Effects of Thyroxine and Thiouracil on Hypothalamic PIF and Pituitary Prolactin Levels* (33929)

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There are many indications that thyroid hormones can stimulate and that thyroidectomy or goitrogen administration can inhibit pituitary prolactin secretion. An *in vitro* study in this laboratory indicated that both thyroxine and triiodothyronine directly stimulated prolactin release when cultured with rat pituitary for 3 days (1). The galactopoietic action of thyroid substances in ruminants (2) and other species (3) also suggests that pituitary prolactin secretion is enhanced. On the other hand, hypothyroidism has been reported to decrease milk secretion (4) and reduce pituitary prolactin content in male (5) and female (6) rats. Estrogen can promote prolactin secretion both by direct stimulation of the anterior pituitary (7) and by reducing PIF production by the hypothalamus (8). The present study was undertaken in an attempt to elucidate the site of action of thyroxine and thiouracil on pituitary prolactin secretion.

Materials and Methods. A total of 100 mature male Sprague-Dawley rats (body wt. 240–270 g) were used in this experiment. Rats were maintained in a room with automatically controlled lighting (14 hr light daily) and temperature (25 ± 1°). The rats were randomly divided into 4 groups of 25 each, and treated as follows: group I, controls, received subcutaneous (SC) injections of saline daily; group II were fed 0.1% thiouracil in the feed; group III received sc injections of 5 μg of thyroxine/100 g of body weight daily; group IV received sc injections of 25 μg of thyroxine/100 g of body weight daily. Water and food were provided *ad libitum*. All treatments were given for 21 days.

On day 22, all rats were weighed and killed by decapitation. Individual anterior pituitaries (AP) were removed, weighed, and AP from each group were pooled and immediately placed in a freezer at −20°. Hypothalami from each group were pooled and placed in 0.1 *N* HCl in −20° until ready for assay. The testes and seminal vesicles were also weighed.

Hypothalamic inhibiting factor (PIF) was assayed by incubating neutralized hypothalamic acid extracts with male AP, according to the method of Kragt and Meites (9). For each incubation, the AP from a mature male rat was separated from the posterior lobe, halved, and each half was placed in a separate 25-ml Erlenmeyer flask. Each flask contained 4 AP halves and 4 hypothalamic equivalents (2 hypothalamic/AP). For each group, 3 flasks were used. All incubations were carried out in a Dubnoff metabolic shaker (60 cycle/min) under constant gassing with humidified 95% O_2–5% CO_2 at 37.0 ± 0.5°. At the end of incubation the medium was separated from the AP tissue and assayed for prolactin. This procedure was repeated for each group of rats.

Prolactin activity was assayed by the sensitive pigeon crop method by Lyons (10) as modified by Reece and Turner (11) in 4–8-week-old white king pigeons. Pituitary homogenates from each group were prepared at a concentration of 10 mg/ml. Each of 4 daily injections was given in 0.1 ml so that the total pituitary tissue given was 4 mg/pigeon. The paired comparison method was employed for all assays, and 7 pigeons were used per group. Incubated media were assayed by injecting 0.2 ml daily, or a total dose of 0.8 ml was given to each pigeon. Seven pigeons were used for each PIF assay. NIH-P-B1 prolactin[1] was used as a reference standard and assayed at 3 different doses. All calculations and analysis of variance for the *F* test were carried out as described by Steel and Torrie (12). The multiple range *F* test

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[1] NIH-P-B1 prolactin (13 IU/mg) was kindly provided by the Endocrinology Study Section, NIH.

was carried out according to the method of Duncan (13).

Results. Table I shows the effects of thiouracil and thyroxine on AP prolactin content and concentration and hypothalamic tent of PIF. The AP prolactin concentration and content were significantly decreased by 0.1% thiouracil feeding despite an increase in AP weight. On the other hand, thyroxine at both dose levels significantly increased AP prolactin levels despite a decrease in AP weight. Two separate assays of hypothalamic PIF content showed no significant differences in hypothalamic PIF content either as a result of thiouracil or thyroxine treatment.

Control animals given saline injections gained 100.6 g whereas rats fed thiouracil or given injections of thyroxine showed much smaller body weight gains. The most severe inhibition of body growth was produced by injecting 25 μg of thyroxine daily/100 g of body weight. Testes weights were not altered by either thyroxine or thiouracil when compared with controls and only the higher dose of thyroxine decreased seminal vesicle weight ($p<0.05$). During the 21 days of treatment, one rat died in group 1, 2 in groups 2 and 3 and 6 rats in group 4. The latter may reflect the severe degree of hyperthyroidism induced by the high dose of thyroxine.

