SELECTIVE STIMULATION OF RIBONUCLEIC ACID SYNTHESIS IN UTERINE NUCLEI BY ESTRADIOL-17β

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SUMMARY: Nearest-neighbor nucleotides of UMP and base composition of P^{32}-RNA synthesized by the uterine nuclei of castrated rats were altered by the injection of estradiol-17β. Actinomycin D *in vivo* minimized such differences. Administration of estradiol-17β to castrated rats selectively enhanced the *in vitro* synthesis by the uterine nuclei of RNA rich in guanine and adenine and poor in uracil and cytosine. Because of this differential synthesis of RNA in uterine nuclei, it would appear that estradiol-17β may exert some influence at the level of transcription.

INTRODUCTION: The early response to estradiol-17β by the ovariectomized rat's uterus is characterized by a rapid increase in RNA and protein synthesis in that order (Mueller *et al.*, 1961; Noteboom and Gorski, 1963; Ui and Mueller, 1963; Hamilton, 1964; and Hamilton *et al.*, 1965.). Pulse-labeling experiments with several amino acids show a depression of protein synthesis in uterin subcellular fractions of ovariectomized rats by 30 minutes of estrogen action followed by increases at 2, 4, and 8 hours (Means and Hamilton, 1966a; 1966b). During the period of depression of protein synthesis, nuclear RNA synthesis, assayed by pulse-labeling with uridine-H^3, increases rapidly by 30 minutes (actually as early

as 2 minutes), then decreases as the rates of nuclear and cytoplasmic protein synthesis rise.

The finding that nuclear RNA synthesis is stimulated within 2 minutes of estrogen's acting on the uterus of the ovariectomized rat (Means and Hamilton, 1966c), leaves little doubt that one of the earliest effects of this hormone occurs at the transcription level of the uterine cell. Available information suggests that this effect may involve RNA polymerase (Gorski, 1964), inactivation of repressors (Talwar et al., 1964), nuclear membrane permeability (Szego, 1965; Means and Hamilton, 1966c), chromatin template activity (Barker and Warren, 1966), or a multiple effect involving some combination of these factors. The present studies were carried out to analyze the effect of the hormone on the qualitative nature of RNA produced by uterine nuclei.

MATERIALS AND METHODS: Three hundred female rats of the Sprague-Dawley strain weighing 270-320 g. each were ovariectomized and the certainty of anestrus was established by following the vaginal smears for at least two weeks. The animals were killed by decapitation 24 hours after a tail-vein injection of 10.0 μg of estradiol-17β in 1.0 ml of 1% ethanol in isotonic saline. Control animals received only the vehicle. One half of the control and experimental animals also received an intraperitoneal injection of 100 μg of actinomycin D in 0.5 ml of 10% ethanol in isotonic saline, 1 hour prior to the I.V. administration. Each batch of nuclei was prepared from the uteri of at least 75 ovariectomized females by a modification of the Chauveau method (Chauveau et al., 1965). The final nuclear pellets

were suspended in 30 times their volume of 0.25 M sucrose containing 4 mM $MgCl_2$ and tris buffer (0.01 M, pH 7.8).

α-P^{32}-UTP was obtained from the International Chemical and Nuclear Corporation. Ribonucleoside triphosphates were products of the Nutritional Biochemical Corporation. Actinomycin D was produced by the Merck, Sharp and Dohme Research Laboratories, while the estradiol-17β was from Calbiochem.

ANALYSIS FOR RELATIVE POSITIONS OF RIBONUCLEOTIDES IN RNA SYNTHESIZE BY UTERINE NUCLEI: Reactions were carried out in a final volume of 1.0 ml containing 1.0 μmole each of ATP, CTP, and GTP; 0.1 μmole of α-P^{32}-UTP (180 μc per μmole); 1.0 μmole of $MnCl_2$; 4.0 μmoles of $MgCl_2$; 20.0 μmoles of dithiothreitol (DTT); 6.0 μmoles of KF; 300 μmoles of $(NH_4)_2SO_4$; 70 μmoles of KCl; 6.0 μmoles of 2-phosphoenolpyruvate (PEP); 8 μg of pyruvate kinase; 250 μmoles of sucrose; 10.0 μmoles of Tris-HCl buffer, pH 7.8; and an amount of uterine nuclear preparation containing approximately 100-200 μg of DNA. Reactions were carried out for 45 minutes at 37°C. and terminated by the addition of 5% trichloroacetic acid (TCA). The acid-insoluble precipitates formed after centrifugation were washed with 5% TCA twice, and ethanol-ether (3:1) twice, and dried with ether. The dried powders were incubated with 0.2 ml of 0.25N KOH for 18 hours at 37°C. to hydrolyze RNA. The mixtures were acidified with 1N $HClO_4$ and the hydrolyzed nucleotides were separated by paper electrophoresis in 0.25 M citrate buffer of pH 3.5 for 3 hours at 400 v. The radioactivity of each 2'(3')-ribonucleotide was measured in a liquid scintillation counter (Packer Tri-Carb Model 574), after separation by paper electrophoresis.

