Active Amino Acid Transport in Plasma Membrane Vesicles from Simian Virus 40-transformed Mouse Fibroblasts

CHARACTERISTICS OF ELECTROCHEMICAL Na⁺ GRADIENT-STIMULATED UPTAKE*

(Received for publication, August 9, 1976)

JULIA E. LEVER

From the Imperial Cancer Research Fund Laboratories, Lincoln's Inn Fields, London WC2A 3PX, England

Selectively permeable membrane vesicles isolated from Simian virus 40-transformed mouse fibroblasts catalyzed Na⁺ gradient-coupled active transport of several neutral amino acids dissociated from intracellular metabolism. Na⁺-stimulated alanine transport activity accompanied plasma membrane material during centrifugation in discontinuous dextran 110 gradients. Carrier-mediated transport into the vesicle was demonstrated. When Na⁺ was equilibrated across the membrane, countertransport stimulation of t-[³H]alanine uptake occurred in the presence of accumulated unlabeled L-alanine, 2-aminoisobutyric acid, or L-methionine. Competitive interactions among neutral amino acids, pH profiles, and apparent Kₐ values for Na⁺ gradient-stimulated transport into vesicles were similar to those previously described for amino acid uptake in Ehrlich ascites cells, which suggests that the transport activity assayed in vesicles is a component of the corresponding cellular uptake process.

Both the initial rate and quasi-steady state of uptake were stimulated as a function of a Na⁺ gradient (external Na⁺ > internal Na⁺) applied artificially across the membrane and were independent of endogenous (Na⁺ + K⁺)-ATPase activity. Stimulation by Na⁺ was decreased when the Na⁺ gradient was dissipated by monensin, gramicidin D or Na⁺ preincubation. Na⁺ decreased the apparent Kₐ for alanine, 2-aminoisobutyric acid, and glutamine transport. Na⁺ gradient-stimulated amino acid transport was electrogenic, stimulated by conditions expected to generate an interior-negative membrane potential, such as the presence of the permeant anions NO₃⁻ and SCN⁻. Na⁺-stimulated t-alanine transport was also stimulated by an electrogenic potassium diffusion potential (K⁺ internal > K⁺ external) catalyzed by valinomycin; this stimulation was blocked by nigericin. These observations provide support for a mechanism of active neutral amino acid transport via the "A system" of the plasma membrane in which both a Na⁺ gradient and membrane potential contribute to the total driving force.

Investigations of energy transduction and active transport across such diverse biological membranes as mitochondrial, chloroplast, bacterial, intestinal, and tumor cell membranes have focused on the role of electrostatic and chemical concentration gradients (1-5). For example, Riggs et al. (1) and Crane (2) have proposed a "Na⁺ gradient hypothesis" in which Na⁺ gradients generated by active Na⁺ extrusion via the (Na⁺ + K⁺)-ATPase system are utilized to drive Na⁺-dependent active transport in certain cases (6-8). Similarly, respiration-coupled transport in Escherichia coli has been interpreted (4, 5, 9) in terms of Mitchell's chemiosmotic hypothesis (3) which proposes that proton gradients generated by proton extrusion via the respiratory chain can be utilized to drive active transport of a variety of solutes. In these conceptually parallel hypotheses, active transport is coupled to metabolism by means of an electrochemical ion potential across the membrane, the sum of two thermodynamic components, an electrical term which is the membrane potential and a chemical component. The relative contributions of each component to the energy coupling of a transport system would depend on the characteristics of each system.

Membrane vesicles with transport catalytic activity have provided a simplified system for studying energy coupling of transport dissociated from intracellular components and in the presence of a defined electrochemical driving force (10-16). Using membrane vesicles isolated from untransformed and Simian virus 40 (SV40)-transformed mouse fibroblasts, I have previously reported evidence that both an interior-negative membrane potential and an electrochemical Na⁺ gradient imposed artificially across the plasma membrane can provide a driving force for electrogenic accumulation of 2-aminoisobutyric acid (iso-Abu) against its concentration gradient (16). Also, alteration of iso-Abu uptake by change of cellular proliferative state and by transformation by SV40 was expressed in isolated membrane vesicles (16). Further characteristics of the coupling between electrochemical Na⁺ gradients and active amino acid transport in plasma membrane vesicles isolated from SV40-transformed mouse fibroblasts are described here.

EXPERIMENTAL PROCEDURES

Cell Culture—SV40 A31E7, a clone line of SV40-transformed Balb/c 3T3 A31 mouse fibroblasts, was obtained from Dr. Y. Ito of this

* This investigation was conducted during tenure of Grant 5F32CA05174-02 from the United States National Cancer Institute, National Institutes of Health. This is the second paper in a series on transport mechanisms in mammalian cells. Ref. 16 is the preceding paper in this series.

† The abbreviations used are: iso-Abu, 2-aminoisobutyric acid; SV40, simian virus 40; Me₂SO, dimethylsulfoxide.
Amino Acid Transport in Plasma Membrane Vesicles

**Materials and Methods**

**Amino Acid Transport in Plasma Membrane Vesicles**



**Characteristics of Membrane Vesicle Transport Activity**

- **Dependence on Na⁺ gradient:**
  - The Na⁺ gradient-stimulated influx of isoleucine into vesicles was measured at temperatures ranging from 21°C to 37°C. The uptake was maximal at 21°C, and a slower rate was observed at 37°C. The half-life of isoleucine uptake at 37°C was approximately 42 s.

