Transport of L-tyrosine by B16/F10 melanoma cells: the effect of the intracellular content of other amino acids

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Summary

The uptake of L-Tyr by B16/F10 malignant melanocytes in culture has been studied. These melanoma cells can either be depleted of amino acids by 1 h preincubation in Hanks' isotonic medium or preloaded with a specific amino acid by 1 h preincubation in the same solution containing 2 mM of the amino acid to be preloaded. By means of these pretreatments, it is shown that the rate of L-Tyr uptake is greatly dependent on the content of other amino acids inside the cells. The L-Tyr uptake is higher in cells preloaded with amino acids transported by the L and ASC systems than in cells depleted of amino acids or preloaded with amino acids transported by the A system. It is concluded that L-Tyr is mainly taken up by an exchange mechanism with other amino acids mediated by the L1 system, although the ASC system can also participate in the process. In agreement with that, the homo-exchange performed by cells preloaded with unlabelled L-Tyr is more efficient than any other hetero-exchange, although L-Dopa, the product of tyrosine hydroxylation in melanin synthesis, is almost as efficient as L-Tyr. Apart from aromatic amino acids, melanoma cells preloaded with L-Met and L-His also yield a high initial rate of L-Tyr uptake. The results herein suggest that melanoma cells do not have transport systems specific for L-Tyr, even if this amino acid is needed to carry out the differential pathway of this type of cells, melanosynthesis.

Key words: melanoma cells, L-tyrosine, amino acid uptake.

Introduction

Previous studies have characterized a number (eight or nine) of distinct systems for the transport of amino acids inside mammalian cells (Kilberg, 1982; Harvey and Ellory, 1989). There are three major neutral amino acid transport systems in animal cells, named systems A, L and ASC (Shotwell and Oxender, 1983). These systems appear to be virtually ubiquitous and differ from each other in their affinity for the substrate transported and other characteristics. Aromatic amino acids are thought to be transported mainly by the system L (Oxender *et al.* 1977), but tyrosine has been the least studied amino acid, perhaps because its low solubility in comparison to phenylalanine and tryptophan (3 mM *versus* 195 mM and 30 mM at 30°C respectively, Sober, 1970) has hampered some experiments, such as the saturation of the transport systems involved in the process. Thus, phenylalanine has been widely used as a model to study the transport systems for the aromatic group (Shotwell *et al.* 1981; Petronini *et al.* 1982). However, some papers have been published pointing out differences between the transport of phenylalanine and tyrosine or tryptophan in erythrocytes (Rosenberg *et al.* 1980) and hepatocytes (Weissbach *et al.* 1982).

On the other hand, melanocytes possess a specific pathway consuming L-tyrosine, melanosynthesis (Prota and Thomson, 1976; Wick *et al.* 1986). Thus, the study of L-Tyr uptake by this type of cell might be very interesting to explore the possible existence of specific transport systems for tyrosine. Moreover, it is also known that amino acid transport often has peculiarities associated with each tissue.

There have been some reports dealing with tyrosine transport by melanoma cells, but they have been designed to distinguish the metabolic utilization of this amino acid in two distinct cellular processes, protein synthesis and melanogenesis (Farishian and Whittaker, 1979, 1980) rather than to elucidate the characteristics of the transport systems participating in tyrosine uptake. So far, the data reported about the time course of Tyr uptake by cultured malignant melanocytes differ, very probably because the conditions related to cell culture and the experimental design were not carefully considered and, as it is known, the type of medium used to culture cells may greatly affect the amino acid uptake. Thus, studies using Cloudman S91 malignant melanocytes described a linear uptake of tyrosine for at least 90 min (Wick and Frei, 1977). However, RPMI 3460 hamster melanoma cells yielded a hyperbolic curve reaching the maximal plateau in only 5–10 min (Farishian and Whittaker, 1979, 1980). These studies used different lines of melanoma cells, different initial content of L-tyrosine and also different tracers to study the uptake, DL-[2-^{14}C]Tyr and L-[3,5-^{3}H]Tyr respectively. The presence of the D-Tyr isomer and the formation of tritiated water by the action of tyrosinase, respectively, might produce some bias in those experiments.

The initial amino acid content of cells may greatly affect the uptake of a particular amino acid, because elevated

cellular levels of amino acids affect trans-stimulation and trans-inhibition phenomena concerning the accumulation of carriers in a cytoplasmic orientation (Kilberg, 1982; Harvey and Ellory, 1989). Therefore, this paper aims to characterize L-Tyr transport in melanoma cells as a function of the initial amino acid content of the cell. Thus, the correlation between the initial rate of L-tyrosine uptake and the intracellular level of amino acids in melanoma cells is investigated. The similarities between the time courses of the L-Tyr uptake by malignant melanocytes and the ubiquitous transport systems described in other cell types are discussed.

