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ABSTRACT

Precursor incorporation into uterine lipids was examined *in vitro* after estradiol-17β administration in immature female rats. The effect of adding supplemental substrates, glucose or gluconate, to the incubation medium on the labeling pattern of pyruvate-3-^{14}C into uterine lipids was studied. Presence of supplemental substrate in the medium enhanced the incorporation of pyruvate into uterine lipids after two hours of *in vivo* estrogen treatment. It is suggested that estrogen's acceleration of pyruvate incorporation into lipid may be due to a concomitant effect on glucose metabolism.

INTRODUCTION

The growth and differentiation of the rat uterus after estrogen treatment is accompanied by many physiological and biochemical changes. With physiological doses of estrogen the uterus of the immature or ovariectomized rat changes from an atrophic organ to a rapidly growing one. Estrogen facilitates the entrance of substrate materials and ions into the uterus with as little as two hours of treatment (Smith 1967, Smith and Stultz 1971).

The works of Aizawa and Mueller (1961) showed that increased lipid synthesis is one of the earliest and most dramatic responses in the uterus treated with estrogen. Measurements of incorporation of various labeled compounds into uterine lipid after estrogen treatment have been used to evaluate the stimulation of lipid synthesis in uteri of immature or ovariectomized rats.

This investigation was undertaken to examine the effect of estradiol-17-β on pyruvate-3-^{14}C incorporation into uterine lipids and the relationship of this process to other aspects of uterine carbohydrate metabolism.

MATERIALS AND METHODS

Immature (21-23 day old) female Holtzman rats were injected intraperitoneally with either 5 μg of estradiol-17-β dissolved in 5 ml of 0.15 M NaCl or with 5 ml of 0.15 M NaCl alone (controls). Animals were decapitated at the end of each specified time period (1-16 hr) after injection. Uteri were removed and trimmed of fat and connective tissue.

Whole uteri were incubated *in vitro* at 37C in a shaking water bath, after gassing with a mixture of 95% 0_2 and 5% CO_2. Incorporation of pyruvate-3-^{14}C (10.3-21.0 mCi/mM, New England Nuclear) was carried out in Robinson's medium. After incubation, flasks were placed on ice, then the uteri were removed and washed twice in cold saline solution.

Washed uteri were placed in 3 ml of 5% TCA in centrifuge tubes. Each tube contained from two to six pooled whole uteri. The contents of each tube was homogenized in a glass Duall homogenizer. Total lipids were extracted by sequential centrifugation with 100% ethanol, chloroform:ethanol (2:1), and anhydrous ether (twice). The washes (5 ml each) were collected and allowed to evaporate in a stream of air for 6-12 hours. Total lipid fractions were transferred in ether to scintillation vials and allowed to evaporate prior to the addition of fluor for counting. For lipid separation into phospholipid and neutral lipids, 0.5 ml of chloroform:absolute methanol (1:1) was added, then the extracts were flushed with nitrogen and stored overnight at -20C for use in thin-layer chromatography (TLC) experiments. TLC experiments were carried out by the procedure of Freeman and West (1966). Liquid scintillation counting procedures were done in a Beckman LS 100 counter.

RESULTS

The data in Table I show the effects of length of estrogen treatment on pyruvate metabolism. Pyruvate incorporation into lipid increased significantly at all time periods. Tables II and III show the effect of estrogen on stimulating the incorporation of pyruvate-3-^{14}C into uterine lipid when exogenous glucose or gluconate, respectively, was present in the incubation medium. Neither substrate alone was effective in elevating pyruvate incorporation into lipid. However, *in vivo* estrogen treatment in combination with *in vitro* glucose or gluconate in the medium gave significant results.

Total lipids extracted from rat uteri were analyzed by TLC. The percentage of total lipid radioactivity appearing in phospholipid and neutral lipid fractions is given in Table IV. The data for controls (0 hr) and two-hour estrogen pretreatment show an approximate 1:1 ratio, but by six hours there was an apparent increase in the proportion of labeling of neutral lipids.

Table V presents data showing the effects of glucose and gluconate on pyruvate labeling of uterine phospholipid and neutral lipid fractions after estrogen treatment. After two hours of hormone treatment neither estrogen nor added substrate had significantly altered the labeling pattern of neutral or phospholipid fractions. However, after six hours of hormone treatment, the phospholipid fraction showed a 10% decrease under all conditions, whereas the neutral lipids showed more than a 10% increase under all conditions.

DISCUSSION

Information obtained from these studies demonstrates that *in vivo* estrogen treatment in immature rats stimulated subsequent *in vitro* incorporation of pyruvate-3-^{14}C into uterine lipids. As suggested by earlier work, the time course of the estrogen effect on lipid synthesis began as early as two hours and continued through the longest time studied (16 hr).

The addition of supplemental substrates, glucose or gluconate, to the incubation medium tended to enhance the incorporation of pyruvate into uterine lipids after two hours of estrogen pretreatment. The combined effects of the nutrient substrates with estrogen in the immature rat uterus caused elevations above control of 137% for glucose and 161% for gluconate. These findings suggest that carbohydrate substrate availability for lipid synthesis is associated with the effect of estrogen on uterine tissue.

TLC analysis of total uterine lipids showed equal stimulation of pyruvate into neutral and phospholipids after two hours of estrogen treatment. These findings are interpreted to mean that the estrogen effect responsible for the increases observed occurs in the metabolism of pyruvate at a step common to all classes of lipids. Although such an effect might implicate acetyl-CoA carboxylase activity, other workers have shown that the activity of this enzyme is not affected by estrogen. An increase in acetyl-CoA pool size is also unlikely because of the differential effect of estrogen on pyruvate oxidation to CO_2 and its incorporation into lipids.