- **pH Dependence:**
  - The pH of the incubation medium significantly affected the uptake of isoleucine. The optimal pH range for transport was between 7.0 and 7.4.

- **Na⁺/K⁺ Transport:**
  - The uptake of isoleucine was inhibited by the presence of K⁺, indicating a dependence on the Na⁺/K⁺ pump.

- **Temperature Dependence:**
  - The transport activity was highest at 21°C and decreased at 37°C.

- **Inorganic Phosphate Release:**
  - The release of inorganic phosphate from the vesicles was proportional to the rate of amino acid uptake.

- **NADH Oxidase Activity:**
  - The NADH-oxidase activity was highest at 21°C and decreased at 37°C.

- **Effect of Temperature:**
  - The effect of temperature on the transport activity was studied by incubating the vesicles at different temperatures and measuring the uptake and release of isoleucine.

**Results**

- **Temperature Effect:**
  - The transport activity was significantly lower at 37°C compared to 21°C.

- **pH Effect:**
  - The transport activity was highest at pH 7.2 and decreased at lower and higher pH values.

- **Na⁺/K⁺ Dependence:**
  - The transport activity was inhibited by the presence of K⁺ in the incubation medium.

**Discussion**

- **Na⁺ Gradient:**
  - The Na⁺ gradient is an important factor in the transport activity of the vesicles.

- **pH Optimization:**
  - The pH of the incubation medium is critical for optimal transport activity.

- **Temperature Sensitivity:**
  - The transport activity is sensitive to temperature changes.

**Conclusion**

- The transport activity of plasma membrane vesicles is significantly affected by temperature, pH, and Na⁺/K⁺ gradients. These factors should be considered in future studies to optimize the transport activity.

---

**Fig. 1.** Effect of temperature on Na⁺ gradient-stimulated influx (A) and efflux (B) of isoleucine. To 140 µg of vesicles from Swiss SV40-transformed BALB/c 3T3 cells, 1.69 mM 1-'Cl-isoleucine (61 fmol/cell) and 50 mM NaCl were added at zero time. A, incubation was at 37°C ( ), 21°C ( ), and 2°C ( ) for 20 min to remove precipitated protein before reading absorbance at 890 nm. B, efflux was measured at 37°C ( ), 21°C ( ), and 2°C ( ) at the indicated times after addition of 1 ml of 0.15M sucrose, 10 mM Tris/ phosphate (pH 7.5), 5 mM MgCl₂, and 50 mM NaCl to mixtures incubated 15 min at 21°C.
Amino Acid Transport in Plasma Membrane Vesicles

The temperature and composition of the dilution and wash steps of the filtration assay were chosen to maximize solute retention. Almost no leakage was observed when dilution and wash in 0.8 M NaCl, 0.01 M Tris/HCl, pH 7.5 (wash buffer) were carried out at 2°C as shown in Fig. 2A. Loss of vesicle contents was extensive at 37°C. Use of hypotonic wash buffers caused loss of accumulated iso-Abu.

The initial rate of iso-Abu uptake under standard assay conditions was proportional to the amount of vesicle protein up to 250 μg and not affected by addition of 80 μg of nonspecific protein (Fig. 2B). The same solute retention was observed at low membrane concentrations using either 0.2-μm or 0.45-μm pore size nitrocellulose filters.

Evidence for Carrier-mediated Amino Acid Transport into Vesicles—Convincing evidence that membrane vesicles enclose a selectively permeable compartment and that both alanine uptake and efflux are mediated by specific carriers was obtained. Fig. 3 shows that exchange diffusion (28) can be demonstrated under conditions where Na⁺ is equilibrated across the membrane both by increased uptake of [³H]alanine when vesicles were preloaded with unlabeled alanine (Fig. 3A) and by increased efflux of labeled alanine (Fig. 3B) when unlabeled L-alanine was added to the external solution.

This stimulation was specific for substrates of this transport carrier. Internal iso-Abu was much more effective in stimulating alanine uptake by exchange diffusion than leucine, glycine, or methionine (not shown). Additional evidence inconsistent with binding to fixed sites, the somatic sensitivity of accumulated iso-Abu, has been presented earlier using this system (16).

Transport did not require metabolic conversion of substrates as shown by chromatography of L-[2,3-³H]alanine, L-[4,5-³H]leucine, L-[G-³H]glutamine, or L-[methyl-³H]methionine extracted after accumulation in mixed vesicles.

Na⁺ Gradient-stimulated Iso-Abu Uptake Activity as Function of Plasma Membrane Purification—Evidence that Na⁺ gradient-stimulated iso-Abu uptake activity is associated with plasma membrane was obtained. Table I compares the enrich-

![Figure 2](http://www.jbc.org/)

![Figure 3](http://www.jbc.org/)

| Membrane fraction | Specific activity | Distribution |
|-------------------|------------------|--------------|
|                   | nmol/min/mg | % |
| Homogenate          | 0.19 (100) | 100 |
| Crude mixed vesicles | 0.47 (72) | 38 |
| Plasma              | 1.16 (20) | 2.6 |
| Membrane            | 0.023 (1.4) | 16 |

Downloaded from http://www.jbc.org/ by guest on March 20, 2020
ment of Na\(^+\) gradient-stimulated iso-Abu uptake specific activity with that observed for 5'-nucleotidase activity, a marker enzyme bound to plasma membrane fractions (19, 29) and resolvable in multiple forms (30). The plasma membranes fractionated after dextran 110 discontinuous gradients contained 20% of the 5'-nucleotidase activity, 21-fold increased in specific activity with respect to the homogenate, and 20% of the Na\(^+\) gradient-stimulated iso-Abu uptake activity, 9.2-fold increased in specific activity. The crude endoplasmic reticulum fraction contained only 1.4% of the total transport activity, 5.0-fold reduced in transport specific activity compared with the homogenate and 50-fold reduced with respect to the plasma membrane fraction. No Na\(^+\) gradient-stimulated transport activity could be detected in the nuclear or mitochondrial fractions but these fractions contained Na\(^+\)-independent iso-Abu transport activity.

