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## THE EFFECT OF ESTROGEN ON AMINO ACID TRANSPORT IN RAT UTERUS

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## SUMMARY

1. Some characteristics of amino acid transport in the isolated rat uterus have been studied using the non-metabolized amino acid analogue  $\alpha$ -aminoisobutyric acid. The effect of estrogen injection into ovariectomized rats on the rate of transport of  $\alpha$ -aminoisobutyric acid was also determined.

2. A single injection of estrogen increased the initial rate of  $\alpha$ -aminoisobutyric acid transport into uterine cells. The effect was evident by 1 h and maximal at 8 h; it persisted for more than 24 h. Control values were reached again by 48 h. The rate of  $\alpha$ -aminoisobutyric acid uptake *in vitro* was the same in the presence or absence of glucose.

3. Several natural amino acids inhibited transport of  $\alpha$ -aminoisobutyric acid to a greater or lesser extent, whereas others were without effect.

4. It is concluded that estrogen increases the rate of uptake of  $\alpha$ -aminoisobutyric acid by the isolated rat uterus, and that it may increase the rate of transport of a number of the natural amino acids.

## INTRODUCTION

In 1957 NOALL *et al.*<sup>1</sup> demonstrated that the concentration of the non-metabolized amino acid analogue,  $\alpha$ -aminoisobutyric acid, in the immature rat uterus was increased 280% 20 h after estrogen injection<sup>1</sup>. DANIELS AND KALMAN<sup>2</sup> reported that this effect of estrogen was detectable after 2 h and was maximal at 12 h. NOALL AND ALLAN<sup>3</sup> were able to demonstrate an increased rate of  $\alpha$ -aminoisobutyric acid uptake in the rabbit uterus *in vitro* within 30 min after hormone treatment was given *in vivo*. HALKERSTON *et al.*<sup>4</sup> reported that  $\alpha$ -aminoisobutyric acid uptake in the uterus of the ovariectomized rat was unchanged 1.5 h after estrogen injection, but was increased after 6 h. RIGGS and co-workers<sup>5</sup> found that uptake *in vivo* of the model amino acid 1-aminocyclopentanecarboxylic acid by the immature rat

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uterus was increased after estrogen injection. In addition, KALMAN AND LOMBROZE<sup>6</sup> demonstrated an increase in the concentration of many of the free amino acids in the rat uterus 4 h after estrogen injection.

Uterine cell membrane transport has been discussed in several recent reviews<sup>7-9</sup>. The data from all of the studies mentioned, except those of NOALL AND ALLAN, demonstrate the ability of the uterus to develop and/or maintain a concentration of the amino acid but do not necessarily define a maximal rate of uptake. In the present study, the rate of transport of  $\alpha$ -aminoisobutyric acid was measured during relatively short periods of incubation *in vitro* in an effort to approximate an initial, maximal, value for the rate of uptake of the analogue.

#### METHODS AND MATERIALS

##### Chemicals

The  $\alpha$ -amino[1-<sup>14</sup>C]isobutyric acid (1 mC/mmol) obtained from the New England Nuclear Corp. was kindly supplied by Dr. I. G. WOOL. Unlabeled amino acids were purchased from Calbiochem Corp. The sources of the other materials were listed previously<sup>10</sup>.

##### Incubation medium

Uterine horns were incubated in Krebs-Ringer bicarbonate buffer<sup>11</sup> equilibrated with O<sub>2</sub>-CO<sub>2</sub> (95:5, v/v) (pH 7.4) at 37°. Each ml of incubation medium contained 0.10  $\mu$ C of  $\alpha$ -amino[1-<sup>14</sup>C]isobutyric acid (100  $\mu$ M) and 0.05  $\mu$ C of D-[1-<sup>3</sup>H]-sorbitol. Carrier D-sorbitol was added to make its final concentration 1.0 mM. Additions to the medium were made in 10- $\mu$ l aliquots to give the final concentration. L-Aspartate, L-glutamate, L-tyrosine, and L-tryptophan were added to the buffer as crystalline solids to give the desired final concentrations. Each uterine horn was incubated in 0.5 ml of medium for the specific time period.

##### $\frac{[\text{Intracellular } \alpha\text{-aminoisobutyric acid}]}{[\text{Extracellular } \alpha\text{-aminoisobutyric acid}]}$

Uterine <sup>14</sup>C and <sup>3</sup>H uptake was measured as described previously<sup>10</sup>. The extracellular distribution of  $\alpha$ -aminoisobutyric acid was assumed to equal the distribution of D-sorbitol.

$$\frac{[\text{intracellular } \alpha\text{-aminoisobutyric acid}]}{[\text{extracellular } \alpha\text{-aminoisobutyric acid}]} = \frac{\text{total } \alpha\text{-aminoisobutyric acid} - \text{extracellular } \alpha\text{-aminoisobutyric acid}}{\text{extracellular } \alpha\text{-aminoisobutyric acid}}$$

Details pertaining to the preparation of animals and procedures for incubation of the uteri, measurement of radioactivity, calculation of results, and administration of hormone have been described elsewhere<sup>10</sup>. The dose of estrogen employed was 1.0  $\mu$ g of 17 $\beta$ -estradiol per animal.

