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CHARACTERISTICS OF PROLINE TRANSPORT INTO R3230AC MAMMARY TUMOR CELLS

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Summary

Cells separated by enzyme treatment of the R3230AC mammary carcinoma were used to characterize the entry of proline. These cells showed minimal changes in cell viability and intracellular volume and were found to be suitable for transport studies, since the v_i of proline was maintained for at least 4 h when cells were stored at 37 or 4°C, or when transport was measured in the presence or absence of Na^+.

Proline was actively transported by these tumor cells, reaching a distribution ratio ([proline] intracellular/[proline] extracellular) of 20 after 2 h. Proline entry consisted of two processes, one saturable (carrier mediated) and the other, non-saturable. The carrier-mediated entry, K_m = 0.83 mM and $V = 151 \cdot 10^{-5}$ μmol/min per $5 \cdot 10^6$ cells, was Na^+-dependent, sensitive to pH and metabolic inhibitors, and completely inhibited by α-(methylamino)-isobutyric acid (K_i = 0.34 mM). Proline entry in the absence of Na^+ was 20% that in the presence of Na^+ and was found to be due to a non-saturable process, since (a) v_i of proline uptake in the absence of Na^+ increased linearly with increasing proline concentration and (b) was not suppressed by either 20 mM α-(methylamino)-isobutyric acid, 50 mM glycine + 20 mM phenylalanine, or 50 mM serine + 20 mM phenylalanine when proline uptake was measured in the presence or absence of Na^+. Therefore, under the conditions studied, we conclude that proline transport appears to be restricted to the A (alanine-preferring) system. Furthermore, these cells should provide a suitable model to study the effect of hormonal manipulations on the amino acid transport process.

Introduction

The R3230AC mammary adenocarcinoma, a transplantable tumor of Fischer rats, is autonomous, hormone-responsive neoplasm [1,2]. In diabetic animals, tumor growth was enhanced as compared to that in intact animals and adminis-

tration of insulin caused inhibition of tumor growth [2]. Recently we reported that tumor cells obtained by enzymatic dissociation possessed receptors for insulin with binding characteristics similar to those reported in other tissues [3]. These cells also transported glucose by a carrier-mediated process, which responded to insulin in a time- and dose-related manner [4,5]. Thus, this mammary carcinoma could be a valuable neoplastic model to study those parameters considered to be insulin-responsive.

Insulin was reported to stimulate amino-acid transport in a number of tissues, such as the rat diaphragm [6—9], isolated rat thymocytes [10], fetal rat calvaria [11], intact levator ani muscle [12,13], chick embryo heart cells [14—17], mesenchymal cells from avian and mammalian tissues [18] and in primary cultures of adult rat liver parenchymal cells [19]. Various studies have shown that the mode of entry for the neutral amino acids was mediated by three transport systems [20—23]: the A (alanine-preferring), the ASC (alanine, serine, cysteine) and the L (leucine-preferring) systems. Insulin was found to enhance the entry of those amino acids transported by the A system but not those that utilize the ASC or L systems [9,16—18]. Therefore, in order to examine the effect of insulin on the initial event in the metabolism of amino acids, i.e., the transport process, it was necessary first to characterize the various amino-acid transport systems present in this tumor.

This paper describes the kinetic characteristics of proline entry into these isolated tumor cells. The data show that proline is transported primarily by the A system and further, that the experimental conditions established provide a suitable system to study the effects of insulin.

Materials and Methods

Female Fischer rats (80—90 g), obtained from Charles River Breeding laboratory (Wilmington, Mass.) had the R3230AC tumor implanted subcutaneously in the axillary region on both sides by a sterile trochar technique as described by Hilf [24].

Diabetes was induced by intravenous administration of streptozotocin two days prior to tumor implantation [2]. Streptozotocin was dissolved in 0.9% NaCl and rapidly adjusted to pH 4.5 with 0.025 M citric acid; injections (60 mg/kg) were made within 30 min. Blood glucose was determined by the glucose oxidase method (Glucostat, Worthington) and urinary glucose was estimated by Clinistix (Ames, Indiana). Animals were classified as diabetic if blood glucose levels were greater than 250 mg/100 ml and urine glucose greater than 0.5 gm/100 ml.

