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ACTION OF INSULIN ON AMINO ACID UPTAKE BY THE IMMATURE RAT UTERUS IN VITRO

TETSURO MOHRI, HARUO KITAGAWA and THOMAS R. RIGGS*

Department of Biological Chemistry, University of Michigan, Ann Arbor, Mich. (U.S.A.) and Department of Biochemical Pharmacology, Faculty of Pharmaceutical Sciences, University of Chiba, Chiba (Japan)

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SUMMARY

1. The uptake of α -aminoisobutyric acid by the immature rat uterus was studied kinetically in vitro, and the stimulatory action of insulin on uptake was characterized. Other amino acids were also studied for comparison.

2. α -Aminoisobutyric acid uptake was increased if the uteri were pretreated with insulin for 20 min or more. The V of the saturable transport was increased by 19% by insulin treatment, but no K_m change was apparent.

3. Puromycin eliminated the insulin action on α -aminoisobutyric acid uptake within 40 min of treatment.

4. The effect of insulin on α -aminoisobutyric acid uptake was abolished either by a preliminary 10-min incubation of the uterus with a high concentration (1 mM) of *N*-ethylmaleimide; or by incubation, concurrent or subsequent, with a low concentration of *N*-ethylmaleimide (0.05 mM).

5. The uptake of L-proline, L-methionine and glycine, but not that of L-leucine or L-valine, was also increased by insulin treatment. Insulin acted on a Na^+ -dependent uptake; it was without effect on the Na^+ -independent uptake of either α -aminoisobutyric acid or methionine. The evidence suggests that insulin acts on the A system of amino acid transport in the uterus.

INTRODUCTION

Insulin has been shown to increase the transport of a number of amino acids into various muscle tissues in vitro [1–12]. Its effect on the uterus has not, however, been previously tested. This latter tissue is of interest because its transport of the short-chain, more polar, amino acids in vitro can be increased by as much as 100% by the injection of estradiol-17 β into rats before the uteri are removed [13]. These and other characteristics of the uptake suggest that the steroid hormone

* Address reprint requests to: Dr T. R. Riggs, Department of Biological Chemistry, The University of Michigan, Ann Arbor, Mich. 48104, U.S.A.

acts on the A system of transport in this tissue [13–15]. Insulin, on the other hand, also acts on the A system, at least in the rat diaphragm [16]. It is of special interest, therefore, to determine if insulin also affects the A system in the uterus; and, if so, to compare the actions of the two hormones on this system.

The present paper reports results showing that insulin can increase transport by the uterus of the same amino acids that are affected by estradiol. Some of the characteristics of this stimulation by the peptide hormone are also presented, and some implications of the mechanism of hormone action on transport are discussed.

MATERIALS AND METHODS

Chemicals. α -[1- 14 C]Aminoisobutyric acid (0.05 Ci/0.6 g) and L-[14 C₅]valine (200 Ci/mole) were purchased from New England Nuclear Corp., L-[14 C₅]proline (200 Ci/mole) and L-[14 C₆]leucine (200 Ci/mole) from Isolab Inc., and L-[Me- 14 C]-methionine and [1- 14 C]glycine (45 Ci/mole) from Daiichi Pure Chemicals Co., Ltd. (Japan). Concentrated water solutions of these radioactive amino acids were stocked deep-frozen. Bovine insulin (crystalline, 24 I.U./mg) and *N*-ethylmaleimide were purchased from Sigma Chemical Co., cycloheximide (Acti-dione) from Nutritional Biochemicals Corp., and puromycin dihydrochloride from the American Cyanamide Company.

Animal and tissue preparation. Sprague Dawley rats of body weight ranging from 60–75 g were sacrificed after feeding ad libitum, or after fasting overnight in some experiments indicated in the text. Rats were decapitated, the uterus was excised rapidly away from ovary and adjacent tissues, and the two horns were cut apart and slit longitudinally. Four horns were put into each 50-ml Erlenmeyer flask, the two horns from each animal being paired between control and experimental flasks.

