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# TRANSPORT OF AMINO ACIDS INTO THE ESTROGEN-PRIMED UTERUS

## I. GENERAL CHARACTERISTICS OF THE UPTAKE *IN VITRO**

THOMAS R. RIGGS, MARY W. PAN AND HSIEN W. FENG
*Department of Biological Chemistry, The University of Michigan, Ann Arbor, Mich. (U.S.A.)*
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SUMMARY

The uptake of free amino acids by the immature rat uterus has been studied *in vitro*.

1. The uptake of the model amino acid, α-aminoisobutyric acid, showed properties of active transport. It was saturable; it occurred against a gradient of the free amino acid; it could be decreased by metabolic inhibitors such as cyanide, 2,4-dinitrophenol and lack of $O_2$; and it was temperature-sensitive. It was also decreased by the presence of high levels of other amino acids or of $K^+$, and by the absence of extracellular $Na^+$.

2. Under appropriate conditions, 1 μg estradiol injected into the rats could increase the uptake *in vitro* of α-aminoisobutyric acid, L-serine, L-alanine, glycine, L-proline, L-lysine, 1-aminocyclopentanecarboxylic acid, L-valine and DL-norleucine. As short a time as 1 h *in vivo* was sufficient for this level of the hormone to stimulate uptake of α-aminoisobutyric acid, 1-aminocyclopentanecarboxylic acid, L-alanine and L-proline. A $10^{-6}$ M level of estradiol added *in vitro* stimulated α-aminoisobutyric acid uptake if incubation periods were extended to 6 h.

3. Estradiol increased uptake at all α-aminoisobutyric acid levels tested (0.3–10 mM). Approximation of kinetic constants showed that the hormone increased the $v_{max}$ of α-aminoisobutyric acid uptake without altering its $K_m$.

## INTRODUCTION

Estradiol-17β has been shown to increase the transport of the model amino acids, α-aminoisobutyric acid and 1-aminocyclopentanecarboxylic acid, into the uterus of the rat or rabbit *in vivo*[2–6]. Attempts to show consistent stimulation when the tissue is exposed to the steroid *in vitro* have up to the present proved unsuccessful. NOALL AND ALLEN[3] found, however, that if the hormone is injected *in vivo* and the uterus subsequently incubated *in vitro* with α-aminoisobutyric acid, estradiol can produce a large increase in the uptake of the amino acid[3]. The isolated uterus can therefore be used to study the characteristics of amino acid uptake by this tissue and their modification by estrogen. The initial results of such a study using the rat uterus are reported here.

* Part of this work has been reported previously in abstract[1].

## MATERIALS AND METHODS

Amino acid uptake was measured *in vitro* in uteri removed from female rats weighing 50–90 g. Care was taken to note that the vagina was still closed in the larger animals. In most cases, 2 uteri were paired for one sample so as to provide 40–80 mg of tissue. The routine procedure was to inject the animals intraperitoneally with $\beta$-estradiol (1 $\mu$g in 0.1 ml 25 % ethanol in saline) or with 0.1 ml of solvent, kill them at the appropriate time later, and remove the uteri rapidly, leaving the ovaries and any connective tissue attached. Samples were then placed in a 50-ml erlenmeyer flask containing a radioactively-labeled amino acid and buffer, in most cases Krebs–Ringer bicarbonate (pH 7.4), with 95 % $O_2$–5 % $CO_2$ as the gas phase. In a few experiments, Krebs–Ringer phosphate buffer with 100 % $O_2$ was used instead. Incubations were carried out in a shaker bath at 37° unless otherwise specified. Ordinarily, 2 uteri from control rats and 2 from estradiol-treated animals were placed in the same flask, with control tissue being identified by a small piece of black surgical thread tied to the end of one horn. Total volume of buffer fluid added to such flasks was 6.0 ml. At the end of the appropriate incubation time, uteri were removed to a filter paper, blotted to remove surface buffer, and trimmed free of attached ovaries and other extraneous tissue. The two control samples were then pooled and weighed on a tissue balance, and the estrogen-primed uteri treated similarly. One volume of 0.1 M acetic acid and 19 vol. of water were added, the samples were homogenized in glass homogenizers and deproteinized by heating in a boiling-water bath for 10 min. Aliquots of the filtrate and of the undiluted incubation fluid were plated and counted on the Geiger–Mueller counter. Corrections were made for self-absorption; for the extracellular fluid, the self-absorption curves were made by using solutions of the same composition and approximate amino acid concentration that was present in the incubation mixture.