#### RESULTS

##### *Uptake of $\alpha$ -aminoisobutyric acid by uterine horns from untreated and estrogen-treated (2 h) ovariectomized rats*

The upper curve (Fig. 1) shows the rate of  $\alpha$ -aminoisobutyric acid uptake by uteri from rats that received estrogen 2 h before sacrifice and the lower curve, the

rate from rats which received only the vehicle. The same values are obtained in the presence or absence of 8 mM glucose; therefore, the increased uptake of  $\alpha$ -aminoisobutyric acid is independent of sugar transport. In both cases, the ratio of intracellular to extracellular  $\alpha$ -aminoisobutyric acid after incubation exceeds 1.0. Since  $\alpha$ -aminoisobutyric acid is not metabolized by this tissue, this ratio indicates that it is concentrated in the cells. It follows that entry occurs, at least in part, by means of an active process, rather than by simple diffusion.

#### *Time course of the $\alpha$ -aminoisobutyric acid transport response to estrogen*

Animals were sacrificed at various intervals after estrogen injection (Fig. 2). The control group received vehicle 2 h before sacrifice. The  $\alpha$ -aminoisobutyric acid uptake was measured after incubation for 30 min *in vitro*. The results show a 50% increase after 1 h of hormone treatment ( $P < 0.02$ ). The maximum rate occurs at 8 h; the rate remains elevated for at least 24 h and returns to the control level by 48 h.

#### *Effect of amino acids on $\alpha$ -aminoisobutyric acid uptake*

Uterine  $\alpha$ -aminoisobutyric acid uptake was measured for 30 min in the presence of 3.5 mM concentrations of the specified amino acids (Fig. 3).  $\alpha$ -Aminoisobutyric acid was present in a concentration of 100  $\mu$ M. The concentration of the test amino acid is approx. 10–20 times that of plasma<sup>12</sup>. L-Methionine, L-alanine,

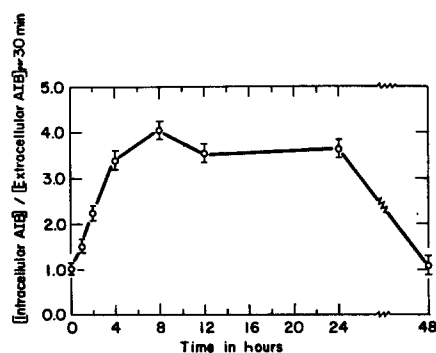
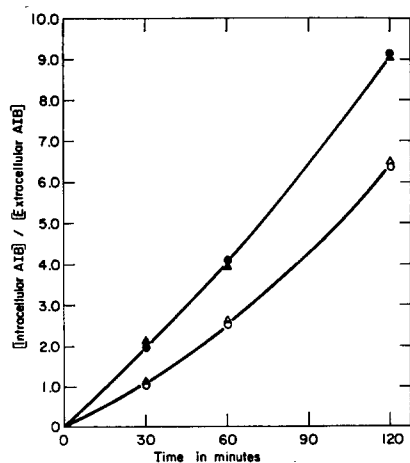


Fig. 1. (AIB) uptake in uteri from estrogen-treated (2 h) and control ovariectomized rats. The incubation medium contained a 100  $\mu$ M concentration of  $\alpha$ -amino[<sup>14</sup>C]isobutyric acid and 1.0 mM D-[1-<sup>3</sup>H]sorbitol. The calculations were carried out as described in METHODS AND MATERIALS. Each point represents the mean of four determinations. Solid symbols are determinations from the uteri of 2-h estrogen-treated animals and open symbols determinations from the uteri of control animals. The standard error for each point is less than ( $\pm 0.15$ ). ●—●, glucose-free medium; ▲—▲, 8 mM glucose.

Fig. 2. Time course of the estrogen-induced  $\alpha$ -aminoisobutyric acid (AIB) transport increase. Experimental animals received 1.0  $\mu$ g of 17 $\beta$ -estradiol in 20% ethanol at zero time. They were sacrificed at the time stated, and the rate of uterine  $\alpha$ -aminoisobutyric acid transport was determined during a 30-min incubation period. Controls received vehicle only for 4 h. Each point represents the mean of the results from five animals. The vertical lines represent 2 standard errors of the mean.