Animals were killed by cervical dislocation two to three weeks after tumor implantation. Tumors were excised quickly, placed in ice-cold 0.9% NaCl and connective and necrotic tissues removed. 3 g of tumor tissue were minced into 1×1 mm pieces on a McIlwain tissue slicer (Brinkman Instruments). The minced tissue was incubated with 10 ml of Hank's balanced salt solution (Ca^{2+}- and Mg^{2+}-free), containing 0.1% hyaluronidase (Sigma) and 0.05% collagenase (Type II, Worthington), for 85 min at 37°C in a Benco shaking water bath (approx. 50 cycles/min). Details of the procedure for preparing cells were published earlier [3,5]. Cell viability was estimated by trypan blue exclusion, and

cell number was determined by the use of hemocytometer. Cell preparations used had more than 90% viability [5].

Transport studies

Transport was measured on $5 \cdot 10^6$ cells in plastic (Falcon) tubes in a final volume of 1 ml of HEPES buffer, pH 7.5, containing 25 mM HEPES (*N*-2-hydroxymethylpiperazine-*N'*-2-ethanesulfonic acid)/10 mM $NaHCO_3$/130 mM NaCl/3 mM K_3HPO_3/1 mM $MgSO_4$/1 mM $CaCl_2$/11 mM glucose/1 mg per ml bovine serum albumin with a final osmolarity of 310 mosM. In experiments where Na^+ was omitted or varied, NaCl and $NaHCO_3$ were replaced by choline chloride and choline bicarbonate at equimolar concentrations.

Initial velocity measurements were determined by incubating cells with labeled and unlabeled proline for 15, 30, 45, and 60 s at 37°C in a shaking water bath. Transport was terminated by adding 8 ml of ice-cold 0.9% NaCl followed immediately by centrifugation for 1 min at 3°C (900 × *g*). The cells were washed again with ice-cold 0.9% NaCl and centrifuged for 2.5 min (900 × *g*). The tubes were inverted for 15 min to drain. The cell pellet was dissolved in 10 ml of Aqueous Counting Scintillant (Amersham/Searle) and radioactivity was counted in a liquid scintillation counter (Nuclear Chicago, Isocap 300). The efficiency for counting ^{14}C was 60% and for ^3H, 40%.

Intracellular and extracellular space measurements were determined with 3-*O*-[^{3}H]methylglucose and [^{14}C]inulin in the same preparation. Cells were incubated as described above for 45 min; [^{3}H]3-*O*-methylglucose was shown by Harmon and Hilf [4] to be transported into these tumor cells by facilitated diffusion reaching equilibrium at approx. 30 min at 20°C. When prolonged incubation was required, the cells were incubated with 3-*O*-methylglucose and inulin for the total period under study. The tubes were centrifuged for 5 min, and 100-μl aliquots of the supernatant were taken and the remaining supernatant was decanted. The tubes were wiped and radioactivity (^3H and ^{14}C) in the pellet was counted.

The 'space' measurements were calculated according to the following formula:

$$(1)\quad \frac{\text{cpm } ^3\text{H (pellet)} \times 100\ \mu\text{l}}{\text{cpm } ^3\text{H (supernatant)}} = \text{intracellular + extracellular space } (\mu\text{l per } 5 \cdot 10^6 \text{ cells})$$

$$(2)\quad \frac{\text{cpm } ^{14}\text{C (pellet)} \times 100\ \mu\text{l}}{\text{cpm } ^{14}\text{C (supernatant)}} = \text{extracellular space } (\mu\text{l per } 5 \cdot 10^6 \text{ cells})$$

$$(1) - (2) = \text{intracellular space } (\mu\text{l per } 5 \cdot 10^6 \text{ cells})$$

After obtaining the intracellular volume, the concentration of labeled amino acid taken up by the cells was calculated and a distribution ratio was calculated by the following formula:

$$\text{Distribution Ratio} = \frac{\text{concentration of the amino acid in the intracellular space}}{\text{concentration of the amino acid in the medium at the end of the incubation}}$$

Uptake is reported either as distribution ratio or as μmol/min per $5 \cdot 10^6$ cells.

