Regulation of Active Amino Acid Transport by Growth-related Changes in Membrane Potential in a Human Fibroblast*

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Indirect measurements of membrane potential in human diploid fibroblast cells indicate that there is a growth-related difference in membrane potential between subconfluent, growing cells (−49 mV) and serum-deprived, quiescent cells (−22 mV). The elevated membrane potential in growing cells appears to be solely responsible for the 2-fold higher α-aminoisobutyric acid accumulating capacity that we observed previously in growing cells, as compared to quiescent cells (Villereal, M. L., and Cook, J. S. (1977) J. Supramol. Struct. 6, 179-189). When the membrane potentials of quiescent and growing cells are set equal, using valinomycin and varying [K+]o, α-aminoisobutyric acid accumulation is dependent on the magnitude of the potential, but it is independent of the growth state of the cell. Initial α-aminoisobutyric acid influx measurements in growing and quiescent cells show that when cells become quiescent the Km increases and the Vmax of the transport system decreases. For growing cells, Km = 1.2 ± 0.09 mM and Vmax = 15.6 ± 0.9 μmol/g of protein/min. For quiescent cells, Km = 2.1 ± 0.18 mM and Vmax = 8.5 ± 0.8 μmol/g of protein/min. Serum stimulation of quiescent cells for 1 h results in a decrease in Km (to 1.2 ± 0.13 mM) and no change in Vmax (7.8 ± 0.8 μmol/g of protein/min). Hyperpolarization of quiescent cells by valinomycin also induces a decrease in Km (to 1.2 ± 0.13 mM) without a change in Vmax. This suggests that the immediate response of initial α-aminoisobutyric acid transport to serum stimulation is mediated by a membrane hyperpolarization. These data indicate that both the accumulation of α-aminoisobutyric acid in human diploid fibroblast cells and the Km for their transport are sensitive to experimentally induced changes in membrane potential, and furthermore, that the observed growth-related difference in membrane potential between growing and quiescent cells can account for their growth-associated differences in α-aminoisobutyric acid accumulation and Km.

The kinetics of amino acid transport have been studied in a variety of cell systems to determine whether alterations in amino acid transport rate coincide with changes in the state of cell growth. The Na+-dependent, A system for amino acid transport (1) has been of special interest because, by utilizing the energy stored in the Na+ electrochemical gradient, it can maintain a high concentration gradient (in excess of 20-fold) of its transported substrates (including glycine, alanine, and the nonmetabolizable α-aminoisobutyric acid). In general, it has been observed that when cells enter a state of quiescence, there occurs either a reduction in the maximal transport rate of the Na+-dependent, A system, an increase in its Km, or both, relative to the values for growing cells (2, 3). The mechanism(s) by which these kinetic parameters change with alterations in cell growth state is as yet not clear. The decrease in Vmax may result from a reduction in the number of available transport sites during quiescence, while an increase in Km during quiescence could be due either to a replacement of high affinity sites with those of lower affinity or to a growth related conformational change in existing transport sites.

However, recent evidence from our laboratory suggests that growth-associated changes in amino acid transport may involve more than alterations in the number or affinity of transport sites. We have demonstrated that in human fibroblasts, there is a higher AIB-accumulating capacity in growing cells than in quiescent cells (4). This observation suggests that there is either more energy available for this accumulation in growing cells or that growing cells utilize the available energy more efficiently. We have presented evidence (4) that the higher accumulation capacity in growing cells is due to a higher membrane potential which serves to energize the additional accumulation. In the present investigation, we seek to determine more accurately the magnitude of this growth-associated change in potential and the extent to which it is responsible for the growth-associated change in AIB accumulation. Also, since a recent report (5) indicates that the magnitude of an experimentally induced membrane potential is an important factor in determining the Km and Vmax of amino acid transport systems, it becomes important to investigate the possible role of a physiologically changing membrane potential in mediating growth-related changes in Km and Vmax of amino acid transport.

