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TRANSPORT OF α-AMINOISOBUTYRIC ACID IN RABBIT DETRUSOR MUSCLE

I. GENERAL CHARACTERISTICS OF THE UPTAKE *IN VITRO*

FIKRY H. OSMAN AND DAVID M. PATON

Department of Pharmacology, University of Alberta, Edmonton 7, Alberta (Canada)

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SUMMARY

1. The uptake of α-aminoisobutyric acid by isolated rabbit detrusor muscle was studied.

2. Uptake of the amino acid at low concentrations exhibited properties of active transport. Transport occurred against a concentration gradient, was decreased by metabolic inhibitors and was temperature sensitive. The process had an energy of activation of 13.1 kcal/mole.

3. Ouabain inhibited transport of the amino acid at concentrations which inhibited Na^+ pumping suggesting that a functioning Na^+ pump was required for amino acid transport to occur.

4. Transport of α-aminoisobutyric acid was ion dependent being very markedly reduced in the absence of external Na^+ or K^+ and, to a lesser extent, by the omission of Mg^{2+}.

INTRODUCTION

There have been many studies *in vitro* of the transport of amino acids in various tissues[1–7]. Comparatively little information is available however on the mechanism of transport of amino acids in isolated smooth muscles. The transport of amino acids into estrogen-primed uterine horns has been studied[8–12] but in these studies the transport into the myometrium alone was not characterised since the endometrium was not removed. The objective of the study reported here was to examine the mechanism of transport *in vitro* of the model amino acid, α-aminoisobutyric acid, in detrusor muscle obtained from male rabbits. An advantage of this preparation is that it is relatively easy to free the muscularis layer from the overlying mucosa thus ensuring that it is mainly transported into smooth muscle that is being studied. A portion of this work has been reported previously in abstract form[13].

METHODS AND MATERIALS

Preparation of tissues

Male rabbits (New Zealand) weighing 2–3 kg, were killed by a blow on the neck. After removal of the urinary bladder, the detrusor portion of the bladder was dissected

free of surrounding tissue, opened longitudionally and immersed in Krebs solution at 37°. The mucosa was removed as rapidly as possible by careful dissection through the plane of cleavage between the mucosa and the muscularis. The remaining muscle layer was then cut into 10–14 pieces and allowed to equilibrate for one hour at 37° in Krebs solution equilibrated with 95 % O_2–5 % CO_2.

Measurement of tracer uptake

After the preincubation period, the tissues were transferred on fine hooks, two to a hook, to test tubes containing 5–10 ml of solution and 10 μM α-amino [3-^{14}C]isobutyric acid. The tissues were then incubated further for varying time periods. At the end of the period of incubation the tissues were removed, blotted on filter paper, rinsed rapidly in Krebs solution and reblotted. The tissues were then placed in preweighed liquid scintillation vial and weighed. The tissues were dissolved using NCS solubilizer (a quaternary ammonium base obtained from Amersham/Searle Corp.), 1 ml/100 mg tissue wet wt., at 37° for at least 6 h. When tissue dissolution was complete, BRAY's phosphor[14], 10–15 ml/vial, was added and the total ^{14}C content measured after the vials had been cooled and dark adapted. Duplicate 1.0-ml portions of media were counted for ^{14}C content after the addition of BRAY's phosphor[14], 15 ml/vial. The ^{14}C content of tissues and media was determined using a Picker Nuclear Liquid Scintillation Counter (Liquimat 110) with a counting efficiency for ^{14}C of about 95 %. All vials were counted for a period sufficient to give a precision of statistical error of counting of 1 % or less. Quenching was corrected for using the channels ratio technique.

Expression of results

The uptake of α-aminoisobutyric acid was expressed in one of the following ways:

(1) μmoles/g wet wt. tissue

$$= \frac{\text{disintegrations/min per g wt. tissue}}{\text{disintegrations/min per } \mu\text{mole } \alpha\text{-amino[3-}^{14}\text{C]isobutyric acid}}$$

(2) distribution ratio (%)

$$= \frac{\text{disintegrations/min per ml tissue water}}{\text{disintegrations/min per ml incubation medium}} \times 100$$

(3) μmoles/ml intracellular water

$$= \frac{X_t - X_e V_e}{V_t - V_e}$$

where X_t = total amount of α-aminoisobutyric acid in tissue (μmoles/g), X_e = concentration of α-aminoisobutyric acid in extracellular water (μmoles/ml), V_e = volume of extracellular water (ml/g), and V_t = total tissue water (ml/g). A total tissue water (V_t) of 85 ml/100 g wet wt. was used in all such calculations except when either the external osmolarity or the ionic composition of the medium was altered. In all such cases V_t was determined in separate experiments. The volume of the extracellular water (V_e) was taken as 42 ml/100 g based on measurements made in this laboratory using [^{14}C]inulin and [^{14}C]dextran.