Incubation and determination of uptake of radioactive amino acids. Tissue preparations were incubated at 37 °C with 6 ml per flask of preincubation or incubation medium as specified in the results. The medium was Krebs–Ringer bicarbonate salt solution or modifications of it made by replacing all of the Na⁺ with choline ions. The gas phase was 95 % O₂–5 % CO₂. Other additions to the medium for various experiments included insulin (0.1 I.U./ml), puromycin (0.12 mM), cycloheximide (0.5 mM) or *N*-ethylmaleimide (1 or 0.05 mM). In the incubation for the measurement of the amino acid uptake, radioactive amino acid was added to a level of 0.02–0.04 μ Ci/ml, and to a total concentration of 1.0 mM unless otherwise mentioned. Extraction of the free amino acid in the tissue after incubation was performed as previously [13]. Aliquots of the extract and incubation medium were analyzed by liquid scintillation counting.

Calculation of the intracellular amino acid concentration was made on the bases of the total water content and the extracellular space of the tissue, determined as described earlier [13, 17]. The water content and the extracellular space of the tissues were obtained in the preliminary experiments for uteri of the ages used (3–4 weeks old) incubated for various periods, and under the conditions of treatment with the hormone and the inhibitors studied in the present work. Statistical analyses were carried out by the calculation of values for paired or non-paired samples, as appropriate.

RESULTS

Insulin effect on the uptake of α -aminoisobutyric acid and its kinetics

Fig. 1 shows the time course of uptake of α -aminoisobutyric acid by the uterus over 50 min in both the presence and absence of insulin. Stimulation of α -aminoisobutyric acid uptake by insulin appeared at 30 min of incubation, with a value of 23% above the control ($P < 0.01$), and was maintained thereafter. Insulin stimulation could be shown in 10-min uptakes, however, if the tissue was incubated with hormone for 20 min or more before amino acid was added (Table I). 50-min of preincubation without insulin, however, could not be substituted for periods of preincubation with insulin (Expt IV, Table I); and a somewhat smaller effect was found if the insulin was omitted from the final incubation medium (compare Expts III and V). These findings indicate that approximately 20 min of pretreatment with insulin is essential for the subsequent stimulation of α -aminoisobutyric acid uptake by the hormone.

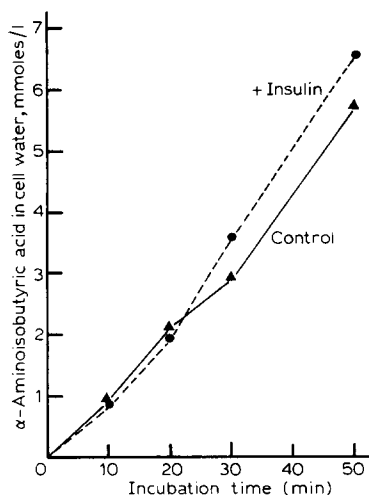


Fig. 1. The insulin effect on the α -aminoisobutyric acid uptake by the uterus. Each two uteri, cut into horns and paired, were incubated in 6 ml of Krebs-Ringer bicarbonate solution with labeled α -aminoisobutyric acid at an initial concentration of 1.0 mM in the presence or absence of insulin (0.1 I.U./ml) for the periods indicated. Each point shows the mean of the intracellular concentration for α -aminoisobutyric acid for 3-9 replications.

Kinetic constants of transport were obtained with the uterus incubated for 10 min with varying concentrations of α -aminoisobutyric acid after 50 min of preincubation in the presence and absence of insulin. The uterine samples (3 or more replications) were paired between the control and the insulin-treated groups. The non-saturable entry of α -aminoisobutyric acid was estimated to be 0.435/h for both control and insulin-treated uteri by the procedure of Akedo and Christensen [3]. The values of K_m , 1.28 and 1.23 mM, V , 12.4 and 14.8 $\text{mmoles} \cdot \text{l}^{-1} \cdot \text{h}^{-1}$ for the saturable transport in control and insulin-treated uterus, respectively, show that the change of V rather than that of K_m may be responsible for the increase of α -aminoisobutyric acid uptake induced by insulin treatment in the uterus (Fig. 2).

