Amino Acid Transport in Lymph Node Cells*

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(Received for publication, February 19, 1962)

Recent studies of antibody synthesis and secretion by lymph node cells (1, 2) and of hormonal regulation of protein metabolism in striated muscle (3, 4) directed our attention to the functional significance of amino acid transport in protein synthesis (5). As an initial step in defining this relationship, the mechanism of amino acid transport in lymph node cells has been examined.

Amino acid entry in these cells is mediated by two mechanisms: (a) a process characterized by saturation kinetics, high temperature coefficient, and functional irreversibility and (b) a first order process with the characteristics of passive diffusion. Amino acid exit, at physiological concentrations, is primarily by diffusion, although carrier-mediated efflux is demonstrable at very low intracellular amino acid levels. The intracellular-extracellular concentration gradient represents a steady state (e.g. equal rates of entry and exit), which can be altered by independent or concomitant changes in rates of either entry or exit. A kinetic analysis of amino acid transport, formulated on the basis of a carrier model, is presented that indicates that the lymph node cell membrane is asymmetric on its outer and inner surface with respect to the kinetic properties of the amino acid carrier. These data are discussed in the light of current concepts of amino acid transport.

EXPERIMENTAL PROCEDURE

General Lymph node cells obtained from sensitized guinea pigs (Hartley strain, Tumblebrook Farm, Brant Lake, New York) were used routinely in the present study. The sensitization procedure, consisting of injecting Freund's adjuvant with and without additional defined antigenic materials into the guinea pig footpads, and the procedure for isolating cells from regional lymph nodes have been described previously (7).

The nonmetabolizable amino acid analogue, o-aminoisobutyric acid, introduced by Christensen and Riggs (8) for the study of amino acid transport, was selected for the present work for the following reasons. (a) It is concentrated by a variety of mammalian cells (9); (b) it shares a common transport system with several naturally occurring amino acids, such as glycine, alanine, serine, methionine, and valine (10, 11); (c) it is not metabolized (9) and therefore permits measurements of the transport process independent of subsequent utilization; and (d) net influx and net efflux can be measured directly with the isotopically labeled compound, whereas similar measurements with labeled natural amino acids represent unidirectional fluxes consisting of two components, net influx and exchange diffusion.

Incubation Procedure Lymph node cells were suspended at a final concentration of approximately 1 × 10⁶ cells per ml in Krebs-Ringer solution buffered with Tris-HCl at pH 7.4. This cell concentration corresponds to an intracellular to extracellular fluid volume ratio of 1:60. Incubations were carried out with 3 to 4 ml of cell suspension in silicone-coated vessels in a Warburg apparatus and 100% O₂ as the gas phase unless otherwise specified. After an initial 10 minutes of incubation to allow for equilibration of temperature and the gas phase, the experiment was initiated by introducing the labeled amino acid from a side arm. The cell suspension was shaken gently throughout the equilibration period, but only intermittently during the experimental period in order to minimize cell damage. For experiments requiring preloading with either labeled or unlabeled amino acid, cells were incubated for 60 to 90 minutes with the appropriate amino acid, separated by centrifugation at 140 × g for 10 minutes at 4°C, washed twice with ice-cold buffer, and resuspended in fresh medium under the conditions specified. Experiments were terminated by rapidly transferring the cell suspension into MoNaught centrifuge tubes precooled to 0°C and separating the cells by centrifugation at 1300 × g for 5 minutes at 4°C. Packed cell volumes could be read with an accuracy of ±5 μl. The supernatant fluid was removed for analysis, and the tubes were washed twice with ice-cold buffer. The cell pellets were lysed completely by adding 0.02 ml of 0.008 M acetic acid per mg, wet weight, of packed cells, heating the digest for 10 minutes at 60°C, and squeezing the cell debris through a thin glass capillary or a 24-gauge needle. The cell debris was removed by centrifugation at 1300 × g for 10 minutes, and aliquots of the clear cell extract were withdrawn for analysis. The recovery of the intracellularly contained o-aminoisobutyric acid was complete, since the cell debris did not contain radioactivity.

The terms, exchange flux or exchange diffusion, are used as defined by Christensen (12) and indicate the exchange of labeled and unlabeled molecules of the same (heteroexchange) or similar species (autoexchange) across the cell membrane without concomitant net flow.

The steady state concentration ratios of cells incubated in bicarbonate-buffered media were 20 to 30% greater than those suspended in Tris-buffered solutions. Lymph node cells, however, are exquisitely sensitive to changes in pH. This buffer, therefore, was used routinely since pH could be more accurately controlled under the experimental conditions employed.

