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Amino Acid and $^{22}Na^+$ Uptake in Membrane Vesicles from Confluent Simian Virus 40 Transformed Balb/c3T3 and Balb/c3T3

Thomas Q. Garvey, III*, and Anita Babcock

Gastrointestinal Unit, Massachusetts General Hospital,
and Laboratory of Theoretical Biology, National Cancer Institute,
Bethesda, Maryland 20014

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Summary. The studies reported here were carried out to characterize further previously described changes in membrane localized amino acid transport associated with simian virus 40 transformation of the mammalian cell line, Balb/c3T3. Membrane vesicles were prepared from confluent cultures of both simian virus 40 transformed Balb/c3T3 (SV3T3) and the untransformed parent line, Balb/c3T3 (3T3). An initial, externally imposed out > in, 100 mM Na^+ gradient produces acceleration of early ingress of α-aminoisobutyric acid (AIB) in vesicles from both cell lines, but transient, concentrative uptake (overshooting) only in SV3T3 vesicles. Early ingress of L-leucine is also accelerated in SV3T3 vesicles by a Na^+ gradient, and overshooting is also demonstrable.

Na^+-gradient independent AIB permeability of SV3T3 and 3T3 membranes was estimated using uptake data, a first order rate equation and measurements of vesicle size derived from quasi-elastic light-scattering studies. AIB permeability of SV3T3 membranes is greater than that of 3T3 membranes (113 Å/min and 43 Å/min, respectively), suggesting that overshooting in 3T3 vesicles is not attenuated by a Na^+-independent AIB "leak". Na^+ permeability of the two membranes is similar, ruling out the possibility that a slower rate of Na^+ equilibration across the SV3T3 membrane allows development of the overshoot.

In SV3T3 vesicles the height of a Na^+-gradient dependent overshoot varies with the initial $[Na^+]_o/[Na^+]_i$ ratio, and $\ln[Na^+]_o/[Na^+]_i$ is linearly related to $\ln \frac{\text{AIB uptake at overshoot peak}}{\text{AIB uptake at equilibrium}}$, consistent with the possibility that for $[Na^+]_o/[Na^+]_i$ ratios in the range studied, AIB overshoot is energized by a constant proportion of the energy available from the initial electrochemical gradient for Na^+.

These results are consistent with the possibility that Na^+-gradient dependent overshooting in SV3T3 vesicles is produced by Na^+-amino acid carrier interactions resulting in either an increase in maximum transport velocity or an increase in carrier affinity for AIB.

Simian virus 40 transformation of the mouse cell line, Balb/c3T3[1] results in major changes in cellular morphology and growth orientation,

* *Address for reprint requests:* Bldg. 10, Room 4B-56, National Institutes of Health, Bethesda, Maryland 20014.

[1] *Abbreviations used:* 3T3, Balb/c3T3; SV3T3, simian virus 40 transformed Balb/c3T3; AIB, α-aminoisobutyric acid.

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loss of contact inhibition of growth [25], increases in maximal rates of nutrient absorption [3, 10] and loss of cell-density dependent modulation of absorption of certain nutrients [22]. Many of these virus transformation-associated changes are thought to be mediated by changes in the plasma membrane of the cell similar to those characteristic of malignant cells *in vivo* [3]. Hence, viral transformation has been considered by some investigators to be an appropriate model for studying abnormalities of cellular nutrient absorption and growth control associated with cancer and thought to be mediated by changes in the plasma membrane [7].

We have studied amino acid uptake into membrane vesicles prepared from SV3T3 in an effort to simplify the task of determining whether transformation-related changes in amino acid absorption in these cells are the result of changes in the cell membrane. The membrane vesicle system is an advantageous one in which to pursue this question since it is free of post-transport, metabolic influences, is independent of energy requiring mechanisms (e.g., $Na^+ - K^+$ ATPase), and allows study of the effects of variable and well-defined composition of both the intra- and extra-vesicular spaces [6]. Using vesicles from SV3T3, we have previously demonstrated Na^+-gradient stimulated, mediated transport of L-leucine and AIB into an osmotically sensitive space [23]. The results with AIB have been confirmed in several subsequent reports [4, 12]. We have also examined uptake of L-leucine and AIB into vesicles prepared from the untransformed parent line, Balb/c3T3, and reported that the transient, concentrative accumulation of L-leucine and AIB in response to an initial Na^+-gradient produced by NaCl, characteristic of vesicles from SV3T3, is much less prominent in 3T3 vesicles [21].

In the studies reported here, we have further characterized Na^+-dependent and Na^+-independent uptake of AIB and L-leucine by SV3T3 vesicles and compared these processes to Na^+-dependent and Na^+-independent uptake of AIB by membrane vesicles prepared from 3T3 cells.

Materials

Dulbecco's modified Eagle's medium was obtained from Microbiological Associates. Fetal calf serum was obtained from Grand Island Biological Company. L-(4,5-^{3}H)leucine, (Methyl-^{3}H)-AIB, and ^{22}NaCl were obtained from New England Nuclear. The amino acids were adjusted to a specific activity of 500 counts per min per pmol and ^{22}NaCl to 500 counts per min per nmol. Nitrocellulose filters 0.2 μM were obtained from the Millipore Corporation.

Methods

Balb/c3T3 (3T3) and SV40-transformed Balb/c3T3 (SV3T3) were obtained from Dr. Howard Green of the Massachusetts Institute of Technology and grown in 490 cm^2 plastic roller bottles at 37° in Dulbecco's modified Eagle's medium (DMEM) supplemented with 2 mM glutamine and 10% (vol/vol) fetal calf serum. Cells were harvested at a density of about 5×10^5 cells per cm^2, and membrane vesicles were prepared as previously described [6]. Following preparation, vesicles could be kept at 4° for at least two days without appreciable effect on uptake characteristics, or detectable bacterial contamination. Freezing at −20° or −70° resulted in almost complete loss of uptake activity.