TABLE I
 α -AMINOISOBUTYRIC ACID UPTAKE BY THE UTERUS FOLLOWING PERIODS OF PREINCUBATION WITH INSULIN

Each two uteri, cut into horns, were placed in 6 ml of Krebs-Ringer bicarbonate salt solution and preincubated for the periods indicated. Fresh medium was used for the incubation and contained 1.0 mM of labeled α -aminoisobutyric acid. The tissue was blotted on filter paper after preincubation. Each value of uptake is the mean of the intracellular concentration of α -aminoisobutyric acid of 4-9 replications \pm SEM.

Expt no.	Insulin addition		α -Aminoisobutyric acid uptake		P
	Preincubation (min)	Incubation with α -aminoisobutyric acid (10 min)	(mmoles/l)	% difference due to insulin	
I	—	—	1.64 \pm 0.08		< 0.02
	+	—	1.97 \pm 0.04	20	
II	—	—	2.38 \pm 0.07		< 0.01
	+	—	2.77 \pm 0.10	17	
III	—	+	2.49 \pm 0.04		< 0.01
	+	+	3.14 \pm 0.13	26	
IV	—	—	2.38 \pm 0.08		(n.s.)
	—	+	2.30 \pm 0.08	3	
V	—	—	2.88 \pm 0.20		< 0.05
	—	+	3.32 \pm 0.08	15	

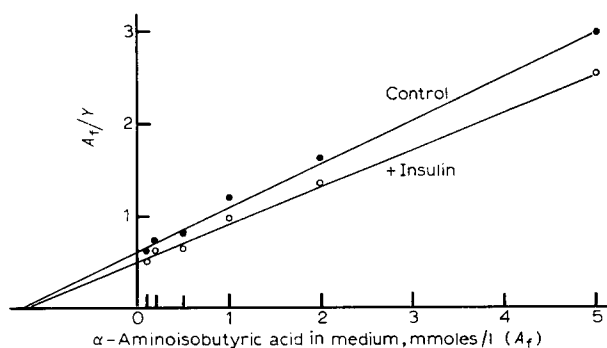


Fig. 2. The insulin effect on the kinetics of the saturable transport of α -aminoisobutyric acid. Each four uterine horns, paired between the control and insulin treatment, were preincubated for 50 min and incubated for 10 min with α -aminoisobutyric acid in Krebs-Ringer bicarbonate solution with or without insulin. Migration of α -aminoisobutyric acid into the uterus was assumed to consist of the saturable and non-saturable fractions, and the following equation was proposed for the total uptake of the amino acid (cf. [3]).

$$dA_c/dt = Y + K_D(A_f - A_c)$$

A_c and A_f show the intra- and extracellular concentrations (mmoles/l), respectively, and Y (mmoles/l of cell water/h) the velocity of uptake by the saturable process. K_D is defined as a rate constant for the non-saturable process and was estimated as 0.435/h, common to the control and insulin-stimulated uptake of α -aminoisobutyric acid, by the procedure of Akedo and Christensen [3]. The values of Y or A_i/Y were calculated in varying concentrations of α -aminoisobutyric acid in medium using the value of K_D . Each point is the average of the values of 3 or more replications.

TABLE II

INFLUENCE OF PUROMYCIN ON THE INSULIN STIMULATION OF α -AMINOISOBUTYRIC ACID UPTAKE BY THE UTERUS

Each four horns of the uterus, paired between two groups in each experiment, were placed in 6 ml of preincubation or incubation medium. Rats were fasted overnight before sacrifice. The dotted line shows the addition of neither insulin nor puromycin. See legends of Table I for other conditions.

