The Influence of Adrenal Steroids on Thyroid Function and Serum-Free Thyroxine in Tumor-Bearing Rats* (33693)

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Studies from this laboratory have demonstrated no correlation between the suppression of thyroid gland uptake of radioactive iodine and the amount of free thyroxine in the serum of rats bearing hormone-secreting pituitary tumors (1, 2).

Galton and Ingbar (3) reported an increase in percent free thyroxine (PFT) and an increase in absolute free thyroxine in sera of rats bearing the nonendocrine Walker 256 carcinoma. They suggested that this may be either a nonspecific response to illness or a more specific response to the tumor. The present study attempted to delineate the effects of the prolactin- and ACTH-secreting rat pituitary tumor 7315a on thyroid function and on serum free thyroxine levels. We will show that in intact rats, 7315a tumor decreased the uptake of radioactive iodine by the thyroid gland and increased the percent free thyroxine in serum. Evidence is presented that this increase was apparently mediated by adrenal corticosteroids.

Materials and Methods. Adult Buffalo and Sprague-Dawley female rats were fed Purina lab chow and tap water *ad libitum*. The 7315a tumor was implanted into the right subscapular area and the animals used for experimentation 6–7 weeks after implantation, when the tumors measured 2 × 4 cm. Thyroid function was studied by injecting subcutaneously 2 μCi of $Na^{131}I$/100 g of body weight and 20 hr later the radioactivity in the weighed thyroid tissue was determined. Adrenalectomy or mock operation was performed at the time of the tumor implantation in the experiments using tumor-bearing animals. Nontumor-bearing animals were adrenalectomized or mock operated 4–5 weeks prior to sacrifice. Adrenalectomized rats were maintained on isotonic saline in place of water. The rats were anesthetized with ether and blood was collected by cardiac puncture. Serum from 2–4 rats was pooled to provide sufficient volume for the free thyroxine assay.

Corticosteroid-treated rats were injected subcutaneously with 1.0 mg of prednisolone *tert*-butylacetate (Merck) daily for 6 days. On the seventh day the rats were injected subcutaneously with 5 mg of hydrocortisone acetate and were sacrificed approximately 2 hr later. Control rats received an identical series of injections of isotonic saline.

Percent free thyroxine was determined by the method of Ingbar *et al.* (4). Thyroxine labeled with ^{125}I (0.25 mCi/ml) or ^{131}I (0.2 mCi/ml) was purchased from Nuclear Chicago. Immediately upon receipt, 5 g of bovine serum albumin/100 ml was added to the thyroxine solution.

Results. The data presented in Table I show that the *in vivo* uptake of $Na^{131}I$ by thyroid tissue of intact female rats bearing the pituitary tumor 7315a, which secretes ACTH and prolactin, is approximately 70% less than that of the controls. Since these animals were pseudopregnant, it was advisable to investigate if tumor suppressed thyroid function in intact male or ovariectomized female rats. In intact male rats bearing this tumor, the thyroid glands contained much less radioactive iodide and qualitatively similar results, although statistically insignificant, were produced by the tumor hormones in ovariectomized rats. Tumor hormones, however, did not decrease thyroid function in adrenalectomized male or female rats. Further experiments were designed to investigate

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TABLE I. Effect of Adrenalectomy and Ovariectomy in Preventing Thyroid Suppression in Rats with an ACTH Secreting Pituitary Tumor.

	Rats/group	Body wt. (g)	Thyroid gland (mg)	Thyroid (mg/100 g of body wt.)	^{131}I (cpm/thyroid gland)	^{131}I (cpm/mg of thyroid)	p
Female controls intact	13	165 ± 12	9.54 ± 0.45	5.72	74,000 ± 6200	7870 ± 643	—
7315a female intact	12	184 ± 9	6.94 ± 0.37	3.77	15,800 ± 2000	2352 ± 347	<.0005
Female controls spayed	4	180 ± 6	10.74 ± 0.53	5.96	88,200 ± 9800	8225 ± 1344	—
7315a female spayed	3	175 ± 12	7.48 ± 0.23	4.27	38,600 ± 4800	5173 ± 666	NS
Female controls spayed-ADX	4	179 ± 9	9.59 ± 0.79	5.36	70,500 ± 9000	7344 ± 481	—
7315a female spayed-ADX	5	185 ± 10	9.60 ± 0.20	5.19	70,000 ±12,500	7302 ± 1344	NS
Male controls intact	8	335 ± 20	29.30 ± 1.89	8.74	100,400 ±23,000	3452 ± 693	—
7315a male intact	7	229 ± 16	26.66 ± 1.78	11.63	32,500 ± 5800	1219 ± 154	<.005
Male controls ADX	9	283 ± 18	22.15 ± 0.90	7.84	91,000 ± 8900	4121 ± 406	—
7315a male ADX	9	313 ± 15	19.10 ± 1.01	6.09	66,500 ± 9000	3469 ± 464	NS

Adult rats of the Buffalo strain were used in all cases; 2 μCi of Na^{131}I/100 g of body weight were injected 20 hr before sacrifice; mean ± SEM.

the effect that adrenal steroid hormones had on thyroid function.

