CONF-770348--1

Possible Role of the Membrane Potential in Serum-Stimulated Uptake of Amino Acid in a Diploid Human Fibroblast*

Mitchel L. Villereal[1] and John S. Cook[2]

The University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences,

and

Cancer and Toxicology Program, Biology Division,

Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830

MASTER

---

*Research sponsored jointly by the National Cancer Institute and the Energy Research and Development Administration under contract with the Union Carbide Corporation.

[1]Postdoctoral Investigator on Carcinogenesis Training Grant #CA 05296 from NCI.

[2]To whom correspondence should be addressed.

Running title: Amino acid accumulation and membrane potential.

DISCLAIMER

SUMMARY

The Na^+-dependent accumulation of α-aminoisobutyric acid (AIB), measured in normal growing and quiescent (serum-deprived) HSWP cells (human diploid fibroblast), was found to be 2-fold higher (AIB_{in}/AIB_{out} = 20-25) under normal growing conditions. Serum stimulation of quiescent cells increases their AIB concentrating capacity by approximately 70% within 1 hr. These observations suggest that the driving forces for AIB accumulation may be reversibly influenced by the serum concentration of the growth medium. Addition of valinomycin (Val) to cells pre-equilibrated with AIB causes an enhanced accumulation of AIB, suggesting that the membrane potential can serve as a driving force for AIB accumulation. After pre-equilibration with AIB in 6 mM K^+, transition to 94 mM K^+ with Val results in a marked and rapid net loss of AIB. The effect of Val on the accumulation of AIB is greatest in quiescent cells, with the intracellular AIB concentrations reaching those seen in both Val-stimulated, normal cells and Val-stimulated, serum-stimulated cells. By adjusting $[K^+]_o$, in the presence of Val, the membrane potential of growing cells can be matched to that of quiescent cells or vice versa. The resultant AIB accumulating capacity is characteristic of the membrane potential rather than of the growth state. In summary, these data suggest that the accumulation of AIB in HSWP cells may be influenced by changes in membrane potential and that a serum-associated membrane hyperpolarization could be partly responsible for the increased capacity for AIB accumulation in serum-stimulated cells.

INTRODUCTION

In recent years, many investigators have sought to determine whether alterations in membrane permeability can be correlated with changes in cell growth state. Several studies have demonstrated that a decrease in the transport of several small molecular weight nutrients (P_i, uridine, amino acids) occurs when cells go from a state of growth to one of quiescence. These observations have led to speculation that alterations in membrane permeability to metabolites may, by controlling their intracellular concentrations, play a role in regulating cell growth and transformation (1).

The transport of amino acids, in particular, has been extensively studied as a possible growth regulatory mechanism. The well documented dependence of cell growth on amino acid supply (2), as well as the small margin of safety observed in some cells between maximal transport rate of and metabolic demands for amino acids (3), suggest that this pathway is well suited for such a regulatory function. Investigations of amino acid transport indicate that the rate of amino acid uptake in logarithmically growing, non-transformed cells is substantially higher than in either confluent [3T3 cells (4)], hyperconfluent [chick embryo cells (5)] or serum-deprived, quiescent cells [chick embryo cells (5), human diploid cells (6)]. The enhanced amino acid uptake seen in growing cells suggests the presence of an increased number of transport molecules in the cell membrane. However, in some cases, changes in transport are too rapid to be due to the incorporation of new transport sites into the membrane. Also, observations indicate that the level of amino acid accumulation is elevated in growing cells (4,5). Since, an increase in the number of transporters is not sufficient to increase the

amino acid concentrating capacity of the cell, it appears that when cells enter a growth state there may be either an increase in the driving force for amino acid uptake or a more efficient coupling to existing forces.

The present study attempts to identify the source of energy for the elevated accumulation of amino acids in growing cells. Amino acid transport is studied in a skin-derived, human diploid fibroblast strain (HSWP), using α-aminoisobutyric acid (AIB) as a model substrate for the N_a^+-dependent amino acid transport system. We show that AIB accumulation is greater in growing cells than in serum-deprived, quiescent cells and that the effect is partially reversed within 1 hour after serum-stimulation of quiescent cells. The possible role of the membrane potential in driving the enhanced amino acid accumulation in growing cells is tested using valinomycin to alter the resting membrane potential. Evidence is presented which suggests that the enhanced level of AIB accumulation in growing cells is the result of a growth-related membrane hyperpolarization.

MATERIALS AND METHODS

Cells and Growth

HSWP cells, human diploid fibroblasts, were derived from human foreskin by J. D. Regan (ORNL). They were cultured in Eagle's minimum essential media (KC Biological Inc.) containing 10% fetal calf serum (KC Biological Inc.), and 25 μg/ml gentamicin (Schering Corp.). Stock cultures were maintained at confluence in the same medium with 1% fetal calf serum. Cells were grown at 37°C in a 95% air-5% CO_2 atmosphere, and were used between the 10th and 25th passage. Cells were removed from stock flasks by trypsinization and were seeded onto individual coverslips (11 X 22 mm) in Petri dishes as

originally described by Foster and Pardee (4) 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 2 to 4 days on 0.1% fetal calf serum. Serum-deprived cells are considered to be quiescent when ^{3}H thymidine incorporation into the acid insoluble fraction drops below 10% of growing controls.