Expt no.	Addition				α -Aminoisobutyric acid uptake		<i>P</i>
	Preincubation (min)	Incubation (min)	mmoles/l	% difference			
VI	—	10	—	30	2.35 ± 0.10		(n.s.)
	Puromycin	10	Puromycin	30	2.29 ± 0.13	-3	
VII	—	10	Insulin	30	2.74 ± 0.13		≤ 0.01
	Puromycin	10	Insulin	30	2.20 ± 0.14	-20	
			+ puromycin				
VIII	—	50	—	10	1.28 ± 0.06		< 0.05
	Puromycin	50	Puromycin	10	1.14 ± 0.02	-11	
IX	Insulin	50	Insulin	10	1.41 ± 0.10		< 0.02
	Insulin		Insulin				
	+ puromycin	50	+ puromycin	10	1.11 ± 0.10	-21	
X	Puromycin	60	Insulin	30	2.05 ± 0.05		(n.s.)
	Puromycin	60	+ puromycin				
	Puromycin	60	Puromycin	30	2.04 ± 0.11		

TABLE III
 INFLUENCE OF *N*-ETHYLMALDEIMIDE ON THE INSULIN STIMULATION OF α -AMINOISOBUTYRIC ACID UPTAKE BY THE UTERUS

Each four horns of uterus, paired between two groups in each experiment, were placed in 6 ml of Krebs-Ringer bicarbonate solution for each preincubation and incubation. The tissue was blotted on filter paper in each transfer for change of medium. 1.0 mM labeled α -aminoisobutyric acid was added to the incubation medium. Each value is the mean of intracellular concentration of α -aminoisobutyric acid for 3-9 replications \pm SEM.

Expt no.	Addition		Preincubation		Incubation (10 min)		α -Aminoiso- butyric acid uptake mmoles/l	<i>P</i>
	1st	2nd	(min)	(min)	(min)	(min)		
XI	—	Insulin	10	Insulin	50	Insulin	3.74 \pm 0.27	<0.02
	1 mM	Insulin	10	Insulin	50	Insulin	1.93 \pm 0.36	
XII	—	Insulin	30	Insulin	20	Insulin	2.47 \pm 0.09	(n.s.)
	0.05 mM	Insulin	30	Insulin	20	Insulin	2.34 \pm 0.12	
XIII	—	—	50	—	—	—	2.56 \pm 0.08	(n.s.)
	0.05 mM	<i>N</i> -ethylmaleimide	50	0.05 mM	—	<i>N</i> -ethylmaleimide	2.46 \pm 0.06	
XIV	Insulin	Insulin	50	Insulin	—	Insulin	2.92 \pm 0.12	<0.05
	Insulin+	Insulin+	50	Insulin+	—	Insulin+	—	
XV	—	—	20	—	—	0.05 mM	2.43 \pm 0.13	<0.05
	—	0.05 mM	20	0.05 mM	—	<i>N</i> -ethylmaleimide	2.26 \pm 0.04	
XVI	Insulin	Insulin	20	0.05 mM	—	<i>N</i> -ethylmaleimide	2.41 \pm 0.05	<0.05
	Insulin	Insulin	20	0.05 mM	—	<i>N</i> -ethylmaleimide	2.56 \pm 0.13	
XVII	—	—	20	0.05 mM	—	<i>N</i> -ethylmaleimide	2.08 \pm 0.08	<0.05
	—	0.05 mM	20	0.05 mM	—	<i>N</i> -ethylmaleimide	—	

Influence of puromycin on α -aminoisobutyric acid uptake and action of insulin

Puromycin inhibited the insulin stimulation of α -aminoisobutyric acid uptake when it was present for 40 min over successive preincubation and incubation periods; it did not alter the basal uptake of α -aminoisobutyric acid (Expts VI and VII, Table II). Inhibition in both basal and insulin-stimulated uptake appeared, however, when puromycin was present for a total of 60 min of preincubation and incubation (Expts VIII and IX). The insulin effect was completely abolished when the uterus was incubated in the presence of puromycin for a total of 60 min of preincubation and 30 min incubation (Expt X). The ability of insulin to stimulate proline uptake (Table IV) was also abolished by the presence of cycloheximide added at the same time as insulin over a 50-min preincubation period followed by a 10-min proline uptake.