All uptake studies were made using amino acids labeled with $^{14}C$ in the carboxyl group. Norleucine was used as the DL-form; all other optically active amino acids were of the L-configuration.

Calculation of amino acid level in the uterine cells was made on the basis of cellular water content, with conversion to millimolar concentration being made based on the specific activity of the labeled amino acid present orginally in the incubation medium. Total water content was determined on several control and 3-h estradiol-primed uteri by drying the tissues at 110° to constant weight. Extracellular space was measured on separate uteri by chemical analysis of the distribution of added sucrose[7], thiosulfate[8], thiocyanate[9] or inulin[10]. Amino acid uptake is expressed as distribution ratio, which is the ratio of amino acid level in uterine cellular water to that in the extracellular water.

## RESULTS

### *Water content and extracellular space of incubated uteri*

Seven control uteri had an average water content of 81.4 % of their wet weight after incubation for 1 h at 37° in Krebs–Ringer bicarbonate buffer, while four samples that had been primed with estrogen for 3 h averaged 81.2 %. These

values were not statistically different from one another. The space occupied by either sucrose or thiosulfate was found to reach a maximum value of about 59 % of the total tissue water, as an average, after incubation for 1–2 h at 37° (Fig. 1), and it was the same in both control uteri and those primed with estrogen for 3 h. Thiocyanate gave a somewhat higher figure of about 67 % after 1 h. Inulin, in contrast, was distributed in a space equal to only 33 % of the total tissue water within 1 h, and this value increased over a period of 8 h after which it had reached an apparent maximum approximately equal to that given by sucrose at 1 h (Fig. 1). This latter figure, 58.8 % of total water, was therefore used as the extracellular space value for all calculations in experiments run at 37°.

When uteri were incubated at 1°, inulin and sucrose spaces were found to be about 20 and 35 % of total tissue water, respectively, after 1 h, or only 60 % of the values found at 37°. Thiocyanate, in contrast, gave the same value in 1 h at the two temperatures (68 % at 1° *vs.* 67 % at 37°). After 4 h, the sucrose space at 1° was up to 53 %. After 2 h incubation at 23°, extracellular space was 53 % of total tissue water.

*Effect of shaking rate and method of preparation of uterus on the uptake of amino acid*

α-Aminoisobutyric acid was incubated with the control uteri at a 1 mM level for 2 h at three different shaking rates of the water bath (Labline model No. 3580).

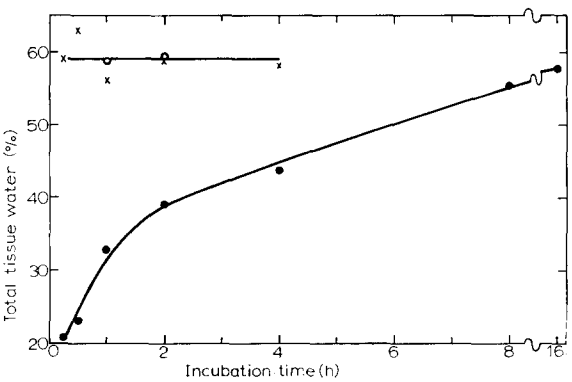


Fig. 1. Spaces occupied by inulin (●), thiosulfate (×) or sucrose (○) in the immature rat uterus incubated for various times at 37°. Krebs–Ringer bicarbonate buffer was used in all cases. Values are those obtained on rats not treated with estrogen.

TABLE I

EFFECT OF OSCILLATION RATE OF SHAKER BATH ON THE UPTAKE OF α-AMINOISOBUTYRIC ACID INTO THE IMMATURE RAT UTERUS *in vitro**

α-Aminoisobutyric acid was added to a level of 1 mM and the incubation time was 2 h at 37°. The distribution ratios recorded are averages for five determinations each ± S.E.

| *Oscillation rate (strokes/min)* | *Distribution ratio* | *P vs. No. 2* |
|---|---|---|
| 70–79 | 6.06 ± 0.40 | <0.001 |
| 106–113 | 8.59 ± 0.18 | |
| 130–136 | 4.84 ± 0.20 | ≪0.001 |

* These experiments were performed by Mr. W. REGAN.