Materials and methods

Chemicals

Radioactive amino acids, L-[U-^{14}C]tyrosine (502 mCi mmol^{-1}) and L-[U-^{14}C]phenylalanine (493 mCi mmol^{-1}), and Aquasol-2 scintillation cocktail were obtained from New England Nuclear. All natural amino acids used, DL-*p*-chlorophenylalanine, L-norleucine, α-aminoisobutiric acid (AIBA) and α-methyl-aminoisobutiric acid (MeAIBA) were purchased from Sigma Co. 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH) was bought from Calbiochem Corp. Culture media, fetal calf serum, Hanks' isotonic salt solution and other reagents for malignant melanocyte culture were from Flow Lab. Inorganic salts and mineral acids were from Merck.

Cell culture

B16/F10 malignant melanocytes were kindly provided by Dr Hearing (NIH, Bethesda, USA). The cells were maintained in minimum essential medium (MEM) (Eagle's, modified with Earle's salts and glutamine without bicarbonate), containing 100 units ml^{-1} penicillin, 100 μg ml^{-1} streptomycin and supplemented with 10% fetal calf serum, at 37°C, in a water-saturated, 5% CO_2 atmosphere. Cells were passaged routinely every four days, and the media were changed every two days. Cells were used between the 3rd and the 30th passage. After this last passage, cells were discarded and new cultures were initiated by thawing further samples from liquid nitrogen.

Uptake assay

Approximately 300 000 cells were seeded into 9.62 cm^2 disposable culture dishes (Nunc, Denmark, 35 mm diameter) and incubated for 24 h in complete growth medium at 37°C. Following medium withdrawal by aspiration, the dishes were rinsed twice with Hanks' solution and the cells were preincubated as required by the experimental design (see below, Depletion or Preaccumulation of specific amino acids inside the cells). To follow the kinetics of uptake, malignant melanocytes maintained in monolayers were incubated for variable times at 37°C in 1 ml Hanks' solution containing 0.5 μCi of the labelled amino acid (L-Tyr or L-Phe) in different isotopic dilutions. Addition of dialysed fetal calf serum during this phase had no effect on the rate of amino acid uptake up to 1 h. Therefore, serum was routinely omitted from the assay medium. The incubations were stopped by rapidly rinsing the monolayers twice with 2 ml of ice-cold Hanks' solution. Then, the cells in each dish were scraped from the plastic surface with a rubber policeman and the suspension transferred to scintillation vials containing Aquasol-2, and counted for radioactivity. Uptake assays were carried out with semiconfluent cells (approx. 500 000 to 800 000 cells per culture dish). Each point is the mean of three dishes. Further details are given in figure or table legends.

Depletion of cellular amino acid content

Malignant melanocytes were depleted of intracellular amino acids by the following treatment: after withdrawing the standard culture medium, cells were washed with 2 ml of Hanks' solution at 37°C for 2 min. Then, the solution was aspirated again and the cells were further incubated for different times up to 1 h with 2 ml of Hanks' solution.

Preaccumulation of specific amino acids inside the cells

To study the trans-effects of preaccumulated amino acids on the influx of L-Tyr into melanoma cells, monolayers of malignant melanocytes were washed with 2 ml of Hanks' solution at 37°C for 2 min and then preloaded by incubation for different times up to 1 h in 2 ml of this medium containing 2 mM of the amino acid to be accumulated into the cells. After the incubation period, the monolayers were rinsed and immediately used to determine amino acid concentration or incubated with 0.5 μCi of labelled L-Tyr as described for the uptake assay.

Amino acid determination

Amino acid content was determined by withdrawing the medium and washing with 2 ml of Hanks' solution at 4°C for 15 s. Then the liquid was aspirated and cells were collected. Samples were immediately deproteinized by treatment with 5% trichloroacetic acid and centrifugation at 13 000 ***g*** in an Eppendorf centrifuge. An aliquot of the supernatant was mixed (1:1) with L-NorLeu (0.02 mM) as internal standard, and injected into an amino acid autoanalyzer (Rank Hilger Chromaspeck) equipped with a fluorometric detector for *o*-phthadialdehyde derivatives. Response factors for each amino acid were calculated by injecting a 0.01 mM AA-S-18 amino acid calibration standard mixture from Sigma.

