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AMINO ACID TRANSPORT IN THE CORNEA

I. 3-AMINOISOBUTYRIC ACID UPTAKE IN THE TOAD

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SUMMARY

1. We have examined the toad cornea, an avascular layer of connective tissue bounded on one surface (epithelial or tear) by an epithelium several cell layers thick and on the other surface (endothelial or aqueous) by a single layer of cells, to determine whether this tissue actively accumulates amino acids.

2. 3-Aminoisobutyric acid is accumulated by the cornea of *Bufo marinus in vitro*. The uptake of 3-aminoisobutyric acid is saturable and is inhibited by iodoacetate but not by anaerobiosis nor by cyanide.

3. Leucine, alanine, glycine, and serine, but not arginine, compete with 3-aminoisobutyric acid for uptake. Leucine and glycine reduce the uptake of 3-aminobutyric acid by approximately 30 and 40%, respectively, and the inhibitory effects of these two amino acids are additive. Serine, in the presence of leucine and glycine, further reduces 3-aminoisobutyric acid uptake by 10–15%. The maximal uptake of 3-aminoisobutyric acid and alanine, but not that of leucine, is dependent upon sodium.

4. Arsenite and lactate stimulate the uptake of 3-aminoisobutyric acid and, to an even greater extent, leucine. Alanine uptake is inhibited by both of these agents.

5. We conclude that 3-aminoisobutyric acid is accumulated by at least two, and perhaps three, neutral amino acid transport systems having markedly different properties. Therefore, the interpretation of data obtained in the cornea with this amino acid must take this limitation into account.

INTRODUCTION

The cells of the corneal epithelium normally have a life span of approximately 1 week[1]. Any loss of corneal epithelium is followed by rapid cell division and replacement of the lost tissue[2]. Damage to the stroma is also readily repaired. These biosynthetic properties require an effective protein synthetic apparatus and a ready supply of the requisite amino acids. The source of these amino acids is not known, but both the tears[3] and the aqueous humor[4] contain significant concentrations of

Abbreviations: TES, *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; MES, 2-(*N*-morpholino)ethanesulfonic acid; Bicine, *N,N'*bis(2-hydroxyethyl)glycine.

amino acids (*i.e.* concentrations approximating or surpassing those in plasma). Reddy[5] has shown that the concentration of every free amino acid but arginine, lysine, ornithine, methionine, and hydroxyproline is higher in the corneal epithelium than in the stroma or aqueous humor. Reddy's data show that the cornea is capable of maintaining concentration gradients of amino acids with respect to the aqueous humor, and indicate the tissue may be capable of transporting amino acids against these gradients.

The studies reported herein were designed to identify and partially characterize the mechanisms by which the cornea accumulates amino acids. Our studies show that the cornea, an avascular tissue, has two or three transport mechanisms to accumulate neutral amino acids from the fluids (tears or aqueous humor) normally bathing the two surfaces of the tissue. One of these transport systems is markedly stimulated by lactate.

MATERIALS AND METHODS

The entire cornea and a ring of sclera was excised from the globe of *Bufo marinus* (Tarpon Zoo, Tarpon Springs, Fla.) and incubated at room temperature (about 25 °C) in 5 ml of a modified Conway Ringer's solution consisting of 83.5 mM NaCl, 17.7 mM $NaHCO_3$, 4 mM KCl, 0.8 mM $MgSO_4$, 0.8 mM KH_2PO_4, 11 mM glucose, and 1.5 mM $CaCl_2$. The medium was bubbled with a mixture of O_2–CO_2 (99:1, v/v). In the studies of the pH dependence of 3-aminoisobutyric acid accumulation, bicarbonate was replaced with either 17.7 mM *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES), *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), 2-(*N*-morpholino)ethanesulfonic acid (MES), or *N,N'*-bis(2-hydroxyethyl)glycine (Bicine), depending upon the pH desired. For amino acid accumulation experiments, the incubation media also contained 1.0 μCi of the requisite amino acid (^{14}C-labeled 3-aminoisobutyric acid, leucine or alanine), 5.0 μCi of [^{3}H]inulin (4.21 Ci/g) or [^{3}H]mannitol (2.65 Ci/mole), and unlabeled amino acids in concentrations ranging from 10 μM to 10 mM. After a period of incubation, the tissue was removed from the medium and a piece of cornea 6 mm in diameter was excised from the center of the tissue with a Paton trephine. The excised corneal fragment was briefly rinsed in Ringer's and dissolved in 2 ml of Soluene, a quaternary amine (Packard Instrument Co.). 15 ml of a toluene-base scintillant were added and the samples counted in a Packard Model 3320 Tri-Carb liquid scintillation counter. In each sample, quenching of ^{3}H and ^{14}C was corrected by means of quenched standards (Nuclear-Chicago) using appropriate channels-ratio quench correction curves derived from two channels of the scintillation counter. In every case, one cornea from each toad was treated as an experimental tissue, while the contralateral cornea of the same animal was used as its control. The extracellular space was calculated for each tissue by means of the [^{3}H]inulin or [^{3}H]mannitol content and this value was used to correct for extracellular ^{14}C-labeled amino acid in each tissue. Sodium and chloride were measured potentiometrically using a Radiometer pHM 52 electrometer and sodium (Orion, Inc., Cambridge) and chloride (Radiometer, Copenhagen) electrodes.