* This work was supported in part by Research Grants E-3765, A-1921, and E-3231 from the National Institutes of Health, United States Public Health Service.
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1 The cell yield from sensitized guinea pigs is 5 to 10 times greater than from nonsensitized animals. Although sensitized cells concentrated amino acids to a lesser extent (10 to 15%) than normal cells, no other metabolic differences have been noted (6).
Radioactivity Measurements—Aliquots of the incubation medium and the acetic acid cell extract were diluted with equal volumes of a water solution of egg albumin (100 mg per ml), plated, and counted in a Nuclear-Chicago D46 gas flow counter. Counting rates were corrected for self-absorption with an empirical curve.

Calculation of Intracellular Amino Acid Content—The extracellular space ("trapped volume") of the cell pellets packed under the conditions described above has been determined previously by a variety of methods and found to be 45 ± 2% of the total cell pellet volume (7). The intracellular water content was 78% of the corrected packed cell mass. The intracellular amino acid concentration was calculated as follows

$$C_i = \frac{C_p - (C_e \times V_e)}{(1 - V_o) \times 0.78}$$

where $C_i$ = amino acid content of the intracellular water, $C_p$ = amino acid content of the total packed cell pellet, $C_e$ = the extracellular concentration, and $V_e$ = the extracellular volume fraction of the packed cell pellet. Results have been expressed as counts per minute per ml or micromoles per ml (millimolar concentration) of intra- or extracellular water.

Materials—Glycine-2-C¹⁴ (specific activity, 18 mc per mmole) was obtained from the New England Nuclear Corporation, Boston, and α-aminoisobutyric acid-1-C¹⁴ (specific activity, 2 mc per mmole), from the Isotope Specialties Corporation, Burbank, California.

RESULTS

Entry of α-Aminoisobutyric Acid into Lymph Node Cells—α-Aminoisobutyric acid is readily concentrated by lymph node cells, the final distribution ratio between intra- and extracellular water varying in an inverse fashion with the extracellular level of the amino acid (Fig. 1). Concentration ratios greater than 50 were observed at low α-aminoisobutyric acid levels (<1 × 10⁻⁶ M), whereas no gradient was observed when the extracellular concentration exceeded 3 × 10⁻² M. This type of relationship between the final distribution ratio and the extracellular concentration of the amino acid does not differentiate between an active transport mechanism and binding to a cellular constituent. However, the latter possibility is unlikely, because intracellular amino acids are osmotically active (13) and their rate of influx is not decreased by preloading cells (e.g., saturation of cellular binding sites; see below). In view of this, it is reasonable to conclude that the final concentration ratio represents a steady state at which rates of amino acid influx and efflux are equal.

The initial rates of α-aminoisobutyric acid transport, $V_o$, were calculated from the velocity of α-aminoisobutyric acid uptake observed during the first 5 to 10 minutes of incubation when uptake was a linear function of time. During this period, the intracellular accumulation of α-aminoisobutyric acid was less than 10 to 15% of that attained at steady state, and hence efflux of this acid from the cell was negligible with respect to influx. The initial velocity increased hyperbolically with increasing extracellular levels of amino acid up to 1 × 10⁻⁴ M but did not attain a maximal rate (Fig. 2A); instead, the rate continued to increase in a linear fashion at α-aminoisobutyric acid levels greater than 0.5 to 1 × 10⁻⁴ M. A maximal rate of net influx ($V_{max}$) of 0.042

![Figure 1: Relation of steady state concentration ratio to extracellular concentration](http://www.jbc.org/

![Figure 2: Kinetics of α-aminoisobutyric acid influx](http://www.jbc.org/)

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amole of α-aminoisobutyric acid per ml of intracellular water per minute at 37° and an apparent Michaelis constant (Km) of 1.1 × 10^-4 m were obtained at 0 × 10^-6 m to 1 × 10^-4 m extracellular α-aminoisobutyric acid concentrations when initial velocities were plotted according to Lineweaver and Burk (Fig. 2B). Similar treatment of initial rates determined at higher α-aminoisobutyric acid concentrations yielded a linear function that intercepted the origin (Fig. 2C), suggesting that α-aminoisobutyric acid entered the lymph node cell by at least two processes: (a) a transport mechanism that exhibits saturation kinetics and is of primary importance at physiological amino acid concentrations and (b) a first order process similar to that of passive diffusion and of functional significance at high amino acid concentrations.

**Influence of Temperature on α-Aminoisobutyric Acid Influx**

The active transport process, measured at low extracellular α-aminoisobutyric acid levels, was temperature-dependent, with a Q10 of 1.93 to 2.33, whereas the diffusion process, measured at high α-aminoisobutyric acid levels, was little affected by lowering the temperature; Q10 = 1.21 (Table I). At low extracellular concentrations, both the initial rate of entry and the steady state concentration ratio were decreased by temperature, suggesting that α-aminoisobutyric acid efflux was relatively independent of temperature and similar to the influx mechanism observed at high external α-aminoisobutyric acid levels (see below).