Results

The effect of depletion or preaccumulation treatments on the intracellular content of amino acids

We first examined the time course of L-Tyr uptake in semiconfluent B16/F10 malignant melanocytes in order to establish the appropriate conditions for measuring initial rates. Fig. 1 shows the results obtained for cells maintained in standard medium. They were washed twice with Hanks' solution and immediately exposed to 0.1 mM labelled L-Tyr. An almost linear uptake can be seen for 30 min. Also, a similar pattern was obtained at different L-Tyr concentrations up to 2 mM.

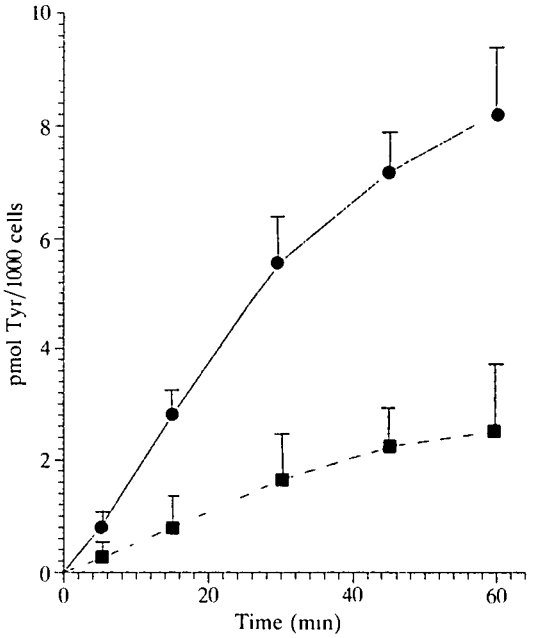

Fig. 1. Time course of uptake of 0.1 mM L-Tyr. (●) Melanoma cells maintained in MEM Eagle's medium. (■) Cells depleted in amino acids by pretreatment for 1 h in Hanks' solution. Each point represents the mean±S.D. of four determinations in the first case and three in the second.

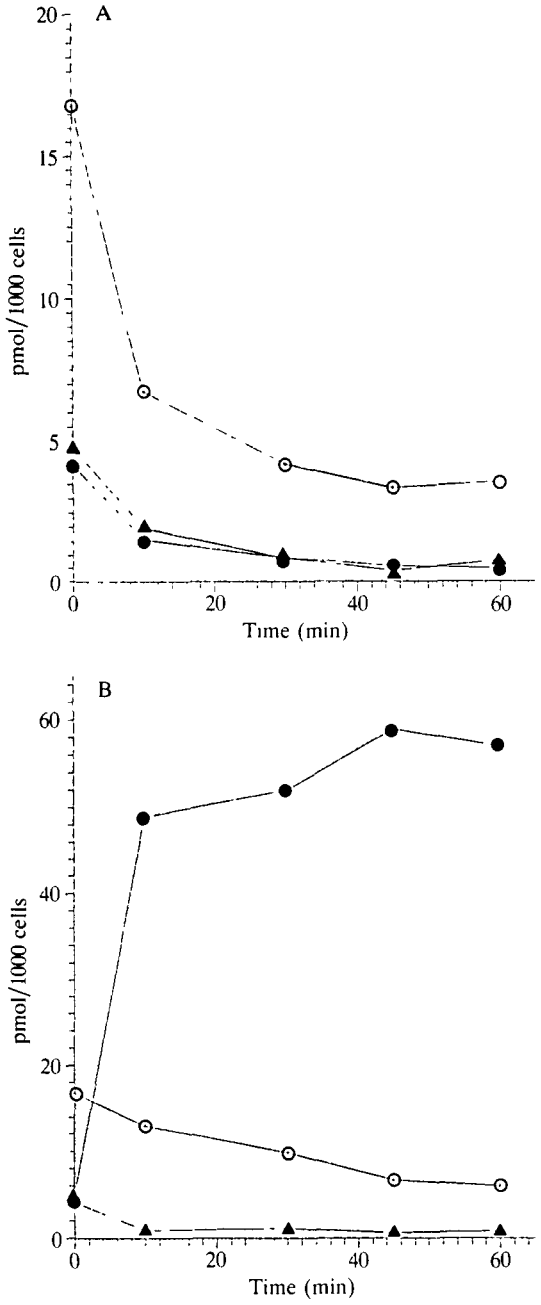

Fig. 2. Time courses of the intracellular content of (●) L-Phe, (○) L-Ala and (▲) L-Leu in melanoma cells after: (A) depletion, (B) preloading with 2 mM L-Phe. Points are single measurements obtained from a pool of three dishes.