RESULTS

3-Aminoisobutyric acid, a non-metabolizable neutral amino acid, is pro-

gressively accumulated by the toad cornea *in vitro*. We measured the apparent extracellular space in each cornea to determine that this accumulation of 3-aminoisobutyric acid represented uptake by the cells in the tissue rather than the diffusion of amino acid into the acellular stroma. Using this value to correct for extracellular ^{14}C-labeled 3-aminoisobutyric acid in each tissue, we found that the uptake of this amino acid by the toad cornea is linear for a period of at least 4 h (Fig. 1).

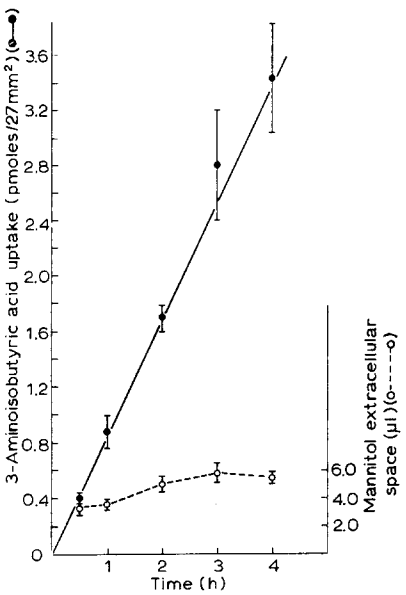

Fig. 1. Time course of 3-aminoisobutyric acid uptake by the toad cornea. Tissues were incubated in modified Conway Ringer's buffered at pH 8.0 with bicarbonate (17.7 mM), containing 1 μCi of ^{14}C-labeled 3-aminoisobutyric acid (20 Ci/mole) and 5 μCi of [^{3}H]mannitol. The amount of radioactivity in the bath and corneas were used to calculate the extracellular space. The results are expressed as the mean $\pm$ S.E.

To determine the optimum pH of 3-aminoisobutyric acid uptake, we replaced the $NaHCO_3$ in the modified Conway Ringer's by an equal concentration of TES, Bicine, MES, or HEPES, titrated the solution to the desired pH, and bubbled the medium with 100% O_2. Replacement of bicarbonate by these sulfonate buffers did not affect amino acid accumulation by the cornea. The uptake of 3-aminoisobutyric acid at pH 8.0 was 0.05 ± 0.01 (mean $\pm$ S.E.) pmoles/mm^2/h in medium buffered with bicarbonate and 0.05 ± 0.01 (mean $\pm$ S.E.) pmoles/mm^2/h in Ringer's solution buffered at pH 8.0 with TES. The optimum pH for accumulation of this amino acid by the toad cornea is approximately 8.0 (Fig. 2).

We next studied the relationship of 3-aminoisobutyric acid uptake to the concentration of amino acid in the bathing media. As shown in Fig. 3, the accumulation of 3-aminoisobutyric acid by toad cornea exhibits a concentration-dependent uptake of amino acid indicating the presence of carrier-mediated transport. Saturation of 3-aminoisobutyric acid uptake in the cornea was achieved at a concentration of approximately 5 mM.