For the transport experiments described below, unless otherwise noted, a purified mixed vesicle population (16, 31) contaminated 20% by endoplasmic reticulum and 1 to 2% by mitochondria as determined by marker enzymes for these organelles was used. Purified plasma membrane vesicles had an internal space of 2 \(\mu\)g of protein accessible to 3-O-methyl-\(\alpha\)-\(\beta\)Hglucose and that of purified mixed vesicles was an internal space of 2 \(\mu\)g of protein accessible to 34-

For the transport experiments described below, unless otherwise noted, a purified mixed vesicle population (16, 31) contaminated 20% by endoplasmic reticulum and 1 to 2% by mitochondria as determined by marker enzymes for these organelles was used. Purified plasma membrane vesicles had an internal space of 2 \(\mu\)g of protein accessible to 3-O-methyl-\(\alpha\)-\(\beta\)Hglucose and that of purified mixed vesicles was an internal space of 2 \(\mu\)g of protein accessible to 34-

For the transport experiments described below, unless otherwise noted, a purified mixed vesicle population (16, 31) contaminated 20% by endoplasmic reticulum and 1 to 2% by mitochondria as determined by marker enzymes for these organelles was used. Purified plasma membrane vesicles had an internal space of 2 \(\mu\)g of protein accessible to 3-O-methyl-\(\alpha\)-\(\beta\)Hglucose and that of purified mixed vesicles was an internal space of 2 \(\mu\)g of protein accessible to 34-

For the transport experiments described below, unless otherwise noted, a purified mixed vesicle population (16, 31) contaminated 20% by endoplasmic reticulum and 1 to 2% by mitochondria as determined by marker enzymes for these organelles was used. Purified plasma membrane vesicles had an internal space of 2 \(\mu\)g of protein accessible to 3-O-methyl-\(\alpha\)-\(\beta\)Hglucose and that of purified mixed vesicles was an internal space of 2 \(\mu\)g of protein accessible to 34-

For the transport experiments described below, unless otherwise noted, a purified mixed vesicle population (16, 31) contaminated 20% by endoplasmic reticulum and 1 to 2% by mitochondria as determined by marker enzymes for these organelles was used. Purified plasma membrane vesicles had an internal space of 2 \(\mu\)g of protein accessible to 3-O-methyl-\(\alpha\)-\(\beta\)Hglucose and that of purified mixed vesicles was an internal space of 2 \(\mu\)g of protein accessible to 34-

| Addition (50 mm) | NaCl | KCl | LiCl | RbCl | Choline Cl | Tris/HCl, pH 7.4 | NaSO\(_4\) | NaSCN | NaCl minus Tris/phosphate | KSCN |
|------------------|------|-----|------|------|------------|-----------------|---------|-------|----------------------------|------|
|                  |      |     |      |      |            |                 |         |       |                            |      |
| 30 s             | 590  | 210 | 300  | 210  | 210        | 220             | 630     | 680   | 580                        | 50   |
| 10 min           |      |     |      |      |            |                 |         |       |                            |      |
| 15 min           |      |     |      |      |            |                 |         |       |                            |      |
NaSCN gradient. At decreased Na⁺ gradients but at constant SCN⁻ concentration, the $K_m$ value increased to 1.7 mM using 10 mM NaSCN plus 40 mM KSCN and $V_{\text{max}}$ was 14.0 nmol/min/mg; the $K_m$ was 2.4 mM using 1 mM NaSCN plus 50 mM KSCN and $V_{\text{max}}$ was 11.9 nmol/min/mg. Similar effects of Na⁺ on the apparent $K_m$ and $V_{\text{max}}$ for L-glutamine and iso-Abu uptake were observed (not shown).

Both the initial rate (Fig. 6A) and steady state accumulation (Fig. 6B) of L-alanine increased as a function of increasing NaSCN gradients at constant SCN⁻ concentration. After addition of monensin to dissipate Na⁺ gradients, the initial rate of alanine uptake maintained a decreased degree of stimulation as a function of Na⁺ concentration, but saturation became evident at NaSCN concentrations above 50 mM with half-maximal stimulation at 10 mM NaSCN. By contrast, Fig. 6B shows that monensin prevented Na⁺ stimulation of steady state accumulation at all Na⁺ concentrations. The inset in Fig. 6B shows that an alternate plot of the same data converted to logarithmic form after subtraction of Na⁺-independent uptake gave a straight line with a slope of 0.7.

**Effect of Zonophores on Na⁺ Gradient-stimulated Uptake**

Fig. 7 shows that creation of a potassium diffusion potential (internal K⁺ > external K⁺) in the presence of valinomycin, a K⁺-specific electrogenic ionophore (36), caused a marked transient increase in Na⁺ gradient-stimulated accumulation of L-alanine. No stimulation of uptake under these conditions was observed in the absence of Na⁺ (data not shown). Fig. 8 shows that this stimulation was dependent on valinomycin concentration, with maximal response at 20 μg/ml of valinomycin and no stimulation observed at higher concentrations. No effect on Na⁺ gradient-driven alanine uptake was observed in K⁺-loaded vesicles in the absence of valinomycin or with valinomycin in the absence of K⁺ gradients (Fig. 7). Addition of nigericin, an ionophore which catalyzes a nonelectrogenic K⁺/H⁺ exchange (37), completely abolished the initial stimulation observed with K⁺-loaded vesicles in the presence of valinomycin.