RESULTS AND DISCUSSION: α-P^{32}-labeled UTP was used to study the effect of estradiol-17β and actinomycin D *in vivo* on the relative position of ribonucleotides in RNA synthesized by uterine nuclei. The results tabulated in Table I clearly indicate dissimilarity in the nucleotide sequences of the RNA synthesized by nuclei of control and estrogen-treated castrates. In the nearest-neighbor nucleotides analyses with P^{32}-UTP, estradiol-17β was found to increase the frequency of the base pairs GpU and ApU. Estrogen administration appears to result in a lower frequency of the base pairs UpU and CpU. Apparently P^{32}-RNA synthesized by the uterine nuclei of estrogen-treated castrates is greater in the (C+G/A+U) ratio than that of control castrates. Administration of actinomycin D minimized this difference.

It would appear, when considering the F values in Table II (F distribution in 'analysis of variants') that actinomycin D had no qualitative effect on the synthesis of RNA by the nuclei of the castrated uteri, whereas the type of RNA synthesis stimulated by estradiol-17β was abolished by the inhibitor. The frequencies of the nearest-neighbor nucleotides of UMP were the same (considering the F distributions) for the RNA of the actinomycin D-treated castrates and stimulates. The results are compatible with what has been known for some time now, viz. that actinomycin D blocks various aspects of the early estrogen response (Ui and Mueller, 1963; Hamilton, 1964; Means and Hamilton, 1966b; Talwar and Segal, 1963; and Nicolette and Gorski, 1964). Greenman and Kenney (1964) observed that there are changes in activity of uterine ribosomes

TABLE I

NEAREST-NEIGHBOR NUCLEOTIDES OF UMP IN P^{32}-RNA SYNTHESIZED BY RAT UTERINE NUCLEI†

CASTRATES

BASE PAIRS	CONTROL		ACTINOMYCIN-D	
	C.P.M.	FRACT.*	C.P.M.	FRACT.
CpU	6599±1701‡	0.308±0.036	3153±749	0.294±0.018
ApU	1076± 180	0.061±0.012	1101±306	0.103±0.021
GpU	4247± 978	0.232±0.042	1759±242	0.192±0.022
UpU	8514±1822	0.399±0.019	4208±899	0.411±0.039
GpU+CpU / ApU+UpU		1.19±0.072		0.98±0.091

ESTROGEN-TREATED CASTRATES

BASE PAIRS	CONTROL		ACTINOMYCIN-D	
	C.P.M.	FRACT.	C.P.M.	FRACT.
CpU	7934±1576	0.193±0.031	4586±1399	0.245±0.054
ApU	6155±3243	0.157±0.049	2689± 801	0.155±0.033
GpU	20404±4532	0.440±0.036	4243±1103	0.266±0.050
UpU	8203±2832	0.210±0.055	5992±1492	0.334±0.030
GpU+CpU / ApU+UpU		1.74±0.086		1.08±0.133

†Radioactive Precursor α-P^{32}-UTP *GpU+CpU+ApU+UpU=1.0

‡The figures in all columns are the average of seven experiments
The numbers in each column are mean values ± standard error of mean

TABLE II

ANALYSIS OF VARIANTS - DISTRIBUTION OF F FOR CONTROL, ACTINOMYCIN-D TREATMENT, CASTRATES AND ESTROGEN TREATMENT

BASE PAIR	CONTROL VS. ACTINOMYCIN-D TREATMENT F	CASTRATE VS. ESTROGEN TREATMENT F
CpU	0.2446	5.0212
ApU	0.4000	5.3428
GpU	7.5714	13.3333
UpU	3.2277	12.1584
CpU+GpU / ApU+UpU	19.7406	11.4098

$F(1/24,0.05)=4.26$
$F(1/24,0.01)=7.82$

due to estrogen indicating changes in messenger RNA, as well as increases in number of ribosomes which would indicate ribosome synthesis. Hamilton et al. (1965) have noted that the uterine nuclear RNA synthesized by 20 minutes of estrogen stimulation exhibits sedimentation profiles characteristic of ribosomal RNA.

Analogous studies with the male sex hormone (Liao et al., 1966 and Liao and Lin, 1967) revealed that the RNA-synthesizing capacity at certain sections of prostatic chromatin is preferentially enhanced by injection of testosterone into castrated animals. RNA synthesized at the chromatin section where testosterone has a profound effect was found to be rich in guanine and cytosine.

Elucidation of the early effect of estrogen on transcriptions of DNA, characterization of the RNA molecules thus synthesized, and description of the biological activity of these molecules may provide valuable information on the mechanism of estrogen action.

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