In this paper, we demonstrate that in human diploid fibroblast cells, an approximately 2-fold higher membrane potential in growing cells (−49 mV) than in quiescent cells (−22 mV) seems to be responsible for the growth-associated change in AIB accumulation. In addition, quiescent, serum-deprived HSWP cells have a lower Vmax and a higher Km for AIB transport than do their growing counterparts. Following serum stimulation of quiescent cells for 1 h, the Km falls to the value observed in growing cells. The decrease in Km in response to serum stimulation can be mimicked by hyperpolarizing serum-

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The abbreviations used are: AIB, α-aminoisobutyric acid; HSWP, human diploid fibroblasts.
deprived cells using the K+ ionophore valinomycin. Neither serum stimulation nor valinomycin stimulation of quiescent cells has any immediate effect on the Vmax for AIB transport.

MATERIALS AND METHODS

Cells and Growth—HSWP cells were derived from human foreskin by James D. Regan (Oak Ridge National Laboratory). They were cultured in Eagle's minimum essential medium (KC Biological, Inc.) containing 10% fetal calf serum (KC Biological, Inc.) and 25 μg/ml of gentamicin (Schering Corp.). Cells were grown at 37°C in a 5% air, 5% CO2 atmosphere and were used between the 10th and 20th passages. Cells were removed from stock flasks by trypsinization and were seeded onto individual coverslips (11 × 22 mm) in Petri dishes as originally described by Foster and Pardee (6) and modified by Salter and Cook (7). Twenty-four hours after plating, the cells were fed with growth media containing either 10% fetal calf serum (growing cells) or 0.1% fetal calf serum (serum-deprived cells). Transport measurements were performed on growing cells at subconfluent densities and on serum-deprived cells following 3 to 4 days on 0.1% fetal calf serum. Serum-deprived cells are considered to be quiescent when [3H]thymidine incorporation into the acid-insoluble fraction drops below 10% of growing controls. Approximately 18 to 22 h after serum stimulation (30% fetal calf serum) of previously serum-deprived cells, there is a burst of incorporation of [3H]thymidine into acid-insoluble material.

Transport Studies—Amino acid transport was measured using AIB, a nonmetabolizable substrate of the Na+-dependent, amino acid-concentrating transport system (A system). The assay medium consisted of amino acid-free Eagle's minimum essential medium with Hanks' salts, 20 mM HEPES (4-(2-hydroxyethyl)-1-piperazineneethane-sulfonic acid), and ~2 μCi/ml of [3H]AIB. The concentration of unlabeled AIB ranged from 0.1 to 10 mM. In some experiments, the Na+ and K+ concentrations of the assay media were altered by equimolar substitution of either potassium chloride or choline chloride for sodium chloride in the Hanks' salts. In all media, NaCl + KCl + CHCl = 144 mM. Valinomycin (Sigma Chemical Co.) was added (10 μM final concentration) to some assay media as an ethanol solution with the appropriate amount of ethanol also being added to control media (<0.25% final concentration). In a few transport studies, furosemide (Hoechst-Roussel Pharmaceuticals) was added at 1 mM to block cation exchanges.

Four-milliliter aliquots of assay medium were placed in shell vials (15 × 45 mm) (Kimble Products) and equilibrated at 37°C. Coverslips were removed from their growth media, drained by touching to an absorbent paper towel, and placed in shell vials for the required times. For initial transport measurements, the coverslips remained in the assay medium for 10 min, over which time uptake is linear at all concentrations. To terminate AIB uptake, the coverslips were removed from the assay medium and rapidly washed three times in cold Tris-buffered saline (pH 7.4). They were then drained and placed in a glass scintillation vial containing 1 ml of 0.1 N NaOH to lyse the cells. Following lysis, 0.1 ml of 1 N HCl and 10 ml of Triton X-100/toluene (1:2) counting solution, containing 5.5 g/liter of PermaBLEND I (Packard), were added. Radioactivity was determined in a Nuclear-Chicago Mark I spectrometer.

For each experimental point, triplicate measurements of [3H]AIB uptake were made and averaged. Initial influx, expressed as micromoles/g of protein/min, was calculated from

$$\text{influx} = \frac{dR_{\text{r}}/dt}{SA_{\text{c}}},$$

where $dR_{\text{r}}/dt$ represents the counts per min of [3H]AIB taken up per g of protein in 1 min and $SA_{\text{c}}$, is the specific activity of the extracellular phase (counts per min/pmol).