Transport Studies

Amino acid transport was measured using α-aminoisobutyric acid (AIB), a nonmetabolizable substrate of the Na^{+}-dependent, amino acid concentrating, transport system [A system (8)]. The assay medium consisted of amino acid-free Eagle's minimum essential medium with Hank's salts, 20 mM Hepes (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid), 0.1 mM AIB, and ∿ 1 μCi/ml ^{3}H AIB (ICN). 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 Hank's salts. In all media [NaCl + KCl + ChCl] = 144 mM. Valinomycin (Val; Sigma Chemical Co.) was added to some assay media as an ethanol solution with the appropriate amount of ethanol also being added to control media (< 0.25% final concentration).

Four-ml aliquots of assay medium were placed in 15 X 45 mm shell vials (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. To terminate AIB uptake, the coverslips were removed from the assay media, and rapidly washed 3 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 1N HCl and 10 ml of Triton X-100/toluene counting solution, containing 5.5g/L Permablend I (Packard), were added. Radioactivity was determined in a Nuclear-Chicago Mark II Spectrometer.

Protein Measurement

Protein was measured by the intrinsic fluorescence of tryptophan residues (9). Protein was solubilized by placing each coverslip in a shell vial containing 4 ml of 0.2% sodium dodecyl sulfate (SDS). The emission of the SDS extract was measured at 338 nm, using an excitation wavelength of 286 nm (Perkin Elmer model 204 fluorescence spectrophotometer). Bovine serum albumin dissolved in 0.2% SDS was utilized as a standard.

Water and Electrolyte Measurements

Cell water content was measured using the distribution of 3-O-Methyl glucose, which is passively distributed in HSWP cells (10). The distribution of L-glucose, which is not transported in these cells, was used to measure extracellular medium not removed by washing. The water to protein ratio ($\mu g\ H_2O/\mu g$ protein) was 4.4 ± 0.10 ($\eta = 5$) 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 $[Na^+]_o$, increase in $[K^+]_o$).

Na^+ and K^+ concentrations were determined flame photometrically using Li^+ as an internal standard.

RESULTS

HSWP cells cultured in 10% fetal calf serum grow to confluent densities of 120 ± 15 µg protein per coverslip within 6 to 8 days after plating. In contrast, cells which are deprived of serum, 24 hours after plating, become quiescent within 3 to 5 days. The serum-deprived cells grow to densities of 30 ± 5 µg protein per coverslip and can achieve confluent densities only upon restoration of serum to the growth medium.

AIB uptake was measured in both growing and serum-deprived cells 3 to 5 days subsequent to plating on coverslips, which corresponds to a time of logarithmic growth for the serum-sufficient cells and of quiescence for the serum-deprived cells. Although AIB uptake in growing cells is relatively slow, reaching steady state in about 60 minutes, the AIB concentration ratios (AIB_i/AIB_o) which can be maintained are 20 to 25 (Figure 1). The AIB accumulation in quiescent, serum-deprived cells is considerably lower than that in growing cells, with AIB ratios of only 8 to 10. However, the AIB concentrating capacity of the quiescent cells can be at least partially restored by serum-stimulation (20% fetal calf serum for 1 hour) prior to measurement of AIB uptake. Serum stimulated cells can accumulate AIB to concentration ratios of 12 to 15.

The data in Figure 2 demonstrate that the uptake of AIB in growing HSWP cells is Na^+-dependent. When extracellular sodium concentrations are decreased by equimolar replacement with choline, the accumulation of AIB is also decreased. For example, when the environmental sodium concentration is lowered from 138 mM to 10 mM, the accumulation ratio drops from 20 to 4.

The capacity for concentrating amino acids in growing cells is dependent on the membrane potential as well as on the Na^+ concentration gradient. If cells are equilibrated with 0.1 mM AIB for 75 minutes, in a control medium of 6 mM K^+ and 50 mM Na^+, and then transferred to a 50 mM Na^+, 0.1 mM AIB environment containing the K^+-ionophore valinomycin (Val), the final level of AIB accumulation is markedly influenced by the external concentration of K^+. In the absence of Val, growing cells incubated in this control environment can achieve an AIB accumulation ratio of 8 (Figure 3). Membrane hyperpolarization by the addition of Val to cells in this medium enables an accumulation ratio of at least 10 to be achieved. However, if cells pre-equilibrated in the control environment are transitioned to a Val-containing medium with 94 mM K^+, causing a membrane depolarization, there is a marked and rapid net loss of intracellular AIB.