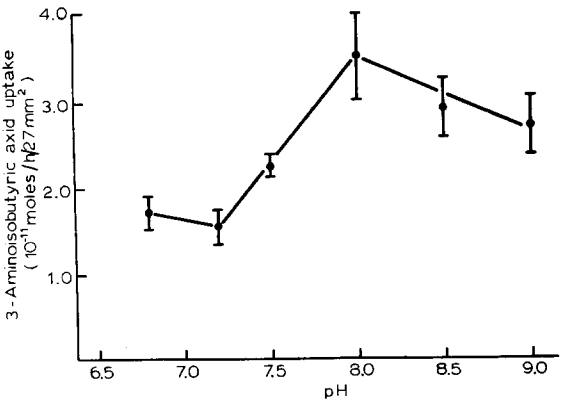

Fig. 2. pH dependence of 3-aminoisobutyric acid uptake. The 'good' buffers were substituted for bicarbonate in modified Conway Ringer's to obtain a trajectory of pH values: 17.7 mM MES (pH 6.8–7.0), TES (pH 7.0–8.0), HEPES (pH 7.0–8.0) or Bicine (pH 8.0–9.0). The incubation media contained 1 μCi of ^{14}C-labeled 3-aminoisobutyric acid (20 Ci/mole) and 5.0 μCi of [^{3}H]-inulin and was bubbled with 100% O_2. Incubations were carried for 1 h. The results are expressed as the mean $\pm$ S.E.

To further characterize the substrate specificity of the transport system(s) responsible for the accumulation of 3-aminoisobutyric acid by the cornea, we measured the effects of a series of neutral amino acids on the uptake of 3-aminoisobutyric acid. Table I shows that leucine, glycine and alanine each significantly inhibited the uptake of 3-aminoisobutyric acid. Moreover, the degree of inhibition of 3-aminoisobutyric acid uptake was related to the concentration of the competing natural amino acid. When 10 mM leucine *plus* 10 mM alanine were added to the incubation media 3-aminoisobutyric acid uptake was decreased by 79.3%. The effects of these two amino acids are apparently additive because the uptake of 3-aminoisobutyric

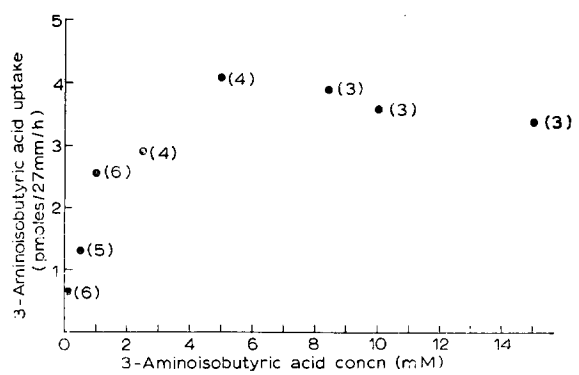

Fig. 3. 3-Aminoisobutyric acid uptake with increasing 3-aminoisobutyric acid concentration in the toad cornea. Corneas were incubated in modified Conway Ringer's, pH 8.0 with 1 μCi ^{14}C-labeled 3-aminoisobutyric acid (20 Ci/mole), 5 μCi [^{3}H]mannitol and cold 3-aminoisobutyric acid; the 3-aminoisobutyric acid concentration ranged from 0.1 mM to 15.0 mM. After 1 h of incubation the corneas were rinsed with Ringers solution and a 6-mm button was excised from the center of the cornea with a Paton trephine. The weight of the excised tissue was 10.6 $\pm$ 0.4 mg (ref. 6) and the mannitol extracellular space was 3.701 $\pm$ 0.189 μl (ref. 12).

