ESTROGEN POTENTIATION OF THE EFFECT OF THYROTROPHIN ON THYROID ¹³¹I UPTAKE IN HYPOPHYSECTOMIZED FEMALE RATS

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SYNOPSIS

The daily administration of β -estradiol benzoate (50 μ g or 1 mg) to hypophysectomized female rats for five consecutive days did not markedly alter ¹³¹I uptake by the thyroid gland. Estrogen or PMS increased thyroid uptake of ¹³¹I in TSH treated hypophysectomized rats. Similar results were obtained with estrogen but not PMS in hypophysectomized and oophorectomized animals. These observations show that estrogen can potentiate the TSH induced uptake of ¹³¹I by an extrapituitary mechanism.

Hypothyroidism, nodular goiter and other types of thyroid diseases occur more often in women than in men. Frequently, goiter manifests itself strikingly during puberty, pregnancy and menopause. Changes in thyroid function also occur following estrogen treatment or castration (Chouke *et al.*, 1935; Amilibia *et al.*, 1936; Muller and Aeppli, 1949; Wolterink *et al.*, 1950; Desclin and Ermans, 1951), and thyroidal ¹³¹I uptake increases significantly in estrus and decreases to the lowest level in proestrus in rats (Feldman, 1956; Soliman and Reineke, 1954). Several investigators found a depletion in the TSH content of the pituitary after estrogen administration (Grumbrecht and Loeser, 1938; Simpson and Evans, 1941; Desclin and Ermans, 1950), and suggested that estrogens bring about TSH release. However, normal levels of TSH in the pituitary of ovariectomized rats also were reported after estrogen administration (Turner and Cupps, 1940). Using various criteria for evaluation of thyroid function, Soliman and Reineke (1959), Noach (1955) and Bogdanove and Horn (1958) observed that estrogens did not produce any significant effect on the thyroid of hypophysectomized rats. Feldman (1956), Donato *et al.* (1958) and Boccabella and Alger (1964), on the other hand that estrogens stimulated ¹³¹I uptake or increased thyroid: serum radioiodine ratio (T:S). Boccabella and Alger (1964) found that after a single injection of estrogen there was a fall in T:S ratio after 18 hrs. and then elevation to a maximum at 60~72 hrs. followed by a second fall. The role played by estrogen in thyroid function, therefore, is not clearly understood. The experiments described in this paper were undertaken to study the effect of estrogens on ¹³¹I uptake by the thyroid of hypophysectomized female rats.

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MATERIALS AND METHODS

Hypophysectomized female rats of Sprague-Dawley strain, weighing 45~55 g, were obtained from Hormone Assay Laboratories, Chicago, 3 days after hypophysectomy. The animals were fed Wayne Lab-Blox (Allied Mills, Inc., Chicago) and received tap water *ad lib.* Sodium iodine ^{131}I for thyroïdial ^{131}I uptake studies was obtained from Abbot Laboratories.

Experiment 1: Ninety-eight hypophysectomized female rats were divided into 8 groups, which were injected subcutaneously (s.c.) for 5 days, on the 14th day after hypophysectomy. Group 1—corn oil, 0.2 ml/rat/day; Group 2—TSH, 2 mg (Thytropar, Armour, 0.1 USP unit) in 0.2 ml saline (0.9 percent aqueous sodium solution)/rat/day; Group 3— 17β -estradiol benzoate (Mann Research Lab., USP) $50\mu\text{g}$ in 0.2 ml corn oil/rat/day; Group 4—estradiol benzoate, 1 mg in 0.2 ml corn oil/rat/day; Group 5—estradiol benzoate, $50\mu\text{g}$ in 0.2 ml corn oil/rat/day and TSH, 2 mg in 0.2 ml saline/rat/day; Group 6—estradiol benzoate, 1 mg in 0.2 ml corn oil/rat/day and TSH, 2 mg in 0.2 ml saline/rat/day; Group 7—pregnant mares' serum (PMS, Equinex, Ayerst) 10 IU in 0.2 ml saline/rat/day; and Group 8—PMS, 10 IU and TSH, 2 mg in 0.2 ml saline/rat/day.

The animals were sacrificed on the 6th day and their thyroid glands were weighed and fixed for histological examination. Some of the animals from each group were subcutaneously injected with $0.2\mu\text{C}$ of ^{131}I in 0.2 ml saline 24 hrs. before sacrifice. After weighing the thyroid glands, the radioactivity was measured using a Tracer-lab well-type scintillation counter for 1 min. and the thyroïdial ^{131}I uptake measured as 24 hrs. thyroid uptake (percent)

$$= \frac{\text{rat thyroid cpm-background cpm}}{\text{cpm of injected } ^{131}\text{I-background cpm}} \times 100$$

Corrections for radioactive decay over the experimental period were incorporated in the calculations.