### Table III

**Dissociation of Na⁺ gradient-stimulated alanine transport from endogenous (Na⁺ + K⁺)-ATPase activity**

| NaSCN concentration (mM) | Initial rate of ATP hydrolysis a | Initial rate of ATP hydrolysis a |
|--------------------------|---------------------------------|---------------------------------|
| Control                  | 380 470 580 500                 | 260                             |
| + KCl                    | 300 490 550 180                 | 260                             |
| + Ouabain, 1 mM          | 330 470 530 140                 | 260                             |

a Average of duplicate determinations with a range of ±15%.

**Fig. 5.** Effect of a Na⁺ gradient on alanine uptake as a function of alanine concentration. At zero time, concentrations of L-[2,3-³H]alanine, 0.4 pmol/cpm, from 0.4 to 4.0 mM were added with 50 mM NaSCN (●), 50 mM KSCN (●), 10 mM NaSCN plus 40 mM KSCN (△), or 1 mM NaSCN plus 50 mM KSCN (○) to aliquots of 110 μg of vesicles (Swiss SV3T3). Uptake was determined after 10 min. Results are averaged from duplicates and represent the sum of Na⁺-dependent and -independent uptake rates.

**Fig. 6.** Effect of a Na⁺ gradient on (A) initial rate of L-alanine uptake and (B) steady state accumulation of alanine, as a function of external Na⁺ concentration. Incubations contained 120 μg of vesicles (Balb SV3T3) and 0.2 mM L-[2,3-³H]alanine, 40 fmol/cpm, added at zero time with concentrations of NaSCN and KSCN as indicated. The total SCN⁻ concentration was maintained at 100 mM by addition of KSCN and the Na⁺/K⁺ ratio was varied. Uptake was determined (A) after 30 s as an estimate of the initial rate and (B) after 10 min as an estimate of steady state accumulation. ○, 1% MeSO; □, 1 μg of monensin in 1% MeSO. Inset, log Na⁺-dependent alanine uptake at 10 min, (pmol/mg) versus log external Na⁺ concentration (mM).

**Fig. 7.** Enhancement of Na⁺ gradient-stimulated alanine uptake by a potassium diffusion potential in the presence of valinomycin. Aliquots of 90 μg of membrane vesicles (Balb SV3T3) were incubated 15 min with 50 mM KCl, 1 μg of valinomycin, and 2% ethanol (○); 50 mM choline chloride, 1 μg of valinomycin, and 2% ethanol (●); 50 mM KCl and 2% ethanol (□); 2% ethanol and 3% MeSO (△); or 0.25 μg of nigericin, 1 μg of valinomycin, 50 mM KCl, 2% ethanol, and 3% MeSO (□). Then, vesicles were diluted 10-fold into 100-μl volumes with 50 mM NaCl and 0.2 mM L-[2,3-³H]alanine, 40 fmol/cpm, added for the times indicated.
valinomycin in 2% ethanol (A). Samples lacking ionophore contained 2% ethanol. The effect of adding 5 pg/ml of nigericin to membranes treated with 10 min after addition of 0.17 mM [l-14C]iso-Abu with 50 mM NaCl. The effect of adding 5 μg/ml of nigericin to membranes treated with 10 μg/ml of valinomycin is shown by the dotted line (○).

Fig. 8. Effect of ionophores on the initial rate of iso-Abu uptake in the presence of an initial Na⁺ gradient. Aliquots of 98 μg of vesicles (Swiss SV3T3) were incubated 15 min with 50 mM KCl then diluted 10-fold in the presence of the indicated concentration of: nigericin in 5% Me₂SO (○), gramicidin D in 2% ethanol (△), or valinomycin in 2% ethanol (▲). Samples lacking ionophore contained 2% ethanol (△, ▲) or 5% Me₂SO (○). Uptake was measured 1 min after addition of 0.17 mM [l-14C]iso-Abu with 50 mM NaCl. The effect of adding 5 μg/ml of nigericin to membranes treated with 10 μg/ml of valinomycin is shown by the dotted line (○).

Interactions among Neutral Amino Acids for Na⁺-dependent Transport—Substrate specificity properties of Na⁺ gradient-stimulated neutral amino acid transport suggested that transport activity assayed in vesicles represents the corresponding in vitro plasma membrane uptake system. Table IV summarizes the effect of addition of several unlabeled amino acids in 25-fold excess on the initial rate of uptake of labeled 0.2 mM alanine, methionine, leucine, and iso-Abu. Alanine and iso-Abu uptake showed a similar pattern of inhibition by the unlabeled amino acids tested, which suggests they share a common carrier for transport into vesicles with the specificity properties described for the "A system" in Ehrlich ascites cells (39-41). Leucine uptake showed the amino acid specificity described for the "L system" (39, 40). Methionine uptake was inhibited by both categories of amino acids. No significant inhibition of l-alanine or iso-Abu uptake by d-amino acids tested was observed, which suggests uptake is stereospecific. By contrast, L-methionine and L-leucine uptakes were inhibited by L-methionine and L-leucine. About 15% of total alanine and methionine uptake activity, 20% of iso-Abu uptake, and 50% of leucine uptake was Na⁺-independent. N-Methyl-dl-alanine inhibited [3H]alanine uptake to almost the same extent as unlabeled L-alanine, which suggests that the "ASC system" (42) does not contribute appreciably to alanine transport in vesicles measured at this pH. Glycine, which is transported by a separate Na⁺-dependent carrier system in some cells (40, 43), showed minimal effectiveness in inhibiting uptake of these amino acids, although its uptake was markedly stimulated by a Na⁺ gradient.

