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Increase in Tracheal Glycoprotein Synthesis with Estrogen Administration (39756)

HENRY YEAGER, JR.,[1] YAEL SHECHTER, AND MARGIT HAMOSH

Departments of Medicine and Physiology and Biophysics, Georgetown University School of Medicine, Washington, D. C. 20007

A number of systemic changes occur in women during the menstrual cycle. Alterations are described in the nasopharyngeal mucous membranes, and in the physical properties of nasal and salivary secretion (1). Little is known, however, about the effect of female sex hormones on the respiratory tract. Cyclic cytomorphologic changes have been seen in the tracheal washes of young women but none have been seen in older women and men (2). The following experiments were designed to assess whether there are any changes in glycoprotein synthesis in the rat trachea following estrogen treatment.

Methods. Caesarian-derived Sprague–Dawley ovariectomized female rats, 125–150 g in weight, were obtained from Charles River Breeding Farms, Winchester, Mass. A dose of estrogen was used similar to that used in other studies of estrogen effect on nontarget tissues (3, 4). Starting 2 weeks after operation, the rats were given daily injections of 25.0 μg of 17β-estradiol (Sigma Chemical Co., St. Louis, Mo.), in corn oil, or of corn oil alone sc for 8 days. They were fed regular lab chow and water *ad libitum*. Animals were sacrificed on the 9th day with intraperitoneal sodium pentobarbital, 75 mg/kg. Estrogen effect was assessed by measuring uterine weights.

The tracheas were dissected out, and connective tissue and blood vessles were removed. They were sliced longitudinally and placed in Earle's medium (Grand Island Biochemical, Grand Island, N. Y.) for biosynthetic studies or frozen in liquid nitrogen for chemical analyses.

Approximately 100 mg wet weight of tracheal slices were placed in 2.0 ml of Earle's medium with 2 μCi of D-[1-^{14}C]glucosamine (New England Nuclear, Boston, Mass.) (final sp act 1 mCi/mole). Flasks were gassed with 95% O_2–5% CO_2 and incubated 3 hr at 37°. Control flasks were kept on ice. After incubation the epithelial tissue was gently abraded off the cartilaginous tissue with a homogenizer. The proteins in the homogenate were precipitated by adding cold trichloroacetic acid (TCA) and phosphotungstic acid (PTA) to give a final concentration of 10% TCA–1% PTA. The precipitates were washed twice with 10% TCA–1% PTA; lipids were extracted with ether:acetone:chloroform (2:2:1) for 30 min at 50°. The precipitate was air-dried and made soluble by heating in 1 *N* NaOH at 80° for 30 min (5). An aliquot was placed in a cocktail of 23% Triton X-100, 4.7% Liquiflor (New England Nuclear) in toluene, and counted in a Mark I liquid scintillation counter (Nuclear Chicago, Despalines, Ill.). Total protein in the homogenate was estimated (10) from another aliquot. The cartilaginous tissue was rinsed in a 0.9 *N* NaCl solution, made soluble in 0.5 ml of NCS (Amersham–Searle, Arlington Heights, Ill.), and counted in the same scintillation fluid.

For biochemical analyses, 25–40 mg of tissue were homogenized in 0.40 ml of cold saline in a 1-ml Kontes glass conical homogenizer. Proteins and nucleic acids were precipitated by the addition of a mixture of cold TCA and PTA to bring the final concentrations to 10% TCA and 1% PTA. The tubes were kept on ice 30 min followed by centrifugation at 2800*g* for 15 min in the cold. The supernatant fluid was decanted and aliquots were taken for measurement of free aminosugars (glucosamine and galactosamine) by the method of Elson and Morgan (6) as modified by Gatt and Berman (7). DNA and protein were measured in the TCA–PTA pellets. DNA was extracted with 5%

[1] Send reprint requests to Henry Yeager, Jr., M.D., Department of Medicine, Georgetown University School of Medicine, 3800 Reservoir Road, N. W., Washington, D. C. 20007.

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TCA (8) and quantitated (9). The protein was solubilized in dilute NaOH (5), followed by quantitation by the Lowry *et al.* method (10). Statistical comparisons were made with the *t* test for paired observations and group differences (11).