To investigate the effect of the initial content of amino acids inside cells on the activity of the different transport systems, we studied the time course of L-Tyr uptake by malignant melanocytes after different pretreatments. Simultaneously, in order to test the efficiency of the pretreatments, parallel cultures of melanoma cells under the same conditions were washed, harvested and lysed to determine their amino acid content.

Fig. 2 shows the cellular content of three illustrative amino acids, L-Ala, L-Leu and L-Phe, after different times of depletion or preloading with L-Phe. In both cases the equilibrium state was reached rapidly, and there was almost no variation in the content of these amino acids after the first 30 min. Most of the amino acids yielded similar patterns. Thus, 1 h was the time routinely used for both types of pretreatments in order to prepare melanoma cells at equilibrium to study L-Tyr uptake.

Table 1. *Amino acid content (pmol/10^3 cells) of B16/F10 melanoma cells*

Amino acid	Control	Depleted	Preloaded with Phe	Preloaded with Leu	Preloaded with Ala	Preloaded with Met
Asp	6.2±3.1	4.8	5.7	3 7	3.7	5.2
Ser+Thr	5.3±1.6	4.9	7.5	5.1	3.3	8.0
Glu	19.2±5.0	3.8	4.3	6.3	4.3	4.1
Gln	4.5±5.2	n.d.	n.d.	2.0	n.d.	n.d.
Gly	13.2±8.9	7 9	16.7	10.3	8.4	16.4
Ala	16 8±4.4	2.3	3.0	4.3	38.6	2.8
Val	3.8±1.3	n.d.	0.4	1.3	n.d.	1.0
Met	2.2±0.4	1 3	1.0	n.d.	n.d.	28.6
Ile	3.9±1.3	0 4	n.d.	0.4	0.3	n.d.
Leu	4.8±0.8	n.d.	n.d.	21 4	0.8	0.7
Tyr	3.2±1.1	n.d.	n.d.	n.d.	n.d.	n.d.
Phe	4.2±1.9	1.9	57.6	0.5	0.4	0.9
His	4.8±0.9	n.d.	n.d.	1.0	1.5	1.0
Arg	6.7±2.7	n d.	n.d.	4.4	1.5	3.5
Lys	4 5±2.8	n.d.	n.d.	n d.	n.d.	n.d.
Total	103.3±41.4	27.3	38.6+ Phe	39.8+ Leu	23.7+ Ala	44.1+ Met

Depletion and preloading treatments were made by incubating cells for 1 h in the appropriate media. Control cells were maintained in MEM Control: mean±S.D. of six measurements from pools obtained from three cell dishes In all other cases, a single measurement was performed from a pool of three dishes. Preloading of melanoma cells with other amino acids (not shown) always yielded a content above 20 pmol/10^3 cells. n.d., not detectable.

Table 1 shows the intracellular amino acid levels for control cells maintained in the culture medium, and also for cells depleted in amino acids or preloaded with four amino acids representative of the main transport systems. Preloading with other amino acids gave a similar pattern. The total amino acid concentration decreases to approximately 25% in depleted cells. Similar results have been described for cultured fibroblasts, where the intracellular concentration of all amino acids tested was lowered by more than 80% in less than 90 min by the same depletion treatment (Gazzola *et al.* 1980). For preloaded cells, the total amino acid concentration (apart from the amino acid specifically preloaded) is similar to the amino acid concentration in depleted cells, and significantly lower than in control melanoma cells. Logically, the intracellular concentration of the preloaded amino acid after the treatment is much higher than its concentration in control cells, Phe being the amino acid most efficiently accumulated by melanoma cells.

Effect of the initial amino acid content on the rate of L-Tyr uptake

Fig. 1 also shows the results obtained for B16/F10 melanoma cells depleted of amino acids by incubation in Hanks' solution for 1 h. Depletion of internal amino acids resulted in a decrease of L-Tyr uptake, suggesting that the transport of L-Tyr by B16/F10 malignant melanocytes is susceptible to trans-stimulation.

We also examined the time course of uptake of L-Tyr by B16/F10 malignant melanocytes preloaded with several amino acids. Figs 3–7 show the time courses of L-Tyr uptake by cells previously preloaded with amino acids representative of different transport systems. The cells

were incubated for 1 h with Hanks' solution containing the corresponding amino acid at a 2 mM final concentration. Remarkable changes in the pattern of the L-Tyr transport were observed, depending on the nature of the amino acid preloaded inside melanoma cells.