Effect of pH—pH profiles for Na⁺-stimulated alanine and iso-Abu transport were almost identical with those obtained for in vivo uptake in Ehrlich ascites cells (39). When the initial rate of Na⁺-stimulated transport into vesicles was measured as a function of external pH, uptake of both iso-Abu (Fig. 9A) and alanine (Fig. 9B) was optimal at pH 7.4. The ratio of activity at pH 7.4 to that of pH 6 was 2.3 for alanine uptake, and 6.3 for iso-Abu uptake. The initial rate of Na⁺-independent alanine uptake did not vary appreciably over this range.

Effect of Inhibitors—Several uncouplers and inhibitors of oxidative phosphorylation tested for effect on Na⁺-stimulated iso-Abu uptake (Table V) had minimal or no effect. Na⁺-stimulated alanine uptake (not shown) was not inhibited by addition of 10 mM sodium arsenate or 50 μg/ml of oligomycin.

The lack of inhibition by proton conductors, such as carbonyl cyanide p-trifluoromethoxyphenylhydrazone and 2,4-di-
Amino Acid Transport in Plasma Membrane Vesicles

**FIG. 9.** Effect of pH on the initial rate of Na\(^{+}\) gradient-stimulated (A) iso-Abu and (B) alanine uptake. At zero time, aliquots of 110 \(\mu\)g of vesicles (Swiss SV3T3) were diluted into 100 \(\mu\)l of 0.125 M sucrose, 5 mM MgCl\(_2\), and either Tris/phosphate (\(\cdot\)), Tris/HCl (\(\bullet\)), potassium phosphate (\(\mathcal{A}\)), or 2-[bis(2-hydroxyethyl)aminopropyl]ethanesulfonic acid (\(\mathcal{W}\)). A, 50 mM buffer with 0.17 mM \([l-\text{[2,3-3H]}\text{]iso-Abu}(40 \text{fmol/cpm})\) and 90 mM NaSCN. The Na\(^{+}\)-independent rate, determined using 50 mM choline chloride in \(A\) or 50 mM KSCN (open symbols) in \(B\) has been subtracted. Results are averaged from duplicates. pH was measured at room temperature in each incubation mixture minus vesicles.

nitrophenol, suggests that H\(^+\) gradients are not coupled to this transport system as has been proposed (44).

**DISCUSSION**

Active neutral amino acid transport in vesicles was unequivocally linked to an electrochemical Na\(^{+}\) gradient. When a Na\(^{+}\) gradient (external Na\(^{+}\) > internal Na\(^{+}\)) was artificially imposed across the membrane amino acid uptake against its concentration gradient occurred. Although in whole cells this Na\(^{+}\) asymmetry is provided by active Na\(^{+}\) extrusion via the \(\text{(Na}^+ + \text{K}^+)\)-ATPase transport system (45), endogenous (Na\(^{+}\) + K\(^{+}\))-ATPase activity of vesicles was not directly involved, in confirmation of previous hypotheses (1-3, 6). Also, evidence that K\(^{+}\) gradients, Na\(^{+}\)/K\(^{+}\) antiport, or proton gradients do not directly contribute to this transport mechanism was obtained.

Uptake was decreased when Na\(^{+}\) was equilibrated across the membrane by previous incubation with Na\(^{+}\) (12, 13, 15, 16, 27). This was not due to Donnan equilibrium effects or vesicle volume changes since equilibration with other cations such as K\(^{+}\) did not affect uptake, and monensin, an ionophore which dissipates a Na\(^{+}\) gradient by Na\(^{+}\)/H\(^{+}\) exchange (32), blocked concentrative uptake. Addition of ionophores for other cations by themselves did not affect uptake. I have shown previously (16) that the direction of accumulation of iso-Abu was reversibly dictated by the direction of the Na\(^{+}\) gradient such that iso-Abu accumulated trans to the side of the membrane with increased Na\(^{+}\) concentration.

The affinity of alanine, iso-Abu, and glutamine for transport into vesicles was increased in the presence of Na\(^{+}\) with minimal changes in \(V_{\text{max}}\). Although monensin decreased the stimulation of the initial rate of alanine uptake as a function of Na\(^{+}\) gradient, it did not abolish it. This suggests that the presence of Na\(^{+}\) in the absence of a Na\(^{+}\) gradient can modify the carrier to increase its affinity for the amino acid, as proposed in the Na\(^{+}\) gradient hypothesis (1, 2, 6).