Unless otherwise noted in the legends to figures, amino acid uptake was assayed in a 200 µl reaction mixture containing 50 mM potassium phosphate buffer pH 7.4, 125 mM sucrose and 500–800 µg of membrane protein preincubated for 10 min at 37°. Uptake was initiated by the addition of labeled amino acid with or without NaCl after pre-equilibration of the vesicles with or without NaCl. Aliquots of 15 µl were withdrawn at the time points described, diluted into 1 ml of 0.8 M NaCl at 37°, filtered, washed once with 3 ml of 0.8 M NaCl at 37° (this process was completed within 10 sec), placed in scintillation vials, dried, covered with 10 ml of Triton-Toluene-Liquifluor counting mixture, and assayed for radioactivity. In preliminary experiments it was determined that loss of amino acid associated with the vesicles into the hypertonic wash solution was negligible within 15 sec at 37°. The washing procedure was patterned after that used with bacterial vesicles for which abrupt lowering of temperature can result in "leakiness" [6]. In experiments where ^{22}Na uptake was assayed, tritiated amino acid and ^{22}Na were added to the assay mixture simultaneously, the assay carried out as described and ^{22}Na and ^{3}H counts assayed simultaneously. ^{3}H counting efficiency was about 50%, and ^{22}Na efficiency was about 80%. Zero-time uptake values, (presumably reflecting nonspecific adherence of counts to the millipore filter and vesicle binding, rather than uptake) were obtained by adding an appropriate quantity of radioactive substrate to a preincubated, prediluted aliquot of the complete assay mixture (containing vesicles) in 1 ml 0.8 M NaCl and filtering and washing immediately. Results of uptake experiments are expressed as pmol amino acid or nmol Na^+ accumulated per mg of membrane protein. Protein was estimated by the method of Lowry *et al.* [15] with bovine serum albumin as standard. Expression of vesicle uptake data as changes in intravesicular solute concentration might be of considerable value. However, attempts to calculate vesicle solvent water volume, using impermeant radioactive markers, were frustrated by what appeared to be binding of these markers to both the vesicles and the nitrocellulose filters.

Results

Figures 1 and 2 show the time courses of uptake of AIB and L-leucine, respectively, in membrane vesicles from confluent SV3T3. Uptake of both amino acids was rapid, and when no NaCl was present, or when the vesicles were pre-equilibrated for 20 min with NaCl, intravesicular accumulation had attained levels 1/3 to 1/2 that at equilibrium by 30 sec. When NaCl (100 mM) and amino acid were added simultaneously to initiate the uptake, a pronounced "overshoot" was produced with both amino acids. Transient, concentrative uptake to levels 3 times the equilibrium value for the vesicles could be produced consistently by this technique. A similar phenomenon

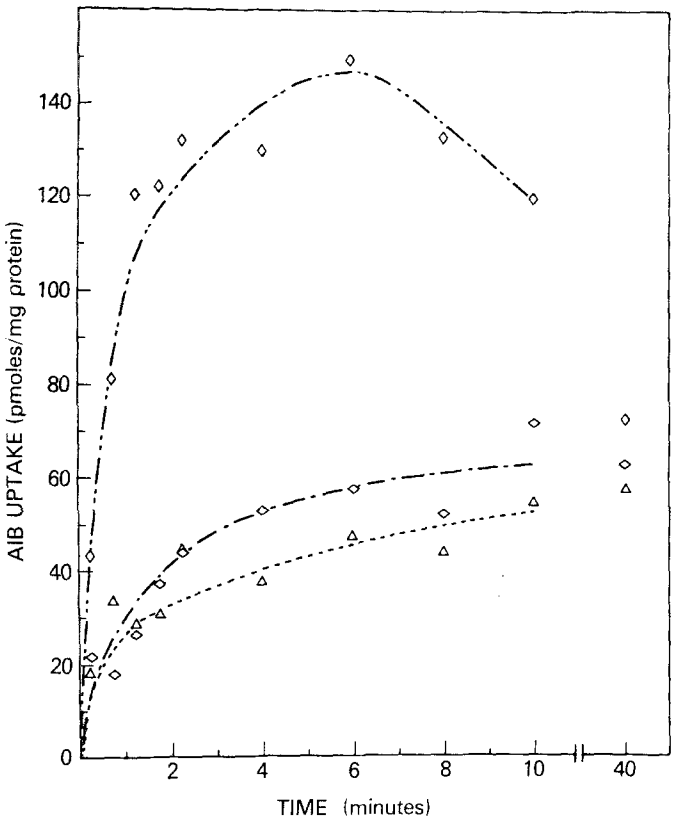

Fig. 1. The effect of 100 mM NaCl on AIB uptake in SV3T3 vesicles. Uptake was initiated by the addition of 100 μM ^{3}H-AIB either in the absence of NaCl (△), after 20 min preincubation with 100 mM NaCl (◇), or simultaneously with 100 mM NaCl (◊)

has been demonstrated in vesicles derived from renal and small bowel brush border [2, 5, 11, 24, 26], pancreatic acinar cells [27], Ehrlich ascites tumor cells [1], and has also been confirmed in SV3T3 vesicles [18, 13]. The effect in SV3T3 vesicles is quite specific for Na^+ (Li^+ shows 1/3 the potency of Na^+, and K^+, Mg^{++}, and Ca^{++} are all ineffective at 100 mM) and, as in vesicles from renal and intestinal brush border, can be enhanced by substitution of SCN^- for Cl^- (data not shown).