Table I shows that maximum uptake of amino acid occurred at the intermediate speed studied, that is, at around 110 oscillations/min. The stroke of the movement of the flask was about 1 cm in all cases.

Amino acid uptake was compared in uteri prepared by three methods: without alteration as they were removed from the rat; with the ends of the horns tied off with surgical thread to prevent free access of bathing fluid to the interior; and after the horns had been slit longitudinally and laid open. 1-Aminocyclopentanecarboxylic acid at a 1 mM level was used as the test amino acid in a 2-h incubation. No significant differences were found in uptake into tissues prepared in these three ways. The first procedure was used routinely in all other work.

*Test for possible binding of amino acid by uterine cell fragments*

Three uteri were homogenized in buffer, placed in a cellophane bag and immersed in the same buffer contained in an erlenmeyer flask. [1-$^{14}$C]1-Aminocyclopentanecarboxylic acid was present originally both inside and outside the bag at a 1 mM level. After a 4-h incubation at 37°, the contents of the bag were precipitated and the supernatant fluids analyzed for radioactivity. The fluid inside the bag gave 1123 counts/min per ml original solution, while that on the outside gave 1156 counts per min per ml.

*Other factors affecting the uptake of α-aminoisobutyric acid in vitro*

One μg β-estradiol given intraperitoneally either 3 or 5 h earlier produced a 50% increase in the subsequent uptake from a 1 mM solution of α-aminoisobutyric acid *in vitro* (Fig. 2; see also Table IV). A smaller but significant increase was found in tissues taken 1 h after hormone injection, but no consistent effect was found in these studies after 0.5 h *in vivo*. A maximum of 3 h *in vivo* was used in other experiments reported here because no major shifts in uterine water content were observed after these tissues had been incubated (see above).

Fig. 3 shows the time course of α-aminoisobutyric acid uptake by the uterus from a 1 mM solution. Cellular amino acid levels increased linearly or better for at

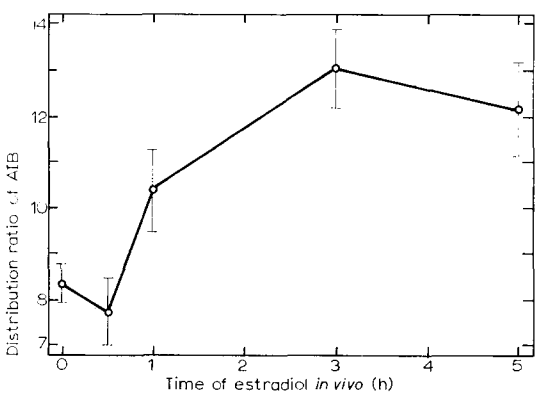


Fig. 2. Effect of time of estradiol *in vivo* on subsequent uptake of α-aminoisobutyric acid (AIB) by the immature rat uterus *in vitro*. One μg of the hormone was injected intraperitoneally at the time indicated, except zero time, before uteri were removed. Tissues were then incubated with 1 mM AIB for 2 h at 37°. Four to eight values for each point. Each parallel line shows ± 2 S.E. The values after 1, 3 and 5 h are significantly higher than at shorter times by statistical test.

least 2 h in control samples, and for 4 h in tissues primed with estradiol for 3 h. The 3 h-primed uteri gave distribution ratios greater than the controls at all times studied. Adding glucose to the medium to a level of 5–10 mM did not increase uptake in incubation times at 8 h or less, although it did at longer times.

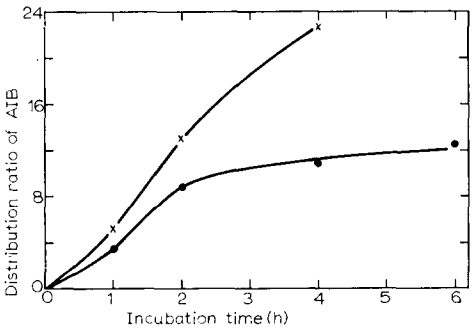


Fig. 3. Uptake of α-aminoisobutyric acid (AIB) by the immature rat uterus with time of incubation; effect of β-estradiol. AIB added to 1 mM. In estradiol-treated samples (×), 1 μg of the steroid was injected 3 h before tissues were taken. Four to nine values averaged for each point. Control (●).

