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Effects of Hypo- and Hyperthyroidism on 5-Hydroxytryptophan and Chlorpromazine-Induced Prolactin Release in the Rat[1] (39297)

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Pituitary prolactin (PRL) content was reported to be decreased in hypothyroid animals (1, 2). Serum levels of PRL as measured by radioimmunoassay were observed to be unchanged by hypothyroidism in rats and human subjects (3, 4). Recently, the response of PRL secretion to thyrotrophin-releasing hormone (TRH) was shown to be higher in hypothyroid than in euthyroid animals, and the stimulating action of TRH on prolactin release was abolished by treatment with thyroid hormones (4, 5). Onishi *et al.* (6) reported that PRL release in response to TRH and to chlorpromazine (CPZ) was eliminated in hyperthyroid humans. The precursor of 5-hydroxytryptamine (5-HT), 5-hydroxytryptophan (5-HTP), and CPZ, an adrenergic receptor blocker, both have been observed to greatly increase PRL secretion (7–9), perhaps by decreasing prolactin release-inhibiting factor (PIF) or by increasing prolactin releasing factor (PRF) in the hypothalamus (9). The purpose of this study was to determine the effects of hypo- and hyperthyroidism on pituitary release of PRL in response to 5-HTP and CPZ in rats.

Materials and methods. Mature male Sprague–Dawley rats weighing 200–225 g were obtained from Spartan Research Animals (Haslett, Mich.). The rats were housed in a light-(14 hr light and 10 hr darkness daily) and temperature-controlled (26 ± 1°) room, and received Wayne Lab Blox rat pellets (Allied Mills, Chicago, Ill.) and tap water *ad libitum*.

The rats were grouped as follows: (1) intact controls, (2) thyro-parathyroidectomized (THX), (3) THX and 2.5 μg thyroxine (T_4) per 100 g body wt, (4) THX and 10 μg T_4 per 100 b body wt. All surgically treated rats were given 0.2 ml Longicil (Fort Dodge Laboratories, Inc., Fort Dodge, Iowa), an antibiotic, by im injection to avoid infection. The sodium salt of L-T_4 (Nutritional Biochemical Co.) was dissolved in slightly alkaline saline (pH 8.0) and given sc to groups of three and four rats once daily. Rats in the intact control and THX groups were injected with alkaline saline (pH 8.0) only. After operation, the THX rats were given 1% calcium lactate solution instead of tap water to prevent tetany.

Ten days after THX or T_4 treatment, rats in each group were given a single ip injection of either 5-HTP (10 mg/100 g BW) or CPZ (2 mg/100 g BW) at 10 AM. The HCl ethyl ester of L-5-HTP (Lot No. 110118, Calbiochem Co.) and CPZ-HCl (KS&F No. 2601-A, Lot No. 93-0-TA) were each dissolved in 0.87% NaCl solution. Individual blood samples (0.8 ml) were collected from each rat by cardiac puncture under light ether anesthesia 1 day before (at 9–10 AM), and 30, 60, and 90 min after injection.

After 5-HTP or CPZ treatment, the animals were permitted 10 days for recovery from the drugs and then were killed. The anterior pituitaries (AP) were removed, weighed, and homogenized with a sonifier cell disruptor (Model W140D, Heat System–Ultrasonics, Inc.) in phosphate buffer saline (PBS). Individual serum samples and AP homogenates were frozen and kept at −20° until assayed. PRL in serum and AP samples was measured by radioimmunoassay (10), and values were expressed in terms of NIAMDD-rat prolactin-RP-1. Least significant differences was used to determine the significance of difference between control and experimental groups.

Results. The effects of a single injection of

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5-HTP on serum PRL in male rats with different thyroid states are shown in Table I. The controls showed an at least threefold increase in serum PRL in response to 5-HTP at all time periods. The average increase of serum PRL in the THX rats was lower than that in the controls at all time periods, but only the difference at 60 min after 5-HTP injection was significant ($P < 0.01$). The average increase of serum PRL in response to 5-HTP in the THX rats given 2.5 μg T_4/100 g BW/day was not different from that in controls. On the other hand, the average increase of serum PRL in the THX rats given 10.0 μg T_4/100 g BW/day was significantly higher than that in controls ($P < 0.001$) at 30 min after 5-HTP injection. In this study, some animals did not respond to 5-HTP and their serum PRL level remained at basal levels during the whole period of experiment.