Figure 3 shows the time course of AIB uptake in membrane vesicles prepared from confluent 3T3. In the absence of NaCl the pattern is similar to that for SV3T3 vesicles with rapid attainment of uptake values 1/3 to 1/2 that at equilibrium. When uptake is initiated by simultaneous addition of AIB and NaCl (100 mM) significant acceleration of initial velocity of uptake

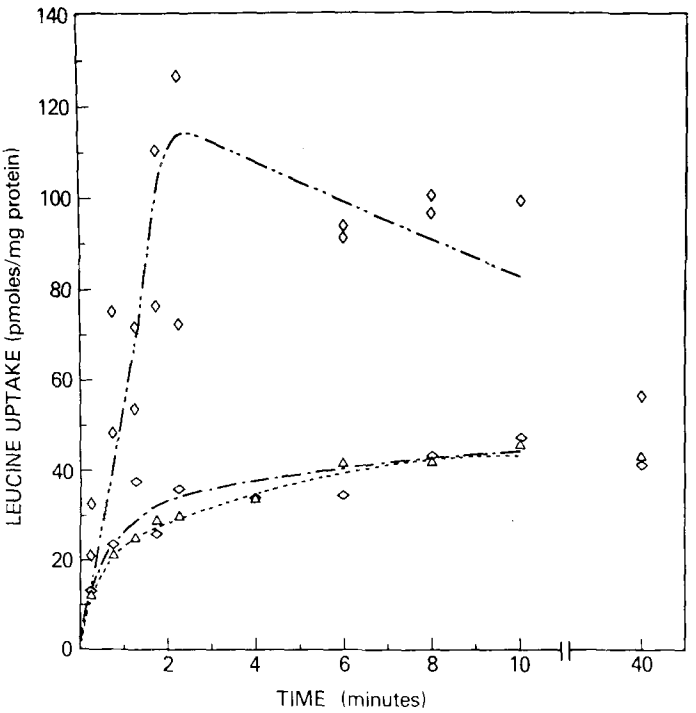

Fig. 2. The effect of 100 mM NaCl on L-leucine uptake in SV3T3. Uptake was initiated by the addition of 100 μM ^{3}H-L-leucine either in the absence of NaCl (△), after 20 min preincubation with 100 mM NaCl (◇), or simultaneously with 100 mM NaCl (◊)

of amino acid is apparent, but the overshoot consistently observed with SV3T3 vesicles under similar experimental conditions is not apparent. A previous series of experiments produced similar results [21] and, in addition, showed that density-related modulation of rates of amino acid uptake seen in many nontransformed whole cells [3] was demonstrable in experiments with vesicles prepared from subconfluent and confluent 3T3.

Data similar to those shown in Figs. 1, 2 and 3 have been used to estimate initial velocities of uptake in vesicles [4, 12, 13, 18]. Vesicles from both SV3T3 and 3T3, however, have accumulated about 1/3 the equilibrium uptake value by 15 sec, the earliest technically possible time point obtainable by the method used in these studies. Amino acid egress from vesicles 1/3 full will be significant and the uptake rate at 15 sec will not represent the initial velocity of amino acid ingress. Another factor complicating comparison of uptake data from different vesicle systems may be introduced by significant heterogeneity in vesicle size [8].

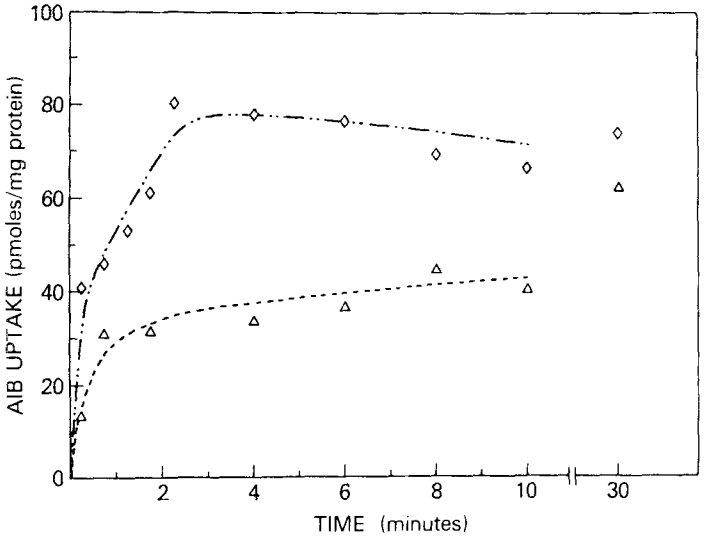

Fig. 3. The effect of 100 mM NaCl on AIB uptake in 3T3 vesicles. Uptake was initiated by the addition of 100 μM ^{3}H-AIB either in the absence of NaCl (△) or simultaneously with 100 mM NaCl (◊)

If it is assumed that the vesicles used in these experiments are spherical and that Na^+-gradient independent amino acid uptake by the vesicles is a symmetrical, diffusion-driven process during which intravesicular amino acid concentrations are within a linear range for efflux via a facilitated diffusion mechanism, then the transmembrane movement of amino acid can be described by a simple first-order rate equation:

$$\frac{ds}{dt} = 4\pi R^2 \phi (C_0 - C) \tag{1}$$

where C_0 and C are the extra- and intra-vesicular amino acid concentrations respectively, ϕ a diffusion, or rate constant with units Å/min, and R the vesicle radius. If this expression is divided through by the vesicle volume $4\pi R^3/3$, and integrated, Eq. (2) is obtained, which describes the intra-vesicular amino acid concentration as a function of ϕ, R, and t:

$$C = C_0 \left(1 - e^{\frac{-3\phi}{R} t}\right). \tag{2}$$

For this model, the time to half-equilibration of the intravesicular space $t_{1/2} = 0.23R/\phi$, which has been proposed as a measure of rate of transmembrane substrate movement varies directly with the vesicle radius and cannot

be used to compare amino acid permeabilities for vesicles from SV3T3 and 3T3 cell membranes in the absence of estimates of the vesicle radii.