Determination of H_2O, Na^+ and K^+ contents of tissues

After weighing, tissues were dried in an oven at 105° for 48 h and reweighed to obtain the total tissue water. The samples were then dissolved in 0.2 ml conc. HNO_3 and 0.1 ml H_2O_2 at 200° and dried to a white powder. The residue was dissolved in 25 ml double distilled water and the ion content determined by flame photometry using an EEL flame photometer.

Solutions

The Krebs solution used had the following composition (mM): NaCl, 116; KCl, 4.6; $CaCl_2$, 1.5; $MgSO_4$, 1.2; $NaHCO_3$, 22; NaH_2PO_4, 1.2; D-glucose, 50. The medium was equilibrated with 95 % O_2–5 % CO_2 except when the effects of anoxia were studied; in such cases 95 % N_2 was substituted for 95 % O_2. All incubations were at 37° unless otherwise specified. In those experiments where Na^+, K^+, Ca^{2+} or Mg^{2+} were omitted from the solution, iso-osmolarity was maintained with sucrose. In Na^+-free solutions, the solution was buffered with Tris and the solution aerated with 100 % O_2. All solutions had a pH of 7.3–7.5.

Chemicals used

α-Amino[3-^{14}C]isobutyric acid with a specific activity of 2.72 mC/mmole was obtained from the New England Nuclear Corporation. Radiochemical purity was checked by thin-layer chromatography using *n*-butanol–acetic acid–water (25:4:10, by vol.) as a solvent system. In all experiments, 10μM α-amino[3-^{14}C]isobutyric acid was used. Where higher concentrations of the amino acid were required, the ^{14}C-labelled compound was diluted with unlabelled α-aminoisobutyric acid obtained from the British Drug House Ltd., Poole, England.

Statistical analysis

The variability of samples is expressed as mean ± standard error of the mean. The significance of differences between samples was determined using Student's *t* test. The difference was regarded as significant when $p < 0.05$. Kinetic analysis were carried out by plotting $1/v$ against $1/[S]$, the straight line being fitted by linear regression using an Olivetti desk-top computer.

RESULTS

Influence of tissue size on uptake of α-aminoisobutyric acid

SMITH AND SEGAL[15] have shown that careful attention should be paid to the influence of size on amino acid uptake by kidney fragments since they found significantly higher accumulation of both dibasic and monoaminomonocarboxylic amino acids by small kidney segments. In the present study changing the weight of the pieces of detrusor muscle from 10 to 100 mg did not alter the uptake of α-aminoisobutyric acid. In all subsequent studies only pieces of tissue weighing 30–90 mg were used.

Time course of α-aminoisobutyric acid uptake

The uptake of 10 μM α-aminoisobutyric acid increased approximately linearly for about 120 min, uptake approaching saturation at 4–8 h at a distribution ratio of more than 300 % (Fig. 1). The uptake of 10 mM α-aminoisobutyric acid was not only

considerably slower after the first 5 min than that at 10 μM but also only attained saturation at a distribution ratio of about 160 % after 6–8 h. At both 10 μM and 10 mM a distribution ratio of 60 % was attained after 5 min. This may mainly reflect rapid equilibration of α-aminoisobutyric acid with the extracellular space and if so, would indicate an extracellular space for α-aminoisobutyric acid of about 50 ml/100 g wet wt.

Effect of concentration on α-aminoisobutyric acid uptake

Tissues were incubated for 8 h with 10 μM–5 mM α-aminoisobutyric acid (Fig. 2). As the concentration was increased, the uptake of the amino acid increased progressively with no indication of a maximum being approached. The initial portion of the uptake curve was however clearly curvilinear. A consideration of the distribution ratios achieved is also informative: at 10 μM the ratio was 360 %; at 1 mM, 220 %; at 2 mM, 160 %; and, at 5 mM, 130 %. These findings suggested that, as for