Protein Measurement—Protein was measured by the intrinsic fluorescence of tryptophan residues (8). Protein was solubilized by placing each coverslip in a shell vial containing 4 ml of 0.2% sodium dodecyl sulfate. The emission of the sodium dodecyl sulfate extract was measured using an excitation wavelength of 286 nm (Perkin-Elmer model 204 fluorescence spectrophotometer). Bovine serum albumin dissolved in 0.2% sodium dodecyl sulfate was utilized as a standard.

Water and Electrolyte Measurements—Cell water content was passively distributed in HSWP cells (1). Glucose, which is not transported in these cells, was used to measure extracellular medium not removed by washing. The water to protein ratio (micromolars of H2O/μg of protein) was 4.4 ± 0.10 (n = 5) for growing cells in the 6 mM K+, 138 mM Na+ assay medium. This ratio was not appreciably altered by any of the experimental manipulations (i.e. serum deprivation, valinomycin addition, decrease in [Na+]), increase to [K+]).

Na+ and K+ concentrations were determined flame photometrically using Li+ as an internal standard. Growing HSWP cells contain 140 ± 5 mM K+. This concentration does not change on quiescence and, in contrast to 3T3 cells (9), is not altered by serum stimulation. Addition of valinomycin leads to approximately an 8-fold enhancement of Nm (K+) analogs exchange rate in cells whose normal exchange mechanisms are inhibited by ouabain and furosemide, suggesting a large increase in K+ conductance. For cells in 1 mM furosemide and 0.1 mM ouabain, K+ influx is 0.96 μmol/g of protein/min, giving a rate constant for this component of the influx of 0.036 min−1 in 10 μM valinomycin, this rate constant is increased to 0.30 min−1. In the presence of valinomycin, there is a net loss of only about 10% of the internal K+ content over a period of 1 h. In the calculation of K+ equilibrium potentials, a value of [K+]o = 125 mM was used to reflect this small loss of K+ in the presence of valinomycin. This slow net loss measured in the presence of a very high exchange flux supports the assumption that HSWP cells in valinomycin are behaving essentially as a K+ electrode and further suggests that the intracellular K+ is maintained at high levels because of very low conductances of the cation channels.

Statistical Analysis—All error values are reported as ± S.E. For the double reciprocal plots, the error values for the slope and the intercept were determined (10) and used to calculate the S.E. for Vmax and Km. Since $Km = k_2 / k_1$, the S.E. for Vmax was determined first and compounded with the error of the slope to give S.E. for Kmax.

RESULTS

HSWP cells cultured in 10% fetal calf serum grow to 10-fold greater cell number in 8 days than in 6 days to 8 days after plating. In contrast, cells deprived of serum 24 h after plating (i.e. serum reduced to 0.1%) become quiescent within 3 to 5 days. Cells in serum-poor medium grow to densities of 12 ± 2 μg of protein/cm2 and can achieve confluent densities only upon restoration of normal serum levels to the growth medium. AIB transport was measured in both growing and serum-deprived cells 4 to 5 days subsequent to plating on coverslips, which corresponds to a time of quiescence for the serum-deprived cells and of logarithmic growth for the serum-sufficient cells.

In a recent publication (4), we showed that the steady state accumulation capacity of HSWP cells for AIB not only has the usual dependence on the external Na+ concentration observed in many other cell types (1), but in addition shows a dependence on the growth state of the cells. When assayed in a 138 mM Na+ environment, growing cells concentrate AIB 25-fold while serum-deprived, quiescent cells concentrate this nonmetabolized amino acid analog only 10-fold. For cells in either growth state, if the Na+ concentration is reduced from its normal 138 mM to 50 mM by substitution with choline, this accumulating capacity is substantially reduced to 8- and 5-fold, respectively. We suggested in this earlier publication that the growth-related difference in AIB accumulation, at this external Na+ concentration, might be due to a growth-related difference in membrane potential between quiescent and growing cells. In the present study, we further tested this hypothesis by systematically altering the membrane potential of both growing and quiescent cells and comparing the levels of AIB accumulation for cells in these two growth states. These experiments were based on the assumption that in the presence of the K+ ionophore, valinomycin the cell membrane behaves like a K+ electrode so that

$$E_m = \frac{RT}{F} \ln \left[\frac{[K^+]}{[K^+]_o}\right],$$

where $E_m$ is the membrane potential, [K+] and [K+]o are the
external and internal K⁺ concentrations, respectively, and R, T, and F have their usual meanings. Thus, by varying the [K⁺] in the presence of valinomycin, the membrane potential of either growing or quiescent cells could be set at any desired value.