Cells incubated under those conditions described above maintained their ability to transport and accumulate proline against its concentration gradient in the presence or absence of Na^+, at either 37 or 4°C, for at least 4 h with minimal changes in cell viability (approx. 3—4%) or intracellular space (4.3 ± 0.13 μl/$5 \cdot 10^6$ cells).

Chemicals

HEPES, NaCl, choline chloride, choline bicarbonate, serine, phenylalanine, glycine, α-aminoisobutyric acid and hyaluronidase were obtained from Sigma Chemical Co., St. Louis, Mo.; α-(methylamino)-isobutyric acid from Aldrich, Metuchen, N.J.; bovine serum albumin from Kupits, Forked River, N.J. (lot 55); and collagenase (type II) from Worthington Biochemical, Freehold, N.J.

L-[U-^{14}C]Proline, (specific activity 274 mCi/mmol); 3-*O*-[*Me*-^{3}H]methyl-D-glucose, (4—5 Ci/mmol); Inulin[*carboxyl*-^{14}C]carboxyl, (1—3 mCi/g), were obtained from New England Nuclear, Boston, Mass.

Results

Kinetic analysis of transport

(a) Effect of time, temperature and pH on uptake. Transport of proline (1 mM) across the membrane of these tumor cells was linear for at least 5 min at 37°C, and at lower temperatures, uptake was linear for longer times (at least 10 min). The rate of uptake at 37, 30 and 20°C was 54.9, 35.0 and $12.6 \cdot 10^{-5}$ μmol/min per $5 \cdot 10^6$ cells, respectively. At higher concentrations of proline (10—50 mM), the initial rate of uptake at 37°C was linear for 1.5—2 min. Consequently, initial velocity measurements, when proline was studied at concentrations of 5 mM or less, were obtained at 15-s intervals for up to 1 min; for concentrations of proline greater than 5 mM, samples were obtained at 10, 20, 30, 40 and 50 s.

The uptake of proline was measured over a pH range of 6.5 to 8.0. In the presence of Na^+, uptake was maximum at pH 7.5. The uptake of proline in the absence of Na^+ was not affected by pH.

(b) Effect of Na^+ on proline uptake and accumulation. Fig. 1 shows that proline entry in the absence of Na^+ was negligible (approx. 20%) and that entry of proline increased with increasing [Na^+]. The uptake of proline in the absence of Na^+ could be due to a non-saturable process, since this entry was not suppressed by either 20 mM α-(methylamino)-isobutyric acid, 50 mM glycine + 20 mM phenylalanine, or 50 mM serine + 20 mM phenylalanine; the level of non-suppressible entry of proline was equal in the presence or absence of Na^+. When this non-suppressible portion of proline entry was subtracted from the total entry, the uptake of proline in the absence of Na^+ became insignificant (Fig. 1). These results indicate that proline was transported by a Na^+-dependent process. We used 130 mM Na^+ in subsequent experiments, the maximum [Na^+] that could be added to maintain an osmolarity of 310 mosM.

The intracellular accumulation of proline was also Na^+-dependent; at 2 h, the concentration of proline inside the cells showed a distribution ratio of 20. In the absence of Na^+ proline was not concentrated (distribution ratio <1).

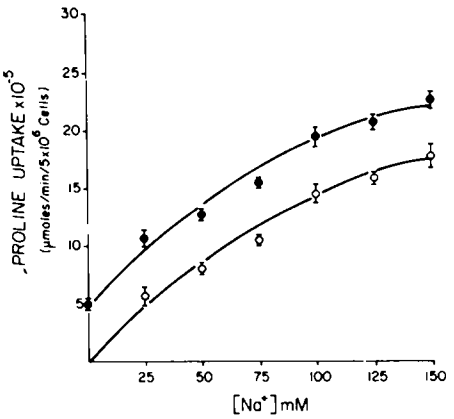

Fig. 1. Effect of Na^+ concentration on proline uptake. The v_i for proline was determined in the absence and presence of increasing [Na^+]. NaCl and $NaHCO_3$ were replaced by equimolar concentration of choline chloride and choline bicarbonate, respectively. •, Total entry; ○, corrected for non-suppressible entry. Each point is the average of triplicate assays ±S.E.M.