**Formulation of Kinetics of Amino Acid Transport**—Net influx is mediated by two mechanisms: (a) an energy-dependent active transport process that saturates at relatively low amino acid concentrations and (b) a first order process characterized by diffusion kinetics. It is assumed that the active transport process is a “carrier”-mediated mechanism which can be described by the equations formulated by Haldane (14) for enzyme-catalyzed reversible unimolecular reactions. Sugar transport in frog sartorius muscle has recently been treated in a similar fashion by Narahara, Özand, and Cori (15). The diffusion component of amino acid entry will be treated as a first order process dependent on the concentration gradient. The net influx, v, can then be represented as

\[ v = \frac{V_1 S_1 - V_2 S_2}{K_1 + \frac{S_1}{K_2}} + k(S_1 - S_2) \]  

where \( K_1 \) and \( K_2 \) are the apparent Michaelis constants for the carrier on the outer and inner cell surfaces, respectively; \( V_1 \) and \( V_2 \) are the maximal velocities of carrier-mediated influx and efflux, respectively; \( S_1 \) and \( S_2 \) are the amino acid concentrations.

**Table I**

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Extracellular concentration</th>
<th>Steady state concentration ratio</th>
<th>Rate constant, net influx</th>
<th>Q10</th>
</tr>
</thead>
<tbody>
<tr>
<td>°C</td>
<td>mmol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>0.029</td>
<td>12.2</td>
<td>0.27</td>
<td>1.95</td>
</tr>
<tr>
<td>27</td>
<td>0.020</td>
<td>6.1</td>
<td>0.14</td>
<td>2.34</td>
</tr>
<tr>
<td>17</td>
<td>0.020</td>
<td>2.5</td>
<td>0.06</td>
<td>2.34</td>
</tr>
<tr>
<td>37</td>
<td>0.095</td>
<td>9.95</td>
<td>0.017</td>
<td>1.21</td>
</tr>
<tr>
<td>27</td>
<td>0.095</td>
<td>0.95</td>
<td>0.014</td>
<td>1.21</td>
</tr>
</tbody>
</table>

In this system, the equilibrium constant for active transport is

\[ K_{eq} = \frac{V_1 K_1}{V_2 K_2} \]  

Substituting Equation 2 into 1 gives

\[ v = \frac{V_1 S_1}{K_1 + \frac{S_1}{K_2}} + k(S_1 - S_2) \]

Initial rates, \( v_0 \), were experimentally determined when \( S_2 \to 0 \). Under these conditions, Equation 4 reduces to

\[ v = \frac{V_1 S_1}{K_1 + S_1} + k(S_1 - S_2) \]

At low concentrations, the diffusion term is negligible, and transport follows Michaelis-Menten kinetics (Fig. 2B). At higher concentrations, \( kS_1 \) becomes large with respect to the active transport component, and the kinetics observed are consequently those of diffusion (Fig. 2C).

A comparison of the experimentally observed rates of net influx at varying periods before the attainment of a steady state with the rates derived from Equation 4 revealed that experimental and derived values were in good agreement if the factor, \( S_2/K_2 \), were neglected (Fig. 3). If \( S_2/K_2 \) is indeed negligible with respect to 1 + \( (S_1/K_1) \), then \( K_2 \gg K_1 \), since \( S_2 \gg S_1 \) under the experimental conditions studied. This would indicate that the kinetic characteristics of the amino acid carrier are so different at the outer and inner surface of the cell membrane as to render carrier-mediated transport operationally irreversible. Equation 4 would then reduce to

\[ v = \frac{V_1 S_1}{K_1 + S_1} + k(S_1 - S_2) \]

which is the formulation used by Heinz for describing net transport of glycine in Ehrlich ascites tumor cells (16).

**Exchange Diffusion**—If \( K_2 \gg K_1 \), with respect to α-aminoisobutyric acid transport in lymph node cells, it might be anticipated that reversibility of carrier-mediated transport of α-aminoisobutyric acid is mediated by two mechanisms: (a) an energy-dependent active transport process saturated at relatively low amino acid concentrations and (b) a first order process similar to that of passive diffusion and of functional significance at high amino acid concentrations.
butyric acid would be difficult to demonstrate. The phenomenon of exchange diffusion has been used as an indicator of the presence and reversibility of a carrier mediated transport system in the cell membrane for cations (17), amino acids (18), and sugars (19). Exchange diffusion has been inferred from the presence and reversibility of a carrier-mediated transport system in lymph node cells. The rate of tracer influx is faster than the maximal net influx rate and (b) that influx of isotopic amino acid is accelerated by preloading the cell with the corresponding unlabeled amino acid. Both experimental conditions have been applied to lymph node cells with the use of α-aminoisobutyric acid, but in neither instance was exchange diffusion observed (Figs. 4 and 5).