When growing or quiescent cells are equilibrated with 0.1 mM AIB for 80 min in a control medium (6 mM K⁺, 50 mM Na⁺) and then transferred to a 50 mM Na⁺, 0.1 mM AIB environment containing valinomycin (10 μM), the final level of AIB accumulation is markedly influenced by the external concentration of K⁺ (Figs. 1 and 2). In these experiments, [Na⁺] was 50 mM so that [K⁺], could be varied over a wide range while the [Na⁺], remained invariant. The total concentration of cations (Na⁺, K⁺, and choline) remained constant at 144 mM. If we plot the log of the AIB accumulation ratio ([AIB]/[AIB]₀) obtained for each value of [K⁺], versus the valinomycin-induced membrane potential calculated for that value of [K⁺], we can compare the effect of membrane potential on AIB accumulation in growing and quiescent cells. The data in Fig. 3 demonstrate that there is a linear dependence of the logarithm of the AIB ratio on the membrane potential for both growing and quiescent cells. Furthermore, all the points for quiescent and growing cells fall on a single line, indicating that when quiescent and growing cells have the same membrane potential (no matter what the magnitude of that potential is), the AIB accumulation becomes independent of the growth state of the cell. This observation indicates that a growth-associated difference in membrane potential is indeed the underlying mechanism for the growth-associated difference in AIB accumulation.

Further analysis of the data from Figs. 1 and 2 allows us to estimate the membrane potential for control cells in either growth state. Note that for each growth state, there is one value of the valinomycin-induced membrane potential that does not alter the AIB accumulation from the control level. This occurs in a (21 mM K⁺ + valinomycin) medium for growing cells (Fig. 1) and a (50 mM K⁺ + valinomycin) medium for quiescent cells (Fig. 2). These null points should occur when the valinomycin-induced membrane potential is equal to the control (pre-valinomycin) membrane potential. Thus, evaluation of these null points gives an estimate of the membrane potential in control growing or control quiescent cells. Referring to the data in Fig. 3 (which are derived from those in Figs. 1 and 2), one can see that accumulation of AIB to the quiescent control level of 5.0 is obtained when the valinomycin-induced membrane potential is −22 mV, while accumulation of AIB to the growing control level of 8.0 is obtained when the valinomycin-induced membrane potential is −49 mV. Correspondingly, we estimate that the control membrane potential for quiescent cells is −22 mV and for growing cells is −49 mV in a 50 mM Na⁺ environment.

Not only the AIB accumulation capacity but also the initial AIB transport rate are higher in growing cells than in quiescent cells, as demonstrated in Fig. 4. Analysis of the double reciprocal plot indicates that in growing cells, the Kₘ is 1.2 ± 0.09 mM and the Vₘₐₓ is 15.6 ± 0.9 pmol/g of protein/min. When serum-deprived cells become quiescent, the Kₘ for AIB transport increases significantly (p < 0.01) to 2.10 ± 0.18 mM and the Vₘₐₓ decreases (p < 0.01) to 8.50 ± 0.8 pmol/g of protein/min, i.e., both the apparent affinity of the transport system and, presumably, the number of transporters decrease with quiescence.

In the next series of experiments, serum-deprived cells were serum-stimulated (20% fetal calf serum) for 1 h prior to the measurement of the initial transport parameters. The data in
the quiescent cells results in a significant (p < 0.01) decrease in 