TABLE II

EFFECT OF ADDITION OF METABOLIC INHIBITORS, AND OTHER CONDITIONS, ON TRANSPORT OF α-[$^{14}$C]AMINOISOBUTYRIC ACID INTO THE IMMATURE RAT UTERUS *in vitro*

α-Aminoisobutyric acid added to a level of 1 mM. Incubation at 37° in Krebs–Ringer bicarbonate under $O_2$–$CO_2$ at pH 7.4 unless stated otherwise. All comparisons are made to the value given under these conditions in absence of inhibitor. At least two determinations made except where indicated.

| *Agent or condition* | *Incubation time (h)* | *% Change in distribution ratio* |
|---|---|---|
| 2,4-Dinitrophenol (0.5 mM) | 2 | −59 |
| Cyanide (5 mM) | 2 | −55 |
| 95% $N_2$–5% $CO_2$ gas phase | 2 | −69 |
| Iodoacetate (5–8 mM) | 1 | −43 |
| Fluoride (20 mM) | 1 | −27* |
| Puromycin (0.2 mM) | 1 | −15 |
| Puromycin (0.25 mM) | 6 | −53 |
| High buffer $K^+$ (124 mequiv/l) | 1 | −79* |
| Buffer $Na^+$ replaced with choline$^+$ | 2 | −64 |
| $Ca^{2+}$–$Mg^{2+}$-free buffer | 2 | −15 |
| 4-h Preincubation in normal buffer | 2** | 83 |
| Stored 23 h at 4° | 2 | 3 |
| Incubation at 23° | 2 | −97 |
| Phosphate buffer (pH 7.4) | 2 | −21 |
| Phosphate buffer (pH 6.8) | 2 | −42 (−27***) |
| Phosphate buffer (pH 5.4) | 2 | −74 (−67***) |

* Single determination.

** Time incubated with α-aminoisobutyric acid in fresh, normal Krebs–Ringer bicarbonate buffer.

*** Change relative to value given in phosphate buffer (pH 7.4), condition of third line from bottom.

Cyanide, 2,4-dinitrophenol, anaerobiosis and iodoacetate strongly inhibited α-aminoisobutyric acid uptake in a 1- or 2-h incubation (Table II). Fluoride was less effective. Puromycin at a 0.2 mM level had only a small effect over a 1-h incubation but was strongly inhibitory over 6 h. Replacing buffer $Na^+$ with $K^+$ resulted in a somewhat larger decrease in uptake than when choline$^+$ was used as the cation. Uteri primed for 3 h *in vivo* with estradiol were inhibited as much or more than were control tissues when tested with the above agents.

Normal α-aminoisobutyric acid uptake was also given by uteri stored for 23 h at 4° before being incubated at 37° with α-aminoisobutyric acid under the usual conditions (Table II). Uphill transport of α-aminoisobutyric acid was not found when tissues were incubated with the amino acid at ice-bath temperature; the amount of radioactivity in the tissue did not exceed the sucrose space even after 4 h at 1°. Even at 23°, very low distribution ratios were found after a 2-h incubation (Table II).

The uteri concentrated α-aminoisobutyric acid to higher levels than normal if they were incubated in normal buffer at 37° for several hours before the α-aminoisobutyric acid was added (Table II). This phenomenon is being studied further. α-Aminoisobutyric acid uptake fell off sharply if the pH of the incubation medium was lowered from the normal value of 7.4 down to 6.8 or 5.4 (Table II, bottom 3 lines). Krebs–Ringer phosphate was used as the buffer in these experiments to reach the low pH values. Uptake was about 20% lower in the phosphate medium at pH 7.4 than from bicarbonate medium of the same pH.

*Uptake of α-aminoisobutyric acid at different extracellular levels*

Uterine cell levels of α-aminoisobutyric acid were increased as the extracellular fluid levels of the amino acid were raised over the range 0.3–10 mM (Fig. 4). The estrogen-primed samples had significantly higher levels than the controls in each case. A 1-h incubation period was used for these experiments because α-aminoisobutyric acid uptake was found to be essentially linear over this time at extracellular fluid levels up to 10 mM.