TABLE I

COMPETITION OF NATURAL AMINO ACIDS FOR THE UPTAKE OF 3-AMINOISOBUTYRIC ACID BY THE CORNEA

Corneas were incubated for 1 h at room temperature in modified Conway Ringer's, pH 8.0, containing 1 μCi of ^{14}C-labeled 3-aminoisobutyric acid (20 Ci/mole), 5 μCi [^{3}H]inulin, 0.5 mM 3-aminoisobutyric acid and unlabelled natural amino acids. In the studies in the upper portion of the table, the concentration of a single amino acid was varied from 0.1 to 10 mM. In the lower portion, the effects of two or three amino acids, each at a concentration of 10 mM, were studied. Paired control corneas were treated in the same manner except that the medium contained no competing natural amino acids. 3-Aminoisobutyric acid uptake was 3.119±0.119 in 104 control experiments. Inhibition of ^{14}C-labeled 3-aminoisobutyric acid uptake is expressed as the mean± S.E. relative to the paired control corneas.

Concentration of competing amino acids (mM)	*% inhibition of 3-aminoisobutyric acid uptake by natural amino acids*		
	Alanine	*Glycine*	*Leucine*
0.1	—1.4±8.0 (7)	3.0±10.6 (8)	10.0±16.8 (3)
0.5	17.6±5.4 (6)	3.6±10.6 (3)	21.2±10.6 (9)
1.0	23.1±6.8 (3)		21.3±11.8 (6)
2.5	54.3±1.7 (3)	40.1± 6.0 (3)	26.8± 8.0 (5)
5.0	40.0±6.0 (4)	41.0± 7.8 (8)	29.0±10.5 (4)
10.0	57.1±5.6 (9)	36.1±14.1 (3)	18.3± 7.9 (5)
	Leucine+glycine	*Leucine+glycine+ serine*	*Leucine+alanine*
10.0	63.8±2.5 (11)	76.7± 1.2 (11)	79.3± 7.8 (5)

acid was reduced only 18.3% by 10 mM leucine and 57.1% by 10 mM alanine. The effects of 10 mM leucine and 10 mM glycine upon 3-aminoisobutyric acid were also additive, inhibiting uptake by 63.8%. The further addition of 10 mM serine to the latter incubation media reduced 3-aminoisobutyric acid uptake to 76.7% of the control value. Thus, 3-aminoisobutyric acid uptake in the presence of 10 mM leucine *plus* 10 mM glycine (1.358±0.063 pmole/mm^2 per h) was reduced by approximately 35% when 10 mM serine was added to the incubation medium (0.803±0.051 pmole/mm^2 per h). In six pairs of corneas 20 mM leucine *plus* 10 mM glycine inhibited 3-aminoisobutyric acid uptake 59.5±6.0% (1.659±0.094 pmole/mm^2 per h). 10 mM leucine *plus* 20 mM glycine inhibited uptake by 53.6±4.5% (1.652±0.213 pmole/mm^2 per h). Therefore, the effects of serine upon this portion of 3-aminoisobutyric acid uptake seem to be rather specific.

We next studied the effects of sodium upon the uptake of 3-aminoisobutyric acid by the cornea. As shown in Table II, replacement of sodium by choline significantly reduced the accumulation of 3-aminoisobutyric acid. The degree of inhibition depended upon the length of time the cornea was pre-incubated in choline Ringer's solution before the addition of radioactively labeled 3-aminoisobutyric acid, but never amounted to more than 50%. When the effects of sodium replacement upon alanine and leucine were studied, alanine uptake was inhibited by 80% whilst the accumulation of leucine was not affected. The relative effects of the longer pre-incubation time upon alanine uptake were similar to those observed with 3-amino-

TABLE II

SODIUM DEPENDENCE OF NEUTRAL AMINO ACID UPTAKE

One cornea from each toad was first incubated for the indicated period in a Conway Ringer's solution in which the sodium was replaced by choline (100 mM). The actual sodium concentration in the choline Ringer's solution was 10 μM. After the initial incubation period, [^{3}H]inulin and either L-[^{14}C]leucine (45 Ci/mole) (0.1 mM), L-[^{14}C]alanine (130 Ci/mole) (0.1 mM), or ^{14}C-labeled 3-aminoisobutyric acid (20 Ci/mole) ($5 \cdot 10^{-6}$ M) was added to the media. The corneas were incubated for an additional 60 min and the amount of accumulated amino acid determined. The paired control corneas were handled identically except that the Ringer's solution contained sodium (100 mM). The results are expressed as the mean ± S.E.