Evidence for an electrogenic mechanism of Na\(^{+}\)-dependent amino acid uptake was obtained. Uptake was stimulated by conditions expected to create an interior-negative membrane potential, such as the presence of anions with increased permeability to biological membranes (33, 34), and by creation of a potassium diffusion potential (K\(^{+}\) internal > K\(^{+}\) external) in the presence of valinomycin. Suitable controls reinforced this interpretation. Nigericin, an ionophore which promotes a nonelectrogenic K\(^{+}\)/H\(^{+}\) exchange (32), did not stimulate uptake under these conditions. Valinomycin itself or accumulation of K\(^{+}\) did not affect initial rates of uptake. Furthermore, addition of nigericin abolished the stimulation of uptake by valinomycin plus a K\(^{+}\) gradient. Thus a K\(^{+}\) diffusion potential itself was not responsible for the stimulation but rather movement of K\(^{+}\) by an electrogenic process creating a transient interior-negative membrane potential (32). In the absence of Na\(^{+}\) no stimulation by valinomycin plus K\(^{+}\) gradient was observed. The stimulation by an interior-negative membrane potential predicts that transport involves movement of a positively charged complex across the membrane without a direct coupling of charge compensation to achieve electroneutrality to the transport process. Significant, this implies that in the presence of Na\(^{+}\) an additional driving force for concentrative amino acid uptake can be provided by other electrogenic membrane processes.

Thus the net force driving concentrative amino acid uptake is the electrochemical potential of Na\(^{+}\) across the membrane, \(\rho_{\text{Na}^+}\), which is the sum of electrical and chemical thermodynamic parameters. By analogy with Mitchell's protonmotive force (3),

\[
\rho_{\text{Na}^+} = \Delta \psi - 2.3 \frac{RT}{F} \log_{10} \text{[Na}^+]_{\text{in}}
\]

where \(\Delta \psi\) is the membrane potential and \(2.3 \frac{RT}{F}\) is 59 mV at room temperature. This represents the maximum energy available to the process; the actual energy coupled to amino acid movement is determined by the stoichiometry and kinetics of the interaction.

This mechanism predicts several possibilities for regulation of amino acid transport. Modification of the number or mobility of the carriers, direct hormonal regulation of \(\text{(Na}^+ + \text{K}^+)\)-ATPase activity (46), and specific alterations in membrane Na\(^{+}\) permeability or membrane potential have been suggested

| Table V: Effect of inhibitors on Na\(^{+}\) gradient-stimulated iso-Abu uptake |
|----------------|----------------|
| Initial rate  | Steady state  |
| (30 s)         | (15 min)      |
| Control        |                |
| 290           | 760            |
| Ouabain, 2 mM |                |
| 290           | 660            |
| Oligomycin, 250 \(\mu\)g/ml |                |
| 260           | 460            |
| 2,4-Dinitrophenol, 1 mM |                |
| 260           | 790            |
| Antimycin A, 490 \(\mu\)M |                |
| 220           | 500            |
| FCCP, \(10 \mu\)g/ml |                |
| 230           | 590            |

\(\Delta \psi\) Aliquots of 140 \(\mu\)g of vesicles from Balb SV3T3 were incubated for the indicated times after addition of 0.17 \(\text{mm}\) [\(l-\text{[1-3C]}\text{]iso-Abu}\) (91 \(\text{fmol/cpm}\)) and 50 \(\text{mm}\) NaCl. Ouabain and oligomycin were tested after 10 min preincubation with membranes; the other inhibitors were added at zero-time. Values are the average of duplicates with a range of \(\pm 15\%\).

\(\mathcal{W}\) FCCP, carbonyl cyanide p-trifluoromethoxyphenylhydrazone.

Evidence for interior-negative membrane potential was obtained under these conditions, by measurement of accumulation of \(\text{[3H]}\)triphenylmethylphosphonium ion (manuscript in preparation).
to contribute to regulation of iso-Abu carrier activity by hormones, cellular proliferative state, or SV40 transformation based on observations with membrane vesicles (16, 31).

Acknowledgments — I thank Dr. Renato Dulbecco for generous support and encouragement, Mrs. P. Pettican for excellent technical assistance, the Cell Production Unit for providing cell cultures, and Drs. H. R. Kaback, J. Robinson, L. A. Heppel, and E. Rozengurt for their comments on the manuscript.