The method of AKEDO AND CHRISTENSEN[11] was used to calculate the non-saturable entry of α-aminoisobutyric acid, and the $K_m$ and $v_{max}$ of the saturable

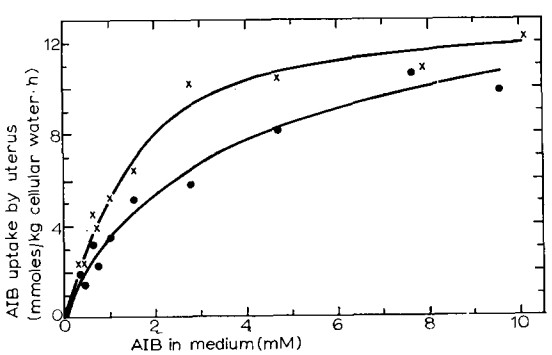


Fig. 4. Uptake of α-aminoisobutyric acid (AIB) by the immature rat uterus *in vitro* from different AIB levels in the extracellular fluid; effect of estradiol. Incubation time 1 h. Estradiol-treated animals (×) given 1 μg of hormone intraperitoneally 3 h before tissues were removed. Levels in the uterus are calculated for cellular water. Two to six values averaged for each point. The lines are drawn by inspection. Control (●).

component, for uptake by both control and estrogen-primed uteri in 1 h. Entry by the non-saturable route was about 0.2–0.4 mmole/kg cellular water per h, and no consistent differences were found between values obtained in control and estrogen-primed tissues. Estradiol priming was found to increase the $v_{max}$ from a normal of 6–7 mmoles/kg cellular water per h to a value as high as 12 (2 separate determinations). The $K_m$ was about 1 mM in each case (see ref. 1). Our recent results suggest, however, that the method used to calculate these values does not apply strictly for α-aminoisobutyric acid uptake by the uterus. The data show that the mediated transport of α-aminoisobutyric acid and other amino acids into the isolated rat uterus does not follow simple Michaelis–Menten kinetics, perhaps because of the involvement of $Na^+$ in the uptake process. In addition, the uptake is complicated by the fact that the uterus increases its ability to concentrate amino acids when it is incubated alone with buffer (Table II). The effects of these two factors will be considered further in subsequent papers in this series.

*Inhibition of α-aminoisobutyric acid uptake by amino acids*

Table III records data showing the inhibition of α-aminoisobutyric acid uptake produced by 20 times the molar concentration of different amino acids. L-Methionine produced nearly complete inhibition of uptake of α-aminoisobutyric acid, and strong inhibition was also given by α-aminoisobutyric acid, L-proline, L-serine, L-alanine and 1-aminocyclopentanecarboxylic acid. Glycine, L-valine and L-phenylalanine showed much more inhibition in 2 h than in 1 h (Table III). L-Lysine produced consistent stimulation of α-aminoisobutyric acid uptake, a phenomenon that has been observed for neutral amino acid uptake in Ehrlich ascites tumor cells *in vitro*[12].

*Which amino acids have their transports stimulated by estradiol-17β?*

The uptake of 11 individual amino acids from 1 mM solutions was tested in uteri of rats injected 3 h earlier with 1 μg estradiol each. Varying degrees of stimulation were found in 1-h incubations with L-serine, glycine, L-alanine, L-proline,

TABLE III

INHIBITION OF UTERINE UPTAKE OF α-AMINOISOBUTYRIC ACID BY AMINO ACIDS

α-[$^{14}$C]Aminoisobutyric acid was added to the extracellular fluid to a level of 1 mM, other amino acids to 20 mM. Three to nine trials were made with each amino acid except those indicated.

| *Inhibiting amino acid* | % *Change in distribution ratio* | |
|---|---|---|
| | *1-h Incubation* | *2-h Incubation* |
| L Methionine | −98 | −99 |
| α-Aminoisobutyric acid | −91 | −87 |
| L-Proline | −93 | −80 |
| L-Serine | −87* | |
| L-Alanine | −69* | |
| 1-Aminocyclopentanecarboxylic acid | −63* | |
| Glycine | −46 | −73 |
| L-Valine | −32 | −68 |
| L-Leucine | −38 | −27 |
| L-Phenylalanine | − 1 | −42 |
| L-Lysine | 61 | 37 |

* Single determination.

