SHORT COMMUNICATIONS

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Cycloheximide and actinomycin D inhibition of estrogen-stimulated sugar and amino acid transport in rat uterus

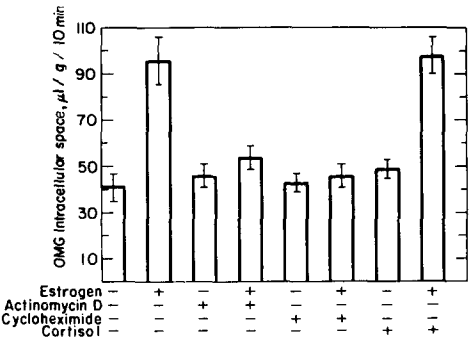
Estrogen stimulates anabolic activity in the uterus of the ovariectomized and of the immature rat. This activity includes increased glucose uptake by the isolated rat uterus 4 h after estrogen injection[1] and increased incorporation of [$^{14}C_6$]glucose into uterine lipid, RNA, protein, and CO_2 1 to 2 h after estrogen injection[2]. These observations suggest that estrogenic hormones increase the rate of glucose transport into the uterine cells. However, HALKERSTON and co-workers observed no difference in the distribution after 1.5 h of the glucose analogue D-xylose 1.5 or 6 h after estrogen injection *in vivo*[3]. NOALL *et al.* observed that the concentration of the amino acid analogue α-aminoisobutyric acid increased in the rat uterus 20 h after estrogen and α-aminoisobutyric acid injection[4]. RIGGS, SANDERS AND WEINDLING found an increased concentration of 1-aminocyclopentane carboxylic acid 24 h after injection of estrogen and this amino acid analogue[5].

In the above-mentioned studies, the distribution of the test compound was measured a fairly long time after its injection, and therefore the results represent steady-state distribution values. In the experiments to be described, brief incubation periods were used in an effort to measure initial rates of uptake.

Young female Sprague–Dawley rats were ovariectomized and used 7 to 30 days later. 1.0 μg of 17β-estradiol in 20% ethanol was injected subcutaneously as indicated. The animals were killed and the uterus was removed. One horn was incubated for 10 min in glucose-free Krebs–Ringer bicarbonate buffer containing 3-*O*-methyl-D-glucose, 1 mM (which contained 3-*O*-methyl-D-[^{14}C]glucose, 0.10 μC), and D-sorbitol, 1 mM (which contained 0.50 μC D-[1-^{3}H]sorbitol), to measure glucose transport[6]. The contralateral uterine horn was incubated in the same buffer with α-aminoisobutyric acid, 100 μM (with 0.10 μC α-amino[1-^{14}C]isobutyric acid), and D-sorbitol, 1 mM (with 0.50 μC D-[1-^{3}H]sorbitol) for 30 min. After incubation the uterine horns were rinsed in saline, incised longitudinally, weighed, and boiled for 15 min in water. They were then homogenized and protein was removed with 0.15 M $Ba(OH)_2$ (0.10 ml) and 5% $ZnSO_4$ (0.10 ml). An aliquot of the supernatant solution was added to BRAY's solution (15.0 ml)[7] for determination of radioactivity in a liquid-scintillation counter. The counts due to each isotope were calculated by the method of OKITA *et al.*[8]. The 3-*O*-methyl-D-glucose or α-aminoisobutyric acid space, that is, the volume of tissue required to contain the compound in a concentration equivalent to that of the medium, was calculated as follows:

$$\text{3-}O\text{-methyl-D-glucose space, } \mu\text{l/g} = \frac{\text{counts/min 3-}O\text{-methyl-D-glucose per g tissue}}{\text{counts/min 3-}O\text{-methyl-D-glucose per } \mu\text{l medium}}$$

The sorbitol space was taken to represent the extracellular space. The intracellular 3-*O*-methyl-D-glucose space is the difference between the 3-*O*-methyl-D-glucose space and the sorbitol space, and the intracellular α-aminoisobutyric acid space is the difference between the α-aminoisobutyric acid space and the sorbitol space. The ratio of the intracellular α-aminoisobutyric acid to the extracellular α-aminoisobutyric acid