Amino acid	*Pre-incubation time (h)*	*Uptake (pmoles/27 mm²/h)*		*% change in amino acid uptake*
		Conway Ringer's	*Choline Ringer's*	
3-Aminoisobutyric acid	0.5	0.027 ± 0.003	0.019 ± 0.003	—27.6 ± 14.6 (4)*
3-Aminoisobutyric acid	2.5	0.034 ± 0.008	0.014 ± 0.004	—55.3 ± 15.2 (6)*
Alanine	0.5	0.656 ± 0.078	0.340 ± 0.019	—43.8 ± 4.7 (5)**
Alanine	2.5	0.697 ± 0.058	0.115 ± 0.026	—80.0 ± 6.0 (9)**
Leucine	0.5	0.427 ± 0.085	0.365 ± 0.054	+ 2.5 ± 19.6 (4)

* $P < 0.05$.
** $P < 0.005$.

isobutyric acid. We also found that the small residual uptake of 3-aminoisobutyric acid in the presence of saturating concentrations of both leucine and glycine (0.033 ± 0.007 pmole/mm^2/h) (Table II) was not reduced further when sodium was replaced by choline (0.044 ± 0.003 pmole/mm^2/h).

TABLE III

EFFECT OF METABOLIC INHIBITORS UPON THE ACCUMULATION OF 3-AMINOISOBUTYRIC ACID

Paired corneas were incubated in modified Conway Ringer's, pH 8.0; the incubation medium of the experimental cornea of each pair also contained 2 mM of the indicated inhibitor or was rendered anoxic. After 30 min, 5 μCi of [^{3}H]inulin and 1.0 μCi of ^{14}C-labeled 3-aminoisobutyric acid, L-[^{14}C]leucine, or L-[^{14}C]alanine were added to the media. The corneas were incubated for an additional 60 min in this mixture, after which the amount of amino acid accumulated was determined (*cf.* legend to Table I). The amino acid uptake of each cornea exposed to inhibitor was compared to its paired control and the values are expressed as the mean ± S.E.

Amino acid	*Inhibitor*	*% change in amino acid uptake*
3-Aminoisobutyric acid	iodoacetate	—48.9 ± 8.5 (4)*
Alanine	iodoacetate	—41.2 ± 3.3 (5)*
Leucine	iodoacetate	+ 8.6 ± 13.5 (4)
3-Aminoisobutyric acid	anaerobiosis	+ 4.0 ± 2.5 (6)
3-Aminoisobutyric acid	cyanide	— 3.1 ± 18.5 (5)
Alanine	cyanide	+ 9.0 ± 13.7 (5)
Leucine	cyanide	+11.9 ± 6.5 (5)

* Results that differ from zero at the level of $P < 0.01$.

The effects of anaerobiosis and certain metabolic inhibitors upon the accumulation of 3-aminoisobutyric acid were also examined. Neither anaerobiosis nor cyanide (2 mM) had an apparent effect upon the rate of 3-aminoisobutyric acid uptake (Table III). To rule out the possibility that cyanide had paradoxical effects on two systems transporting 3-aminoisobutyric acid with only an apparent lack of effect on 3-aminoisobutyric acid uptake, we also measured the uptake of radioactive alanine and leucine in the presence of cyanide. The uptake of both these amino acids was unaffected. Iodoacetate (2 mM) markedly inhibited the uptake of both 3-aminoisobutyric acid and alanine but had no significant effect upon leucine uptake by the cornea. When the cornea was pre-incubated in sodium arsenite (2 mM) for 30 min, the uptake of 3-aminoisobutyric acid was increased 45% over the paired

TABLE IV

THE EFFECT OF ARSENITE UPON AMINO ACID UPTAKE

Corneas were pre-incubated in modified Conway Ringer's (pH 8.0) containing 2 mM arsenite. The tissues were transferred to media containing 2 mM arsenite. [^{3}H]inulin, and 1 μCi of ^{14}C-labeled alanine (0.1 mM), leucine (0.1 mM), or 3-aminoisobutyric acid ($5 \cdot 10^{-6}$ M). The amino acid uptake was determined after 1 h incubation in this mixture. Arsenite was omitted from the media bathing paired control corneas. The results are expressed as the mean $\pm$ S.E. of the arsenite-treated corneas relative to the paired controls.