1-aminocyclopentanecarboxylic acid, and L-lysine, as well as with α-aminoisobutyric acid (Table IV). No significant effect was found for DL-norleucine, L-phenylalanine, L-valine or L-leucine in this short incubation period (Table IV). L-Leucine uptake was likewise not stimulated by estradiol priming when the measurement was made in the presence of 0.2 mM puromycin—a level that inhibited incorporation of $^{14}C$ from L-leucine into protein by about 95 %. Distribution ratios of the leucine were higher in the presence of puromycin than in its absence, perhaps because of the greater accumulation of intracellular leucine. Inhibiting the incorporation did not unmask an effect of estradiol on transport, however, as has been found for the insulin stimulation of amino acid uptake by the perfused rat heart[13].

Uptakes of 1-aminocyclopentanecarboxylic acid, L-alanine and L-proline, as well as α-aminoisobutyric acid, could be stimulated by a 1-h priming with estradiol (Table V; Fig. 2 and Table VI). When valine and norleucine uptakes were measured after 4-h incubations, significantly higher values were found in the 3-h primed tissues

TABLE IV

THE EFFECT OF ESTRADIOL-17β *in vivo* 3 h ON THE UPTAKE OF VARIOUS AMINO ACIDS BY THE IMMATURE RAT UTERUS *in vitro*

One μg of the hormone was injected intraperitoneally 3 h before uteri were removed. Control and primed tissues were incubated in the same flasks for 1 h at 37° in Krebs–Ringer bicarbonate buffer with a 1 mM level of the amino acid. Distribution ratios are averages ± S.E. Cycloleucine, 1-aminocyclopentanecarboxylic acid. n.s. means: difference from control not significant statistically ($P > 0.05$).

| *Amino acid* | *Distribution ratio* | | *% Change* | *Degrees of freedom* | *P for estradiol effect* |
|---|---|---|---|---|---|
| | *Control* | *+ Estradiol* | | | |
| L-Serine | 8.70 ± 0.38 | 10.61 ± 0.43 | 23 | 12 | <0.01 |
| D-Alanine | 6.97 ± 0.32 | 9.08 ± 0.13 | 30 | 10 | <0.001 |
| Glycine | 6.77 ± 0.17 | 8.16 ± 0.15 | 21 | 6 | <0.001 |
| L-Proline | 3.93 ± 0.36 | 5.76 ± 0.50 | 47 | 7 | <0.05 |
| α-Aminoisobutyric acid | 2.22 ± 0.28 | 4.43 ± 0.94 | 100 | 12 | <0.01 |
| Cycloleucine | 2.12 ± 0.15 | 3.33 ± 0.14 | 57 | 10 | <0.001 |
| L-Lysine | 1.24 ± 0.10 | 1.59 ± 0.08 | 28 | 8 | <0.05 |
| DL-Norleucine | 1.73 ± 0.09 | 1.58 ± 0.14 | − 9 | 8 | n.s. |
| L-Phenylalanine | 1.37 ± 0.07 | 1.34 ± 0.09 | − 2 | 6 | n.s. |
| L-Valine | 1.21 ± 0.16 | 1.12 ± 0.09 | − 7 | 7 | n.s. |
| L-Leucine | 0.39 ± 0.14 | 0.21 ± 0.17 | −46 | 6 | n.s. |

TABLE V

ESTRADIOL STIMULATION OF AMINO ACID UPTAKE BY UTERI PRIMED WITH HORMONE 1 h *in vivo*

Amino acid added to 1 mM, incubation time 2 h. 4–14 values per group. Cycloleucine, 1-aminocyclopentanecarboxylic acid.

| *Amino acid* | *Distribution ratio* | | *% Change* | *P* |
|---|---|---|---|---|
| | *Control* | *+ Estradiol* | | |
| Cycloleucine | 1.84 ± 0.17 | 2.71 ± 0.32 | 47 | <0.01 |
| L-Alanine | 10.19 ± 0.41 | 11.48 ± 0.45 | 13 | <0.05 |
| L-Proline | 5.03 ± 0.34 | 6.46 ± 0.20 | 28 | <0.02 |