*Influence of *N*-ethylmaleimide on α -aminoisobutyric acid uptake and insulin action*

Pretreatment of the uterus with 1 mM *N*-ethylmaleimide for 10 min completely suppressed the stimulatory effect of insulin on the α -aminoisobutyric acid uptake, the uptake by the *N*-ethylmaleimide-treated tissue being below that normally found in control uteri (Expt XI, Table III). When the uterus was pretreated with a much lower concentration of *N*-ethylmaleimide (0.05 mM) for 30 min, the insulin stimulation was not hampered (Expt XII). But when the tissue was exposed to the low concentration of *N*-ethylmaleimide for 60 min during the successive preincubation and incubation in the presence of insulin (Expt XIV) or for 30 min during the preincubation after 20 min of insulin treatment (Expt XVI), the stimulation was abolished. The presence of the low concentration of *N*-ethylmaleimide for 60 min throughout the preincubation and incubation was without effect on the basal uptake by the uterus (Expt XIII). It should be noted that the α -aminoisobutyric acid uptake by the tissue was increased somewhat when the low concentration of *N*-ethylmaleimide was present in the medium for 30 min (Expt XV), but not after 60 min (Expt XIII).

Separate experiments were carried out to show that the *N*-ethylmaleimide did not produce its effect by binding the insulin in the solution. Ascending paper chromatography of an insulin-*N*-ethylmaleimide mixture, using butanol-acetic acid-water, showed only one spot after it was sprayed with bromocresol green. This spot had the same R_F (0.08) as given by insulin alone. In addition, a mouse bioassay showed the insulin in an insulin-*N*-ethylmaleimide mixture to be as effective as insulin alone in causing skeletal muscle relaxation, ataxia, and convulsions. The results suggest, therefore, that the *N*-ethylmaleimide may have acted by preventing the insulin from binding to the tissue.

Effect of insulin on the uptake of other amino acids

The uptake of proline and glycine during 10 min of incubation was increased by about 20 and 63 %, respectively, by treatment with insulin for a total of 60 min of successive preincubation and incubation (Table IV). Methionine uptake was elevated 17 % under these conditions (Table V). The uptake of leucine and valine, however, was not stimulated by the same treatment, either in the presence or absence of an inhibitor of protein synthesis (Table IV). Control uptake of the latter two amino acids was higher than normal in the presence of cycloheximide or puromycin, however (Table IV, Expts XIX-XXII).

TABLE IV
EFFECT OF INSULIN ON THE UPTAKE OF DIFFERENT AMINO ACIDS BY THE UTERUS

Each four horns of the uterus, paired between two groups in each experiment, were placed in 6 ml of Krebs-Ringer bicarbonate solution in preincubation and incubation. The tissue was blotted on filter paper after preincubation. The medium was renewed for the final incubation and contained 1.0 mM of labeled amino acid indicated at the far left of the table. Each value of the uptake is the mean of the intracellular concentration of the labeled amino acid of 3-6 replications \pm SEM.

Labeled amino acid tested	Expt no.	Addition		Incubation (10 min)	Amino acid uptake mmoles/l	P
		Preincubation (50 min)				
Proline	XVII				1.10 \pm 0.07	< 0.02
		Insulin		Insulin	1.33 \pm 0.09	
Glycine	XVIII				1.75 \pm 0.07	< 0.02
		Insulin		Insulin	2.85 \pm 0.12	
Leucine	XIX				0.98 \pm 0.04	(n.s.)
		Insulin		Insulin	1.03 \pm 0.05	
	XX				1.15 \pm 0.01	(n.s.)
		Puromycin Insulin + puromycin		Puromycin Insulin + puromycin	1.18 \pm 0.04	
Valine	XXI				0.95 \pm 0.05	(n.s.)
		Insulin		Insulin	0.94 \pm 0.05	
	XXII				1.52 \pm 0.03	(n.s.)
		Cycloheximide Insulin + cycloheximide		Cycloheximide Insulin + cycloheximide	1.51 \pm 0.08	

TABLE V

Na⁺-DEPENDENCE OF THE UPTAKE OF α -AMINOISOBUTYRIC ACID AND METHIONINE AND INSULIN ACTION

Each four horns of the uterus, paired between the control and insulin treatment in each experiment, were placed in 6 ml of Krebs-Ringer bicarbonate solution in preincubation. The medium was renewed for incubation, and was either Krebs-Ringer bicarbonate solution (in experiments marked Na⁺) or a choline-substituted medium defined in Materials and Methods (in experiments marked Ch⁺) containing 1.0 mM of either α -aminoisobutyric acid or methionine. Each value of the uptake is the mean of the intracellular concentration of the labeled amino acid of 4-8 replications \pm SEM.