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It is important to mention at this point that, since the influx of AIB occurs by co-transport with Na+, the carrier system as a whole behaves as a Na+ ionophore. One must consider how this coupled inward Na+ current affects the magnitude of the membrane potential during the various experimental manipulations. As low external AIB concentrations (as in the accumulation studies), the influx of AIB, and therefore the Na+ current, is low and would not be expected to measurably affect the membrane potential. However, as the AIB concentration is elevated, as is done for the determination of Vmax, the AIB-coupled Na+ influx increases and could act to discharge the membrane potential. Thus, it is conceivable that the reason the Vmax appears to be potential-insensitive is that at high AIB concentrations, the membrane potential has been discharged. However, we have preliminary data on Na+ and K+ conductive fluxes in HSWEP cells which argue against this possibility. Fluxes are measured with 82Rb+ (K+ analog) and 22Na+ in the presence of 10-4 M ouabain and 10-4 M furosemide to block carrier-mediated potassium-sodium, potassium-potassium, and sodium-sodium exchanges so that the (ouabain + furosemide)-insensitive influx serves as an estimate of conductive influx (11). Measurements of Na+ and K+ conductive fluxes in the presence of valinomycin give influx rate constants of 0.007 min-1 for Na+ and 0.30 min-1 for K+; the ratio of rate constants equals 0.023. In 10 mM AIB, the overall rate constant for Na+ influx rises to 0.014 min-1, so that the ratio of rate constants equals 0.047. From these values together with known cell and medium concentrations of the cations, one may calculate a (K+ + Na+) diffusion potential in the absence of AIB of -73 mV, and (K+ + Na+) diffusion potential in the presence of 10 mM AIB of -65 mV. The Na+ current at near maximum AIB influx thus reduces the valinomycin-induced potential by only about 10%. The lack of effect of valinomycin on Vmax indicates that the Vmax is indeed potential-insensitive.

It may of course be inappropriate to use the (ouabain + furosemide)-insensitive fluxes to estimate growth state-associated diffusion potentials and equate them with the membrane potentials. It is quite possible that there is a substantial electrogenic component in the membrane potential (12) and
this component may vary with growth state. We are testing these possibilities at present.

**DISCUSSION**

Early studies in Ehrlich ascites tumor cells (13, 14) demonstrated that an experimentally induced membrane hyperpolarization could provide the energy needed to increase amino acid accumulation capacity. This is further evidenced by recent observations that experimentally induced hyperpolarizations can enhance AIB accumulation in human fibroblasts (Figs. 1 and 2), in membrane vesicles derived from mouse fibroblasts (15), and in cell envelope vesicles prepared from *Halobacterium halobium* (16). Thus, it appears that, thermodynamically, an electrochemical potential difference can be an effective a driving force for amino acid accumulation as a chemical potential difference (i.e. a Na⁺ concentration gradient). In light of this information, it is important to consider whether a membrane potential-related mechanism might be responsible for the observed difference in AIB-accumulating capacity between growing and quiescent HSWP cells (4). Indirect measurements (Fig. 3) indicate that there is approximately a 2-fold difference in membrane potential between growing (−49 mV) and quiescent (−22 mV) HSWP cells incubated in a 50 mM Na⁺ environment. The data in Fig. 3 also indicate that when the growth-associated difference in membrane potential is eliminated, quiescent and growing cells accumulate AIB to the same level, which is dictated by the magnitude of the experimentally induced potential. This observation implies that the difference in membrane potential between growing and quiescent cells is the sole factor responsible for their different AIB accumulation capacities.

It has been shown by Oxender and his associates (17) that in BALB/3T3 cells, the concentration of intracellular amino acids tends to rise when cell growth and protein synthesis are inhibited by the approach to confluence, by serum deprivation, or by inhibitors like cycloheximide. These intracellular pool increases tend to accelerate the uptake of exogenous amino acids that are transported primarily by exchange (L system). The relationship, if any, of endogenous pools to transport on the A system is less clear (18). From Fig. 3, it is apparent that if there are changes in endogenous pools in HSWP cells with growth state, they are not responsible for the growth-associated difference in AIB-accumulating capacity. In preliminary analyses on acid-soluble amino acids in these cells, we find in fact that the total pool of A system substrates tends to decrease slightly, rather than rise, as the cells become quiescent (data not shown).

Recently, studies have demonstrated that the initial transport kinetics (5) as well as the level of accumulation of amino acids (Figs. 1 and 2) can be affected by induced alterations of membrane potential. These studies show that the membrane potential can be expected predominately to affect either the $K_m$ or $V_{\text{max}}$ of the transport system, depending on whether the loaded (Na⁺ + AIB + carrier) or empty carrier is the charged species. It was, therefore, important to consider whether a growth-related change in membrane potential could also be responsible for the observed growth-related differences in the $K_m$ and $V_{\text{max}}$ for AIB transport. Serum stimulation experiments indicated that no single factor is responsible for the growth-related changes in both $V_{\text{max}}$ and $K_m$. The changes in these two transport parameters, following the serum stimulation of previously quiescent cells, are seen to occur with different time courses (Fig. 3). The $V_{\text{max}}$ is not appreciably altered even after 6 to 8 h of serum stimulation.