The experiments depicted in Figure 4 were designed to compare the effects of membrane hyperpolarization on the AIB accumulating capacity of cells in three different growth states. Either growing, quiescent, or serum-stimulated cells were incubated in a medium containing 0.1 mM AIB, 138 mM Na^+, and 6 mM K^+ for 90 minutes, at which time they were transferred to an identical medium containing Val (10 μg/ml). The presence of Val enabled growing cells to increase their accumulation ratio of AIB from 25 to 34 (Figure 4). The effect of Val-stimulation is more dramatic in quiescent cells, causing a 3-fold increase in the previously depressed intracellular AIB concentration, to an AIB accumulation ratio of 34. This corresponds to the same concentrating capacity observed in Val-stimulated, growing cells. It is important to emphasize that the effects of serum-stimulation and Val-stimulation on AIB accumulation in quiescent cells are not additive. The combined treatment with Val and serum drives the internal AIB concentration to the same value observed in quiescent cells treated with Val alone. Thus, Val-stimulation

overrides the differences in AIB accumulation associated with the growing, quiescent, and serum-stimulated growth states.

Since a membrane hyperpolarization could negate the growth associated differences in AIB accumulation, the next series of experiments investigated whether the increased concentrating capacity in growing cells might be accounted for by a higher membrane potential. In this series of experiments, the Na^+ concentration of the assay medium was maintained at 50 mM to allow flexibility in manipulating K^+ concentration. Membrane potentials were estimated by varying the external K^+ concentration in the presence of Val, and determining at what value of $[K^+]_o$ the AIB accumulation equaled the accumulation in the control medium (6 mM K^+, no Val). This method is based on the "null point" technique used with the fluorescent dyes (11). After AIB accumulation has reached steady state in the controls (6 mM K^+, no Val), $[K^+]_o$ is adjusted to a series of values in the presence of Val and the change in AIB is determined for each value of $[K^+]_o$. The value of $[K^+]_o$, in the presence of Val at which there is no change in AIB concentration ratio, is determined and the membrane potential calculated from the equation for a K^+ electrode ($Em = \frac{RT}{F} \ln \frac{[K^+]_o}{[K^+]_i}$). Since cells lose about 10% of their internal K^+ content, after 60 minutes in the presence of Val, the potential was calculated based on the value of $[K]_i$ measured at the end of the experiment. This potential is taken to be the same as the pre-existing potential of the control cells in 6 mM K^+ without Val. The complete data for the determination of the "null point" is presented elsewhere (12).

The data in Figure 5 demonstrate that in the control environment (6 mM K^+, no Val) the cells accumulate AIB to about an 8-fold concentration ratio, and experience little or no change of AIB when cells are transferred to a 21 mM K^+ environment containing Val. Thus, the "null point" value for $[K^+]_o$ is approximately 21 mM, when $[K^+]_i$ = 125 mM (measured at the end of the experiment). This corresponds to a membrane potential of -47mV. The

quiescent cells, on the other hand, have a "null point" of 50 mM K^+, again with $[K^+]_i$ = 125 mM (measured at the end of the experiment), which corresponds to a membrane potential of -24 mV. Thus, in the control environment quiescent cells accumulate AIB to a concentration ratio of approximately 5 and undergo no change in internal AIB concentration when transferred to a 50 mM K^+ environment containing Val (Figure 5).

Quiescent cells in a 21 mM K^+ + Val medium, where their membrane potential is matched to that determined for growing cells, accumulate AIB to the same level as do growing cells in a control environment (Figure 5). Conversely, growing cells in a 50 mM K^+ + Val medium, where their membrane potential is matched to that determined for quiescent cells, decrease their AIB concentration ratio to the same level found in control quiescent cells.

DISCUSSION

Studies of growth-related alterations in amino acid transport have dealt primarily with the kinetics of initial transport rates. The observation that in some cells the maximum amino acid transport rate is higher during growth than quiescence suggests a larger number of transport sites in growing cells. However, since the V_{max} for AIB uptake can be a function of more than the number of transport sites, and under some conditions may even reflect primarily a change in the membrane potential (13), then the growth-associated alterations in transport may be more complex than a simple increase in the number of transport sites. Steady state transport experiments can help clarify this point by determining whether increased transport rates during growth are accompanied by an increased amino acid accumulating capacity. An increase in concentrating capacity would be independent of the number of transport sites, but would instead be dependent on an additional source of energy.

The observations that AIB accumulation in confluent 3T3 cells is 30% lower than in nonconfluent cells (4) and that valine equilibrium uptake (expressed only as cpm/mg protein) into the acid-soluble fraction of chick embryo cells is about 4-fold higher in growing than in hyperconfluent cells (5) suggest that more than an increase in the number of transport sites is required to explain growth-associated alterations in amino acid transport. The above mentioned findings, coupled to the observations that growing HSWP cells can accumulate AIB to a two-fold higher concentration ratio than can quiescent cells (Figure 1) indicate that in growing cells there is either an increase in the driving force for amino acid transport or a more efficient coupling to existing forces.