In Experiment 1 (Fig. 4), the rate of α-aminoisobutyric acid-C\(^{14}\) influx under steady state conditions was compared with the net rate of α-aminoisobutyric acid entry at the same extracellular concentration of amino acid. No significant difference in rates was observed. In Experiment 2 (Fig. 5A), cells were preloaded with either unlabeled α-aminoisobutyric acid or glycine, washed, and resuspended with isotopic α-aminoisobutyric acid. Under both conditions, the rate of tracer amino acid entry into the cell was identical with that observed with nonloaded cells incubated in the same concentration of α-aminoisobutyric acid-C\(^{14}\). It is interesting to note, however, that the rate of glycine-C\(^{14}\) influx is accelerated in preloaded cells (Fig. 5B). These observations are in accordance with reports of exchange diffusion of glycine and other natural amino acids in ascites tumor cells (18). A possible explanation for the failure to demonstrate exchange diffusion of α-aminoisobutyric acid is presented in the "Discussion."

Comparison of α-Aminoisobutyric Acid and Glycine Transport—α-Aminoisobutyric acid and glycine concentration ratios maintained by these cells under identical experimental conditions are shown in Fig. 6. The extracellular amino acid concentration selected for these studies (5.5 × 10\(^{-4}\) m) corresponds to the glycine content of guinea pig plasma.\(^6\) Although the steady state ratios measured isotopically with either α-aminoisobutyric acid or glycine\(^7\) are reliable indices of the amino acid concentration ratios, the observed rates of isotope entry are not measurements of the same process for the two amino acids. The rate of α-aminoisobutyric acid-C\(^{14}\) entry is a direct measure of net influx, whereas glycine-C\(^{14}\) entry represents a unidirectional flux measurement composed of net influx and exchange diffusion.

It is evident that α-aminoisobutyric acid is transported into these cells more rapidly and concentrated to a greater extent than glycine; the initial rates of influx in these experiments were 0.12 μmole per ml per minute for α-aminoisobutyric acid-C\(^{14}\) and 0.06 μmole per ml per minute for glycine-C\(^{14}\). Heinz and Walsh (18) have presented data indicating that the rate of net influx of glycine in Ehrlich ascites tumor cells is approximately 15% of the rate of unidirectional flux. If such were the case in lymph node cells, the rate of net influx of glycine would be 0.009 μmole, compared with a flux rate of 0.06 μmole per ml per minute, and it would thus follow that α-aminoisobutyric acid transport is 10 to 20 times faster than glycine net influx at comparable concentrations.

\(^6\) David M. Kipnis, unpublished observations.

\(^7\) Over 90% of the radioactivity extracted from cells incubated in glycine-1-C\(^{14}\) for 60 minutes remained as glycine-C\(^{14}\).
external concentrations. It is of interest to note that the $K_m$ for glycine transport ($3 \times 10^{-5}$ M) appears to be much larger (see below) than that for $\alpha$-aminoisobutyric acid ($1.1 \times 10^{-4}$ M), suggesting that the rate of active net transport reflects the affinities of these amino acids for the carrier.

The steady state concentration ratio for glycine, which is lower than that for $\alpha$-aminoisobutyric acid (Fig. 6), appears to be a consequence of a slower rate of net influx, since the efflux coefficient for glycine is comparable to that for $\alpha$-aminoisobutyric acid in the lymph node cell.\(^9\)

**Competition Between $\alpha$-Aminoisobutyric Acid and Glycine for Transport---$\alpha$-Aminoisobutyric acid and glycine compete for cellular entry in lymph node cells (Table II). The former is an effective inhibitor of glycine entry, but glycine is a relatively poor inhibitor of $\alpha$-aminoisobutyric acid transport, indicating a greater affinity of $\alpha$-aminoisobutyric acid for the transport carrier.\(^9\) That $\alpha$-aminoisobutyric acid and glycine compete for the active transport component is further corroborated by the effect of temperature. For example, after 5 minutes of incubation in $5.8 \times 10^{-5}$ M $\alpha$-aminoisobutyric acid-1-C\(^14\) with and without $2 \times 10^{-2}$ M glycine, the respective intracellular levels were 1500 c.p.m. per ml and 4200 c.p.m. per ml at 37°C and 670 c.p.m. per ml and 1800 c.p.m. per ml at 27°C. The inhibitor constant ($K_i$) for glycine was calculated according to

$$K_i = \frac{K_m I}{(S + K_m) \left( \frac{v_i}{v_f} - 1 \right)}$$

where $K_m$ is the Michaelis constant of $\alpha$-aminoisobutyric acid for the carrier, $I$ = the concentration of glycine, $S$ = the concentration of $\alpha$-aminoisobutyric acid, and $v_i$ and $v_f$ = the uninhibited and inhibited rate of $\alpha$-aminoisobutyric acid influx, respectively. $K_i$ values of 2.35, 2.95, and $3.26 \times 10^{-5}$ M for 23, 37, and 86% inhibition of $\alpha$-aminoisobutyric acid transport were obtained. As was expected (see Table II), $K_i$ for glycine was larger than $K_m$ for $\alpha$-aminoisobutyric acid ($1.1 \times 10^{-4}$ M) but closely approximated the $K_m$ for glycine transport in ascites tumor cells (16). If one assumes that $K_i$ of glycine is an approximation of its $K_m$, the $K_i$ calculated for $\alpha$-aminoisobutyric acid is $1.1$ to $3.5 \times 10^{-4}$ M, which agrees well with its experimentally determined $K_m$.\(^9\)