Discussion. The study shows that thyroxine increased and thiouracil decreased anterior pituitary prolactin levels, in agreement with earlier work on this subject (1). Hypothalamic PIF content was not altered by administering either thyroxine or thiouracil, suggesting that their effects on AP prolactin levels are not exerted via the hypothalamus.

Since thyroxine had no effect on hypothalamic PIF content, its stimulating effect on prolactin production is believed to be exerted directly on the AP. Earlier work from this laboratory demonstrated that thyroxine and triiodothyronine acted directly on the rat AP *in vitro* to increase prolactin release (1). The reduction in pituitary prolactin levels by thiouracil can be attributed to a lack of direct action of thyroxine or triiodothyronine on the AP.

TABLE I. Effects of Thyroxine and Thiouracil on Pituitary Prolactin and Hypothalamic PIF Levels.

	Group and treatment	No. of rats	Av body wt gain (g)	Av sem. ves. wt (g)	Av AP wt (mg)	AP prolactin		Hypothalamic PIF	
						(IU[a]/100 mg of AP)	(IU[a]/AP)	Assay no.	Prol. rel. into med. (IU[a]/100 mg of AP)
1	Control	24	100.58 ± 5.10[b] (256.16) (356.74)[c]	0.86 ± 0.05[b]	9.55 ± 0.22[b]	1.34 ± 0.31[b]	0.13 ± 0.01[b]	1	0.96 ± 0.20[b]
								2	0.95 ± 0.30
2	Thiouracil, 0.1%	23	54.39 ± 2.38 (261.40) (315.79)[c]	0.81 ± 0.06	11.37 ± 0.31	0.82 ± 0.20	0.09 ± 0.01	1	1.08 ± 0.33
								2	1.25 ± 0.35
	Thyroxine								
3	5 μg/100 g	23	69.87 ± 4.86 (248.80) (318.67)[c]	0.74 ± 0.02	8.83 ± 0.35	1.91 ± 0.42	0.17 ± 0.01	1	1.01 ± 0.47
								2	1.00 ± 0.48
4	25 μg/100 g	19	20.16 ± 4.34 (245.50) (265.66)[c]	0.36 ± 0.07[d]	7.75 ± 0.22	5.34 ± 0.53[d]	0.41 ± 0.01[d]	1	1.26 ± 0.37
								2	1.25 ± 0.38

[a] Expressed as equivalent of NIH-P-B_1.
[b] Mean ± standard error of the mean.
[c] Original and final body weight.
[d] Significantly different from other groups ($p<0.001$).

There is other evidence that thyroxine can exert a direct effect on the AP without necessarily involving the hypothalamus. Sinha and Meites (13) reported that thyroxine inhibits AP release of TSH by acting directly on the AP *in vitro*, and observed that *in vivo* injection of thyroxine did not alter hypothalamic TRF content. Other workers (14, 15) also found that thyroid hormones directly inhibit TSH secretion by the AP rather than via the hypothalamus. Agents other than thyroid hormones and estradiol appear to act exclusively through the hypothalamus to promote prolactin secretion. These include the suckling stimulus, reserpine, epinephrine, acetylcholine, progesterone, testosterone, and cortisol, all of which reduce hypothalamic PIF content [cf. (16)]. High circulating levels of prolactin or implants of prolactin into the median eminence increase hypothalamic content of PIF (17, 18). Only the thyroid hormones appear to act exclusively on the AP to promote prolactin secretion.

Summary. The effects of thyroxine or thiouracil on pituitary prolactin and hypothalamic prolactin inhibiting factor (PIF) levels were studied in rats. Thyroxine injected in doses of 5 or 25 μg/100 g of body weight daily for 21 days significantly increased AP prolactin content and concentration despite a significant decrease in AP weight. Thiouracil feeding (0.1%) for 21 days reduced pituitary prolactin content and concentration although these decreases were not significant. Neither thyroxine nor thiouracil had any effect on hypothalamic PIF content. These results suggest that thyroxine stimulates and thiouracil depresses prolactin secretion by a direct action of thyroxine or of the lack thereof on the AP. They do not provide any evidence that thyroxine or thiouracil influence prolactin secretion via the hypothalamus.

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