One possible source of energy for an enhanced amino acid concentrating capacity in growing cells, is the electrochemical potential energy stored in the cation gradients. A growth-related increase in the Na^+ electrochemical potential could provide an increased driving force for the accumulation of Na^+-dependent amino acids. The response of AIB uptake in growing cells to alterations in the Na^+ concentration gradient (Figure 2) is consistent with this idea. However, not only a change in the Na^+ concentration gradient, but also an alteration in the membrane potential affects the Na^+ electrochemical gradient. Thus, a growth associated increase in the membrane potential could provide the necessary energy for the enhanced AIB accumulation observed in growing cells.

Experiments to test this hypothesis were based on the assumption that in the presence of Val the cell membrane behaves like a K^+ electrode so that $Em = \frac{RT}{F} \ln \frac{[K^+]}{[K^+]_i}o$. It has been determined that Val specifically increases the K^+ conductance of Amphiuma red cell membranes (11), artificial lipid membranes (14,15), and many other systems. Although no comparable electrical measurements are available in HSWP cells, we have found that Val induces a 4-fold increase in K^+ exchange (measured with ^{86}Rb) when the normal K^+

exchange components are inhibited by ouabain and furosemide (unpublished observations). This occurs presumably by electrical coupling of influx and efflux through a conductive pathway.

The accumulation of AIB in growing cells was demonstrated to be sensitive to Val-mediated alterations in the membrane potential (Figure 3). When the membrane is hyperpolarized by adding Val to cells in a 6 mM K^+ medium there is a 36% increase in the AIB concentrating capacity. In contrast, a dramatic decrease in AIB accumulation is observed when the membrane is depolarized by transferring cells to a 94 mM K^+ medium containing Val. Thus, the response of AIB accumulation to changes in membrane potential indicates that a growth-associated alteration in membrane potential could provide the energy required for an enhanced AIB accumulation in growing HSWP cells.

If the enhanced AIB concentrating capacity in growing cells is due solely to an increase in membrane potential, then one would predict that AIB accumulation could be driven to the same level in quiescent and growing cells by hyperpolarizing both to the same membrane potential. However, if the energy were provided by some other source (i.e., an increase in the Na^+ concentration gradient) or if the enhancement were due to tighter coupling to existing energy sources, then one would expect that a hyperpolarization would increase the concentrating capacity in both quiescent and growing cells, but that accumulation would still be higher in the growing cell. Therefore, the observation that a membrane hyperpolarization drives the AIB accumulation ratio to 34 in both quiescent and growing cells suggests that a growth-associated difference in membrane potential does exist and is responsible for the higher AIB accumulation observed in growing cells (Figure 4). Also, the observation that Val-stimulation of serum-stimulated cells again drives the AIB accumulation

ratio to 34 is consistent with this hypothesis, and suggests that the membrane potential in serum-stimulated cells is somewhere intermediate between that of growing and quiescent cells.

An attempt was made to measure indirectly the membrane potential of growing and quiescent cells by varying the external K^+ concentration in the presence of Val, and determining at what value of $[K^+]_o$ the AIB accumulation corresponded to control accumulation (6 mM K^+ environment, without Val). The "null point" value of $[K^+]_o$ for growing cells is approximately 21 mM which predicts a membrane potential of -47 mV. (Figure 5). In contrast, the "null point" value of $[K^+]_o$ for quiescent cells is about 50 mM, which corresponds to a membrane potential of -24 mV.

It must be pointed out that, although this method is adequate for measuring differences in membrane potential between the two growth states, the actual values of the potentials measured in a 50 mM Na^+ medium may deviate from those that exist in a normal growth environment. Since we have no data on how the actual cytoplasmic Na^+ concentrations (16,17) vary with the reduction of extracellular Na^+ concentration, we cannot estimate the contribution of the Na^+ gradient to the potential. However, since the membrane potentials of both growing and quiescent cells were measured in the same environment it appears that a real difference in potential does exist. If anything, the difference in potentials may be underestimated in a 50 mM Na^+ medium. In a 138 mM Na^+ environment, growing cells accumulate more than twice as much AIB as do quiescent cells (Figure 1), while in a 50 mM Na^+ environment, AIB accumulation is only 1.5 times higher in growing cells (Figure 5). A lower potential in quiescent cells implies that the Goldman equation (18), describing the potential in quiescent cells,

may be weighted more in favor of the Na^+ potential than in growing cells. Thus, reducing the external Na^+ concentration might cause a larger increase in the potential in quiescent cells than in growing cells, thereby decreasing the difference between them.

The most convincing evidence that an increased membrane potential is responsible for the enhanced AIB accumulation in growing cells is the observation that accumulation in quiescent cells can be matched to that of growing cells by increasing the membrane potential of the quiescent cells to -47 mV. Conversely, the accumulation of AIB in growing cells can be matched to that in quiescent cells by decreasing the membrane potential of growing cells to -24 mV. Thus, by adjusting their membrane potentials one can mimic the growth-associated alterations of AIB accumulation seen in HSWP cells.