We have previously proposed that a hyperpolarization occurs within 1 h following serum stimulation (4). Our argument was based on the observation that differences in AIB-accumulating capacity among growing (25-fold), quiescent (3-fold), and serum-stimulated (17-fold) cells could be abolished if the cells were all clamped at the same membrane potential by the addition of valinomycin, suggesting that the only difference among them in mediating AIB-accumulating capacity was the growth state-associated membrane potential. In other words, since cells serum-stimulated for 1 h have already developed almost twice the AIB-accumulating capacity of the quiescent cells, serum stimulation must lead to a rapid hyperpolarization. On the other hand, since $V_{\text{max}}$ does not increase until many hours later, it is unlikely that the higher $V_{\text{max}}$ in growing cells is directly mediated by this hyperpolarization. This is supported by the observation that the $V_{\text{max}}$ is not measurably altered by a valinomycin-induced hyperpolarization of quiescent cells. The change in $K_m$, on the other hand, occurs within 1 h following serum stimulation of quiescent cells, and therefore could well be mediated by the membrane hyperpolarization. This contention is strongly supported by the observation that a valinomycin-induced hyperpolarization of quiescent cells (Fig. 6) produces a response in $K_m$ which is indistinguishable from that produced by serum stimulation.

Geck and Heinz (5) have provided the most thorough kinetic analysis of several transport models relating the effects of membrane potential on various transport parameters. Our results and those in the Ehrlich cells most closely correspond to what is termed the “velocity” model, with the unloaded amino acid carrier uncharged and the (Na⁺ + AIB)-loaded carrier bearing the positive charge of the bound Na⁺ ion. The changes in $K_m$ induced by hyperpolarization are not ascribed to a direct effect of potential on the affinity of the AIB transport site for its substrate. Rather, the potential is considered as affecting the transition probabilities for the movement of carriers from the inner to the outer surface of the membrane and back. We present here a simplified description of an initial uptake kinetics in a velocity model and show qualitatively how the principal effect of the membrane potential is on the $K_m$ term. For simplicity, the product $[\text{Na}^+]_0\cdot[AIB]_0$ is taken as $S_0^+$; at constant $[\text{Na}^+]_0$, $S_0^+$ is a linear function of [AIB]. The substrate $S_0^+$ combines with externally orientated carrier, $C_0^-$.

**Initial transport, $V$, is the translocation of $CS_0^+$:**

$$k_1 S_0^+ + C_0^- \xrightarrow{k_2} CS_0^+$$

Since $CS_0^+$ is positively charged, owing to the co-transported Na⁺, $k_1$ is sensitive to the transmembrane electric field; $k_2$ increases as the membrane potential increases (cell interior negative). For initial uptake, the translocation is taken to be unidirectional and the back reaction (efflux) is neglected.

Unloadable carriers can change their external-internal orientation:

$$C_0^- \xrightarrow{k_3} C_0$$

completing the carrier cycle. The externalization coefficient $k_3$ may, in fact, be compound. One component (probably major, see below) is the return of unloaded, electrically neutral carrier to its external orientation. A second (probably minor) component is the return of carrier associated with the efflux of endogenous, A system amino acids coupled with intracellular Na⁺. Although it is operationally easy to assay uptake from a
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ponent of \( k_z \) will be against the transmembrane electric field, and will be reduced if the cell membrane is hyperpolarized.