TABLE VI

ESTRADIOL STIMULATION OF AMINO ACID UPTAKE BY THE IMMATURE RAT UTERUS INCUBATED FOR EXTENDED TIMES

Amino acid added to a 1 mM level in each case. Estradiol added *in vitro* was dissolved in 25% ethanol; a similar volume of 25% ethanol (0.14 ml/6.0 ml) was added to control flasks. Other conditions were as described in Table IV. 7–9 values per group.

| *Amino acid* | *Dose estradiol* | *Incubation time (h)* | *Distribution ratio* | | *P* |
|---|---|---|---|---|---|
| | | | *Control* | *+Estradiol* | |
| L-Valine | 1 μg *in vivo*; 3 h | 4 | 1.99 ± 0.13 | 2.63 ± 0.25 | <0.05 |
| DL-Norleucine | 1 μg *in vivo*; 3 h | 4 | 2.25 ± 0.08 | 2.87 ± 0.11 | ≪0.01 |
| α-Aminoisobutyric acid | 1 μg *in vivo*; 1 h | 6 | 12.6 ± 1.2 | 16.8 ± 1.1 | <0.05 |
| α-Aminoisobutyric acid | $10^{-6}$ M *in vitro* only | 6 | 12.6 ± 1.2 | 16.9 ± 0.7 | <0.02 |

than in their controls (Table VI). Incubation times up to 6 h also resulted in stimulation of α-aminoisobutyric acid uptake when estradiol was added *in vitro*. The increase given by a $10^{-6}$ M level of the steroid *in vitro* was the same as that produced by 1 μg estradiol given *in vivo* 1 h before uteri were removed for incubation (Table VI). The hormone *in vitro* did not alter uptake in a 3-h incubation, however.

## DISCUSSION

The isolated uterus of the immature rat has several advantages over other tissues that have been used *in vitro* for studying the effect of hormones on transport. The tissue can be removed rapidly without trauma or the necessity of cutting it; it transports amino acids to substantial levels during an incubation of 1 h or more; its ability to transport continues for long incubation periods (up to 16 h have been tested) and after such adverse conditions as storage for a day at refrigerator temperatures. In addition, the tissue is thin, so that a relatively large portion of its cells are bathed directly by the incubation medium, and penetration should be rapid into the tissue interior. It responds within 1 h to estradiol injected *in vivo* as measured by the subsequent uptake of amino acids *in vitro*. Duplicate incubations show good reproducibility in all the circumstances so far studied, with somewhat better results if the incubation period is 1 h or more. An additional advantage is presented by the fact that control and estrogen-primed uteri can be incubated in the same flasks, eliminating when necessary the variable factor of slightly different extracellular media in evaluating the effect of the hormone. A complexity results, however, from the fact that the uterus is a multiple tissue. It is not possible to say at present whether estradiol produces its major change in amino acid uptake by an action on the endometrium or on the myometrium.

The space occupied by thiosulfate, sucrose, or inulin in the uterus *in vitro* was found to be the same (around 59% of the total tissue water) as that reported by DANIEL AND DANIEL[14] for inulin space in the rat uterus *in vitro*, and by NOALL AND ALLEN[3] for sucrose space in rabbit uterus. Thiocyanate was distributed in a larger space, giving a value equal to that found by NOALL AND ALLEN[3] for chloride space. In our experiments, inulin was much slower to fill this space than were the other substances used (up to 16 h as compared with 0.5–1 h for thiosulfate or sucrose). We

have assumed that the amino acids would be more like the smaller thiosulfate and sucrose molecules in equilibrating with the interstitial fluid, and in fact they would probably fill the space more rapidly since they could penetrate into the interior of the tissue by going through the cells as well as between them.

The movement of α-aminoisobutyric acid into the uterine cells shows the characteristics of active transport. The uptake is saturable and can be inhibited with other amino acids. High gradients can be obtained, and the uptake is greatly decreased or eliminated by metabolic inhibitors, lack of oxygen, lowering the temperature, or altering the pH. Radioactive amino acid was not bound significantly by uterine cell fragments, suggesting that amino acids are in the free state in these cells as they are in other types of cells.