The rate of accumulation of substrate for this model is:

$$\frac{ds}{dt} = 4\pi R^2 C_0 \phi \, e^{\frac{-3\phi}{R}t}. \tag{3}$$

In practice, as in the results reported here, uptake is usually expressed as moles of substrate accumulated per mg of protein *vs.* time. The protein associated with these vesicles is within a thin membrane enclosing a relatively large, spherical volume. The amount of protein associated with each vesicle is, then, nearly proportional to the surface area of the vesicle, i.e., protein per vesicle $= P \cdot 4\pi R^2$ (where P is the quantity of protein per unit of vesicle surface area). If data are expressed as substrate accumulated per mg of protein *vs.* time, the initial velocity will be $\phi C_0/P$, i.e., inversely proportional to P, which may well vary for vesicles from different sources. For a facilitated diffusion system in vesicles from one source, studied under uniform conditions, P will be constant. If, however, experiments with vesicles from different sources are analyzed by conventional initial velocity techniques using data expressed as substrate accumulated per mg of vesicle protein, the influences of differing vesicle radius and protein content remain unaccounted for and may compound those associated with inaccurate initial velocity determinations.

Rearranging Eq. 2 and taking the natural logarithm of both sides:

$$\ln\left[\frac{C_0 - C}{C_0}\right] = \frac{-3\phi}{R}t. \tag{4}$$

Although C_0, the extravesicular concentration of amino acid is known, C, the amino acid concentration prior to equilibrium cannot be conveniently estimated. If it is assumed, however, that the equilibrium uptake value is that at which the transmembrane amino acid concentration gradient has dissipated, resulting in equivalent intra- and extra-vesicular concentrations, then $(C_0 - C)/C_0$ for the Na^+-independent uptake shown in Figs. 1, 2 and 3 may be approximated by $(U_e - U)/U_e$ (where U_e is uptake in pmol/mg protein at equilibrium, and U is uptake in pmol/mg protein at various time points prior to equilibrium) and the fit of the data to Eq. 4 tested. In Fig. 4 data from Figs. 1, 2 and 3 are replotted according to Eq. (4). In each of the three cases the data points are fitted well by a straight line. All three fitted lines, however, have nonzero intercepts. This may indicate a significant component of binding of amino acid to the vesicles not accounted for by the estimate of binding described under *Methods.*

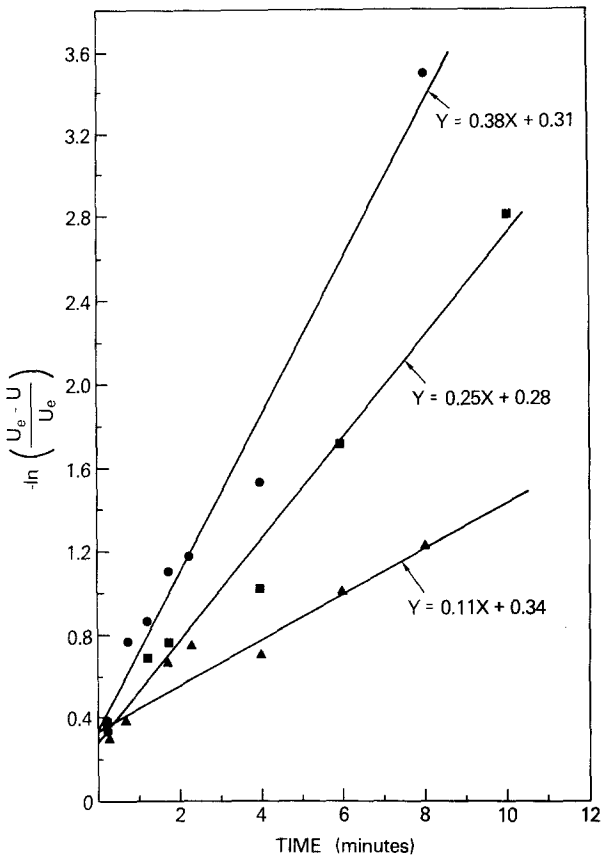

Fig. 4. Na^+-independent uptake of AIB and L-leucine in SV3T3, and AIB in 3T3 vesicles. The data are from the experiments shown in Figs. 1, 2, and 3 and are plotted as $-\ln (U_e - U/U_e)$ *vs.* time. (■) AIB and (●) L-leucine in SV3T3; (▲) AIB in 3T3

Previously reported experiments with SV3T3 in which the osmolarity of the extra-vesicular medium was systematically varied with sucrose also produced evidence of significant binding [22]. Replots of data derived from studies of Na^+-independent AIB uptake in SV3T3 vesicles reported by other investigators [12, 18] according to Eq. (4) also give nonzero intercepts similar in magnitude to those in Fig. 4.

Another explanation for the nonlinearity of these replots is heterogeneity of vesicle size. One consequence of such heterogeneity would be a pattern of progressively decreasing slope in the log replot, a pattern which might be reasonably well fitted by a straight line with a nonzero intercept.

The composite time constant $-3\phi/R$ in Eq. (2) can be derived directly from an uptake *vs.* time plot (e.g., Fig. 1), using $T_{1/2}$, or may be calculated