(c) Effect of proline concentration on uptake. The kinetic parameters of proline uptake were determined by measuring the v_i at various proline concentrations, from 0.05 to 25 mM. The uptake of proline in the absence of Na^+ increased linearly with increasing proline concentration (Fig. 2a) and when extrapolated to zero concentration, passed through the origin as expected for a non-saturable process. In the presence of Na^+, proline entry demonstrated

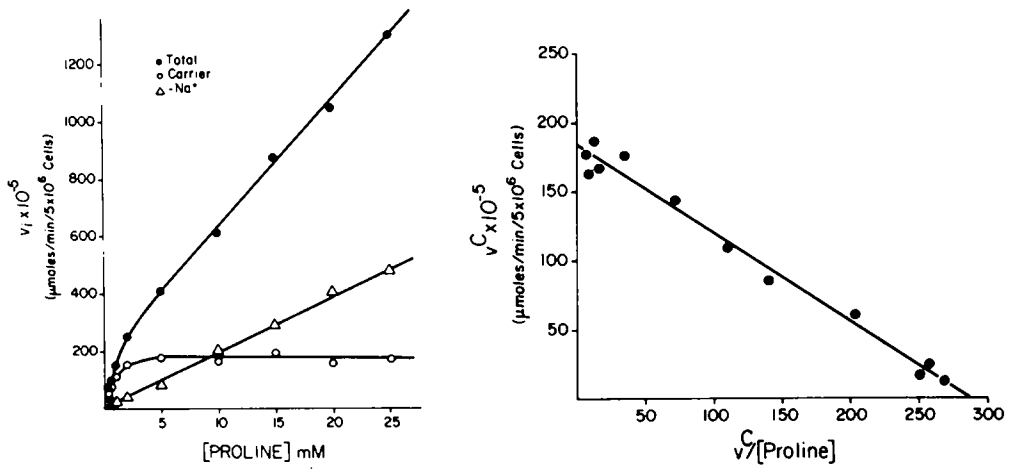

Fig. 2. a. Initial velocity of proline transport as a function of proline concentration and the effect of Na^+ on the saturable and nonsaturable entry of proline. The v_i was determined at various proline concentrations (0.05—25 mM) in the absence or presence of Na^+ at 37°C. The rate of proline entry at high concentration (5—25 mM) in the presence of Na^+ was used to calculate the diffusional coefficient (K_D). The K_D in the presence of Na^+ was 44.8 min^{-1}, and in the absence of Na^+, the K_D was 20 min^{-1}. Using the K_D in the presence of Na^+ to correct for non-saturable entry, a saturable process was observed. When the K_D in the absence of Na^+ was used, saturation was not observed (data not shown). v_i, Initial velocity; •, total entry (saturable and non-saturable) in the presence of Na^+; ○, saturable entry in the presence of Na^+ (v^C); △, proline entry in the absence of Na^+. Each point represents measurements made in duplicate at 15, 30, 45, and 60 s. b, Data for the carrier-mediated entry (panel a), plotted according to Eadie-Hofstee. Best-fitting line was determined by linear regression analysis (correlation coefficient 99%). K_m, 0.68 mM; V, $183 \cdot 10^{-5}$ μmol/min per $5 \cdot 10^6$ cells.

