Effect of Thyroxine on RNA Synthesis of Rat Uterus (35294)

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The synthesis of RNA in rat uteri is influenced by 17β-estradiol, insulin, and growth hormone (1, 2). Since thyroxine influences RNA synthesis and various metabolic pathways in the liver (3), it may affect these processes in the uterus. The present study was undertaken to determine whether or not thyroxine influences RNA synthesis in rat uteri.

Materials and Methods. Female overiectomized and ovariectomized–thyroidectomized Sprague-Dawley rats of approximately 200 g body wt were purchased from Hormone Assay Co., Chicago, Ill., and used not earlier than 3 weeks following the operation. Uridine-5-^{3}H (sp act 28.3 Ci/mmole) was purchased from New England Nuclear Corp., Boston, Mass.; tetralithium 5-^{3}H-uridine-5′-triphosphate (sp act 17.1 Ci/mmole) from Schwarz BioResearch Inc., Orangeburg, N.Y.; 17β-estradiol from Ciba Pharmaceutical Products Inc., Summit, N.J.; L-thyroxine and yeast RNA from Sigma Chemical Co, St. Louis, Mo.; actinomycin D from Merck & Co., Inc., West Point, Pa.; Soluene 100 from Packard Instrument Company, Inc., Downers Grove, Ill. Ovariectomized–thyroidectomized rats were given 1% $CaCl_2$ as drinking water. 17β-Estradiol (10 μg/ml of 1% ethanol–0.9% NaCl solution) was administered ip to rats 30 min prior to sacrifice. L-Thyroxine (500 μg/0.2 ml of slightly alkaline saline solution) was administered sc 2 hr prior to sacrifice. Actinomycin D (250 μg/ml water) was administered ip 150 min prior to sacrifice. Control animals received the same solution without hormone. Uridine-5-^{3}H (86.6 μCi/0.5 ml of saline) was administered ip 10 min prior to sacrifice. The assay for the incorporation of uridine-^{3}H into uterine acid-soluble and RNA fractions and radioactivity in blood serum was described in a previous publication (1).

Preparation of uterine nuclei. The rats were killed by guillotine. Blood was drained from the severed jugular veins and allowed to clot. Serum was obtained after centrifugation. Uteri were removed, dissected free of fat and connective tissues, and kept in cold TKM medium (0.25 *M* sucrose; 0.05 *M* Tris–HCl buffer, pH 7.4; 0.025 *M* KCl; 3 m*M* $MgCl_2$). All subsequent steps were performed in the cold. Three uteri were cut finely with scissors, homogenized in a Polytron Type PT-10 (Brinkmann Instruments, Westbury, N.Y.) with 20 vol of the TKM medium for 20 sec and filtered through 2 layers of gauze. The filtrate was centrifuged at 700*g* for 10 min. The pellet was suspended in 5 ml of the buffer and centrifuged. The pellet was resuspended in 5 ml of 0.05 *M* Tris–HCl buffer, pH 8.0, and the procedure was repeated. The nuclear pellet was suspended in 0.6 ml of 0.05 *M* Tris–HCl buffer, pH 8.0. DNA was determined by the method of Schneider (4). The reaction mixture for RNA polymerase contained 25 μmoles of Tris–HCl buffer, pH 8.0; 15 μmoles of KCl; 1 μmole of $MgCl_2$; 5 μmoles of NaF; 0.4 μmoles of mercaptoethanol; 0.4 μmoles of $MnCl_2$; 0.25 μmoles of ATP; 0.025 μmoles of GTP; 0.025 μmoles CTP; 1 μCi ^{3}H-UTP; and 0.1 ml of nuclei suspension in a total volume of 0.25 ml. After incubation at 37° for 10 min, the reaction was stopped by adding 1.0 ml of cold saturated sodium pyrophosphate solution. To the mixture 0.2 ml of serum albumin solution (1 mg/0.2 ml) and 1.45 ml of cold 10% TCA were added. After centrifugation of the mixture the pellet was washed in sequence with 5 ml of cold 5% TCA, 95% ethanol, ethanol–ether 1:1 (v/v), ether, and dried. One ml of Soluene 100 (solubilizer) was added to the pellet and incubated at 37° overnight. The samples were dissolved in 10 ml of liquifluor (2.37

TABLE I. Effect of Thyroxine, 17β-Estradiol and Actinomycin D on Tritiated Uridine Incorporation into Acid-Soluble and RNA Fractions of Rat Uteri.[a]