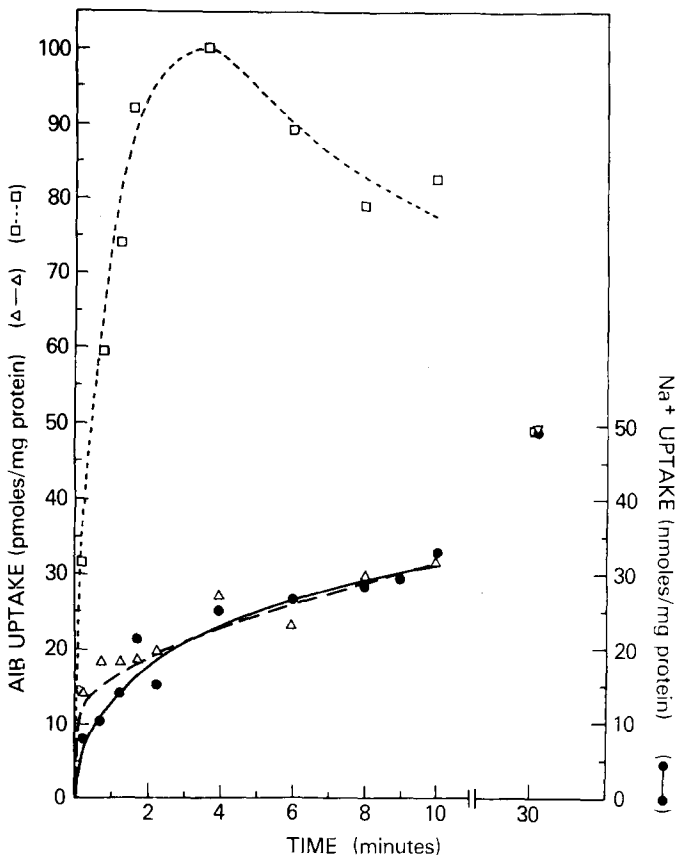

Fig. 5. Uptake of $^{22}Na^{+}$ and AIB by SV3T3 vesicles. Uptake was initiated by addition of 100 μM ^{3}H-AIB either in the absence of NaCl (△) or simultaneously with 100 mM ^{22}NaCl ^{3}H-AIB (□), ^{22}Na (●)

using the slope of the log replot. The slopes of the lines in Fig. 4 are 0.25 min^{-1} and 0.38 min^{-1} for AIB and L-leucine, respectively, in SV3T3 vesicles and 0.11 min^{-1} for AIB in 3T3 vesicles. Using a quasi-elastic light-scattering technique[2] diameters of 2750 and 2365 Å were measured for SV3T3 and 3T3 vesicles, respectively. The distribution of the vesicle diameters was unimodal in both cases with variances of about 35%.

These large variances lend weight to the possibility that the nonlinearity of the replotted data in Fig. 4 is the result of inhomogeneity of vesicle size. It is, however, clear that the mean diameters for SV3T3 and 3T3 vesicles do not differ significantly and that the distribution of these diameters about

[2] G. Williams, and D. Litster, (*manuscript in preparation*).

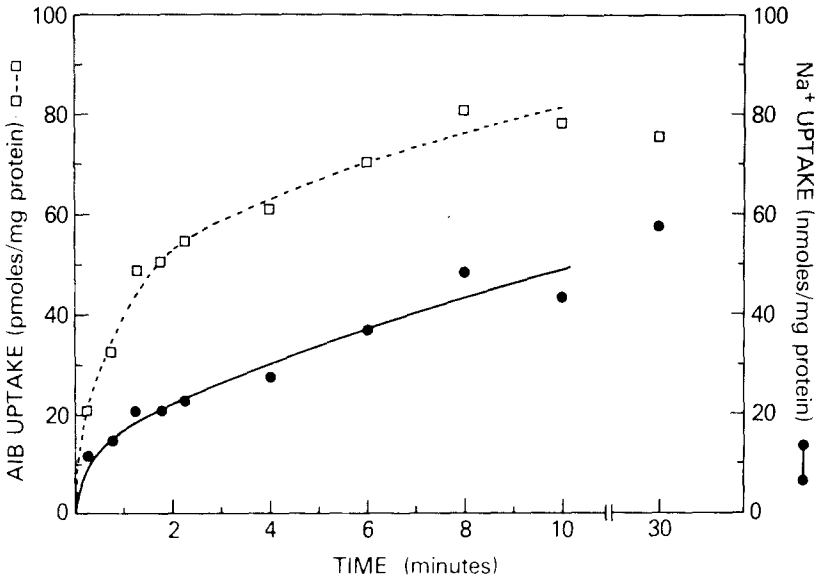

Fig. 6. Uptake of $^{22}Na^+$ and AIB by 3T3 vesicles. Uptake was initiated by the simultaneous addition of 100 μM ^{3}H-AIB and 100 mM ^{22}NaCl. (□) ^{3}H-AIB, (●) $^{22}Na^+$

their respective means are similar. Thus, a rough estimate of the relative permeabilities of the two membranes for AIB may be derived from comparison of the slopes of the lines in Fig. 4. The calculated "flux" constants are 113 Å/min and 171 Å/min for diffusion of AIB and L-leucine, respectively, into SV3T3 vesicles, and 43 Å/min for diffusion of AIB into 3T3 vesicles. These data suggest that the Na^+-independent permeability of SV3T3 membranes to AIB is somewhat greater than the Na^+-independent permeability of 3T3 membranes to the same amino acid, and further that the absence of an overshooting response in 3T3 vesicles is not a consequence of greater efflux of intra-vesicular AIB via a Na^+-independent "leak."

One possible explanation for the much diminished or absent overshooting response of the 3T3 vesicles to a Na^+ gradient is that the 3T3 membrane is more permeable to Na^+ than the SV3T3 membrane, allowing more rapid dissipation of the Na^+ gradient which, putatively, energizes the overshoot. Figures 5 and 6 show the results of double-label experiments in which the ingress of Na^+ and AIB was monitored after simultaneous addition of AIB (100 μM) and ^{22}NaCl (100 mM) to vesicles from SV3T3 (Fig. 5) and 3T3 (Fig. 6). Transient concentration of AIB to a level twice that at equilibrium is apparent in the SV3T3 vesicles in contrast to the absence of an appreciable overshoot in 3T3 vesicles. The pattern of Na^+ ingress appears

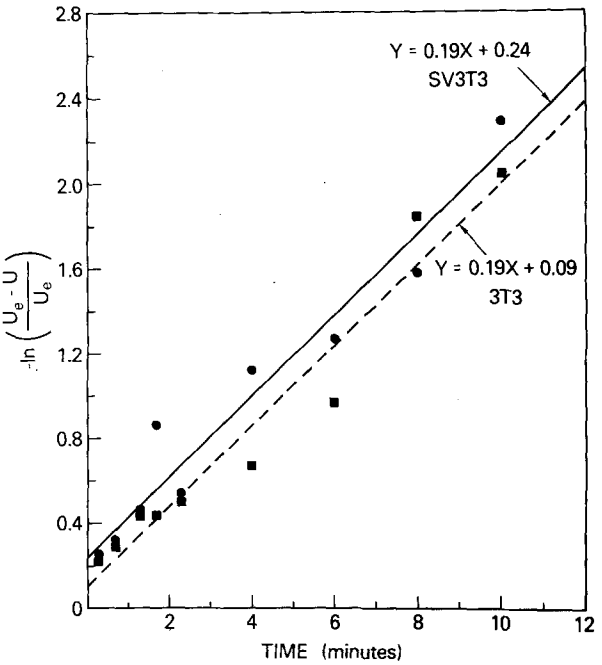

Fig. 7. $^{22}Na^+$ uptake in SV3T3 and 3T3 vesicles. The data are from the experiments shown in Figs. 6 and 7 and are plotted as $-\ln(U_e - U/U_e)$ *vs. t.* (●) SV3T3, (■) 3T3

similar for the two types of vesicles, resembles that for unstimulated AIB ingress, and is suggestive of a diffusion mechanism.