curvilinear characteristics suggesting the involvement of two processes, one which was saturable and another, which was non-saturable. Such relationships could be described by the equation:

$$v_i = [v^C + v^D] = \frac{V \cdot [S]}{K_m + [S]} + K_D \cdot [S]$$

where v_i is the initial velocity, saturable (v^C) and non-saturable (v^D); V, maximum velocity; K_m substrate concentration at half maximum velocity; and K_D, the diffusional coefficient, which represents the rate of the non-saturable process. The non-saturable process was estimated from the rate of entry at high substrate concentrations, where v_i increases linearly with substrate concentration. The K_D for the non-saturable entry (Fig. 2a) in the presence of Na^+ was 44.8 min^{-1} (mean ±S.E.M. for 6 experiments was 43.7 ± 0.85 min^{-1}); in the absence of Na^+, K_D = 20 min^{-1} (mean ±S.E.M. for 4 experiments was 19.1 ± 0.4 min^{-1}). Using the K_D of 44.8 min^{-1} to correct the non-saturable entry (v^D) at each substrate concentration, the entry of proline (v^C) demonstrated a saturable process. When the K_D of 20 was used, however, saturation was not observed (data not shown).

The data for the saturable uptake were treated according to Eadie-Hofstee analysis (v^C vs. $v^C/[S]$). A linear relationship was obtained (Fig. 2b), suggesting that proline entry was mediated by one class of carriers having a K_m of 0.68 mM and a V of 183 · 10^{-5} μmol/min per 5 · 10^6 cells (mean ±S.E.M. of 4 experiments: K_m = 0.83 ± 0.17 mM and V = 151 ± 11.8 · 10^{-5} μmol/min per 5 · 10^6 cells). These results agree with others in which the K_m ranged from 1.0 to 2.0 mM, and in which proline was transported by one class of carrier, the Na^+-dependent A system [15,25—27].

The rate of the non-saturable uptake in the presence of Na^+ was twice that seen in the absence of Na^+. Furthermore, only the non-saturable process in the presence of Na^+ appeared to be pH dependent (data not shown). These findings suggest that at high concentrations, in the presence of Na^+, proline entry may be involving an interaction between proline and Na^+ [28].

Inhibition studies

Since Christensen [30] suggested that inhibition analysis could reveal heterogeneity in transport that might not be detected by kinetic studies, we used several inhibitors to investigate the possible entry of proline by the ASC [26, 29] and/or L system in addition to the A system. The data in Fig. 3 show that α-(methylamino)-isobutyric acid, a non-metabolizable amine acid analog, which is known to be transported only by the A system in other tissues, as well as in this tumor (data not shown), inhibited proline entry completely. Furthermore, α-aminoisobutyric acid, a non-metabolizable amino acid analog which appears to be transported by both A- and L-like systems in this tumor (unpublished observations) to the extent of 70% and 30%, respectively, inhibited proline entry by 70%. Similar results (70% inhibition) were obtained with glycine. Phenylalanine, which was reported [27] to have a high affinity for the L system ($K_m \approx 0.5$ mM) and a low affinity for the A system ($K_m \approx$ 7—13 mM), slightly inhibited proline entry when phenylalanine was present at low concentrations; 40 mM phenylalanine completely inhibited proline entry.

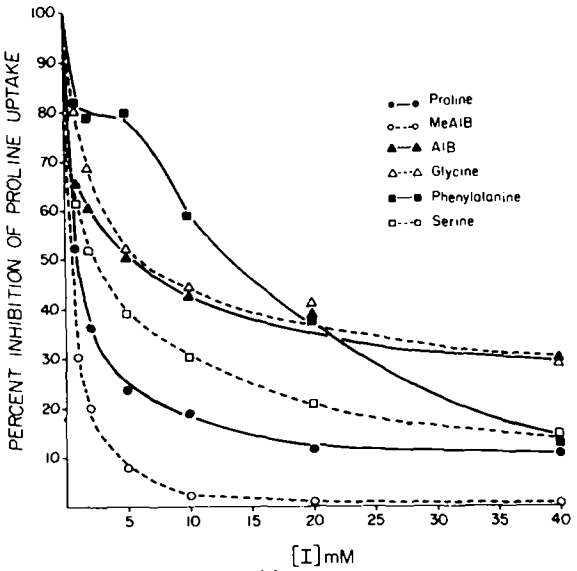

Fig. 3. Inhibition (%) of [^{14}C]proline transport (0.25 mM) by various amino acids (0.1—40 mM). ●, Proline (control); ○, α-(methylamino)isobutyric acid (MeAIB); ▲, α-aminoisobutyric acid (AIB); △, glycine; □, serine; ■, phenylalanine. [^{14}C]Proline and the unlabeled amino acid inhibitor were added simultaneously for the transport determination.