In the CPZ treated rats (Table II), the increase of serum PRL reached a maximum by 30 min after injection and remained at this level at 60 and 90 min. The THX rats showed a significantly smaller response to CPZ than the controls at 60 min after injection ($P < 0.05$). The THX rats given 2.5 μg T_4/100 g BW/day showed the same response to CPZ as compared to the controls at all time periods. On the other hand, the THX rats given 10.0 μg T_4/100 g BW/day always showed a significantly greater response to CPZ than the controls after injection ($P < 0.05$). The pretreatment PRL levels in THX + 10 μg T_4/100 g BW/day rats (75 ± 18 ng/ml) were significantly higher than those in the controls (43 ± 4 ng/ml).

The AP PRL concentration of intact, THX and T_4 treated rats is shown in Table III. Twenty days after THX, AP PRL concentration decreased to about one-third of that in control rats ($P < 0.001$). Both 2.5 and 10.0 μg T_4/100 g BW/day treatments returned AP PRL concentration in the THX rats to the intact control level or even higher ($P < 0.001$).

Discussion. The present study shows that

TABLE III. PITUITARY CONCENTRATIONS OF PRL IN THX AND T_4-TREATED RATS.

Treatment and number of rats	Pituitary PRL (ng/mg)
Intact controls (7)	5047 ± 275
THX (7)	1768 ± 175***
THX + 2.5 μg T_4/100 g BW (6)	5361 ± 163
THX + 10 μg T_4/100 g BW (6)	6349 ± 298***

*** $P < 0.001$ as compared with intact controls.

TABLE I. EFFECTS OF A SINGLE INJECTION OF 5-HTP ON SERUM PRL LEVELS IN THX AND T_4-TREATED RATS.

	Serum PRL (ng/ml)			
		Post 5-HTP treatment		
Treatment and number of rats	Pretreatment	30 min	60 min	90 min
Intact controls (3)	53 ± 4	157 ± 40	269 ± 22	224 ± 47
THX (6)	50 ± 4	137 ± 5	181 ± 12**	181 ± 18
THX + 2.5 μg T_4/100 g BW (5)	52 ± 6	148 ± 16	274 ± 8	190 ± 18
THX + 10.0 μg T_4/100 g BW (6)	66 ± 6	264 ± 11***	285 ± 18	213 ± 22

** $P < 0.01$ as compared with intact controls.
*** $P < 0.001$ as compared with intact controls.

TABLE II. EFFECTS OF A SINGLE INJECTION OF CPZ ON SERUM PRL LEVELS IN THX AND T_4-TREATED RATS.

	Serum PRL (ng/ml)			
		Post CPZ treatment		
Treatment and number of rats	Pretreatment	30 min	60 min	90 min
Intact controls (8)	43 ± 4	186 ± 30	190 ± 23	185 ± 20
THX (7)	24 ± 2	122 ± 14	98 ± 8*	108 ± 11
THX + 2.5 μg T_4/100 g BW (7)	39 ± 13	220 ± 40	219 ± 26	233 ± 27
THX + 10.0 μg T_4/100 g BW (6)	75 ± 18*	308 ± 67*	307 ± 54*	348 ± 60*

* $P < 0.05$ as compared with intact controls.

the increase of PRL release in response to 5-HTP and CPZ in THX rats was smaller than in intact controls. Injection of 2.5 μg T_4/100 g BW to THX rats, a replacement dose of T_4 (11), returned the PRL responses to the two drugs to that of the intact controls, and treatment with 10.0 μg T_4/100 g BW to produce hyperthyroidism (12) increased the PRL response to the two drugs. The lower PRL response to the two drugs in the THX rats may be related to the marked reduction in pituitary PRL content, making less PRL available for release. The increased PRL response to these two drugs in the rats given 10.0 μg T_4/100 g BW may be related to the higher initial pituitary PRL content in these rats. THX and the 10.0 μg dose of T_4 also may alter the metabolism of 5-HTP and CPZ, as well as of catecholamines and 5-HT in the hypothalamus. However, it has been reported that hypothyroidism had very little effect on the metabolism of brain monoamines in adult rats, even though it was important in early neonatal life (13, 14). PRL release is believed to be influenced by both dopaminergic and serotonergic neurons in the hypothalamus. The former has been found to exert inhibitory and the latter stimulatory effects on PRL release (15). The observation that combined treatment with CPZ and 10.0 μg T_4/100 g BW enhanced PRL release in rats differs from the results reported by Onishi *et al.* (6) in human subjects and may represent a species difference in response to these treatments.

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