Conditions	Hormone treatment	Wet wt (mg)	Radioactivity: Serum (10^3 cpm/ml)	Radioactivity: Acid soluble (cpm/mg of tissue)	Radioactivity: RNA (10^3 cpm/mg of RNA)	p value
Ovariectomized	Control	117.5 ± 3.1	1.61 ± 0.08	448 ± 59	2.95 ± 0.56	—
	Estradiol	116.0 ±21	1.62 ± 0.01	468 ± 47	4.37 ± 0.53	<0.05
Ovariectomized–thyroidectomized	Control	115.0 ± 4.6	2.25 ± 0.11	1010 ± 51	6.01 ± 0.80	—
	Estradiol	115.4 ± 2.7	2.15 ± 0.14	850 ±100	8.20 ± 2.50	ns
	Thyroxine	116.6 ± 4.7	2.20 ± 0.20	820 ± 66	11.60 ± 1.50	<0.002
	Estradiol–thyroxine	113.0 ± 3.3	2.02 ± 0.14	910 ±112	15.21 ± 2.20	<0.002
Ovariectomized–thyroidectomized + actinomycin D	Control	110.3 ± 4.9	2.15 ± 0.11	922 ± 37	1.55 ± 0.17	—
	Estradiol–thyroxine	107.5 ± 4.8	2.17 ± 0.06	866 ± 78	1.44 ± 0.29	—

[a] Probabilities are by Student's t test between means of control and hormone-treated rats (9). Values are mean ± SE of 6 to 9 separate experiments.

liter of toluene mixed with 100 ml of Spectrafluor) and radioactivity was measured with a Packard liquid scintillation counter, Model 3003. Control experiments were performed by adding nuclei suspension to the reaction mixture after the addition of sodium pyrophosphate solution. The values of the control runs were subtracted from the experimental values.

Results and Discussion. Table I shows the uterine weight and the amounts of incorporation of tritiated uridine into the acid-soluble fraction and RNA of thyroidectomized–ovariectomized rat uteri following 17β-estradiol and thyroxine administration. The amount of incorporation of uridine-^{3}H into uterine acid-soluble fraction and RNA and the level of radioactivity in blood serum were higher with ovariectomized–thyroidectomized rats in comparison to the values obtained with ovariectomized rats. The elevated blood level of radioactivity may account for the increase in the incorporation of uridine-^{3}H into uterine RNA. However, the blood radioactivity level remained unchanged during the 2-hr period of thyroxine administration (Table I). Although the factors responsible for the increased blood level were not elucidated, the present findings suggests that the permeability, transport, half-life in blood or renal clearance of uridine were altered in ovariectomized–thyroidectomized rats.

17β-Estradiol and thyroxine administered to thyroidectomized–ovariectomized rats increased the incorporation of uridine-^{3}H into uterine RNA. On the other hand, uridine incorporation into the acid soluble fraction remained unchanged (Table I). The highest incorporation was observed following the administration of both hormones. Actinomycin D administered to ovariectomized–thyroidectomized rats with or without hormone treatment inhibited the incorporation of uridine-^{3}H into uterine RNA to a level below that of the control untreated rats (Table I) which suggests that even in the absence of 17β-estradiol and thyroxine, uterine RNA synthesis occurs at a significant rate. This apparent RNA synthesis which is inhibited by actinomycin D may be due to the stimulatory influence of other factors such as insulin and growth hormone which will facilitate RNA synthesis in rat uteri (1). Actinomycin D did not influence the incorporation of tritiated uridine into the uterine acid soluble fraction. The uterine weights remained unchanged in all groups studied. This finding is in contrast to the results obtained with insulin and growth hormone administration (1).

Table II shows the nucleotide requirement of the assay system for uterine RNA polym-

TABLE II. Nucleotide Requirement for the Assay of Rat Uterine RNA Polymerase Activity.[a]

	cpm/μg of DNA
Complete	6.68
—ATP	0.70
—GTP	1.85
—CTP	2.31
—ATP, —GTP	0.02
—ATP, —CTP	0.25
—GTP, —CTP	1.94
—ATP, —GTP, —CTP	—

[a] The assay system is described in the text.

erase activity. The maximum activity was observed at pH 7.5–8.0. Table III shows the RNA polymerase activity of uterine nuclei obtained from ovariectomized and ovariectomized–thyroidectomized rats. Thyroxine administered to ovariectommized rats did not alter the uterine RNA polymerase activity. However, the RNA polymerase activity of ovariectomized rats was lower than that of ovariectomized–thyroidectomized rats ($p<0.05$). Moreover, thyroxine administered to ovariectomized–thyroidectomized rats depressed the RNA polymerase activity of uterine nuclei ($p<0.02$); whereas the combined administration of thyroxine and 17β-estradiol did not influence the activity. The mechanism of the inhibitory activity (Table III) and of its stimulatory effect on the polymerase activity of rat liver (3) remains obscure. It is noteworthy that 17β-estradiol administered to ovariectomized rats increased slightly uterine polymerase activity and did not affect the uterine polymerase activity of ovariectomized–thyroidectomized rats. The present results suggest that the stimulatory influence of 17β-estradiol on uterine RNA polymerase activity may be dependent upon the presence of thyroid hormone.