\(^9\) Paine and Heinz (10) reported that the relative affinity of $\alpha$-aminoisobutyric acid for the transport carrier in ascites tumor cells is approximately 3 times greater than that of glycine.

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**Table II**

<table>
<thead>
<tr>
<th>Extracellular $\alpha$-aminoisobutyric acid concentration</th>
<th>Extracellular glycine concentration</th>
<th>Ratio (inhibitor)/ (substrate)</th>
<th>Inhibition$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>mM</td>
<td>mM</td>
<td></td>
<td>%</td>
</tr>
<tr>
<td>0.03</td>
<td>0</td>
<td>1.7</td>
<td>0</td>
</tr>
<tr>
<td>0.03</td>
<td>0.05</td>
<td>3.3</td>
<td>6</td>
</tr>
<tr>
<td>0.03</td>
<td>0.10</td>
<td>30</td>
<td>23</td>
</tr>
<tr>
<td>0.03</td>
<td>0.90</td>
<td>166</td>
<td>57</td>
</tr>
<tr>
<td>0.006</td>
<td>20</td>
<td>3333</td>
<td>86</td>
</tr>
<tr>
<td>0</td>
<td>0.55</td>
<td>0.002</td>
<td>0</td>
</tr>
<tr>
<td>0.001</td>
<td>0.55</td>
<td>0.018</td>
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<tr>
<td>0.01</td>
<td>0.55</td>
<td>0.09</td>
<td>27</td>
</tr>
<tr>
<td>0.05</td>
<td>0.55</td>
<td>0.18</td>
<td>36</td>
</tr>
<tr>
<td>0.1</td>
<td>0.55</td>
<td>0.91</td>
<td>55</td>
</tr>
</tbody>
</table>

$^a$ Calculated on the basis of initial rates.

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**Efflux of $\alpha$-Aminoisobutyric Acid from Lymph Node Cells---** Amino acid efflux from cells was examined before the attainment of steady state and at steady state (Table III). Since the intracellular content of $\alpha$-aminoisobutyric acid was constantly decreasing throughout the non-steady state experiments, initial velocities were determined during the first 5 to 10 minutes of incubation, when efflux was linear and the net change in intracellular content small. The efflux coefficient remained relatively constant over a wide range of intracellular concentrations ($2 \times 10^{-4}$ to $3.5 \times 10^{-3}$ M) but increased at the very lowest levels ($2 \times 10^{-4}$ M). Similar results were obtained when measurements were made at steady state, which was accomplished by preloading cells with radioactive amino acid until a steady state had been reached and then washing and resuspending them in medium containing unlabeled amino acid at the same concentration utilized for preloading. In these experiments,\(^10\) efflux followed first order kinetics and the efflux coefficient was calculated from the integrated form of the first order reaction rate equation. In one experiment, efflux was measured under non-steady state conditions in the presence of a large excess of extra cellular glycine (e.g. block re-entry of $\alpha$-aminoisobutyric acid). The efflux coefficients of 0.04 min$^{-1}$ and 0.07 min$^{-1}$, observed at intracellular $\alpha$-aminoisobutyric acid concentrations of 0.23 and 0.17 pmole per ml, respectively (compare the first and last experiments in Table III), were considerably greater than those observed at higher intracellular $\alpha$-aminoisobutyric acid levels and indicated carrier-mediated efflux. In accordance with this view, the accelerated rate of $\alpha$-aminoisobutyric acid efflux in the presence of glycine is suggestive of heteroexchange.\(^11\)

It is of interest to note that the efflux coefficients observed at intracellular $\alpha$-aminoisobutyric acid levels of $8 \times 10^{-3}$ M to $3.48 \times 10^{-3}$ M are identical with the efflux coefficients measured at high extracellular $\alpha$-aminoisobutyric acid concentrations (Table I). Furthermore, $Q_s$ of efflux at these levels is 1.36

\(^10\) Glycine efflux was also examined under similar circumstances and at intracellular levels of $3.5 \times 10^{-2}$ M; $k$ was 0.02 min$^{-1}$.