Because pyruvate utilization for lipid synthesis is influenced under *in vitro* conditions by the presence of exogenous sugars, it is evident that the metabolism in uterine tissue is complex. Estrogen alterations in uterine glucose metabolism are known to occur early enough in the hormone's action on the uterus to be capable of causing changes in lipid synthesis secondarily (Smith 1967). It is suggested that estrogen's acceleration of pyruvate incorporation could be due to a

concomitant effect on glucose metabolism. The reported estrogen-induced increases in glycolysis and oxidative glucose metabolism are probably sufficient to supply the increased amounts of substrates, energy, and reducing equivalents necessary to stimulate lipogenesis generally.

LITERATURE CITED

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Table I. Time Course of the Estrogen Effect on Uterine Metabolism of Pyruvate-3-^{14}C

Estradiol-17-β treatment *in vivo* (hr)	$^{14}CO_2$ as % of control	^{14}C-lipid as % of control
0	100.0	100.0
2	96.3 ± 3.5 (40)	132.8 ± 6.0**(27)
4	88.0 ± 3.8*(8)	181.8 ± 27.0**(6)
8	82.2 ± 7.8*(8)	277.0 ± 46.0**(6)
12	84.2 ± 2.5*(7)	538.0 ± 66.0**(4)
16	81.5 ± 3.4*(7)	364.0 ± 39.0**(4)

* indicates significance at $p < 0.05$; ** $p < 0.01$.

Immature rat uteri were incubated for one hour in Robinson's medium containing 0.125 μCi/ml of pyruvate-3-^{14}C. $^{14}CO_2$ and lipids were collected as described in methods section. Data are expressed as percentage deviation ± SEM from control where control is considered 100%. Typical values for control uteri are CO_2 10,278 cpm/uterus and lipid 580 cpm/uterus. Values in parentheses indicate the number of determinations.

Table II. Effect of Estrogen and/or Exogenous Glucose on Incorporation of Pyruvate-3-^{14}C into Uterine Lipid

Treatment or condition	^{14}C-lipid cpm as % of control
Control	100
Glucose (5 mM)	112.4 ± 16.0
Estrogen (2 hr)	116.2 ± 10.0
Estrogen (2) hr) + Glucose Added (5 mM)	137.15 ± 12.0*
	Δ (117.23 ± 10.0)

* indicates significance at $p < 0.01$.
Δ = % change compared with estrogen alone.

Immature rat uteri were incubated for one hour in Robinson's medium containing 0.125 μCi/ml of pyruvate-3-^{14}C, prepared with or without glucose. Lipids were extracted as described in methods section. Typical ^{14}C-lipid value for control uteri is 582 cpm/uterus. Values are the result of nine determinations.

Table III. Effect of Estrogen and/or Exogenous Gluconate on Incorporation of Pyruvate-3-^{14}C into Uterine Lipid

Treatment or condition	^{14}C-lipid cpm as % of control
Control	100
Gluconate (5 mM)	109.5 ± 13.0
Estrogen (2 hr)	151.8 ± 11.3*
Estrogen (2 hr) + Gluconate added (5 mM)	161.1 ± 18.2*
	Δ (109.7 ± 9.0)

* indicates significant at $p < 0.01$.
Δ = % change compared with estrogen alone.

Immature rat uteri were incubated for one hour in Robinson's medium containing 0.125 μCi/ml of pyruvate-3-^{14}C, prepared with or without gluconate. Data are expressed as % of control ± SEM. Typical lipid control value is 318 cpm/uterus. Values are the result of 10 determinations.

Table IV. Effects of Estrogen on Pyruvate Labeling of Phospholipid and Neutral Lipids Recovered in Total Uterine Lipids

Estradiol-17-β treatment in vivo (hr)	% of total lipid radioactivity Phospholipid	Neutral lipid
0	46.5 (6)	53.5 (6)
2	49.0 (4)	51.0 (4)
6	35.8 (2)	64.2 (2)

Immature rat uteri were incubated for one hour in Robinson's medium containing 0.50 μCi/ml of pyruvate-3-^{14}C. Data are expressed as % fraction recovered (phospholipid fraction cpm or neutral lipid fracton cpm/total lipid cpm recovered x 100). Radioactivity measurement and lipid extraction were as described in methods section. Typical control values are 367 cpm/uterus (phospholipid) and 393 cpm/uterus (neutral lipid). Values in parentheses indicate the number of determinations.

Table V. Effect of Estrogen and Added Glucose or Gluconate on Pyruvate Labeling of Uterine Phospholipids and Neutral Lipids

Substrate added to medium	Estrogen treatment in vivo (hrs) 0	2	6
	Phospholipid (% of total lipid radioactivity)		
None	46.5 (6)	49.0 (4)	35.8 (2)
Glucose (5 mM)	47.4 (6)	48.8 (4)	31.0 (2)
Gluconate (5 mM)	41.2 (6)	46.3 (4)	30.0 (2)
	Neutral lipid (% of total lipid radioactivity)		
None	53.5 (6)	51.0 (4)	64.2 (2)
Glucose (5 mM)	52.6 (6)	51.2 (4)	69.0 (2)
Gluconate (5 mM)	58.8 (6)	53.7 (4)	70.0 (2)

Immature rat uteri were incubated for one hour in Robinson's medium containing 0.5 μCi/ml of pyruvate-3-^{14}C plus added substrate where indicated. Phospholipids and neutral lipids were separated and radioactivity measured as described in methods section. Data are expressed as in Table IV. Values in parentheses indicate number of determinations.