The percent of free thyroxine in the sera of adrenalectomized control rats was found to slightly decrease as compared to intact control animals as shown in Fig. 1. The sera of the intact 7315a tumor-bearing rats had a greatly increased PFT, but adrenalectomized rats bearing the 7315a tumor demonstrated no increase in PFT. Tumor 7315a has previously been shown to secrete ACTH (5). Since adrenalectomy prevented the increase in PFT caused by the tumor, it was thought that the elevation of PFT in intact rats might be mediated via ACTH stmulation of adrenal corticosteroid secretion. To test this hypothesis, adrenalectomized rats were injected with prednisolone, a synthetic adrenal corticosteroid.

The data in Table II show that adrenalectomized rats injected with saline had a significantly decreased PFT compared to saline-injected, mock-operated controls. Injection of adrenalectomized rats with prednisolone significantly increased the PFT compared to adrenalectomized saline-treated rats. The PFT in the steroid-treated adrenalectomized

FIG. 1. Percent free thyroxine in sera of intact and adrenalectomized nontumor-bearing Buffalo and 7315a tumor-bearing Buffalo rats. Multiple determinations of pooled sera from 3–7 rats.

TABLE II. Percent Free Thyroxine in Sera of Adrenalectomized Sprague-Dawley Rats Receiving Prednisolone.[a]

	Mock operated	Adrenalectomized
Saline	0.206 ± 0.016	0.120 ± 0.012[b]
Prednisolone	—	0.314 ± 0.053[c,d]

[a] Values are expressed as mean ± SEM; mean of 5 analyses of separately pooled sera from several rats.

[b] Significance of difference from intact saline $p < 0.01$.

[c] Significance of difference from adrenalectomized saline $p < 0.01$.

[d] Not significantly different from intact saline. 1.0 mg of prednisolone *tert*-butylacetate was injected daily for 6 days; 2 hr before sacrifice on the seventh day, 5 mg of hydrocortisone acetate was injected.

rats was not significantly different from the saline-treated, mock-operated controls, indicating that the steroid therapy increased PFT in the adrenalectomized animals to the intact control levels.

Discussion. Previous reports from this laboratory (1, 2) have described the effects of several pituitary tumors on thyroid function. The transplantable tumors MtTW5, which is known to secrete prolactin and growth hormone but not ACTH (6, 7), and 7315a which secretes prolactin and ACTH (8) decreased thyroidal ^{131}I incorporation, while PBI was not significantly altered. It should be noted that 7315a tumor in most cases produced a decrease in the weight of the host's thyroid gland. In addition, thyroid glands from nontumor male rats were larger than those found in nontumor female rats of the Buffalo strain even when the tissue weight was corrected for the differences in body weight. Rats bearing the MtTW5 tumor had PFT values which are not significantly different from nontumor-bearing controls, but the absolute free thyroxine level was significantly decreased. In rats bearing the pituitary tumor StW5, which secretes growth hormone but not prolactin (9), the PFT was also not significantly different from nontumor-bearing controls.

The present study demonstrates that incorporation of radioactive iodide into thyroid glands was decreased and that the PFT was elevated in rats bearing the prolactin and ACTH secreting tumor 7315a. This increase in PFT caused by 7315a was completely blocked by adrenalectomy and thyroidal ^{131}I uptake remained at normal values. This suggests that the effect of the tumor is mediated by adrenal steroids, following adrenal stimulation by tumor ACTH.

The effects of ACTH and corticosterone on thyroid function were extensively studied in humans and animals (10, 12). These studies showed that cortisone decreases radioiodine uptake by the thyroid and decreases PBI. The question of whether this is a real decrease in PBI or a dilution phenomenon has been raised (13). The present study demonstrates that adrenalectomy caused a significant decrease in the PFT of rats and that corticosteroid therapy prevented this fall, causing the PFT to return to levels equal to those of normal intact control rats. Since the PFT is an indirect measure of the total thyroxine-binding capacity of the serum, these results suggest a decrease in the thyroxine-binding proteins in the 7315a tumor-bearing animals and in the adrenalectomized rats treated with corticosteroid. Oppenheimer and Werner (14) reported decreased binding of thyroxine to thyroxine-binding globulin (TGB) and increased binding to thyroxine-binding prealbumin (TBPA) in humans following prednisolone administration. They found no change in free thyroxine levels, presumably due to the increase in thyroxine binding by prealbumin equalizing the decreased thyroxine bound by TGB. Since TBPA is not present in the rat, this equalizing system is absent, presumably allowing an increase in PFT during corticosteroid-induced decrease of thyroxine binding proteins in the serum. This may allow for increased feedback by free thyroxine of thyroid function at the hypothalamo–pituitary level.

Summary. The correlation between the suppression of thyroidal uptake of radioactive iodide and the serum percent free thyroxine (PFT) in rats with hormone-secreting pituitary tumors was studied. Thyroid glands

in intact male or female rats bearing the pituitary tumor 7315a, which secretes prolactin and ACTH, took up 30 and 34% of $Na^{131}I$ as compared with nontumor intact controls. The serum PFT of these tumor-bearing rats was 72% higher than controls. Adrenalectomy resulted in complete restoration of thyroidal function to normal levels and was accompanied by a fall in serum PFT. Serum PFT was significantly decreased in the sera of adrenalectomized nontumor-bearing rats compared with intact controls, and this decrease was prevented by prednisolone treatment. The PFT in sera of prednisolone-treated adrenalectomized rats was not significantly different from intact controls. It is suggested that in the 7315a tumor-bearing animals the decreased thyroid function is related to the increase in serum PFT. This increase in PFT may reflect a decrease in total binding of thyroxine to serum proteins and this effect may be mediated by the elevated serum corticosteroids caused by the ACTH secreted by the pituitary tumor.

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