Experiment 2: Thirty-two female hypophysectomized rats were injected subcutaneously 14 days after hypophysectomy: Group 1—TSH, 2 mg in 0.2 ml saline/rat/day; Group 2—estradiol benzoate, $50\mu\text{g}$ in 0.2 ml corn oil/rat/day and TSH, 2 mg in 0.2 ml saline/rat/day and Group 3—estradiol benzoate, 1 mg in 0.2 ml corn oil/rat/day and TSH, 2 mg in 0.2 ml saline/rat/day. Six controls and 9 other animals in various groups died during the experimental period. All the rats were injected with $0.2\mu\text{C}$ of ^{131}I and the thyroïdial uptake calculated.

Experiment 3: Fifty-six female hypophysectomized rats were injected subcutaneously 14 days after hypophysectomy: Group 1—corn oil, 0.2 ml/rat/day; Group 2—estradiol benzoate, $50\mu\text{g}$ in 0.2 ml corn oil/rat/day; Group 3—estradiol benzoate, 1 mg in 0.2 ml corn oil/rat/day and Group 4—PMS, 10 IU in 0.2 ml saline/rat/day. All the rats were injected with $0.2\mu\text{C}$ of ^{131}I and the thyroïdial uptake calculated.

Experiment 4: Twenty-three female hypophysectomized rats were castrated 4 days after hypophysectomy. Ten days after castration the animals were treated by subcutaneous injections for 5 days: Group 1—corn oil, 0.2 ml/rat/day; and Group 2—PMS, 10 IU in 0.2 ml saline/rat/day. All rats were subcutaneously injected with $0.2\mu\text{C}$ of ^{131}I in 0.2 ml saline 24 hrs. before being sacrificed. The thyroid was removed and weighed and thyroïdial uptake was determined.

Experiment 5: Twelve hypophysectomized-castrated rats similar to those used in Experiment 4 were injected subcutaneously for 5 days: Group 1—PMS, 10 IU in 0.2 ml saline/rat/day and Group 2—PMS, 10 IU in 0.2 ml saline/rat/day and estradiol benzoate, 1 mg in 0.2 ml corn oil/rat/day. Thyroïdial uptake was then calculated.

RESULTS

In hypophysectomized rats, the administration of estrogen ($50\mu\text{g}$ and 1 mg of estradiol benzoate) resulted in no marked change in thyroid weight or ^{131}I uptake

Table 1. Influence of estrogen and PMS on thyroidal ^{131}I uptake in hypophysectomized rats—I

Treatment	No. of rats	Body wt (g)	Thyroid wt		Thyroidal ¹³¹ I uptake 24 hr. per mg thyroid
			(mg)	(mg/100 g B wt)	
Expt 1					
Control (oil)	12	67.4±1.1*	5.5±0.3*	8.2±0.3*	1.0±0.1*(6)
TSH 2 mg/day	12	70.3±1.4	6.6±0.2	9.4±0.2	5.3±0.3 (6)
estrogen 50 µg/day	12	67.5±1.4	5.3±0.3	7.8±0.4	1.0±0.1 (6)
estrogen 1 mg/day	12	65.4±1.3	5.2±0.3	7.7±0.3	1.4±0.1 (6)
TSH 2 mg/day estrogen 50 µg/day	13	66.3±2.8	5.9±0.2	8.9±0.4	7.1±0.5 (6)
TSH 2 mg/day estrogen 1 mg/day	13	64.8±1.3	6.0±0.3	9.4±0.4	8.6±0.4 (6)
PMS 10 IU/day	12	66.3±1.6	5.2±0.3	7.8±0.3	2.6±0.2 (6)
TSH 2 mg/day PMS 10 IU/day	12	68.2±1.1	6.3±0.2	9.3±0.4	8.6±0.4 (6)
					0.21±0.04*
					0.83±0.06
					0.20±0.05
					0.24±0.04
					1.51±0.15
					1.68±0.10
					0.53±0.08
					1.47±0.10

* Represents standard error of mean. () Indicates number of rats measured ^{131}I uptake.