	Insulin addition		Amino acid uptake (mmoles/l)	% Difference	P
	Preincubation (50 min)	Incubation (10 min)			
α -Aminoisobutyric acid	-	Na ⁺	1.96 \pm 0.14		
	+	Na ⁺	2.41 \pm 0.15	+ 23	<0.05
	-	Ch ⁺	0.85 \pm 0.05		
	+	Ch ⁺	0.88 \pm 0.03		
Methionine	-	Na ⁺	2.43 \pm 0.15		
	+	Na ⁺	2.83 \pm 0.24	+ 17	<0.05
	-	Ch ⁺	1.46 \pm 0.04		
	+	Ch ⁺	1.49 \pm 0.04		

Na⁺ sensitivity of insulin action on amino acid uptake

Replacing the Na⁺ of the medium with choline ion eliminated the insulin stimulation of the uptake of α -aminoisobutyric acid and methionine (Table V). In these experiments, normal Krebs-Ringer bicarbonate medium was used for the preincubation with insulin, and the Na⁺ was replaced only during the 10-min period during which the amino acid was present. It should be noted that methionine was taken up concentratively into the tissue even in the absence of sodium in the incubation medium (Table V).

Effect of insulin on α -aminoisobutyric acid transport in the estrogen-primed uterus

Since insulin and estradiol showed many characteristics in common in their stimulation of α -aminoisobutyric acid uptake, the two hormones were tested together at optimal levels of each. Insulin was added to the incubation medium of uteri taken from rats given 1 μ g estradiol *in vivo* 3 h earlier (a condition shown to give a maximum response in α -aminoisobutyric acid uptake [13]). The results are shown in Table VI. A significant increase was given by the insulin, beyond that given by estradiol, whether the insulin was preincubated with the tissue or present during the entire period with α -aminoisobutyric acid.

TABLE VI

EFFECTS OF ESTRADIOL AND INSULIN ALONE AND TOGETHER ON THE α -AMINOISOBUTYRIC ACID UPTAKE BY THE IMMATURE UTERUS

Estradiol (1 μ g in 25 % ethanol in saline), or solvent alone was injected intraperitoneally into rats 3 h before the uteri were removed. Experiment A: All tissues were preincubated for 50 min before the α -aminoisobutyric acid uptake was measured for 10 min. Samples with insulin contained 0.1 I.U./ml during both periods. Experiment B: The α -aminoisobutyric acid uptake was measured for 60 min. The insulin was present at 0.8 I.U./ml over the entire period. α -Aminoisobutyric acid was at 1 mM in each case. There were 4-12 values per group.

Expt	Condition	α -Aminoisobutyric acid in cell water (mmoles/l)	P
A	Control	2.49 \pm 0.04	0.01
	+ insulin	3.14 \pm 0.13	
	Estradiol-primed	3.51 \pm 0.12	
	Estradiol + insulin	3.79 \pm 0.11	
B	Control	1.06 \pm 0.29	0.001
	Estradiol-primed	3.70 \pm 0.26	
	Estradiol + insulin	4.65 \pm 0.29	

DISCUSSION

The above results show that insulin significantly increases the short-term uptake of α -aminoisobutyric acid, proline, methionine and glycine by the immature rat uterus *in vitro*. All of these amino acids have been found to have largely Na⁺-dependent transport in tissues [18] (cf. [15]). On the other hand, uptake by the uterus of leucine and valine, which prefers consistently a Na⁺-independent system for transport in Ehrlich ascites tumor cells [18, 19] and in the rat uterus [15], was not affected by treatment with insulin. The Na⁺-independent component of the methion-

ine transport, on the other hand, was not accelerated by insulin. From these results, it is reasonable to conclude that insulin differentially accelerates the Na^+ -dependent transport of amino acids in the uterus.