If the Na^+ uptake data in Figs. 5 and 6 are replotted as $\ln(U_e - U)/U_e$ *vs. t*, and straight lines fitted to the data by the least squares method, as shown in Fig. 7, the slopes of these lines are identical and the nonzero intercepts nearly identical. Although vesicle diameters were not obtained for the preparations used in these experiments, measurements made on other vesicle preparations, such as those used in the experiments of Figs. 1 and 2, show that SV3T3 and 3T3 vesicle diameters differ little. These data indicate that the permeabilities of the SV3T3 and 3T3 membranes to Na^+ are of similar magnitude, making unlikely the possibility that the absence of overshooting response to Na^+ in 3T3 vesicles is related to more rapid collapse of the Na^+ gradient.

The data in Fig. 8 indicate that overshooting uptake of AIB in SV3T3 is correlated with the magnitude of a transmembrane $[Na^+]_o/[Na^+]_i$ ratio as opposed to an initial Na^+ concentration difference. In these experiments, vesicles from SV3T3 were pre-equilibrated with either buffer, NaCl (100 mM) or KCl (100 mM) for 20 min, and uptake of AIB initiated by the

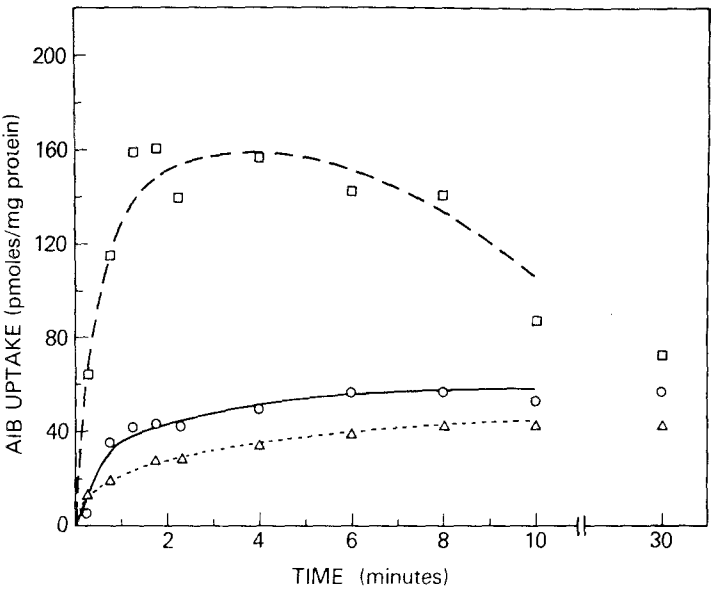

Fig. 8. Effect of pre-equilibration of vesicles with 100 mM NaCl on Na^+-stimulated AIB uptake. Uptake was initiated by addition of 100 μM ^{3}H-AIB in the absence of NaCl (△), simultaneously with 100 mM NaCl after pre-equilibration with 100 mM KCl (□), or simultaneously with 100 mM NaCl after pre-equilibration with 100 mM NaCl (○)

simultaneous addition of 100 μM AIB and 100 mM NaCl in each case. An overshoot was produced in vesicles pre-equilibrated in buffer or 100 mM KCl (where the initial transmembrane Na^+ concentration difference was 100 mM) and was also seen after initiating uptake by simultaneous addition of 100 μM AIB and 200 mM NaCl to vesicles pre-equilibrated in buffer (data not shown). In vesicles pre-equilibrated with 100 mM NaCl, where the initial transmembrane Na^+ concentration difference after simultaneous addition of 100 mM NaCl and 100 μM AIB to initiate uptake was also 100 mM, no overshoot occurred. These data indicate that a transmembrane Na^+ concentration difference of 100 mM is not sufficient to produce an overshoot, and are consiststent with dependence of the overshoot on the initial $[Na^+]_o/[Na^+]_i$ ratio (infinite for the case of pre-equilibration of the vesicles with buffer or KCl, and 2 for the case of pre-equilibration with 100 mM NaCl).

Figure 9 shows the results of a series of experiments in which vesicles from SV3T3 were pre-equilibrated with increasing concentrations of NaCl, and uptake initiated by simultaneous addition of 100 mM NaCl and 100 μM AIB. The height of the overshoot peak clearly decreases with increasing initial intravesicular sodium concentration. At an initial in-