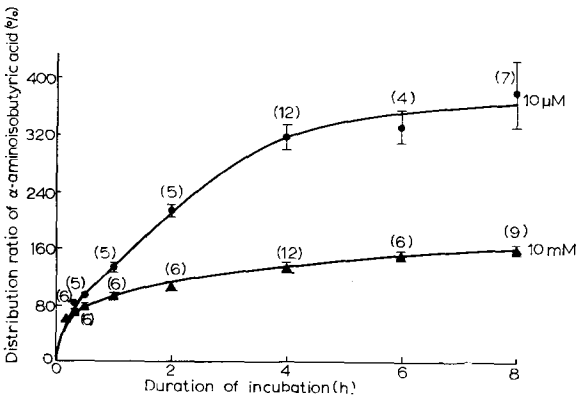

Fig. 1. Effect of time on α-aminoisobutyric acid uptake by rabbit detrusor muscle. Tissues were incubated at 37° in Krebs solution containing 10 μM (●) or 10 mM (▲) α-aminoisobutyric acid. Mean values ± S.E. Number of observations indicated in parentheses. The two curves differ significantly ($p < 0.05$) except after 10 min.

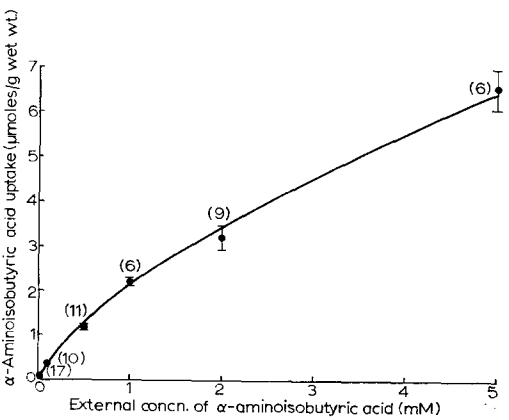

Fig. 2. Effect of concentration on α-aminoisobutyric acid uptake by rabbit detrusor muscle. Tissues were incubated for 8 h at 37° in Krebs solution containing 10 μM–5 mM α-aminoisobutyric acid. Mean values ± S.E. Number of observations indicated in parentheses.

amino acid transport in other preparations[16–20], the uptake of α-aminoisobutyric acid in detrusor muscle is a complex phenomenon that could involve at least two components: a transport mechanism that exhibits saturation kinetics and a linear process rather similar to passive diffusion. In order to investigate this possibility further tissues were incubated with 10 μM–200 mM α-aminoisobutyric acid for 8 h and uptake determined (Fig. 3). The osmotic strength of all solutions was kept equal by the addition of sucrose. It can be seen that uptake was again clearly curvilinear at the lowest concentrations increasing in a linear fashion at concentrations greater than 10 mM.

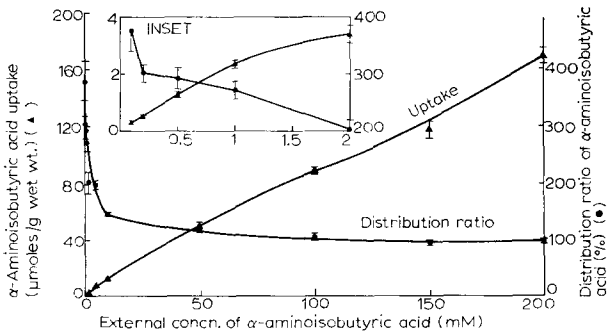

Fig. 3. Effect of concentration on α-aminoisobutyric acid uptake by rabbit detrusor muscle. Tissues were incubated for 8 h at 37° in Krebs solution containing 10 μM–200 mM α-aminoisobutyric acid, iso-osmolarity being maintained by the addition of sucrose. Mean values ± S.E. Number of observations was 5–9 at each point. Plotted as α-aminoisobutyric acid uptake (▲) or distribution ratio (●). Inset shows uptake and distribution ratio at 10 μM–2 mM α-aminoisobutyric acid.