It is of interest to note that cell cycle dependent variations in AIB accumulation ratios have been observed in Ehrlich ascites tumor cells (19). Minimum AIB accumulation is seen in early M phase with maximum AIB ratios, representing a 3-fold increase, occuring in S phase. Since it has been demonstrated that Na^+-dependent amino acid accumulation in ascites cells responds to Val-mediated alterations in membrane potential (20,21), one can speculate that cell cycle dependent variations in membrane potential could be responsible for the observed changes in AIB accumulation ratios. Although no measurements of membrane potential throughout the ascites cell cycle have been reported, comparable measurements in cultured Chinese hamster cells indicate that their membrane potential is at a maximum during S phase (22). Also, the ouabain-insensitive, furosemide-insensitive component of K^+ flux, presumably the diffusional K^+ flux, in the ascites cell approximately triples during S phase (23). This finding is consistent with a hyperpolarization during S phase in the ascites cell.

At present we have no information concerning the mechanism for growth associated alterations of membrane potential in the HSWP cell. The possibility that changes in ionic conductances, comparable to those seen in the ascites cell, may occur is currently being investigated. Also under investigation is the possibility that alterations in membrane potential are responsible for the differences in amino acid transport observed between transformed and nontransformed cells.

REFERENCES

1. Holley, R. W., Proc. Nat. Acad. Sci. U.S.A. 69:2840 (1972).
2. Eagle, H., Science 148:42 (1965).
3. Hempling, H. G., In "Transport and Accumulation in Biological Systems", E. J. Harris (Ed.), University Park Press, Baltimore, p. 271 (1972).
4. Foster, D. O., and Pardee, A. B., J. Biol. Chem. 244:2675 (1969).
5. Bhargava, P. M., and Vigier, P., J. Memb. Biol. 26:19 (1976).
6. Birckbichler, P. J., Whittle, W. L., and Dell'orco, R. T., Proc. Soc. Expt. Biol. and Med. 149:530 (1975).
7. Salter, D. W., and Cook, J. S., J. Cell. Physiol. 89:143 (1976).
8. Christensen, H. N., In "Na^+-Linked Transport of Organic Solutes," E. Heinz (Ed.), Springer-Verlag, New York, p. 39 (1972).
9. Avruch, J., and Wallach, D. F. H., Biochim. Biophys. Acta 233:334 (1971).
10. Salter, D. W., personal communication (1976).
11. Hoffman, J. F., and Laris, P. C., J. Physiol. 239:519 (1974).
12. Villereal, M. L., and Cook, J. S., (in preparation).
13. Geck, P., and Heinz, E., Biochim. Biophys. Acta 443:49 (1976).
14. Henderson, P. J. F., Annu. Rev. Microbiol. 25:393 (1971).
15. Haydon, D. A., and Hladky, S. B., Quart. Rev. Biophys. 5:187 (1972).
16. Pietrzyk, C., and Heinz, E., Biochim. Biophys. Acta 352:397 (1974).
17. Smith, T. C., and Adams, R., J. Memb. Biol. (in press).
18. Goldman, D. E., J. Gen. Physiol. 27:37 (1943).
19. Tupper, J. T., Mills, B., and Zorgniotti, F., J. Cell Physiol. 88:77 (1976).

20. Gibb, L. E., and Eddy, A. A., Biochem. J. 129:979 (1972).

21. Reid, M., Gibb, L. E., and Eddy, A. A., Biochem. J. 140:383 (1974).

22. Sachs, H. G., Stambrook, P. J., and Ebert, J. T., Exp. Cell Res. 83:362 (1974).

23. Mills, P., and Tupper, J. T., J. Cell. Physiol. 89:123 (1976).

FIGURE LEGENDS

Figure 1 Effect of serum deprivation and serum stimulation on AIB uptake in subconfluent HSWP cells.

For assay, cells were removed from the indicated growth medium and placed in a serum-free, amino acid-free, Eagle's minimum essential medium (6 K^+, 138 Na^+) containing 0.1 mM AIB. AIB uptake is plotted versus time of accumulation for data compiled from 4 experiments. Mean ± SE of at least three determinations at each time point is shown.

Figure 2 Na^+ sensitivity of AIB uptake in subconfluent, growing HSWP cells.

Cells were removed from their normal growth medium and placed in a serum-free, amino acid-free medium containing 0.1 mM AIB, 6 mM K^+, and $Na^+ + Ch^+ = 138$ mM. AIB uptake versus time of accumulation is plotted for a representative experiment. Mean ± SE of at least three determinations is shown.

Figure 3 Modification of AIB accumulation by valinomycin and its dependence on the K^+ diffusion gradient.

Cells were removed from their normal growth medium and incubated in a serum-free, amino acid-free, 6 mM K^+, 50 mM Na^+ medium for 75 minutes. They were then transferred to either (i) the same medium, (ii) a 6 K^+, 50 mM Na^+ medium containing Val (10 µg/ml), or (iii) a 94 mM K^+, 50 mM Na^+ medium containing Val. The intracellular AIB concentration is plotted versus time of accumulation for data from 3 experiments. Mean ± SE of 9 determinations is shown.

Figure 4 Effect of valinomycin on AIB accumulation by HSWP cells in three growth states.