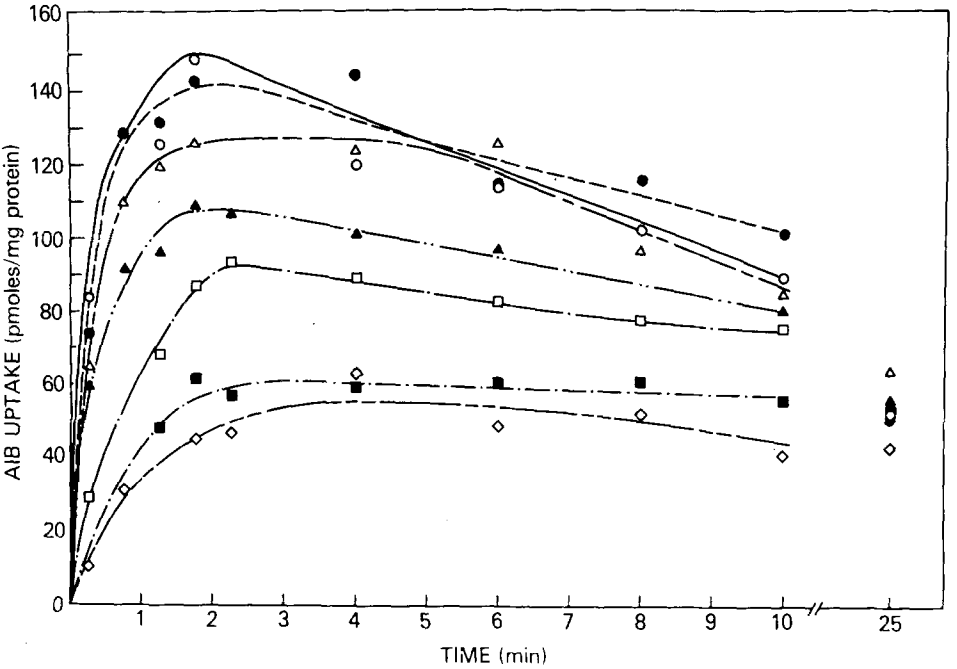

Fig. 9. Effect of pre-equilibration of vesicles with varying concentrations of NaCl on Na^+-stimulated AIB uptake. Uptake was initiated by the simultaneous addition of 100 μM ^{3}H-AIB and 100 mM NaCl to vesicles pre-equilibrated with varying concentrations of NaCl: 1 mM (○) 2 mM (●), 4 mM (△), 10 mM(▲), 20 mM (□), 50 mM (■) and 100 mM (◇)

travesicular NaCl concentration of 50 mM no overshoot is present, while at an initial intravesicular NaCl concentration of 20 mM a small overshoot can be discerned. These findings are consistent with the data in Fig. 5 which show the AIB ingress curve slowing significantly 1 min 15 sec after initiation of uptake, at a point where the Na^+ ingress curve indicates a $[Na^+]_o/[Na^+]_i$ ratio in the range of 3 to 4. Although no overshooting occurs with initial intravesicular Na^+ concentrations of 50 or 100 mM, the early rate of AIB ingress appears faster for 50 mM. Inspection of Fig. 6 also indicates that, although an initial $[Na^+]_o/[Na^+]_i$ ratio of 2 does not produce overshooting, the early rate of AIB uptake is more rapid than in the absence of a Na^+ gradient.

In Fig. 10 data from the experiments shown in Fig. 9 are replotted as

$$\ln \frac{\text{AIB uptake at overshoot peak}}{\text{AIB uptake at equilibrium}} \text{ vs. } \ln \frac{[Na^+]_o}{[Na^+]_i}.$$

The data replotted in this way can be fitted to a straight line. The fit is good and is consistent with transduction of a constant proportion of the

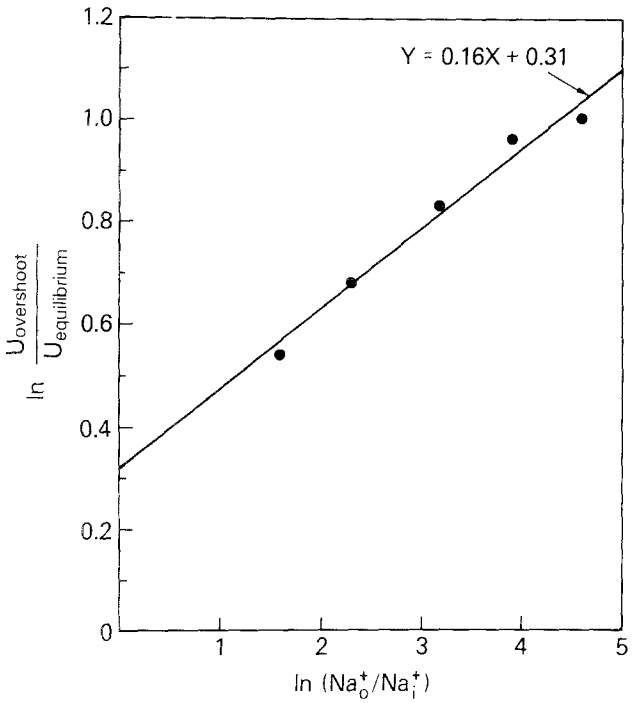

Fig. 10. The effect of varying initial $(Na^+)_o/(Na^+)_i$ ratios on overshoot of AIB in SV3T3 vesicles. The data are derived from the experiment shown in Fig. 9, and are plotted as $\ln \frac{\text{AIB uptake at overshoot peak}}{\text{AIB uptake at equilibrium}}$ *vs.* $\ln[Na^+)_o/[Na^+]_i$. The Na^+ concentrations are those prevailing at the initiation of ^{3}H-AIB uptake

energy available from the initial concentration gradient for Na^+, $RT \ln \frac{[Na^+]_o}{[Na^+]_i}$, into a concentration gradient for AIB, formation of which, if energy loss involved in membrane localized transport processes is assumed to be negligible, requires energy equivalent to $RT \ln \frac{[AIB]_i}{[AIB]_o}$. Analysis of these data by fitting to a straight line does not, of course, rule out the possibility that a fit to a nonlinear function, consistent with a different mechanism, might more accurately describe the uptake process.

An estimate of the tightness of coupling of AIB ingress to the energy of the Na^+ gradient, which would be of considerable interest, cannot be obtained from these data since the initial Na^+ gradient decrements too rapidly for the maximal $[AIB]_i/[AIB]_o$ ratio, which is needed to estimate coupling, to be achieved.