\(^11\) It was not possible to carry out efflux measurements at lower $\alpha$-aminoisobutyric acid levels because of the experimental limitations imposed by the low specific activities of $\alpha$-aminoisobutyric acid-C\(^14\) available at the time of these studies.
Table III

<table>
<thead>
<tr>
<th>Experimental conditions</th>
<th>Intracellular (\alpha)-aminoisobutyric acid concentration (mM)</th>
<th>Efflux coefficient (\text{min}^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-steady state</td>
<td>0.25</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>0.88</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>8.20</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>20.0</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>34.8</td>
<td>0.010</td>
</tr>
<tr>
<td>Steady state</td>
<td>3.0</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>0.011</td>
</tr>
<tr>
<td>Non-steady state; glycerol ((2 \times 10^{-2} \text{ M})^a)</td>
<td>0.17</td>
<td>0.07</td>
</tr>
</tbody>
</table>

* At 27°C.

** See the text.

(Table III), similar to that of influx at high \(\alpha\)-aminoisobutyric acid concentrations (Table I).

Effect of Succinate on Transport Mechanisms—In the presence of \(1 \times 10^{-4} \text{ M} \) succinate, the oxygen uptake of lymph node cells was increased 70% (Fig. 7), and concomitantly the \(\alpha\)-aminoisobutyric acid steady state concentration ratio rose 30 to 50% (Table IV). Neither the endogenous oxygen consumption nor the increased respiration in the presence of succinate was affected by \(3 \times 10^{-3} \text{ to } 3 \times 10^{-2} \text{ M} \) \(\alpha\)-aminoisobutyric acid (Fig. 7).

Since the initial rates of \(\alpha\)-aminoisobutyric acid influx measured at low extracellular concentrations remained unchanged in the presence of succinate, it was evident that the increased steady state concentration ratio was not caused by an accelerated active transport of \(\alpha\)-aminoisobutyric acid into the cell. At high extracellular concentrations, however, the initial rate of \(\alpha\)-aminoisobutyric acid entry was decreased 25 to 30% in the presence of succinate. Since \(\alpha\)-aminoisobutyric acid entry at high concentrations is by diffusion, it was anticipated that efflux, which also appears to be primarily a diffusion process at physiological concentrations, would also be decreased and thereby account for the increased steady state gradient seen with succinate. As can be seen from Table IV, this was actually the case. Although efflux at high intracellular \(\alpha\)-aminoisobutyric acid concentrations was decreased \(\sim 40\%\), an even greater depression, \(\sim 75\%\), was observed at lower amino acid levels. It is interesting to note that, in accordance with the results in Table III, the efflux coefficient was larger at the lower intracellular concentrations and, correspondingly, that the effect of succinate was greater. We interpret these observations to indicate that carrier-mediated efflux of \(\alpha\)-aminoisobutyric acid is suppressed by succinate oxidation.

A rabbit lymph node cell suspension, prepared in the same manner as the guinea pig cell preparation, was used for these experiments. We had replaced the guinea pig cells with rabbit cells at this stage of our investigations because of the greater cell yield per animal. Unpublished studies indicate that the qualitative and quantitative aspects of amino acid transport are quite similar in both cell species.

Table IV

<table>
<thead>
<tr>
<th>Extracellular (\alpha)-aminoisobutyric acid concentration</th>
<th>Conditions</th>
<th>Steady state concentration ratio</th>
<th>Rate constant, net influx (\text{min}^{-1})</th>
<th>Efflux coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1 \times 10^{-8})</td>
<td>Succinate</td>
<td>16.7</td>
<td>0.40</td>
<td>0.035</td>
</tr>
<tr>
<td>(1 \times 10^{-3})</td>
<td>Succinate</td>
<td>24.3</td>
<td>0.42</td>
<td>0.009</td>
</tr>
<tr>
<td>(1 \times 10^{-4})</td>
<td>Succinate</td>
<td>12.0</td>
<td>0.06</td>
<td>0.009</td>
</tr>
<tr>
<td>(1 \times 10^{-5})</td>
<td>Succinate</td>
<td>16.6</td>
<td>0.07</td>
<td>0.009</td>
</tr>
<tr>
<td>(1 \times 10^{-6})</td>
<td>Succinate</td>
<td>0.9</td>
<td>0.021</td>
<td>0.009</td>
</tr>
<tr>
<td>(1 \times 10^{-7})</td>
<td>Succinate</td>
<td>1.0</td>
<td>0.015</td>
<td>0.009</td>
</tr>
<tr>
<td>(1 \times 10^{-8})</td>
<td>Succinate</td>
<td>3.0</td>
<td>0.014</td>
<td>0.009</td>
</tr>
<tr>
<td>(1 \times 10^{-9})</td>
<td>Anaerobic cyanide</td>
<td>8.3</td>
<td>0.26</td>
<td>0.05</td>
</tr>
<tr>
<td>(1 \times 10^{-10})</td>
<td>Anaerobic cyanide</td>
<td>7.2</td>
<td>0.24</td>
<td>0.06</td>
</tr>
</tbody>
</table>

* Sensitized rabbit lymph node cells were used in these experiments.