Table 2. Influence of estrogen and PMS on thyroid ^{131}I uptake in hypophysectomized rats—II

Treatment	No. of rats	Body wt (g)	Thyroid wt		Thyroidal ^{131}I uptake 24 hr	
			(mg)	(mg/100 g B wt)	per thyroid	per mg thyroid
Expt 2						
TSH 2 mg/day	5	61.0 $\pm$ 1.6*	6.4 $\pm$ 0.2*	10.5 $\pm$ 0.5*	5.5 $\pm$ 0.1	0.86 $\pm$ 0.07*
TSH 2 mg/day Estrogen 50 $\mu\text{g/day}$	5	59.8 $\pm$ 2.3	7.4 $\pm$ 0.1	12.4 $\pm$ 0.7	8.6 $\pm$ 0.9	1.19 $\pm$ 0.13
TSH 2 mg/day Estrogen 1 mg/day	5	55.8 $\pm$ 0.9	6.7 $\pm$ 0.3	12.0 $\pm$ 0.5	9.3 $\pm$ 1.2	1.36 $\pm$ 0.13
Expt 3						
Control (oil)	11	66.2 $\pm$ 1.1*	5.7 $\pm$ 0.2*	8.5 $\pm$ 0.2*	0.7 $\pm$ 0.07*	0.11 $\pm$ 0.01*
Estrogen 50 $\mu\text{g/day}$	10	57.8 $\pm$ 0.9	5.0 $\pm$ 0.0	8.6 $\pm$ 0.1	0.6 $\pm$ 0.06	0.12 $\pm$ 0.01
Estrogen 1 mg/day	14	59.0 $\pm$ 0.8	5.7 $\pm$ 0.2	9.6 $\pm$ 0.4	0.7 $\pm$ 0.03	0.12 $\pm$ 0.01
PMS 10 IU/day	11	57.6 $\pm$ 1.1	6.4 $\pm$ 0.4	11.1 $\pm$ 0.6	1.9 $\pm$ 0.3	0.29 $\pm$ 0.06

* Represents standard error of mean.

Table 3. Influence of estrogen and PMS on thyroidal ^{131}I uptake in hypophysectomized and oophorectomized rats

Treatment	No of rats	Body wt (g)	Thyroid wt		Thyroidal ^{131}I uptake 24 hr.	
			(mg)	(mg/100 g B. wt.)	per thyroid	per mg thyroid
Expt 4						
Control (oil)	11	70.4 $\pm$ 1.0*	5.9 $\pm$ 0.3*	8.4 $\pm$ 0.3*	0.6 $\pm$ 0.03*	0.10 $\pm$ 0.10*
PMS 10 IU/day	12	68.8 $\pm$ 0.7	6.4 $\pm$ 0.2	9.2 $\pm$ 0.2	0.6 $\pm$ 0.03	0.08 $\pm$ 0.10
Expt 5						
PMS 10 IU/day	6	62.0 $\pm$ 1.5*	7.5 $\pm$ 0.1*	12.0 $\pm$ 0.2*	0.6 $\pm$ 0.08*	0.09 $\pm$ 0.10*
PMS 10 IU/day Estrogen 1 mg/day	6	59.1 $\pm$ 1.0	7.3 $\pm$ 0.2	12.3 $\pm$ 0.3	1.2 $\pm$ 0.15	0.18 $\pm$ 0.02

* Represents Standard error of mean.

(Table 1). No significant histological difference was seen between the thyroids of estrogen treated and control rats. TSH increased the thyroidal ^{131}I uptake significantly over controls ($p < 0.05$) and so did the administration of PMS. Simultaneous administration of PMS or estrogen appeared to potentiate the effect of TSH ($p < 0.05$) on the uptake of ^{131}I by the thyroid (Table 1). However, no histological distinction was observed between the thyroids of TSH, TSH-PMS, or TSH-estrogen treated animals. The absence of the influence of estrogen at the $50\mu\text{g}$ or 1 mg level on thyroidal ^{131}I uptake and the potentiation by estrogen of the TSH induced ^{131}I uptake at these levels were confirmed in Experiments 2 and 3 (Table 2). Increased uptake of ^{131}I after PMS was also noted (Table 2; Expt. 3). This effect could not be demonstrated in hypophysectomized and ovariectomized rats (Table 3; Expt. 4). However, when estrogens were administered simultaneously with PMS, the thyroidal ^{131}I uptake was elevated significantly (Table 3; Expt. 5).