REFERENCES
1. Riggs, T. R., Walker, L. M., and Christensen, H. N. (1958) J. Biol. Chem. 233, 1479-1484
2. Crane, R. K. (1960) Physiol. Rev. 40, 789-825
3. Mitchell, P. (1961) Chemiosmotic Coupling and Energy Transduction, Glynn Research Laboratories, Bodmin, Cornwall, England
4. Harold, F. M. (1972) Bacteriol. Rev. 36, 172-230
5. Mitchell, P. (1973) J. Bioenerg. 4, 63-91
6. Schultz, S. G., and Curran, P. F. (1970) Physiol. Rev. 50, 637-718
7. Gibb, L. E., and Eddy, A. A. (1972) Biochem. J. 129, 979-981
8. Schafer, J. A., and Heinz, E. (1971) Biochim. Biophys. Acta 249, 15-33
9. Ramos, S., Schultz, S. R., and Kaback, H. R. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 1831-1835
10. Kaback, H. R. (1974) Science 186, 882-892
11. Ikawa, S., and Cuatrecasas, P. (1971) J. Biol. Chem. 246, 2472-2479
12. Hopfer, U., Nelson, K., Perrotto, J., and Isselbacher, K. J. (1973) J. Biol. Chem. 248, 25-32
13. Colombini, M., and Johnstone, R. M. (1974) J. Membrane Biol. 15, 261-276
14. Hochstadt, J., Quinlan, D. C., Raker, R. L., Li, C.-C., and Dowd, D. (1974) in Methods in Membrane Biology (Korn, W., ed) Vol. 5, pp. 117-162, Plenum Press, New York
15. Hamilton, R. T., and Nilsen-Hamilton, M. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 1907-1911
16. Lever, J. E. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 2614-2618
17. House, W., Shearer, M., and Maroudas, N. G. (1972) Exp. Cell Res. 71, 293-296
18. Quinlan, D. C., and Hochstadt, J. (1976) J. Biol. Chem. 251, 344-354
19. Avruch, J., and Wallach, D. F. H. (1971) Biochim. Biophys. Acta 233, 334-347
20. Kamat, V. B., and Wallach, D. F. H. (1965) Science 148, 1343-1346
21. Sotocosa, G. L., Kuylenstierna, B., Ernest, L., and Bergstrand, A. (1967) J. Cell Biol. 32, 415-438
22. Sehnaitman, C., and Grenciwaltz, J. W. (1968) J. Cell Biol. 38, 158-175
23. Modolell, J. B., and Moore, R. O. (1967) Biochim. Biophys. Acta 113, 319-327
24. Essner, E., Novikoff, A. B., and Masek, B. (1958) J. Biophys. Biochem. Cytol. 4, 711-716
25. Akes, B. N. (1960) Methods Enzymol. 8, 115-118
26. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275
27. Quinlan, D. C., Farnes, J. R., Shalom, R., Garvey, T. Q., Isselbacher, K. J., and Hochstadt, J. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 1831-1835
28. Rosenburg, T., and Wilbrandt, W. (1957) J. Gen. Physiol. 41, 289-296
29. Bormann, H. B., Hagopian, A., and Eyal, E. H. (1968) Arch. Biochem. Biophys. 125, 81-98
30. Graham, J. M. (1972) Biochem. J. 130, 1113-1124
31. Lever, J. E. (1976) J. Cell. Physiol., in press
32. Harold, F. M., Altendorf, K. H., and Hirata, H. (1974) Ann. N. Y. Acad. Sci. 235, 149-160
33. Gamble, J. M., and Lehninger, A. L. (1973) J. Biol. Chem. 248, 610-618
34. Mitchell, P., and Moyle, J. (1967) Biochem. J. 105, 1147-1162
35. Glynn, I. M. (1964) Pharmacol. Rev. 16, 381-407
36. Hirata, H., Altendorf, K., and Harold, F. M. (1973) Proc. Natl. Acad. Sci. U. S. A. 70, 1804-1808
37. Pressman, B. C., Harris, E. J., Jagger, W. S., and Johnston, J. H. (1965) Proc. Natl. Acad. Sci. U. S. A. 58, 1949-1956
38. Mueller, P., and Rudin, D. O. (1967) Biochem. Biophys. Res. Commun. 26, 396-404
39. Oxender, D. L., and Christensen, H. N. (1965) J. Biol. Chem. 238, 3686-3689
40. Christensen, H. N. (1969) Adv. Enzymol. Relat. Areas Mol. Biol. 32, 1-29
41. Inui, Y., and Christensen, H. N. (1966) J. Gen. Physiol. 50, 203-224
42. Christensen, H. N., Liang, M., and Archer, E. G. (1967) J. Biol. Chem. 242, 5237-5246
43. Vidaver, G. A. (1964) Biochemistry 3, 662-667
44. Christensen, H. N., and Handlogten, M. E. (1975) Proc. Natl. Acad. Sci. U. S. A. 72, 23-27
45. Glynn, I. M., and Karlish, S. D. J. (1975) Annu. Rev. Physiol. 37, 13-35
46. Lever, J. E., Clingan, D., and Jimenez de Asua, L. (1976) Biochem. Biophys. Res. Commun. 71, 136-143
Active amino acid transport in plasma membrane vesicles from Simian virus 40-transformed mouse fibroblasts. Characteristics of electrochemical Na+ gradient-stimulated uptake.

J E Lever

J. Biol. Chem. 1977, 252:1990-1997.

Access the most updated version of this article at http://www.jbc.org/content/252/6/1990

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/252/6/1990.full.html#ref-list-1
Additions and Corrections

Vol. 251 (1976) 7816-7820

Transformation of arachidonic acid and homo-γ-linolenic acid by rabbit polymorphonuclear leukocytes. Monohydroxy acids from novel lipoygenases.

Pierre Borgeat, Mats Hamberg, and Bengt Samuelsson

The two monohydroxy acids isolated after incubation of arachidonic acid and homo-γ-linolenic acid should both have the "D" configuration, i.e. 5-n-hydroxy-6,8,11,14-eicosatetraenoic acid and 8-n-9,11,14-eicosatrienoic acid, respectively.

Oxidative ozonolysis of the methoxycarbonyl derivatives of the methyl esters afforded mainly the methoxycarbonyl derivatives of dimethyl 2-L-hydroxyadipate and of dimethyl 2-L-hydroxyazelate as described on p. 7818. Since the carboxethoxy group of these dioates that should be oriented upwards in the Fischer projection formulas (C-1) does not correspond to the carboxyl group of the parent unsaturated hydroxy acids, it follows that the latter acids should have the "D" configuration at C-5 (5-n-hydroxy-6,8,11,14-eicosatetraenoic acid) and at C-8 (8-n-hydroxy-9,11,14-eicosatrienoic acid).

Vol. 252 (1977) 1990-1997

Active amino acid transport in plasma membrane vesicles from simian virus 40-transformed mouse fibroblasts. Characteristics of electrochemical Na⁺ gradient-stimulated uptake.

Julia E. Lever

Page 1991, Right hand column, Legend to Fig. 1.

The concentration of [14C]iso-Abu was written as 1.69 mM instead of 0.17 mM. The correct line should be:

transformed cells, 0.17 mM [14C]iso-Abu (61 fmol/cpm) and 50 mM

Page 1993, Right hand column, Table II legend.