The complex pattern of inhibition by phenylalanine could be seen more clearly when the data were plotted as $1/(V_0 - V_t)$ vs. $1/[I]$ (Fig. 4), according to the method of Inui and Christensen [31], where the reciprocal obtained from the y intercept represents the inhibitable portion by the competing amino acid. Fig. 4 shows that proline entry was inhibited partially at low phenylalanine concentration (23%), and completely at infinite concentrations of phenylalanine (similar results were obtained when proline was used at 0.25 or 1.0 mM).

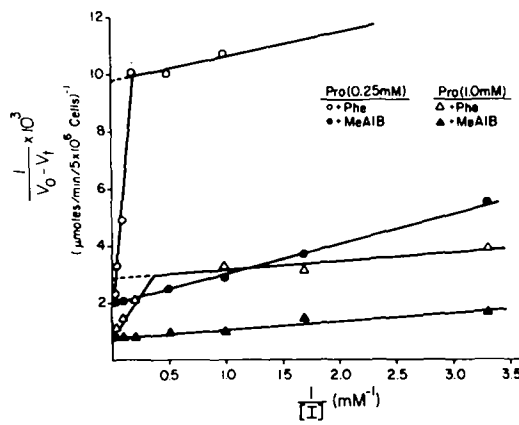

Fig. 4. Inhibition of two concentrations of proline (0.25 and 1.0 mM) by increasing concentrations of either α-(methylamino)-isobutyric acid (MeAIB) or phenylalanine (Phe). Data plotted as $1/V_0 - V_t$ vs. $1/[I]$. V_0 and V_t, the initial velocities of proline in the absence and presence of the amino acid inhibitor, respectively; $[I]$, inhibitor concentration, ○ and △, inhibition of 0.25 and 1.0 mM proline by phenylalanine; ● and ▲, inhibition of 0.25 and 1.0 mM proline by α-(methylamino)-isobutyric acid, respectively. The figure shows the biphasic inhibitory effect of phenylalanine. At concentrations lower than 5 mM, phenylalanine inhibited 23% of proline entry. Infinite concentrations of phenylalanine resulted in complete inhibition of proline entry similar to that observed with α-(methylamino)-isobutyric acid.

Finally, serine, which enters by the A, ASC or L systems, also inhibited proline uptake (Fig. 3) but to no greater extent than that seen with α-(methylamino)-isobutyric acid. Taken together, these data suggest that proline does not appear to enter by either the ASC or L systems under the conditions employed.

The ability of these amino acids and analogues to inhibit proline entry should be related to their respective affinities for the carriers. Thus, estimation of the K_i, the concentration that inhibited proline entry by 50%, should yield a value similar to the K_m, when the inhibitor is used as a substrate and its entry is measured. Experiments were performed using two different concentrations of proline in the presence of increasing concentrations of either α-(methylamino)-isobutyric acid or phenylalanine. The data obtained, analyzed according to a Dixon plot ($1/v^C$ vs. [I]), are shown in Figs. 5a and 5b. The K_i obtained for α-(methylamino)-isobutyric acid was 0.34 mM, identical to the K_m obtained in this tumor (data not shown) and other tissues [27]; for phenylalanine (Fig. 5b), the K_i was 10 mM, a value that is compatible with the K_m for phenylalanine reported by others [27]. Taken together, the above data indicate that proline entry in these tumor cells appears to occur by the Na^+-sensitive A system.

Effect of metabolic inhibitors on proline accumulation

The A system is energy-dependent and can be inhibited by a variety of metabolic inhibitors. We examined the effects of pentachlorophenol, a potent inhibitor of mitochondrial ATP synthesis [32], and found that pentachlorophenol inhibited proline accumulation in a dose-related fashion ranging from 10^{-4} M to 10^{-3} M (data not shown). At 10^{-3} M pentachlorophenol, the distribution ratio for proline was the same as that obtained in the absence of Na^+ (distribution ratio <1). Dinitrophenol, a less potent inhibitor of mitochondrial ATP synthesis, proved to be a less potent inhibitor of proline uptake (data not shown).