Although a thorough study has not been made, the evidence suggests the presence of multiple transport systems in the uterus that have some of the general characteristics of the 'A' and 'L' sites described in Ehrlich ascites tumor cells and other tissues (refs. 15–17; *cf.* refs. 18, 19). α-Aminoisobutyric acid uptake was inhibited more strongly by L-alanine, L-proline, and L-serine than it was by L-leucine, L-phenylalanine and L-valine (Table III). Its uptake was also greatly reduced in the absence of extracellular $Na^+$. These properties are characteristic of amino acids transported to a large degree by the 'A' site. In addition, the amino acids stimulated most easily by estradiol (*i.e.*, in a 1-h incubation) were also those that were transported extensively by the 'A' system of the Ehrlich cell. A more complete examination is needed, however, to establish the number and nature of the amino acid transport systems in this tissue.

The results with α-aminoisobutyric acid, 1-aminocyclopentanecarboxylic acid, L-alanine and L-proline show that a 1-h priming with 1 μg estradiol is sufficient to stimulate amino acid uptake into the uterus *in vitro*. L-Valine and DL-norleucine uptakes, in contrast, were not affected even after a 3-h priming unless samples were incubated for up to 4 h. Amino acid used is undoubtedly one factor that determines the time needed *in vivo* for the hormone to produce stimulation. Even with the same amino acid, however, different results would be expected with variation of factors that might influence the time needed for an effective level of estradiol to reach its site of action (*e.g.*, amount of estradiol given, route of its administration, solvent used). Differences in such factors may explain the failure of HALKERSTON *et al.*[4], and COULSON AND GORSKI[20] to find changes in uterine uptakes of amino acids within 1–1.5 h after estradiol injection; while NOALL AND ALLEN[3], ROSKOSKI AND STEINER[21], and the present work show stimulation within 0.5–1 h. It is evident that effects on amino acid transport can be obtained within relatively short times under the proper conditions. Whether or not these changes are among the first to occur in response to estradiol can probably not be determined unless simultaneous measurements are made of other factors that might be altered.

Even though the kinetic constants calculated for α-aminoisobutyric acid transport undoubtedly do not represent the true $K_m$ and $v_{max}$ of mediated entry, they do show that $v_{max}$ is elevated by estradiol treatment. The hormone may be increasing the amount of postulated α-aminoisobutyric acid carrier, or it may be stimulating the same amount of carrier to function more rapidly. The first possibility seems more likely, especially with the finding that estradiol-stimulated uptake of α-aminoisobutyric acid can be inhibited by actinomycin D or cycloheximide[21]. If these two

agents are preventing formation of new carrier, we may conclude that estradiol-stimulated synthesis of nucleic acid and/or protein is necessary for new carrier formation, and that estradiol stimulates transport secondarily to an action on synthesis of large molecules.

LANGDON AND SLOAN[22] have reported that insulin increases the $v_{max}$ of sugar uptake by adipose tissue cells. Insulin does not increase α-aminoisobutyric acid uptake by diaphragm in the same way that estradiol alters its transport into the uterus, however. The protein hormone has been found to decrease the $K_m$ of α-aminoisobutyric acid uptake by diaphragm without an apparent effect on $v_{max}$[11]. In addition, as ROSKOSKI AND STEINER[21] have pointed out, both actinomycin D and cycloheximide inhibit the estradiol-stimulated uptake of this amino acid into uterus, while similar agents do not reduce insulin-stimulated transport into muscle tissue.

The stimulation of α-aminoisobutyric acid uptake by addition of estradiol *in vitro* provides evidence that the hormone can act directly on the uterus to produce this effect. The level of steroid added *in vitro* was, nevertheless, about 20 times that used *in vivo* to give the same result (0.27 μg/ml *vs.* about 0.01–0.02 μg/g rat). The amount of estradiol that entered the tissue under each condition is not known, but the results of NOALL AND ALLEN[3] suggest that much more would be present after the addition *in vitro* than *in vivo*. NOALL AND ALLEN[3] show, however, that the mere presence of estradiol in the uterus is not enough to stimulate α-aminoisobutyric acid uptake. The present results demonstrate that long incubation periods (up to 6 h) are needed to produce the effect *in vitro*. The evidence suggests either that the action of the hormone on transport is secondary, or that time is needed to modify the structure or location of the steroid molecule within the uterus before an effect is evident. The hormone in every case produced a greater change in amino acid uptake with increased exposure times to estradiol up to 6 or 8 h, whether that exposure was *in vivo*, *in vitro*, or a combination of the two.

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