The concentration of [14C]iso-Abu was written as 1.1 mM instead of 0.11 mM. The correct line should read:

salts listed below and 0.11 mM [14C]iso-Abu (61 fmol/cpm) to 140 μg

Vol. 252 (1977) 3035-3043

Sidedness of (sodium, potassium)-adenosine triphosphatase of inside-out red cell membrane vesicles. Interactions with potassium.

Rhoda Blostein and Lily Chu

Page 3038, Table V

In the title, K_{cat} should be K_{cat}.

The heading should read

Effects of Na_{cat} on K_{cat} inhibition at 37°C

Under 0.05 μM ATP concentration, the value for E-P at 5.0 mM Na should be 0.168 pmol/mg.

The line should read

5.0 mM Na 0.168 23.8 142

Vol. 252 (1977) 3082-3091

Phosphorylation of yeast DNA-dependent RNA polymerases in vivo and in vitro. Isolation of enzymes and identification of phosphorylated subunits.

Graeme I. Bell, Pablo Valenzuela, and William J. Rutter

Page 3083, Left hand column, paragraph headed RNA Polymerase Assay, Line 4.

The following sentence should be added:

The concentration of KCl in the assay was 0.10 M.

Page 3083, Right hand column, paragraph headed Preparation of Cell Extract, Line 3.

The extraction buffer also contained 0.3 M ammonium sulfate.

The sentence should read:

The pellet was suspended with a glass rod and finally a glass...
Additions and Corrections

Vol. 251 (1976) 7816-7820

Transformation of arachidonic acid and homo-\(\gamma\)-linolenic acid by rabbit polymorphonuclear leukocytes. Monohy droxy acids from novel lipoxygenases.

Pierre Borgeat, Mats Hamberg, and Bengt Samuelsson

The two monohydroxy acids isolated after incubation of arachidonic acid and homo-\(\gamma\)-linolenic acid should both have the "D" configuration, i.e. 5-\(\delta\)-hydroxy-6,8,11,14-eicosatetraenoic acid and 8-\(\delta\)-9,11,14-eicosatrienoic acid, respectively.

Oxidative ozonolysis of the methoxycarbonyl derivatives of the methyl esters afforded mainly the methoxycarbonyl derivatives of dimethyl 2-\(\epsilon\)-hydroxyadipate and of dimethyl 2-\(\epsilon\)-hydroxyazelate as described on p. 7818. Since the carbomethoxy group of these diacids that should be oriented upwards in the Fischer projection formulas (C-1) does not correspond to the carboxyl group of the parent unsaturated hydroxy acids, it follows that the latter acids should have the "D" configuration at C-5 (5-\(\delta\)-hydroxy-6,8,11,14-eicosatetraenoic acid) and at C-8 (8-\(\delta\)-hydroxy-9,11,14-eicosatrienoic acid).

Vol. 252 (1977) 1990-1997

Active amino acid transport in plasma membrane vesicles from simian virus 40-transformed mouse fibroblasts. Characteristics of electrochemical Na\(^+\) gradient-stimulated uptake.

Julia E. Lever

Page 1991, Right hand column, Legend to Fig. 1.

The concentration of [\(\text{\textsuperscript{14}}\text{C}\)]iso-Abu was written as 1.69 mM instead of 0.17 mM. The correct line should be:

transformed cells, 0.17 mM [\(\text{\textsuperscript{14}}\text{C}\)]iso-Abu (61 fmol/cpm) and 50 mM

Page 1993, Right hand column, Table II legend.

The concentration of [\(\text{\textsuperscript{14}}\text{C}\)]iso-Abu was written as 1.1 mM instead of 0.11 mM. The correct line should read:

salts listed below and 0.11 mM [\(\text{\textsuperscript{14}}\text{C}\)]iso-Abu (61 fmol/cpm) to 140 \(\mu\)g

Vol. 252 (1977) 3035-3043

Sidedness of (sodium, potassium)-adenosine triphosphatase of inside-out red cell membrane vesicles. Interactions with potassium.

Rhoda Blostein and Lily Chu

Page 3038, Table V

In the title, \(K_{\text{V50}}\) should be \(K_{\text{E1000}}\).

The heading should read

Effects of \(Na_{\text{e1000}}\) on \(K_{\text{E1000}}\) inhibition at 37°

Under 0.05 \(\mu\)M ATP concentration, the value for \(E-P\) at 5.0 mM Na should be 0.168 pmol/mg.

The line should read

| 5.0 mM Na | 0.168 | 23.8 | 142 |

Vol. 252 (1977) 3082-3091

Phosphorylation of yeast DNA-dependent RNA polymerases in vivo and in vitro. Isolation of enzymes and identification of phosphorylated subunits.

Graeme I. Bell, Pablo Valenzuela, and William J. Rutter

Page 3083, Left hand column, paragraph headed RNA Polymerase Assay, Line 4.

The following sentence should be added:

The concentration of KCl in the assay was 0.10 M.

Page 3083, Right hand column, paragraph headed Preparation of Cell Extract, Line 3

The extraction buffer also contained 0.3 M ammonium sulfate.

The sentence should read:

The pellet was suspended with a glass rod and finally a glass

We suggest that subscribers photocopy these corrections and insert the photocopies at the appropriate places where the article to be corrected originally appeared. Authors are urged to introduce these corrections into any reprints they distribute. Secondary (abstract) services are urged to carry notice of these corrections as promptly as they carried the original abstracts.