Cells were removed from their growth medium and incubated in a serum-free, amino acid-free, EMEM (6 K^+, 138 Na^+) containing 0.1 mM AIB for 90 minutes. Valinomycin (10 μg/ml) was added to half the population of cells at 90 minutes, while the other half served as controls. AIB uptake is plotted versus time of accumulation for data compiled from 3 experiments. Mean ± SE of at least 3 determinations is shown.

Figure 5 AIB accumulation in quiescent and growing cells: the effect of matching their membrane potentials.

Cells were removed from their growth medium and incubated in a serum-free, amino acid-free, 6 mM K^+, 50 mM Na^+, 0.1 mM AIB medium for 75 minutes. They were then transferred to either a 21 mM K^+, 50 mM Na^+, Val containing medium or a 50 mM K^+, 50 mM Na^+, Val containing medium. Intracellular AIB concentration is plotted versus time of accumulation for a representative experiment. Mean of at least three determinations is shown.

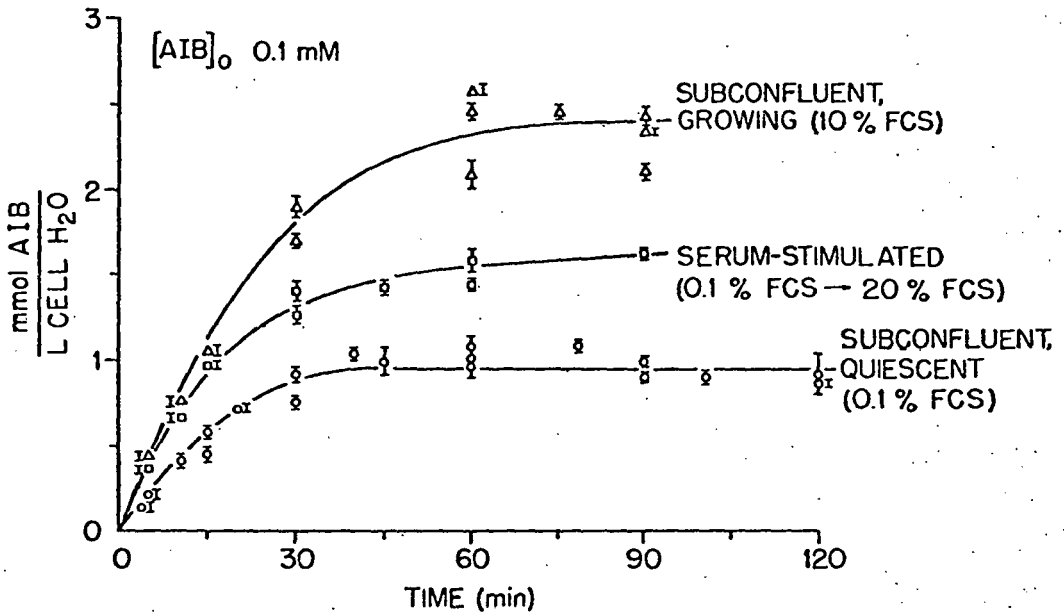

[AIB]o 0.1 mM
SUBCONFLUENT, GROWING (10 % FCS)
SERUM-STIMULATED (0.1 % FCS → 20 % FCS)
SUBCONFLUENT, QUIESCENT (0.1 % FCS)
mmol AIB / L CELL H2O
TIME (min)
0
30
60
90
120
1
2
3