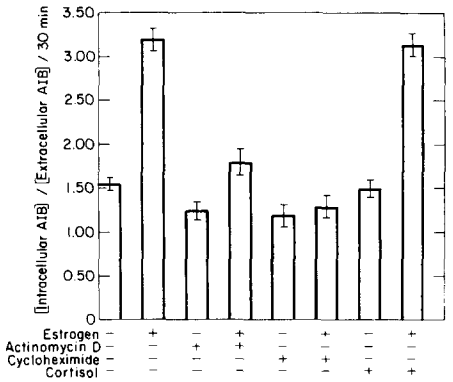


Fig. 1. The effect of actinomycin D, cycloheximide, and cortisol on the estrogen-induced 3-*O*-methyl-D-glucose (OMG) transport response. Each bar represents the mean of 5 determinations. The vertical line represents 2 standard errors of the mean.

Fig. 2. The effect of actinomycin D, cycloheximide, and cortisol on the estrogen-induced α-aminoisobutyric acid (AIB) transport response. Each bar represents the mean of 5 determinations. The vertical line represents 2 standard errors of the mean.

was calculated by dividing the intracellular α-aminoisobutyric acid space by the intracellular water space (340 μl$\pm$20 μl) per g of tissue wet weight.

The rates of transport of both 3-*O*-methyl-D-glucose and α-aminoisobutyric acid increased 2- to 3-fold within 2 h after estrogen injection (Figs. 1 and 2). No change in the uptake of 3-*O*-methyl-D-glucose was detectable 1 h after estrogen, but the uptake of α-aminoisobutyric acid increased 50%. The rate of transport of 3-*O*-methyl-D-glucose returned to the control value by 12 h, and that of α-aminoisobutyric acid by 24 to 48 h (ref. 9).

The effects of actinomycin D and cycloheximide on the transport response were studied. Both antibiotics inhibit many estrogen-induced changes in the uterus[2,10,11]. The effect of cortisol-hemisuccinate on the transport response also was evaluated, as adrenocortical steroids inhibit water-imbibition by the uterus in response to estrogen[1].

Castrate female rats were given an intraperitoneal injection of either (1) physiologic saline (0.5 ml), (2) actinomycin D (360 μg in 0.72 ml saline), (3) cycloheximide (880 μg in 0.5 ml saline), or (4) cortisol-21-hydrogen succinate (4 mg in 0.4 ml saline). One hour later half of each of the 4 groups of animals was given either 1.0 μg of 17β-estradiol or vehicle subcutaneously. The animals were killed 2 h later. None of the compounds appreciably altered the basal rate of uterine transport of 3-*O*-methyl-D-glucose or α-aminoisobutyric acid (Figs. 1 and 2). The dose of actinomycin D, which inhibited the incorporation of [^{3}H]uridine into uterine RNA by 90%, and the dose of cycloheximide, which inhibited the incorporation of [$^{14}C_6$]lysine into protein by 95%, abolished the estrogen stimulation of both α-aminoisobutyric acid and 3-*O*-methyl-D-glucose transport. Cortisol did not block the estrogen stimulation of α-aminoisobutyric acid and 3-*O*-methyl-D-glucose transport and, therefore, the inhibition produced by cycloheximide and actinomycin D could not be secondary to increased secretion of adrenocortical steroids[12].

The transport response to estrogen of uterus differs considerably from that of muscle to insulin in that neither actinomycin D nor puromycin inhibits the increased

uptake by muscle tissue of sugar[13,14,16] or of α-aminoisobutyric acid[13,15,16] in response to insulin. This finding indicates a fundamental difference in the mechanisms by which the two anabolic hormones stimulate transport processes in their respective target tissues. Insulin appears to act more or less directly in accelerating transport, while the effect of estrogen is indirect and apparently mediated through a mechanism that requires both RNA and protein synthesis. It is possible that estrogen stimulates the formation of protein elements necessary for α-aminoisobutyric acid and 3-*O*-methyl-D-glucose transport, and the actinomycin D and cycloheximide exert their influence by inhibiting the transcription and translation that is required for synthesis of the protein elements.

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