** These experiments were performed with bicarbonate-buffered medium containing \(1 \times 10^{-2} \text{ M} \) glucose.

(c) Theoretically derived values calculated on the basis of steady state conditions (Equation 6; see text).

(d) At \(1 \times 10^{-6} \text{ M} \).

Fig. 7. Effect of succinate on oxygen consumption. Curve 1, range of oxygen consumption in the presence of \(1 \times 10^{-4} \text{ M} \) succinate with and without \(\alpha\)-aminoisobutyric acid at \(3 \times 10^{-4} \text{ to } 3 \times 10^{-2} \text{ M} \). Curve 2, range of endogenous oxygen consumption with and without \(\alpha\)-aminoisobutyric acid at \(3 \times 10^{-4} \text{ to } 3 \times 10^{-2} \text{ M} \). \(\alpha\)-Aminoisobutyric acid had no effect on oxygen consumption in either case. Curve 3, endogenous oxygen consumption in the presence of \(1 \times 10^{-4} \text{ M} \) \(\text{CN}^-\) and \(3 \times 10^{-4} \text{ M} \) \(\alpha\)-aminoisobutyric acid. For each experiment, \(1 \times 10^8 \text{ to } 3 \times 10^8 \text{ cells} \) were incubated in \(3 \text{ to } 4 \text{ ml of Tris-buffered media in } 100\% \text{ O}_2 \) at \(37^\circ \). Oxygen uptake was measured directly; \(\text{CO}_2\) was trapped by alkali added to the center well. Oxygen uptake is expressed as microliters of \(\text{O}_2\) per \(1 \times 10^8 \text{ cells}\).
Anaerobiosis or cyanide increased the permeability of the cell membrane and inhibited the active transport process, thereby causing a marked decrease in the steady state concentration ratio (Table IV). Since glucose was present in these experiments, it is evident that glycolysis could sustain active transport to a limited extent. The calculated efflux coefficients for \( \alpha \)-aminoisobutyric acid did not appear to differ markedly from those observed at similar concentrations in aerobic experiments. These results agree in general with observations of Heinz and Mariani (20) on the effects of metabolic inhibitors on glycine transport in ascites tumor cells.

**DISCUSSION**

Most of our knowledge of the mechanism of amino acid transport in mammalian tissues has been derived from observations with the Ehrlich ascites tumor cell (12). In the present work, isolated lymph node cells have proved to be suitable for the study of amino acid transport. In addition, these cells offer several potential advantages over ascites tumor cells. (a) Their metabolism is more representative of normal mammalian tissues \((21, 22)\); (b) the relatively slow rates of amino acid influx and efflux facilitate kinetic measurements; (c) their ability to synthesize antibodies and \(\gamma\)-globulins in vitro \((1, 2)\) allows for concomitant yet independent measurements of amino acid transport and the synthesis of specific proteins; and (d) their responsiveness to hormones \((22)\), *e.g.* adrenoglucocorticoids, growth hormone, known to influence protein synthesis \((3, 4)\) enables examination of amino acid transport as a possible site of hormone action.

The results of this study indicate that amino acids enter and leave the lymph node cell both by diffusion and by an active process capable of transport against a chemical gradient. The characteristics of these two processes are such that at physiological concentrations entry is primarily by active transport, and efflux, by diffusion. Influx by diffusion can be observed at high external levels, and efflux by reversal of active transport, at very low internal levels of amino acid. Various kinetic models have been proposed for the active transport system which assume participation of a "carrier" but differ with respect to the mobility of the carrier amino acid complex. In the carrier model proposed by Christensen \((12)\) and Heinz and Walsh \((18)\), transport proceeds by a series of reversible reactions by which the amino acid combines with the carrier on the outer cell surface to form a complex that dissociates when translocated to the inner cell surface. In order to account for movement against a concentration gradient, it has been proposed that the carrier is inactivated on the inner cell surface and reactivated as it returns to the outer cell surface. Reversibility of the transport system, *i.e.* exchange-diffusion, would then be accomplished by that part of the carrier on the inner cell surface that escaped inactivation. The kinetic formulation presented in this paper has clarified certain aspects of the transport system. For example, the Michaelis-Menten rate equation, which is based on "initial velocities," adequately described \(\alpha\)-aminoisobutyric acid transport throughout its course of entry, although the transport system is assumed to operate as a reversible sequence of reactions. The steady state rate equation (Equation 4) indicates that the extent to which a reaction may proceed and still allow for simple velocity measurements to be used for determining initial velocities is dependent upon the Michaelis constant \((K_2)\) for the product \((S_0)\). Since observed and calculated velocities of \(\alpha\)-aminoisobutyric acid influx \(\text{(based on Equation 6)}\) agreed throughout the course of attainment of steady state, it follows that \((S_0/K_2) < 1\) or that \(K_2 \gg K_1\); consequently, the equilibrium constant of the transport system \(\text{(Equation 2)}\) favors the intracellular accumulation of amino acid and renders active transport functionally irreversible \(\text{(hence following Michaelis-Menten kinetics)}\). Using a similar kinetic treatment, Narahara, Ózand, and Cori \((15)\) have recently concluded that the concept of membrane asymmetry with respect to the carrier's properties on the inner and outer cell surface applies to the glucose transport system in frog sartorius muscle.