Discussion

Membrane vesicles prepared from confluent cultures of the virally transformed cell line, SV3T3, are are capable of transient concentration of AIB and L-leucine in response to an initial out > in concentration gradient for Na^+ as shown in Figs. 1 and 2. This capability is either greatly attenuated or absent in membrane vesicles prepared from confluent cultures of the untransformed parent line, 3T3 (Fig. 3). These results were obtained using vesicles prepared from SV3T3 and 3T3 cultures of similar cell density, under experimental conditions in which membrane associated ion pumps would not be active, and reveal a transformation-associated, cell membrane localized difference between SV3T3 and 3T3 which may be related to differences in sodium-stimulable nutrient uptake rates in whole cells. Overshooting of intravesicular amino acid and hexose concentrations in response to a Na^+ gradient has been demonstrated previously in many systems. Absence of the overshooting response has been documented using amino acids and D-glucose in vesicles prepared from the basolateral membranes of small bowel and renal epithelium [9, 16]. The critical differences between those membranes manifesting overshooting and those which do not have not been identified.

The finding of nonzero intercepts for the fitted lines in Fig. 4 is consistent with either a component of binding in uptake of amino acid by both SV3T3 and 3T3 vesicles, heterogeneity in vesicle size, or a combination of both factors. A relatively rapid binding process resulting in binding of B pmoles amino acid/mg protein would displace the $-\ln\left(\frac{U_e - U}{U_e}\right)$ *vs.* t line upward, giving a positive y-intercept of $\ln\left(\frac{U_e}{U_e - B}\right)$ and would have no effect on the slope. Thus in the presence of binding, the slope of a $-\ln\left(\frac{U_e - U}{U_e}\right)$ *vs.* t replot of uptake data can still be used to estimate membrane permeability. If inhomogeneity of vesicle size accounts for nonlinearity in the replots of Fig. 4, comparison of the slopes of the three fitted lines still may give a reasonable qualitative assessment of the relative permeabilities since SV3T3 and 3T3 vesicles have similar mean radii and similar distributions about these means. The flux constants derived for Na^+-independent AIB uptake suggest that SV3T3 membranes are somewhat more permeable to AIB than are 3T3 membranes. This is consistent with SV3T3 membranes having either a higher density of carrier sites (a higher V_m) or an amino acid carrier with a higher affinity for AIB at a concentration of 100 μM (a lower K_m), but would not alone account for the overshoot in SV3T3 vesicles.

The Na^+-gradient dependent overshoot seen in SV3T3 vesicles with L-leucine as substrate clearly indicates that transport of this amino acid is also a Na^+ accelerated process. Studies in Ehrlich ascites cells have produced evidence for a Na^+-independent "L" system as a major pathway for L-leucine transport in these cells, and documented lower distributions ratios for L-leucine than for AIB [20]. The finding of Na^+ stimulation of L-leucine ingress in SV3T3 vesicles may merely reflect a difference from the Ehrlich ascites cell system. The greater permeability of the SV3T3 vesicles to L-leucine than to AIB may, however, mean that in intact cells L-leucine ingress is indeed Na^+-stimulable as is that of AIB, but that L-leucine moves out of the cell at a rate rapid enough to result in a low net forward flux, and a final distribution ratio lower than that for AIB.

Overshooting in SV3T3 vesicles in response to an initial Na^+ gradient can be greatly attenuated, or abolished by the Na^+ ionophore, gramicidin [19]. This effect is presumably produced by rapid collapse of the initial Na^+ gradient. Greater permeability to Na^+ in the 3T3 membranes could explain the absence of overshoot in 3T3 vesicles. That this is not the case, however, is clear from the data in Figs. 5, 6 and 7 which suggest that the Na^+ permeability of SV3T3 and 3T3 membranes is of similar magnitude. These results appear to exclude the possibility that an initial Na^+ gradient in 3T3 vesicles collapses too rapidly to allow development of an overshoot of intravesicular AIB concentration.

Further investigation of the overshoot phenomenon in SV3T3 vesicles (Figs. 8, 9 and 10) reveals that the maximal level of transient, intravesicular concentration of AIB varies with the magnitude of an externally imposed Na^+ gradient. These data are consistent with the possibility that the overshoot is energized by the chemical potential difference for Na^+ and are in agreement with findings of other investigators using vesicles from SV3T3 and other sources [14, 16, 19].

Although uptake in 3T3 vesicles does not respond to a Na^+ gradient by developing an overshoot (Fig. 4), there is clear evidence of acceleration of AIB uptake. This pattern is similar to that seen in SV3T3 vesicles when amino acid uptake is initiated in the presence of $[Na^+]_o/[Na^+]_i$ ratios which do not produce overshooting (Fig. 9) and suggests that the effects of a Na^+ gradient are qualitatively similar in the two types of vesicles and consist of generation of a transient asymmetry of influx and efflux, presumably by acceleration of influx. The magnitude and duration of this asymmetry determine whether an overshoot occurs. These effects could result from Na^+-related changes in either the V_m or the affinity of a putative amino acid carrier. Previous studies with other Na^+-dependent overshoot-

ing vesicle systems, directed at elucidating the nature of the interaction between Na^+ and a putative amino acid or sugar carrier have produced different results. Some have suggested Na^+-related increases in substrate affinity [4, 12, 14]. Others have indicated Na^+-related increases in V_m [8]. Data derived from studies in membrane vesicles in which K^+ and H^+ ionophores were used [2, 16, 19] are consistent with two roles for Na^+ in vesicles systems manifesting overshooting: Na^+-specific cotransport with nonelectrolyte (possibly as a ternary complex of Na^+-nonelectrolyte-carrier) and, in conjunction with a permeant anion, generation of an in-negative gradient energizing the overshoot.

The data presented here suggest that the major difference between SV3T3 and 3T3 cell membranes in relation to amino acid uptake lies in differences in interactions of the putative amino acid carrier with Na^+. A more precise understanding of how the Na^+-amino acid-carrier interactions differ in these membranes may explain differences in rates of amino acid uptake in the whole cells. A more detailed model of the uptake process is discussed in the accompanying paper. In this model, nonlinear methods are applied to obtain estimates of Na^+ and AIB transport parameters for the SV3T3 and 3T3 vesicle systems under all experimental conditions employed.

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