The amount of thyroxine administered was excessive to assure that the effect of the hormone can be observed within 2 hr. When 20 μg of thyroxine were administered to castrated–thyroidectomized rats, a slight but significant increase in the incorporation of uridine-^{3}H into RNA ($6.98 \pm 1.42 \times 10^3$ cpm/mg RNA, Table III for control value) was observed. The daily requirement of L-thyroxine for the rat is about 1.2 μg/100 g of body wt (5). However, to observe an early effect of thyroxine a larger dose is required. The oxygen consumption of thyroidectomized rats returned to normal value 8 hr after a single injection of 150 μg of thyroxine/100 g of body wt (5). In the present study thyroidectomized rats were killed 2 hr after thyroxine administration (250 μg/100 g of body wt) when the oxygen consumption was probably below normal.

It is not clear how thyroxine increases the incorporation of uridine-^{3}H into uterine RNA while concomitantly depressing RNA polymerase activity. The apparent opposing effects of thyroxine suggest that its action on uterine RNA synthesis is probably indirect as was postulated for its influence on hepatic RNA synthesis (3). It may be secondary to its influence on oxidative phosphorylation. Other factors may influence the incorporation of labeled uridine into RNA under physiological conditions such as pool size of nucleotides (6), ionic conditions within the cell (7,

TABLE III. Effect of 17β-Estradiol and Thyroxine on RNA Polymerase Activities of Rat Uteri.[a]

Treatment	Ovariectomized (cpm/μg of DNA)	Ovariectomized–thyroidectomized (cpm/μg of DNA)	p value
Control	8.03 ± 0.33	11.70 ± 1.03	—
Estradiol	9.52 ± 0.77	10.18 ± 1.42	ns
Thyroxine	8.94 ± 1.20	6.94 ± 0.58	<0.02
Estradiol–thyroxine	—	9.26 ± 1.52	ns

[a] Values are mean $\pm$ SE of 5 to 7 separate experiments consisting of three rats per group. Probabilities are by Student's t test between the means of control and hormone-treated ovariectomized–thyroidectomized rats. The p value of means between control ovariectomized and control ovariectomized–thyroidectomized rats was <0.05.

8), ribonuclease activity, and the conversion of uridine to UTP. These factors may be important in the early stimulation of RNA synthesis by thyroxine which may not be observed by measuring RNA polymerase activity *in vitro*.

Summary. The influence of thyroxine on the incorporation of uridine-^{3}H into uterine acid soluble and RNA fractions and on nuclear RNA polymerase activity of ovariectomized–thyroidectomized rats was studied. The incorporation of uridine-^{3}H into uterine RNA of untreated ovariectomized-thyroidectomized rats was higher than that of untreated ovariectomized rats which was further elevated by thyroxine and 17β-estradiol administration. A potentiating effect was observed following the administration of both hormones which was inhibited by actinomycin D.

The uterine nuclear RNA polymerase activity of untreated ovariectomized-thyroidectomized rats was higher than that of ovariectomized rats. Thyroxine administered to ovariectomized-thyroidectomized rats depressed the polymerase activity and 17β-estradiol administration of these rats did not influence the polymerase activity. The results of the present study suggest that thyroxine influences uterine RNA synthesis and potentiates the stimulatory effect of 17β-estradiol.

We thank Dr. Sheldon J. Segal for his interest, discussion, and support which facilitated this study.

1. Miura, S., and Koide, S. S., Proc. Soc. Exp. Biol. Med. **133**, 882 (1970).

2. Hamilton, T. H., Teng, C-S., and Means, A. R., Proc. Nat. Acad. Sci. U.S.A. **59**, 1265 (1968).

3. Tata, J. R., Biochem. J. **104**, 1 (1967).

4. Schneider, W. C., Methods Enzymol. **3**, 680 (1957).

5. Heming, A. E., and Holtkamp, D. E., Proc. Soc. Exp. Biol. Med. **83**, 875 (1953).

6. Gorski, J., and Mueller, G. C., Arch. Biochem. Biophys. **102**, 21 (1963).

7. Taubert, H. D., and Ilan, J., Experientia **23**, 706 (1967).

8. Gorski, J., J. Biol. Chem. **239**, 889 (1964).

9. Bailey, N. T. J., *in* "Statistical Methods in Biology," p. 47. English Univ. Press, London (1959).

Received Sept. 2, 1970. P.S.E.B.M., 1971, Vol. 136.