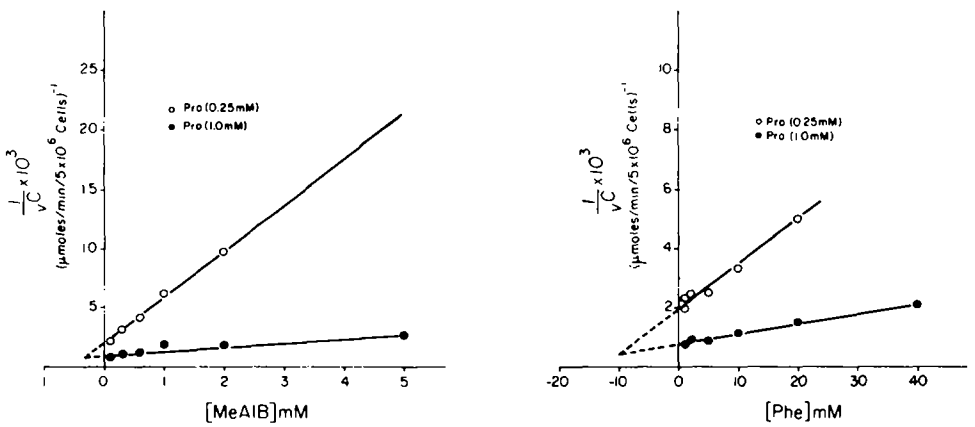

Fig. 5. a. Inhibition of proline entry, 0.25 mM (○) and 1 mM (●) by increasing concentrations of α-(methylamino)-isobutyric acid (MeAIB). A plot of $1/v^C$ vs. [α-(methylamino)-isobutyric acid] (Dixon plot) gave a K_i for α-(methylamino)-isobutyric acid of 0.34 mM. b. Inhibition of proline entry by phenylalanine (Phe). Dixon plot reveals a K_i of 10 mM. Proline concentrations used were the same as in a.

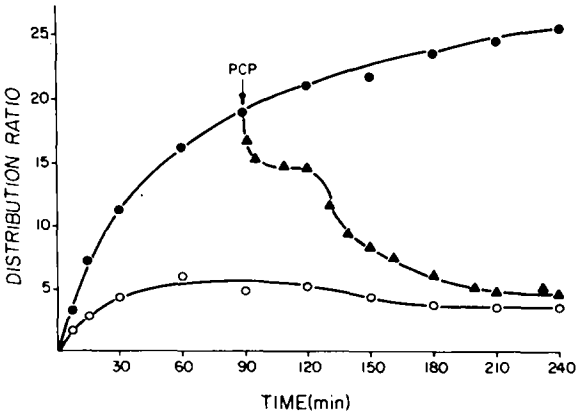

Fig. 6. Effect of pentachlorophenol ($7.5 \cdot 10^{-4}$ M) on the concentrative uptake of proline and on its dissipation. Tumor cells were allowed to accumulate proline for periods up to 4 h in the presence and absence of pentachlorophenol (PCP). To one group of cells, pentachlorophenol was added at zero time. To another group, pentachlorophenol was added after allowing the cells to accumulate proline for 90 min, to the third group of cells, no pentachlorophenol was added. Proline accumulation was measured and distribution ratios calculated (see Materials and Methods). ●, Proline accumulation in the absence of pentachlorophenol; ○, proline accumulation in the presence of pentachlorophenol (added at zero time); ▲, proline accumulation in the presence of pentachlorophenol when it was added after 90 min of proline accumulation.

The effect of pentachlorophenol ($7.5 \cdot 10^{-4}$ M) on proline accumulation (or retention) was examined by addition of pentachlorophenol at 90 min after addition of labeled proline (zero time); the amount of proline was measured in the cells at frequent intervals thereafter. These results (Fig. 6) show that addition of pentachlorophenol resulted in a loss of proline from the cells, the loss with time appearing to be biphasic. Pentachlorophenol caused a rapid initial decrease in the amount of proline that had accumulated during the first 90 min of incubation, and this was followed by a second, more gradual decrease in the amount of proline retained by the cells. At 4 h (2.5 h after addition of the inhibitor), the amount of proline in the cells was about 20% of that found in cells incubated without pentachlorophenol. A similar amount of proline was found in cells that had been incubated for 4 h in the presence of pentachlorophenol. These data indicate that proline uptake and retention were energy-dependent processes.