Kinetics of α-aminoisobutyric acid uptake

The uptake of 1 mM or less α-aminoisobutyric acid was determined after 60 min incubation. The findings were then plotted as $1/v$ *versus* $1/[S]$ and a straight line relationship was obtained (Fig. 4) indicating a saturable rate limiting step in the process

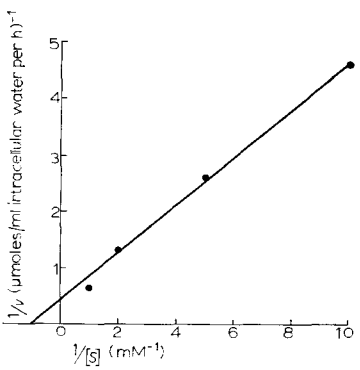

Fig. 4. Determination of K_m and V for α-aminoisobutyric acid transport by rabbit detrusor muscle. Tissues were incubated for 60 min at 37° in Krebs solution containing 0.1–1 mM α-aminoisobutyric acid. $1/v$ is the reciprocal of the net intracellular α-aminoisobutyric acid uptake. $1/[S]$ is the reciprocal of the external α-aminoisobutyric acid concentration. Each point is the mean of 8–12 observations.

of transport. From the plot of $1/v$ against $1/[S]$, the apparent K_m and V were determined and were, at the low concentrations of α-aminoisobutyric acid used, $K_m = 1.1$ mM and $V = 2.6$ μmoles/ml intracellular water per h. It must be emphasised however that these apparent kinetic parameters are based on data following a relatively prolonged period of incubation due to the relatively slow rate of entry of the substrate. During this 60-min period however the reaction velocity was found to be first order with respect to the substrate. The interpretation of values for K_m and V obtained in such studies has been discussed by MATTHEWS AND LASTER[21].

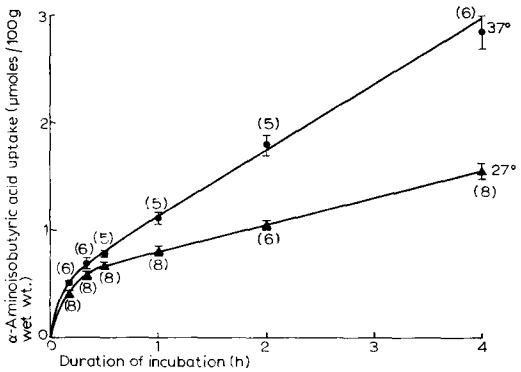

Fig. 5. Effect of temperature on α-aminoisobutyric acid uptake by rabbit detrusor muscle. Tissues were incubated in Krebs solution containing 10 μM α-aminoisobutyric acid at either 37° (●) or 27° (▲). Mean values ± S.E. Number of observations indicated in parentheses. The two curves differ significantly ($p < 0.05$) at all points.

Effect of temperature on α-aminoisobutyric acid uptake

The uptake of 10 μM α-aminoisobutyric acid uptake was significantly greater at 37° than at 27° after 10 min to 4 h incubation (Fig. 5), the calculated temperature coefficient (Q_{10}) being 2.1 when allowance was made for amino acid present extracellularly. In order to calculate the activation energy for transport the effects of temperature on uptake were examined in greater detail. The changes in the apparent V with temperature were determined by plotting $1/v$ *versus* $1/[S]$ after 1 h incubation at 12°, 22°, 30° and 37°. Increasing the temperature from 12° to 37° produced a progressive increase in apparent V from 0.4 to 2.6 μmoles/ml intracellular water per h. The value of the activation energy was obtained by plotting log V against $1/T$ and by measuring the slope of the straight line obtained. The activation energy for α-aminoisobutyric acid transport was found to be 13.1 kcal/mole; this value is similar to those reported by JACQUEZ *et al.*[22] for a number of amino acids transported by Ehrlich ascites cells.

Effect of ouabain on uptake of α-aminoisobutyric acid

Ouabain produced a concentration-dependent inhibition of transport of 10 μM α-aminoisobutyric acid accompanied by a gain in tissue Na^+ and a loss of tissue K^+ (Table I). 10^{-8} M ouabain had no significant effects but 10^{-5} M produced near maximal inhibition of α-aminoisobutyric acid uptake and very marked downhill ion movements.

Effect of metabolic inhibitors on uptake of α-aminoisobutyric acid

The uptake of 10 μM of the amino acid was not reduced by omission of D-glucose if the medium was oxygenated but was greatly reduced if omission of D-glucose was combined with anoxia (Table II). If the tissue was subjected to anaerobic conditions in the presence of D-glucose, uptake was slightly but significantly reduced. Transport was greatly reduced by 1 mM dinitrophenol in the presence of D-glucose but was only significantly reduced by 1 mM iodoacetic acid after 90 min exposure to the inhibitor (Fig. 6).