Fig 1

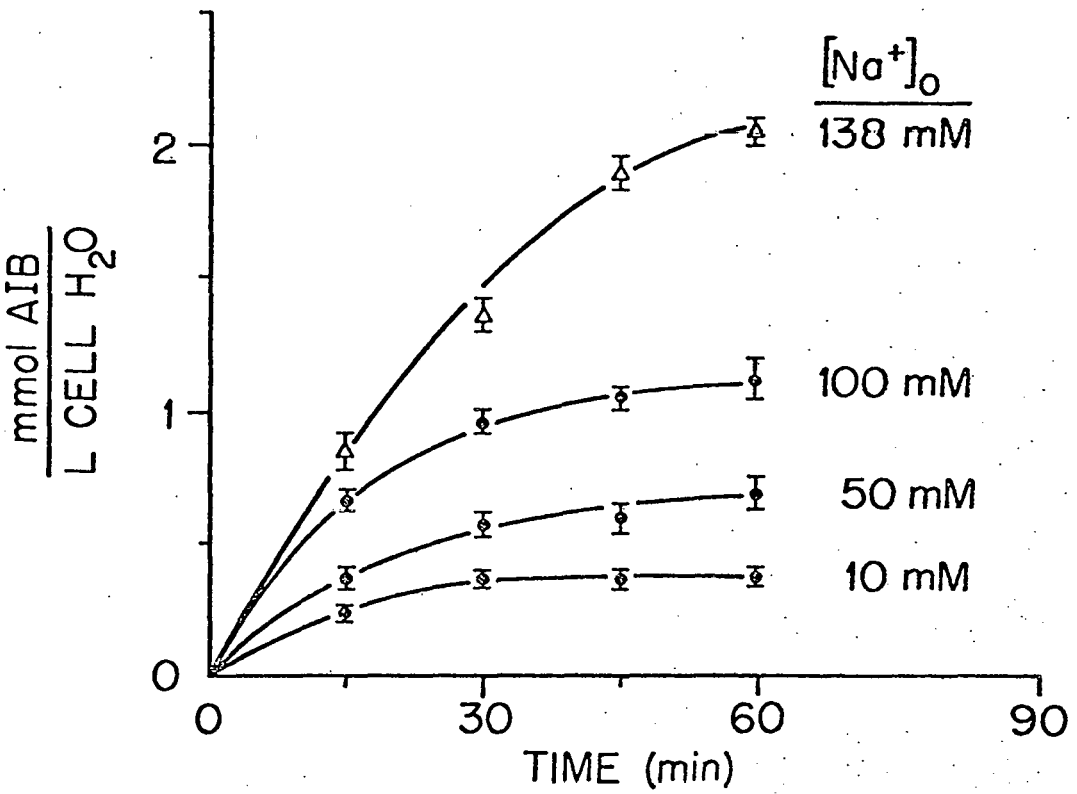

[Na+]o
138 mM
100 mM
50 mM
10 mM
mmol AIB / L CELL H2O
2
1
0
0
30
60
90
TIME (min)

Fig 2

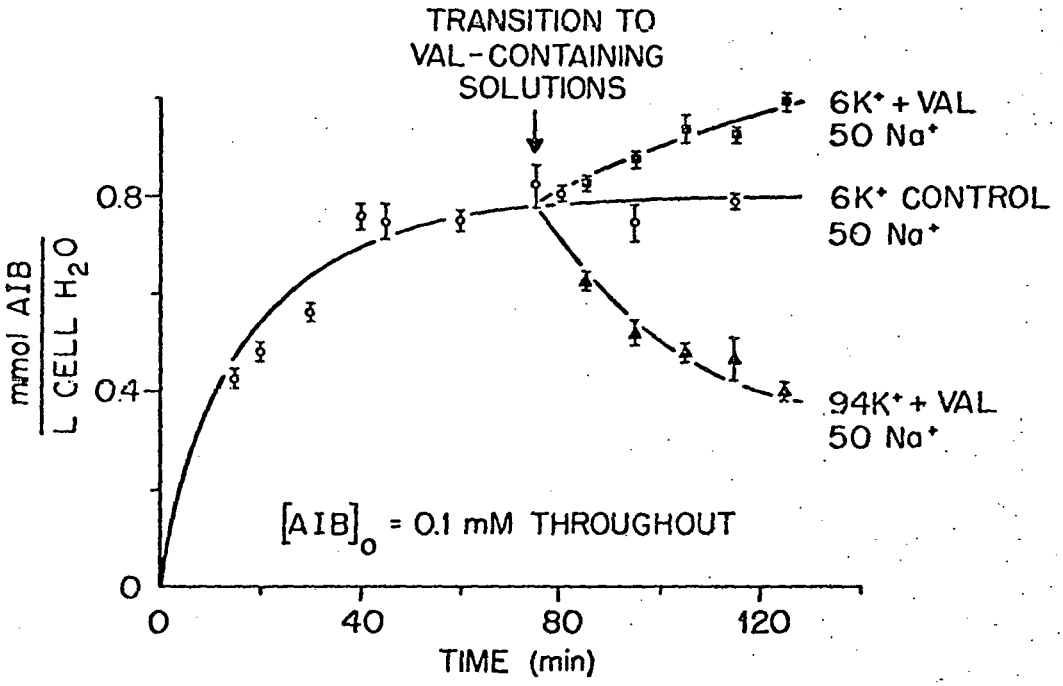

TRANSITION TO
VAL-CONTAINING
SOLUTIONS
6K⁺ + VAL
50 Na⁺
6K⁺ CONTROL
50 Na⁺
94K⁺ + VAL
50 Na⁺
mmol AIB / L CELL H2O
0.8
0.4
0
[AIB]o = 0.1 mM THROUGHOUT
0
40
80
120
TIME (min)