Discussion

From the data presented here, we conclude that proline transport in these tumor cells appears to be restricted to the A system. This conclusion was based on the following findings: (a) dependence of transport on Na^+; (b) specificity for substrate as indicated by inhibition of proline entry by selected amino acids, particularly by α-(methylamino)-isobutyric acid; (c) an estimated K_m of 0.83 mM for proline; (d) transport displayed concentrative characteristics, i.e., active transport as indicated by a high distribution ratio; (e) sensitivity to pH; (f) sensitivity to metabolic inhibitors.

These properties are similar to those described for the A system in Ehrlich ascites tumor cells [20,23]. Since the entry of proline in the absence of Na^+

did not display saturability, nor was it suppressed by amino acids whose entry are mediated by the L system, we concluded that entry of proline in the absence of Na^+ was a non-saturable process. Based on competition analysis, the fact that serine could not suppress proline entry to any greater extent than α-(methylamino)-isobutyric acid indicated that proline was not utilizing the ASC system; if this were the case, serine should have been more effective (serine enters by the A, ASC, and L systems) as an inhibitor of proline entry. Since there are no specific inhibitors of the ASC system, the conclusions reached were by indirect, rather than direct, methods.

It should be noted that the rate of proline entry at high proline concentration in the presence of Na^+ was higher (2-fold) than that in the absence of Na^+. Moreover, this rate in the presence of Na^+ was pH sensitive in contrast to that in the absence of Na^+. This finding suggests that simple diffusion may not be an adequate explanation at high proline concentrations, rather, that a more complex process is involved in which Na^+ is required. This observation may be analogous to the original suggestion by Christensen and Liang [28,33], who postulated the existence of high capacity, low affinity transport carriers that might contribute to the rate of the 'non-saturable' process at high substrate concentrations.

An interesting observation was the biphasic mode of proline efflux (after accumulation for 90 min) when the system was perturbed by pentachlorophenol. These data suggest that the energy required for proline accumulation and retention may be dependent, at least in part, on mitochondrial ATP synthesis. The complexity of the biphasic nature of the results depicted would require more detailed analysis before this process can be elucidated.

A variety of biological systems have been employed for the study of neutral amino acid transport: isolated perfused organs [34—36]; tissue slices [37—40]; naturally-occurring free-cell systems, i.e., erythrocytes [41,42], lymphocytes [43,44] and Ehrlich ascites tumor cells [22,45]; and isolated cells obtained by enzymatic dissociation of the tissue, i.e., adipocytes [46,47], embryonic heart cells [48—50] and hepatocytes [51].

The system utilized in the experiments reported here was dissociated cells obtained by enzymatic treatment of the R3230AC mammary tumor. As compared to tissue slices, a cell suspension system (a) provides more uniformity, which minimizes variability within each experiment; (b) offers a less complex model representing a two-compartment system rather than the three-compartment system in tissue slices, thereby simplifying the estimation of extracellular space considerations; and (c) allows one to examine transport at early and frequent intervals so as to obtain better estimates of initial velocity, since the cell surface is exposed more uniformly to the added amino acid. The results shown above provide evidence for the suitability of these cells for kinetic characterization of transport processes, since these cells, with initial viability of approx. 90% maintained their concentrative capability for at least 4 h with negligible effects on viability and intracellular volume. Since our overall goal was to examine the effects of insulin on amino acid transport in vitro, it appears that the system described here is satisfactory for such studies. Preliminary results have shown that addition of insulin to these cells produced an increase in v_i of proline transport, an increase in the distribution ratio, and an insulin-

induced increase in $^{14}CO_2$ production from uniformly-labeled glucose; a detailed report of these studies is in preparation.

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