Effect of cations on uptake of α-aminoisobutyric acid

The uptake of 10 μM α-aminoisobutyric acid was greatly inhibited by the omission of Na^+ from the medium, no uphill transport occurring (Fig. 7). The omission of K^+ also caused a marked inhibition of uptake. The omission of Mg^{2+} produced a slight but significant inhibition of transport. Omission of Ca^{2+} produced swelling of the tissues but no significant change in amino acid transport.

TABLE I

EFFECT OF OUABAIN ON UPTAKE OF α-AMINOISOBUTYRIC ACID BY RABBIT DETRUSOR MUSCLE

Tissues were preincubated for 60 min at 37° in Krebs solution with or without added ouabain. Tissues were then incubated for 4 h at 37° in Krebs solution containing 10 μM α-amino[3-^{14}C]-isobutyric acid with or without added ouabain. The values shown are the mean ± S.E. The number of observations are shown in parentheses. Values marked with an asterisk differ significantly ($p < 0.05$) from values obtained in the absence of ouabain.

Ouabain (M)	*Tissue H_2O content (g/100 g)*	*Tissue Na^+ content (mmoles/kg wet wt.)*	*Tissue K^+ content (mmoles/kg wet wt.)*	*α-Aminoisobutyric acid uptake (μmoles/kg wet wt.)*
—	86.8 ± 0.6 (6)	91.8 ± 1.8 (6)	46.0 ± 0.7 (6)	37.1 ± 3.0 (8)
10^{-8}	87.1 ± 0.8 (6)	95.0 ± 2.6 (6)	42.7 ± 2.1 (6)	31.2 ± 2.6 (10)
10^{-7}	85.1 ± 0.7 (11)	97.8 ± 2.1* (11)	35.0 ± 1.6* (11)	24.4 ± 1.4* (10)
10^{-6}	86.0 ± 0.2 (6)	106.5 ± 2.4* (6)	24.7 ± 2.6* (6)	21.1 ± 0.5* (10)
10^{-5}	87.3 ± 0.2 (6)	121.4 ± 3.4* (6)	8.9 ± 0.7* (6)	13.2 ± 0.3* (10)

TABLE II

EFFECTS OF SUBSTRATE AND ANOXIA ON UPTAKE OF α-AMINOISOBUTYRIC ACID BY RABBIT DETRUSOR MUSCLE

Tissues were preincubated for 60 min at 37° in Krebs solution and were then incubated for 8 h in Krebs solution containing α-amino[3-^{14}C]isobutyric acid. During both preincubation and incubation tissues were subjected to the treatments shown. Mean values ± S.E. of 9–12 observations. Values marked with an asterisk differ significantly ($p < 0.05$) from values obtained in the presence of D-glucose and 95 % O_2.

Krebs solution		*α-Aminoisobutyric acid distribution ratio (%)*
Containing substrate (50 mM)	*Equilibrated with*	
D-Glucose	95 % O_2–5 % CO_2	428 ± 22
Sucrose	95 % O_2–5 % CO_2	436 ± 27
D-Glucose	95 % N_2–5 % CO_2	316 ± 19*
Sucrose	95 % N_2–5 % CO_2	109 ± 2*