In this context, it should be recalled that reversibility of carrier-mediated transport, *i.e.* exchange-diffusion, could not be demonstrated readily with \(\alpha\)-aminoisobutyric acid, whereas exchange was observed with glycine.\(^{11}\) The competition experiments indicated that glycine and \(\alpha\)-aminoisobutyric acid share a common transport system but that the affinities of these amino acids for the transport carrier differed markedly. The exchange experiments suggested that reversibility may depend on the same factors that determine the equilibrium constant of the transport system; namely, the ratio of the carrier's affinity on the inner and outer cell surface for the particular amino acid \((K_2/K_1)\). The greater the ratio, the more difficult it would be to demonstrate exchange. Since \(K_1\) appeared to be 20 to 30 times greater for glycine than for \(\alpha\)-aminoisobutyric acid, one would expect to demonstrate glycine exchange more readily, and, in addition, the glycine steady state concentration ratio should be less than that of \(\alpha\)-aminoisobutyric acid at comparable concentrations. Both of these predictions are consistent with the experimental data \((\text{Figs. } 5\text{B and } 6)\). Furthermore, in this formulation, the carrier participating in the exchange reaction could be the "inactivated" carrier on the inner cell surface.

The manner by which the energy derived from cellular metabolism is coupled to the active transport process has as yet not been defined. Of interest in relation to this important problem are the effects of succinate addition on \(\alpha\)-aminoisobutyric acid transport in lymph node cells. Succinate oxidation did not influence carrier-mediated influx but markedly decreased carrier-mediated efflux as well as the diffusion of \(\alpha\)-aminoisobutyric acid through the cell membrane measured as either influx or efflux. The steady state concentration ratio consequently increased as a result of decreased efflux. Especially pertinent to these observations is the recent report of Horecker, Thomas, and Monad \((24)\) that succinate did not affect galactose influx in *Escherichia coli* but did decrease its efflux, thereby resulting in a marked increase in the galactose concentration gradient maintained by these cells. These results, obtained with both mammalian and bacterial cells, suggest that the link of cellular metabolism to transport involves the transition of the carrier from a state of high affinity on the outer cell surface to one of low affinity on the inner cell surface for the penetrant. If this were the case, the energetically activated carrier would actually exhibit lower affinity for the penetrant. Furthermore, if the extent of exchange-diffusion is dependent on the ratio, \(K_2/K_1\), it might be anticipated that exchange diffusion with natural amino acids would be decreased in the presence of succinate.

\(^{11}\) Exchange diffusion could not be demonstrated with \(\alpha\)-aminoisobutyric acid but was observed with lysine, valine, and leucine in the intact rat diaphragm preparation \((23)\). (David M. Kipnis, unpublished observations.)
SUMMARY

1. The mechanism of amino acid transport in guinea pig (and rabbit) lymph node cells has been studied with the nonmetabolizable amino acid analogue, α-aminobutyric acid. Amino acid entry and exit were mediated by two mechanisms: (a) an active transport process exhibiting saturation kinetics, high temperature coefficient, and capability of transport against a chemical gradient and (b) a first order process with characteristics of diffusion.

2. At physiological amino acid concentrations, entry is primarily by active transport, and efflux, by diffusion. Influx by diffusion is observed at high extracellular amino acid levels, whereas efflux by reversal of the active carrier-mediated transport system can be observed at very low intracellular amino acid levels.

3. A kinetic treatment of amino acid transport, based on a reversible carrier-mediated system, is formulated which clarifies certain characteristics of the transport process and indicates that the cell membrane is asymmetric on its inner and outer surface with respect to the kinetic properties of the carrier.

4. Exchange diffusion was not readily demonstrable with α-aminobutyric acid but was observed with glycine.

5. Succinate oxidation stimulated oxygen consumption and increased the steady state α-aminobutyric acid concentration ratio maintained by lymph node cells. The permeability of the cell membrane, measured as either influx or efflux, was decreased by succinate. Active influx was not affected, but carrier-mediated efflux was markedly decreased by succinate oxidation.

Acknowledgments—The authors wish to express their sincere appreciation to Dr. Carl Frieden for suggesting the kinetic treatment used in this study and for many stimulating discussions. We are grateful to Miss L. Slonevsky and Mr. R. Sherman for their valuable technical assistance.

REFERENCES