Results. The dose of estrogen employed caused a marked increase in uterine weights. The wet weight of the uteri of 10 treated animals was 227.9 ± 19.8 mg, compared to 29.6 ± 3.3 mg in 10 control animals; the dry uterine weight of treated animals was 36.8 ± 2.0 mg, compared to 7.6 ± 1.9 in control animals.

Tracheas were divided into cartilaginous and epithelial fractions. In preliminary experiments it was found that after a 3-h incubation the cartilaginous fractions of the trachea from hormone-treated and control rats contained a consistent 4–6% of incorporated ratioactivity; therefore radioisotope uptake by the cartilage was not considered further. The epithelial fractions from the tracheas of hormone treated animals showed a significant increase in uptake of [^{14}C]glucosamine (Table I). Our data for total radioisotope uptake include both radioactivity actually in the epithelial layer and that which had been secreted into the medium during incubation.

TABLE I. EFFECT OF ESTROGEN ON [^{14}C]GLUCOSAMINE UPTAKE BY TRACHEAL SLICES.[a]

Group of rats	[^{14}C]Glucosamine uptake (cpm/mg of protein)	
Control (n = 25)	578.2 ± 22.7	
		$P < 0.005$
Estrogen (n = 25)	689.0 ± 28.7	

[a] Tracheal slices (100 mg) from either estrogen-treated or control animals were incubated in 2.0 ml of Earle's medium with 2 μCi of D-[1-^{14}C]glucosamine for 3 hr; radioactive glycoproteins were isolated as described in Methods. Values are means ± SEM.

The concentration of free aminosugars was not affected by estrogen administration. Free aminosugars (glucosamine and glactosamine) were present at concentrations of 0.23 μmole/100 mg of wet weight in tracheas of estrogen-treated rats and 0.25 μmole/100 mg wet weight in tracheas of control animals. There was likewise no difference in aminosugars as expressed per unit weight of protein or desoxyribonucleic acid (DNA) or in total concentration of DNA and protein (Table II).

Discussion. Estrogen can cause increased glycoprotein synthesis in female genital organs (12–14). In view of previous data showing that D-glucosamine is mostly incorporated into glycoprotein by trachea, and not metabolized by other pathways (5), and of the present data showing increased uptake of [^{14}C]glucosamine in tracheal epithelium without any detectable alteration in aminosugar pools, it may be concluded that estrogen can cause an increase in glycoprotein synthesis in airway epithelium as well.

Preliminary observations from this laboratory (15) and elsewhere (16) suggest that changes in levels of female hormone may alter the morphology of the secretory cells of respiratory epithelium. There is evidence that estrogen can directly accelerate mucociliary clearance (17, 18). One might speculate that these hormonal effects on respiratory epithelium may facilitate the clearance of irritating substances by women, and could be in part responsible, in addition to differences in exposure both to tobacco smoke and to environmental pollutants, for the differences in the male and female prevalence rats both of chronic bronchitis (19) and of bronchogenic carcinoma (20).

Summary. Experiments were performed to examine the effect of estrogen adminis-

TABLE II. EFFECT OF ESTROGEN ON COMPOSITION OF RAT TRACHEA.[a]

Group of rats	Aminosugars		Protein (mg/g wet weight)	DNA (mg/g wet weight)
	μg/mg of Protein	μg/mg of DNA		
Control (n = 7)	5.6 ± 0.3	70.0 ± 4.2	84.2 ± 6.0	6.5 ± 0.3
Estrogen (n = 7)	5.1 ± 0.5	67.0 ± 6.3	86.4 ± 7.0	6.4 ± 0.6

[a] Tracheas from control and estrogen-treated rats were homogenized in cold saline. Proteins and nucleic acids were precipitated by the addition of TCA and PCA (10 and 1% final concentrations, respectively) and quantitated as described in Methods. Aminosugars were quantitated in the supernatant solution. Values are means ± SEM.

tration on D-[1-^{14}C]glucosamine uptake of rat trachea. Ovariectomized 125-g rats were given 25 μg of 17β-estradiol daily for 8 days and sacrificed on the 9th day. Treated animals had a 20% increase/3 hr in uptake of radioactivity into acid-insoluble material of trachea. There was no change in aminosugar pool sizes. It was concluded that estrogen can cause changes in rat tracheal epithelium consistent with increased glycoprotein synthesis.

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