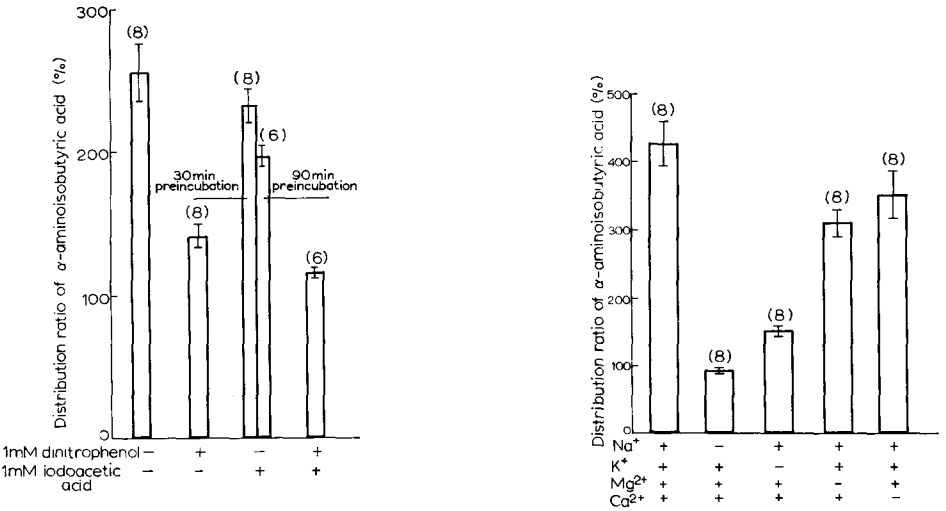

Fig. 6. Effect of metabolic inhibitors on α-aminoisobutyric acid uptake by rabbit detrusor muscle. Tissues were preincubated at 37° in Krebs solution containing 1 mM dinitrophenol (for 30 min) or 1 mM iodoacetic acid (for 30 or 90 min) or both 1 mM dinitrophenol and iodoacetic acid (for 30 min). They were then incubated for 2 h at 37° in Krebs solution containing 10 μM α-amino-[3-^{14}C]isobutyric acid and 1 mM dinitrophenol, if this inhibitor had been present during pre-incubation. Mean values ± S.E. Number of observations indicated in parentheses. All values, except that after 30 min preincubation with 1 mM iodoacetic acid, differ significantly ($p < 0.05$) from the control value obtained in Krebs solution alone.

Fig. 7. Effect of cations on α-aminoisobutyric acid uptake by rabbit detrusor muscle. Tissues were preincubated for 60 min at 37° in Krebs solution or Na^+-free, or K^+-free or Ca^{2+}-free, or Mg^{2+}-free Krebs solution. 10 μM α-amino[3-^{14}C]isobutyric acid was then added and incubation continued for 4 h. Mean values ± S.E. Number of observations indicated in parentheses. All values, except that in Ca^{2+}-free solution, differ significantly ($p < 0.05$) from that obtained in normal Krebs solution.

DISCUSSION

The movement of α-aminoisobutyric acid, at low concentrations, into rabbit detrusor muscle possessed the characteristics of active transport since uptake achieved a distribution ratio of greater than 100%, was energy dependent, was inhibited by ouabain and was temperature sensitive. The process thus has many features in common with the transport *in vitro* of amino acids by other tissues[1–4,6,7,9,16–18]. In addition however it was found that as the concentration of α-aminoisobutyric acid was further increased, uptake became linear with no evidence of saturation up to 200 mM. A similar phenomenon has been observed in other tissues[16–20]. The characteristics of transport at high concentrations of the amino acid have not been further characterised. CHRISTENSEN AND LIANG[20] have shown that the so-called non-saturable uptake of amino acids by Ehrlich ascites tumour cells does not occur by simple diffusion since it shows chemical specificity, has a high Q_{10} and is markedly pH sensitive.

A prolonged period of incubation was required for transport at a given concentration of amino acid to reach equilibrium as has been found in certain other tissues[3,9]. The kinetics of transport of α-aminoisobutyric acid at low concentrations was apparently amenable to analysis by the Michaelis–Menten equation. The K_m

for the process was similar to that found in rat uterus[9] but was lower than that observed in certain other tissues[23]. MATTHEWS AND LASTER[21] have discussed the interpretation of these kinetic relationships and have concluded that they do not demonstrate the nature of the transport mechanism and are only an indication of a saturable rate-limiting step in the process of transport.

Evidence was obtained that transport of α-aminoisobutyric acid in rabbit detrusor muscle may be dependent upon the functioning of the Na^+ pump since uptake of the amino acid was impaired by ouabain in concentrations which caused downhill ion movements. As in many other tissues[2–4,6,7,9,16–18] amino acid uptake was Na^+ and K^+ dependent. How Na^+ and K^+ are implicated in transport in this tissue has not however been established.

Omission of Ca^{2+} caused tissue swelling probably due to an increase in cellular permeability but did not alter transport of α-aminoisobutyric acid. In other tissues Ca^{2+} has been found to variously increase[3,24], decrease[25] or have no effect[26–28] on amino acid transport.

The energy for amino acid transport can apparently be derived from endogenous substrates since the omission of D-glucose did not alter transport provided that the medium was oxygenated. Na^+ pumping in this tissue is similarly affected[29]. Amino acid transport was inhibited by dinitrophenol but not by iodoacetic acid. By contrast contractility[30] and Na^+ pumping[29] in this tissue were abolished by iodoacetic acid and depressed, but not abolished, by dinitrophenol. These differences in susceptibility to metabolic inhibition are under investigation.

This study has demonstrated that isolated detrusor muscle strips can be used to study amino acid transport into smooth muscle. The preparation has the advantage of allowing easy removal of the overlying mucosa. The relationship of Na^+ and K^+ to transport and the structural requirements for transport are currently under investigation.

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