Finally, \( C_{in} = C_i + C_e + C_s \). The solution for initial influx in this system is:

\[
V = \frac{k_h k_0}{k_l + k_h} \left( S_0 - \frac{k_0 + k_l}{k_0 + k_h} \right)
\]

where \( V_{max} = \left[ k_h k_0/(k_l + k_h) \right] C_0 \). Since it is a property of the velocity model that carrier-borne influx of substrate is more rapid than reorientation of unloaded carrier, i.e., \( k_0 \gg k_h \), then \( V_{max} \) reduces to \( k_l C_0 \). In other words, at high external substrate concentrations, all of the carrier \( C_i \) tends to accumulate as \( C_e \), and the return of carrier to \( C_i \) becomes rate-limiting. Given that the unloaded carrier is electrically neutral, one may expect \( k_h \) and therefore \( V_{max} \) to be potential-independent. If \( k_h \) contains a potential-dependent component co-transporting endogenous amino acids and \( Na^+ \), \( V_{max} \) will in fact decrease with hyperpolarization. No such decrease was observed, and we conclude that this is not an important component in \( k_h \).

The apparent \( K_m \) is \( (k_3 + k_{-3}) (k_{-1} + k_2)/k_1 k_2 k_3 \). From considerations given above, only \( k_2 \) in the velocity model depends importantly on membrane potential. Other coefficients remain invariant with changes in potential. The terms containing \( k_h \) may be collected in the factor \( (k_{-1} + k_2)/k_1 k_2 + k_3 \). The model assumes that the association-dissociation reactions of substrate and carrier at the surface are fast relative to any of the translocation or carrier reorientations steps, i.e., \( k_{-1} \gg k_2 \). Therefore, \( (k_{-1} + k_2) = (k_2 + k_3) \), and \( (k_{-1} + k_2)/(k_2 + k_3) \gg 1 \). If \( k_2 \) increases while the other coefficients remain constant, the factor decreases and approaches unity. In other words, as \( k_2 \) increases with hyperpolarization, the factor, and hence the \( K_m \) decrease asymptotically to a limiting value.

The description above of potential-mediated effects on initial transport parameters is, of course, an oversimplification made for the purpose of visualizing how a hyperpolarization might affect the \( K_m \) for transport of a neutral amino acid. A more rigorous analysis allows for four possible states in which the carrier may undergo translocation (unloaded, \( Na^+ \)-loaded, amino acid-loaded, and \( Na^+ \) + amino acid-loaded), all of which may be affected differently by the driving forces for amino acid transport. It serves no useful purpose for this more complex treatment of the problem to be described here; the paper of Geck and Heinz (5) should be consulted for more details. Heinz and Geck (19) have tested some of their derivations in Ehrlich ascites tumor cells and observed, as we have now observed in quiescent HSWP cells, that a valinomycin-induced hyperpolarization reduces the \( K_m \) but does not appreciably alter the \( V_{max} \) of the AIB transport system. In this connection, it is of interest that the studies on Ehrlich cells were carried out in a serum-free medium with results comparable to those we obtain with serum-deficient HSWP cells. We have, however, extended the observation beyond the point of experimental manipulations of membrane potential having an effect on AIB transport parameters and have indicated that growth-associated physiological changes in membrane potential serve a regulatory role in controlling the \( K_m \) of the transport system in HSWP cells.

The kinetic equations derived by Geck and Heinz (5) to include membrane potential effects on amino acid transport as well as the equations derived from our more simplified model predict that the \( K_m \) will not decrease indefinitely with increasing potential but will level off at some value of the potential. This provides an explanation for why in our experiments, \( K_m \) measured in growing or serum-stimulated cells (or both) is not statistically different from that measured in valinomycin-stimulated cells, although the potential must be higher in the latter. The cells have undergone approximately a 2-fold increase in membrane potential in going from a state of quiescence to one of growth, and further hyperpolarization appears to have only a small, if any, additional effect on \( K_m \). Consistent with this idea is the observation that a double reciprocal plot for initial AIB transport in valinomycin-induced, hyperpolarized growing cells (not shown) is not statistically different from that observed for control growing cells (Fig. 4).

Finally, we note that the origins of the different membrane potentials must lie in growth-related alterations in electrolyte fluxes. In principle, these could be due to changes in relative membrane conductances of the principal ions or changes in the electrogenticity in active ion transport. Such growth-related potential changes, which in human cells do not result in any significant change in the intracellular \( Na^+ \) or \( K^+ \) concentration, may play an important role in controlling the flow of nutrients across the cell membrane or in controlling the level of other growth-regulating factors (i.e. cytoplasmic \( Ca^{2+} \) levels). These questions are currently under investigation.

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