Fig 3

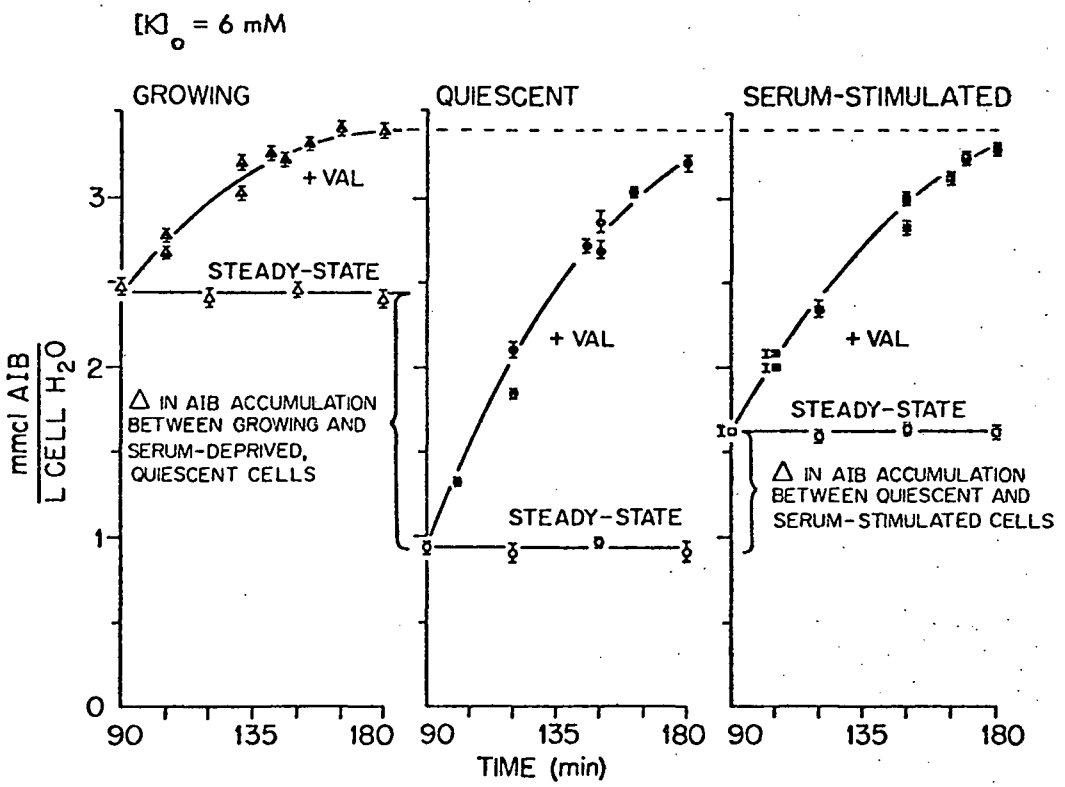
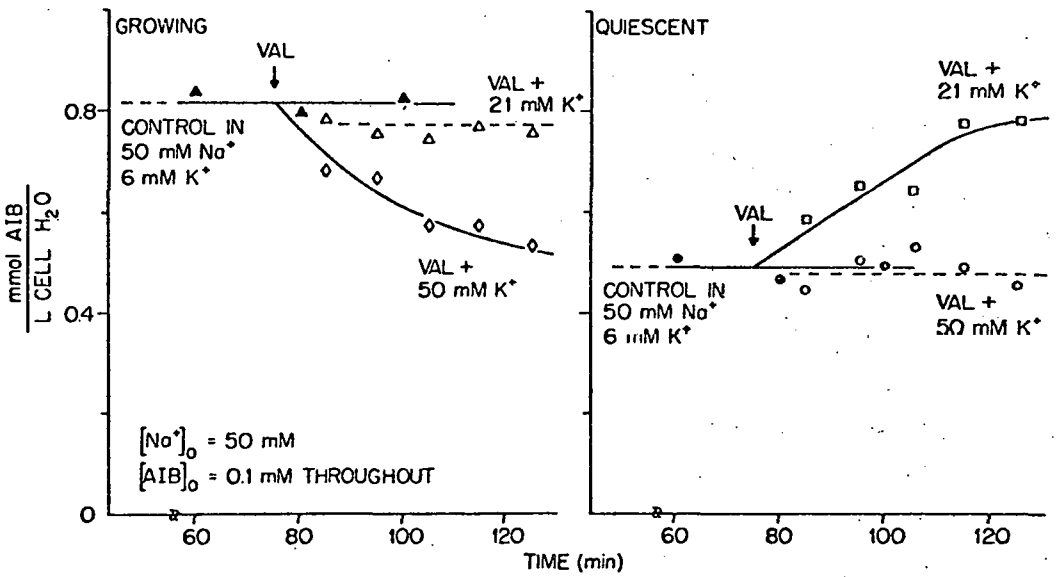

[K]o = 6 mM
GROWING
QUIESCENT
SERUM-STIMULATED
+ VAL
STEADY-STATE
Δ IN AIB ACCUMULATION BETWEEN GROWING AND SERUM-DEPRIVED, QUIESCENT CELLS
+ VAL
STEADY-STATE
+ VAL
STEADY-STATE
Δ IN AIB ACCUMULATION BETWEEN QUIESCENT AND SERUM-STIMULATED CELLS
mmol AIB / L CELL H2O
0
1
2
3
90
135
180
TIME (min)

Fig 4


GROWING
VAL
VAL +
21 mM K+
CONTROL IN
50 mM Na+
6 mM K+
VAL +
50 mM K+
[Na+]o = 50 mM
[AIB]o = 0.1 mM THROUGHOUT
QUIESCENT
VAL +
21 mM K+
VAL
CONTROL IN
50 mM Na+
6 mM K+
VAL +
50 mM K+
mmol AIB / L CELL H2O
0.8
0.4
0
60
80
100
120
TIME (min)