Amino acid	*Pre-incubation time (h)*	*Uptake (pmoles/27 mm²/h)*		*% change in uptake*
		Control	*+Arsenite*	
3-Aminoisobutyric acid	0.5	0.036 ± 0.003	0.046 ± 0.007	$+44.9 \pm 14.2$ (5)*
3-Aminoisobutyric acid	4.5	0.047 ± 0.005	0.069 ± 0.012	$+16.1 \pm 2.1$ (5)*
Leucine	0.5	0.264 ± 0.018	0.404 ± 0.032	$+56.0 \pm 17.1$ (5)*
Alanine	0.5	0.714 ± 0.158	0.395 ± 0.038	-36.3 ± 15.7 (4)*

* $P < 0.05$.

TABLE V

EFFECTS OF LACTATE UPON UPTAKE OF NEUTRAL AMINO ACIDS

The tissues were incubated in modified Conway Ringer's buffered with bicarbonate (17.7 mM) and containing 1 μCi (0.1 mM) of either L-[^{14}C]alanine (130 Ci/mole), L-[^{14}C]leucine (45 Ci/mole), or ^{14}C-labeled 3-aminoisobutyric acid (20 Ci/mole) and 5 μCi of [^{3}H]inulin. 5 mM ($\pm$)-lactate was added to the experimental corneas while their untreated contralateral cornea served as controls. The pH maintained at 8.0, and incubations were carried out for 1 h. The results are expressed as the mean $\pm$ S.E., relative to the paired control corneas, and are all significant by Student's 't' test at the level of $P < 0.01$.

Amino acid	*Uptake (pmoles/27 mm²/h)*		*% change in amino acid uptake*
	Control	*+Lactate*	
3-Aminoisobutyric acid	0.364 ± 0.053	0.414 ± 0.075	$+10.7 \pm 0.1$ (5)
Leucine	0.236 ± 0.054	0.370 ± 0.043	$+81.2 \pm 30.3$ (5)
Alanine	0.527 ± 0.069	0.340 ± 0.036	-34.6 ± 4.8 (4)

controls (Table IV). After longer preincubation times (4.5 h) the uptake of 3-aminoisobutyric acid in the arsenite-treated cornea was maintained at a level significantly greater (16%) than in the paired controls. Uptake studies with radioactively-labeled leucine and alanine showed that arsenite greatly stimulated (56%) the accumulation of leucine but moderately inhibited (36%) the uptake of alanine.

Because exposure of the amphibian cornea to arsenite results in a rise in the lactate concentration in the bathing media[6], we studied the effects of exogenous sodium lactate upon amino acid uptake by the cornea. As shown in Table V, (±)-lactate (5 mM) caused a significant increase in the uptake of 3-aminoisobutyric acid. When the effects of lactate upon leucine and alanine uptake were measured, lactate caused a striking increase in leucine accumulation, and moderately inhibited alanine uptake.

DISCUSSION

The *in vitro* toad cornea exhibits saturable carried-mediated accumulation of 3-aminoisobutyric acid. This amino acid is generally considered to be a specific substrate for the transport system responsible for the sodium-dependent uptake of glycine and alanine (the "A" system)[7], and the accumulation of radioactive 3-aminoisobutyric acid by tissues is often interpreted as a measure of this single transport process[8]. However, in the toad cornea, 3-aminoisobutyric acid uptake is apparently mediated by at least two separate systems. The uptake of this amino acid is reduced only about 60% by the addition of high concentrations of alanine. Glycine, also a preferred substrate for "A" transport systems[9], inhibits 3-aminoisobutyric acid uptake about 40%. Leucine, as well as isoleucine and valine, are usually transported by the "L" system, which does not accumulate 3-aminoisobutyric acid[7]. The presence of leucine in the medium, which has no effect upon alanine uptake in the toad cornea, reduces the uptake of 3-aminoisobutyric acid by about 30%. This result indicates that a large portion of the 3-aminoisobutyric acid is accumulated by a mechanism normally transporting leucine, presumably the "L" system. The effects of alanine, glycine and leucine upon 3-aminoisobutyric acid uptake are related to their concentrations, with a maximum inhibition of 3-aminoisobutyric acid occurring at a five-fold concentration of each of the natural amino acids. The inhibition of 3-aminoisobutyric acid uptake by alanine exhibits a slight decrease at a concentration of 5 mM alanine. Since the inhibition achieved with 2.5 and 10 mM alanine is identical, we feel that maximal inhibition of 3-aminoisobutyric acid occurs at a concentration of 2.5 mM alanine.