The action of the hormone, on the other hand, was found only after a lag of 20 min or so, and this time could not be shortened by preincubation without insulin for as long as 50 min. Insulin-stimulated uptake of α -aminoisobutyric acid was blocked preferentially to give control values by the simultaneous presence of puromycin for 40 min. In addition, the hormone produced an increase in the V of α -aminoisobutyric acid uptake without altering the K_m . Although the change in V was small, it appears to account for the increased uptake produced by the hormone, since other transport parameters were not altered. These facts taken together suggest that insulin acted on the transport by increasing the synthesis of one or more proteins of the A system in the uterus.

The action of insulin on amino acid transport in the uterus appears to be similar to that in the diaphragm [16], in that the hormone acts on the A system in both tissues. Several major differences are found, however, a fact that suggests different mechanisms of action. First, a lag of more than 20 min occurred before stimulation in the uterus was initiated, while the increase induced in the diaphragm was apparent in as short a time as 5 min [20]. The lag time in the uterus, one may speculate, could be the time needed to form new protein of the transport system. Such an interpretation is also consistent with the finding that insulin alters the V of α -aminoisobutyric acid uptake in the uterus, a fact suggesting the formation of an increased amount of a protein component of the carrier system. For the diaphragm, in contrast, it seems unlikely that insulin alters the amino acid transport by increasing the protein synthesis (see discussion in [21], [22]), but instead the hormone apparently alters the affinity between amino acid and carrier [3, 10].

The results with *N*-ethylmaleimide also add evidence regarding the possible mechanism by which insulin increases the transport in the uterus. A relatively low concentration of the agent, 0.05 mM, blocked the insulin-stimulated α -aminoisobutyric acid uptake, regardless of whether it was added at the same time as insulin, or subsequent to treatment with the hormone. The same treatment with *N*-ethylmaleimide produced no effect on the basal α -aminoisobutyric acid uptake, a result consistent with earlier work from this laboratory [23]. *N*-ethylmaleimide did not act, however, by binding with the insulin itself in the medium. These findings suggest that the sulfhydryl groups of the uterus are essential for the action of insulin on amino acid transport. We concluded earlier [23] that *N*-ethylmaleimide alters the amino acid transport by binding to the uterus. Also, the uptake in the presence of insulin is more sensitive than normal to *N*-ethylmaleimide. A similar increased sensitivity to *N*-ethylmaleimide was also found for the estradiol-primed uterus [23]. Estradiol [13, 15] and insulin both appear to increase the activity of the A system; the action of *N*-ethylmaleimide on the A system is inhibitory [23]. It is, therefore, not surprising that the insulin-stimulated uterus should also show a greater sensitivity to *N*-ethylmaleimide than given by normal tissue. A 0.05 mM level of *N*-ethylmaleimide, used in these experiments, was shown before not to inhibit control α -aminoisobutyric acid uptake, while a 1 mM level did [23] (cf. Table III).

Mention should also be made of the fact that a 0.05 mM level of *N*-ethylmaleimide produced a small stimulation in the α -aminoisobutyric acid uptake when

a 30-min exposure was used (Expt XV, Table III). This fact suggests that α -aminoisobutyric acid may be transported to a significant extent by the L system (or some other *N*-ethylmaleimide-sensitive system) in the uterus, since this latter system is sensitive to stimulation by SH agents [23].

Insulin and estradiol show many common characteristics in their actions on amino acid transport in the uterus. Mention has already been made of their response to *N*-ethylmaleimide. In addition, both hormones appear to act on the Na^+ -sensitive A system; in both cases the increase is associated with an elevated V with no change in K_m [13]. A lag period of 30–60 min is needed for the action of estradiol given *in vivo* [13, 24, 25], while a similar delay is seen in the effect of insulin added *in vitro*. The stimulatory actions of both hormones can also be inhibited by the inhibition of protein synthesis [25]. These results suggest that insulin and estradiol may have some common biochemical effect on at least one step in the transport. Nevertheless, some difference in their actions is suggested by the fact that insulin can produce a significant increase in the uptake in uteri in which α -aminoisobutyric acid transport has been maximally stimulated by estradiol (Table VI).

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