L-serine and L-cysteine inhibit  $\alpha$ -aminoisobutyric acid uptake by about 50% ( $P < 0.001$ ). L-Glycine, L-proline, L-leucine, L-isoleucine, L-phenylalanine and L-valine inhibit uptake by 10–30% ( $P < 0.01$ ). L-Arginine, L-threonine, L-aspartate, L-glutamate, and L-lysine inhibit  $\alpha$ -aminoisobutyric acid uptake by approx. 10% ( $P < 0.05$ ). The remaining amino acids do not inhibit  $\alpha$ -aminoisobutyric acid uptake significantly.

## DISCUSSION

Glucose has no effect on the initial rate of  $\alpha$ -aminoisobutyric acid uptake in uteri from animals receiving estrogen, or in the controls (Fig. 1). One might suppose that glucose would stimulate  $\alpha$ -aminoisobutyric acid uptake, for the process is energy dependent. However, in the case of the isolated rat diaphragm KIPNIS AND NOALL<sup>12</sup> report that glucose decreases  $\alpha$ -aminoisobutyric acid uptake in non-fasted and 72-h fasted animals in the presence and absence of insulin. The rate of  $\alpha$ -aminoisobutyric acid uptake is stimulated by insulin in either case.

The stimulation of  $\alpha$ -aminoisobutyric acid uptake by estrogen reported here is in accord with the findings of NOALL and co-workers<sup>1</sup>, who showed that the hormone increased the concentration of  $\alpha$ -aminoisobutyric acid in immature rat uteri 20 h after injections of estrogen and  $\alpha$ -aminoisobutyric acid. In the present study, it was found that the rate of  $\alpha$ -aminoisobutyric acid uptake (measured in isolated uteri) was increased by estrogen for at least 24 h after hormone injection. However, their studies reflect the ability of the uterus to develop and/or maintain a greater concentration of  $\alpha$ -aminoisobutyric acid. The results reported here corroborate those of NOALL AND ALLAN, who reported that estrogen injection into the ovariectomized rabbit increased the rate of  $\alpha$ -aminoisobutyric acid uptake in the isolated uterus.

HALKERSTON and co-workers<sup>4</sup> were able to demonstrate an increase in  $\alpha$ -

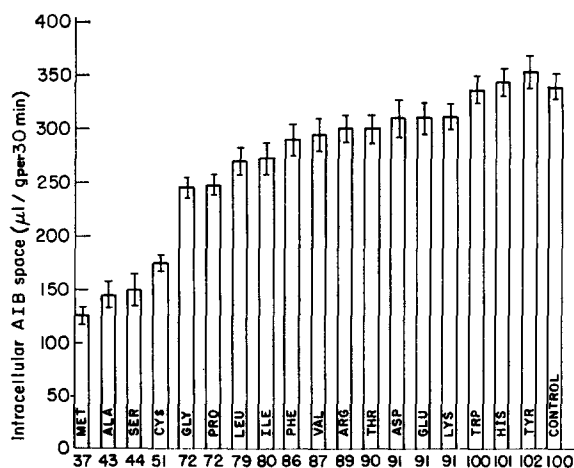


Fig. 3. The effect of various L-amino acids on uptake of  $\alpha$ -aminoisobutyric acid (AIB) by the isolated rat uterus. The test amino acids were present at a concentration of 3.5 mM. The concentration of  $\alpha$ -aminoisobutyric acid was 100  $\mu$ M. The incubation period was 30 min. Each bar represents the mean of four determinations. The vertical line represents 2 standard errors of the mean. The number represents percentage of control value.

aminoisobutyric acid uptake in the uterus of the ovariectomized rat 6 h after hormone injection (1.5 h after  $\alpha$ -aminoisobutyric acid injection). They report that there was no detectable change 1.5 h after hormone and  $\alpha$ -aminoisobutyric acid injection. In the present study the increased  $\alpha$ -aminoisobutyric acid uptake was discernible 1 h after hormone injection. The reasons for this discrepancy between the uptake rate measured *in vitro* as against *in vivo* are not apparent.

Several naturally occurring amino acids inhibited  $\alpha$ -aminoisobutyric acid uptake to a greater or lesser extent in this study. Inhibition here probably depends upon the affinity of the amino acids for the membrane carrier(s) responsible for  $\alpha$ -aminoisobutyric acid transport. A compound with high affinity for the carrier (low  $K_m$ ) would inhibit  $\alpha$ -aminoisobutyric acid uptake to a greater extent than a compound with low carrier affinity (high  $K_m$ ). The inhibition of  $\alpha$ -aminoisobutyric acid uptake by several of the amino acids tested provides presumptive evidence that  $\alpha$ -aminoisobutyric acid is transported by a physiologically important membrane carrier(s), and the increase in  $\alpha$ -aminoisobutyric acid uptake induced by estrogen may be a reflection of increased transport of some of the naturally occurring amino acids from the extracellular to the intracellular space under the anabolic stimulus of the hormone.

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