The inhibitory effects of unlabeled leucine upon 3-aminoisobutyric acid uptake are additive with those of glycine or alanine. The presence of both leucine and glycine does not, however, completely block 3-aminoisobutyric acid accumulation by the cornea. 3-Aminoisobutyric acid uptake in the presence of 20-fold concentrations of both leucine and glycine amounts to 36% of the control value. This suggested the participation of yet another transport system in the accumulation of 3-aminoisobutyric acid by the toad cornea. We found that the further addition of serine (10 mM) to the medium containing saturating concentrations (10 mM) of leucine and glycine reduces this small residual 3-aminoisobutyric acid uptake by approximately 35%. The addition of 20 mM glycine *plus* 10 mM leucine or 10 mM glycine *plus* 20 mM leucine reduced 3-aminoisobutyric acid uptake to the same degree

as did 10 mM leucine *plus* 10 mM glycine. These data suggest the participation of a third transport system, which is inhibited by serine, in the accumulation of 3-aminoisobutyric acid.

These results show that 3-aminoisobutyric acid uptake does not represent the activity of one homogeneous transport system in the cornea and therefore data obtained with this amino acid must be interpreted with this limitation in mind.

The accumulation of amino acids by the "A" system in most tissues is coupled to the movement of sodium into the tissue and is inhibited by lack of sodium in the medium[8,10]. A significant portion of the 3-aminoisobutyric acid uptake by the toad cornea is maintained when sodium is replaced with choline. The degree of inhibition is related to the period of time the cornea is incubated in choline Ringer's, but never exceeds approximately 55%. This degree of inhibition is approximately the same as that observed when alanine is used as a competitive inhibitor of 3-aminoisobutyric acid uptake. We measured the effects of sodium replacement upon the uptake of radioactive alanine and found that the inhibition of this uptake is also related to the time of incubation of the cornea in choline Ringer's. Because the relative inhibition of alanine is approximately 50–75% greater than that of 3-aminoisobutyric acid, and because replacement of sodium has no effect upon the accumulation of radioactive leucine, we interpreted these data as further evidence that 3-aminoisobutyric acid is transported by systems other than the sodium-dependent "A" system. The time-dependent nature of the inhibition, and the fact that alanine uptake is not completely inhibited, may be due to the fact that these processes coupled to sodium are located in a portion of the cornea which retains significant amounts of sodium. For example, these transport processes may be located at the basal margin of the epithelium, in which case the elution of extracellular sodium from the underlying stroma would be time dependent.

The effects of various metabolic inhibitors upon 3-aminoisobutyric acid uptake indicate that glycolysis supplies a significant portion of the required metabolic energy. An unexpected and remarkable finding was the substantial stimulation of 3-aminoisobutyric acid uptake by arsenite. Amino acid uptake initially increased by approximately 50%, was maintained at a significantly higher rate compared to controls even after a pre-incubation period of 4.5 h in arsenite. This sustained increase in amino acid uptake following arsenite is similar to the effects of this agent upon chloride transport[6]. We also found that arsenite markedly stimulates the uptake of radioactive leucine (56%), while the uptake of radioactive alanine is reduced (36%) by this agent. Apparently the large increment in 3-aminoisobutyric acid uptake is an expression of the stimulation of the system normally transporting leucine and a lesser inhibition of the system(s) normally transporting alanine.

Arsenite causes an accumulation of lactate in the media bathing the cornea[6], presumably by inhibiting tissue pyruvate dehydrogenase[11]. The addition of (±)-lactate to the bathing media of the untreated cornea gives results very similar to those found in the arsenite-treated cornea: leucine uptake is almost doubled, while alanine accumulation is significantly reduced. These data indicate that the effects of arsenite upon amino acid uptake by the cornea may be mediated by a rise in tissue lactate levels. They also suggest that the high concentrations of lactic acid normally present in the aqueous humor[12] may have significant effects upon the transport properties of the *in vivo* cornea.

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