DISCUSSION

The experiments reported in this paper demonstrate that the administration of estrogens ($50\mu\text{g}$ or 1 mg) to hypophysectomized rats for 5 days did not alter significantly the thyroid weight or the thyroidal ^{131}I uptake. Soliman and Reineke (1955), after a single injection of estrogens and Noach (1955) after 20 to $50\mu\text{g}$ estrogen administration for 1 week obtained similar results by measuring thyroidal ^{131}I uptake. Using a 5 day treatment period Bogdanove and Horn (1958), also observed no change in thyroid size or T:S ratios following the administration of $8\text{--}150\mu\text{g}$ of α -estradiol benzoate to hypophysectomized female rats. On the other hand, Feldman (1956) and Donato *et al.* (1958), reported stimulation of ^{131}I uptake by estrogen treatment and Boccabella and Alger (1964), found an increase in T:S ratio following the administration of α -estradiol benzoate (1 to $250\mu\text{g}$) for 3 days to ovariectomized and hypophysectomized rats. Furthermore, Boccabella and Alger (1964), showed that following a single injection of estrogens, the T:S ratio was depressed to a minimum after 18 hrs. and elevated to a maximum at $60\sim 72$ hrs., then showing a gradual decrease. Boccabella and Alger (1964), did not study the effect of estrogen 144 hrs. after the first injection as we did. It is felt that the apparent discrepancies in our results with those of Feldman (1956), Donato *et al.* (1958) and Boccabella and Alger (1964) were due to differences in the estrogen employed, in the timing of estrogen administration and evaluation of thyroid function and the different criteria used for measuring thyroid function.

The data in Tables 1 and 2 demonstrate that the concurrent administration of estrogen and TSH to hypophysectomized rats for 5 days, significantly increased the thyroidal ^{131}I uptake as compared to that in animals treated with TSH alone. This indicates estrogen potentiation of the effect of TSH on the thyroid. Similar observations were made in male rats by Noach (1955) (2 Heyl-Laqueur Units daily) who used thyroid weight and ^{131}I uptake as the criteria for thyroid activity. In rabbits, however, Beckers and DeVisscher (1957) obtained lesser stimulation of the thyroid by the simultaneous administration of estrogen and TSH as compared to TSH alone. Delost and Delost (1957) using intact rats did not observe with any changes in the morphology and histopathology of the

thyroid. Feldman (1956) using rather high doses of TSH (0.25 USP units twice daily for 3 days) as compared to our dosage could not demonstrate any influence of estrogen on TSH action and suggested that the effect may have been masked by this dose of TSH.

The potentiation of action of TSH on thyroidal ^{131}I uptake in hypophysectomized rats by PMS could be explained on the basis of PMS stimulation of ovarian estrogens. However, PMS alone, in two separate experiments was able to increase thyroidal ^{131}I uptake to lesser degree and therefore further experimentation was necessary. It was found that PMS alone could not increase ^{131}I uptake in hypophysectomized rats in the absence of the ovaries whereas when a small amount of estrogen was simultaneously administered, a significant increase was observed. On the basis of these observations, it appeared that endogenous or exogenous estrogens accentuated some contaminant possibly TSH like material, in the PMS preparation. The major role, however, of PMS in the potentiation of the action of TSH appears to be mediated through the stimulation of estrogen production by the ovaries. Noach (1955) arrived at similar conclusions of the basis of his study.

REFERENCES

- Amilibia, E., M.M. Mendizabal and Botella-Llusia (1936). *Klin. Wochschr.* **15**, 1001.
Beckers, Ch. and M. DeVisscher (1957). *Ann. Endocrinol.* (Paris) **18**, 1.
Boccabella, A.V. and E.A. Alger (1964). *Endocrinology* **74**, 680.
Bogdanove, E.M. and E. Hörn (1958). *Ibid.* **62**, 97.
Chouke, K.S., H. Friedman and L. Loel (1935). *Anat. Record* **63**, 131.
Delost, P. and H. Delost (1957). *Compt. Rend. Acad. Sci. (Paris)* **245**, 208.
Desclin, L. and A.M. Ermans (1950). *Compt. rend. soc. biol. (Paris)* **144**, 1277.
Desclin, L. and A.M. Ermans (1951). *Ann. Endocrinol.* **12**, 238.
Donato, L., G. Furchetti and A. Zacutti (1958). *bull. soc. ital. biol. sper.* **34**, 265.
Feldman, J.B. (1956). *Endocrinology* **58**, 327.
Grumbrecht, P. and A. Loeser (1938). *Arch. Exptl. Pathol. Pharmacol.* **189**, 345.
Muller, J.H. and H. Aeppelinol. (1949). *Experimentia* **5**, 297.
Noach, E.L. (1955). *Acta Endocrinol.* (Kobenhavn) **19**, 127.
Simpson, M.E. and H.M. Evans (1941). *Anat. Record* **79**, suppl. 2, 57.
Soliman, F.A. and E. Reineke (1954). *Am. J. Physiol.* **178**, 89.
Soliman, F.A. and E.P. Reineke (1955). *Ibid.* **183**, 63.
Turner, C.W. and P.T. Cupps (1940). *Endocrinology* **26**, 1042.
Wolterink, L.F., C.C. Lee, K. Olsen and M. Murray (1950). *Federation Proc.* **9**, 138.