyroid hormones on somatotroph function. However, it is conceivable that other neural pathways may be involved in mediating the response to these hormones, and further examination of this question would seem to be warranted.

It has been reported that steroid hormones, such as estradiol (8) and cortisol (18), have the ability to maintain or restore the GH content of the pituitary in hypothyroid rats. However, Peake *et al.* (3) found that cortisol failed to increase pituitary GH content in rats chronically treated with propylthiouracil. Our findings are similar to those of Peake and coworkers, in that estrogen did not enhance the rate of GH synthesis in THX rats. A single injection of $T_4$, on the other hand, almost completely restored the level of GH synthesis in these animals within 48 hr.

As might be anticipated from the status of

GH synthesis in such animals, the pituitary of the hypothyroid rat contains few, if any, typical somatotrophs. However, there are no changes in the ultrastructural characteristics of lactotrophs to indicate that these cells are likewise adversely affected by thyroid deficiency (19). The effects of several treatments on PRL synthesis reported here are in accord with this observation. Thus, in most instances, thyroid deficiency actually enhanced the ability of lactotrophs to respond to administration of perphenazine, α-methyltyrosine or estrogen with an augmented synthesis of PRL. Furthermore, THX rats had higher serum PRL titers than intact rats following injection of estrogen, while $T_4$ lowered serum PRL in both estrogen- and perphenazine-treated THX animals. In agreement with these latter findings, others have shown that thyroid hormones reduce plasma PRL levels in hypothyroid rats bearing pituitary tumors (3) and diminish the mammotropic effect of pituitary glands implanted in hypophysectomized rats (20) or that produced by perphenazine in propylthiouracil-treated rats (21). Thyroid hormones also suppress TRH-induced PRL release in humans (22, 23) and have a similar influence on rat pituitary cells *in vitro* (5). On the other hand, $T_4$ and $T_3$ have been reported to exert a stimulatory effect on PRL secretion by rat pituitary explants (4).

While thyroid deficiency appears to enhance PRL secretion under some conditions, it is also evident from our data (Fig. 1) that glands from THX rats synthesize significantly less PRL than those from intact or $T_4$-injected THX animals. This inhibitory effect of thyroid deficiency on PRL synthesis, and perhaps on PRL content (1–3) as well, may result from a suppressed rate of cell division within the lactotroph population or cellular precursors, rather than a decline in the activity of existing cells. The unimpaired response of PRL synthesis to the treatments examined in this study is compatible with this hypothesis. We have observed, moreover, that PRL synthesis increases as a function of age in both sham-operated and THX rats (E. C. Augustine and W. C. Hymer, unpublished results). The rate of increase is greater in intact rats, and the ability of glands from these animals to synthesize more PRL largely appears to be a consequence of this differential rate of increase. Thus, although some aspects remain to be elucidated, the diverse changes in PRL metabolism described here and by others suggest the possibility that thyroid hormones have both stimulatory and inhibitory effects on pituitary lactotrophs.

*Summary.* The effects of thyroid hormones on prolactin (PRL) and growth hormone (GH) synthesis by the rat anterior pituitary gland were assessed *in vitro*. A marked reduction (84–87%) in the rate of $H^3$-leucine incorporation into GH was evident 2–4 weeks after thyroidectomy, while incorporation into PRL was 52–71% less than that measured in glands from intact rats. A single injection of $T_4$ (200 μg/kg) administered to thyroidectomized (THX) rats 48 hr before sacrifice significantly increased incorporation into both pituitary hormones, although the stimulation of GH synthesis was much more dramatic.

Perphenazine, α-methyltyrosine and estrogen enhanced the rate of PRL synthesis in intact rats. Thyroid ablation did not affect the response to perphenazine, but significantly increased the response to α-methyltyrosine and estrogen. On the other hand, administration of $T_4$ to THX rats receiving perphenazine, α-methyltyrosine or estrogen diminished the stimulatory influence of these treatments on PRL synthesis.

Perphenazine, α-methyltyrosine and estrogen had no effect on the rate of GH synthesis in THX rats, nor did they alter the ability of $T_4$ to restore GH synthesis in these animals.

These results indicate that GH synthesis in the rat is dependent upon thyroid hormones and support the concept that these hormones exert their stimulatory effect directly on pituitary somatotrophs. Pituitary lactotrophs, however, appear to retain much of their capacity to synthesize PRL under conditions of thyroid deficiency. The changes in pituitary PRL levels and synthesis rate induced by thyroid ablation might reflect differences in the number rather than the activity of these cells.

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