Preloading with most of the amino acids yielded an initial uptake of L-Tyr higher than untreated melanoma cells (Table 2), supporting the trans-stimulation mechanism. The time course of the L-Tyr uptake was different for several groups of amino acids. All aromatic amino acids (Fig. 3) and some other selected amino acids such as His, Met and its analogue NorLeu (Fig. 4), showed a rapid initial uptake reaching a maximum after approx. 15 min.

Table 2. *Initial rate of L-Tyr uptake by B16/F10 melanoma cells*

Pretreatment		V_0
None (cells maintained in MEM)		0.78±0.40
Amino acid depletion		0.36±0.09
Preloaded with	L-Tyr	3.27±0.86
	L-Dopa	2.33±0.19
	L-Trp	1.62±0.37
	L-Phe	1.12±0.52
	DL-ClPhe	0.73±0.19
	L-Met	2.59±0.23
	L-His	2.47±0.55
	L-NorLeu	1.85±0.14
	L-Ile	1.08±0.26
	L-Leu	0.87±0.21
	L-Pro	0.04±0.02
	L-Thr	1.50±0.28
	L-Ser	0.63±0.15
	L-Ala	0.41±0.19
	BCH	1.51±0.11
	AIBA	0.58±0.20
	MeAIBA	0.20±0.08

In all cases, cells were preloaded by 1 h incubation in Hanks' solution containing 2 mM of the respective amino acid, except DL-*p*-ClPhe, which was 4 mM. Results are expressed as pmol L-Tyr/10^3 cells min^{-1} (V_0), and they are the mean ±S.D. of three measurements for preloaded cells, and six for untreated and depleted cells.

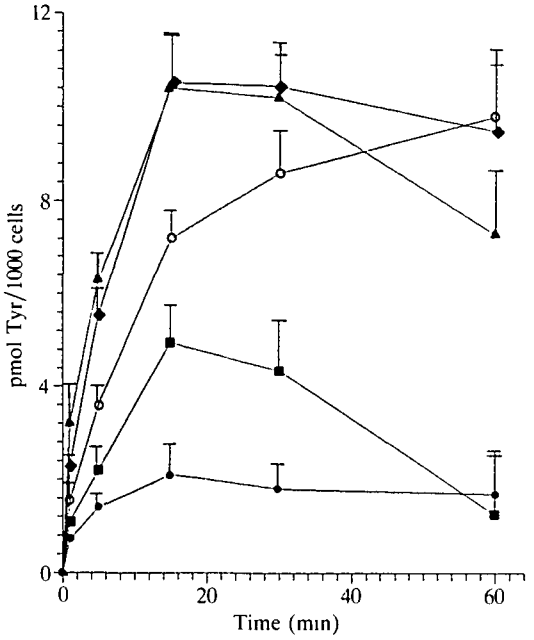

Fig. 3. Time courses of uptake of 0.1 mM L-Tyr by melanoma cells preloaded by 1 h incubation time with a 2 mM solution of (○) L-Trp, (◆) L-Dopa, (▲) L-Tyr, (■) L-Phe or (●) 4 mM DL-ClPhe.

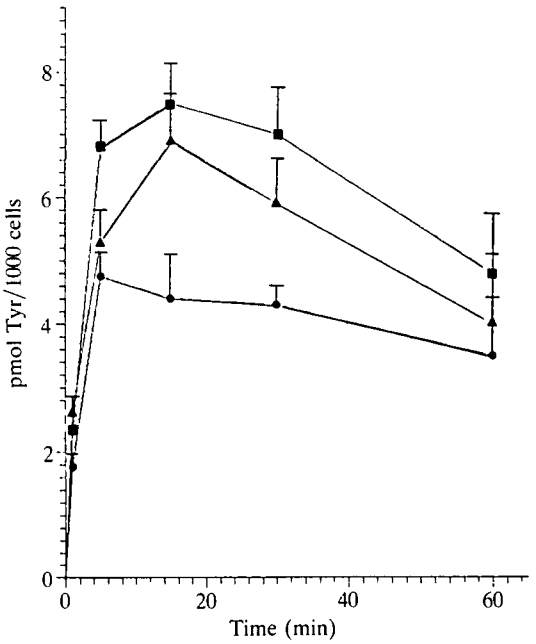

Fig. 4. Time courses of uptake of 0.1 mM L-Tyr by melanoma cells preloaded with (■) L-His, (▲) L-Met or (●) L-NorLeu. Other details as Fig. 2.

Thereafter, a slight decrease in the amount of the L-Tyr accumulated inside melanoma cells was observed. Maximal accumulation of radioactive L-Tyr was reached in melanoma cells preloaded with unlabelled L-Tyr, suggesting that homo-exchange of this amino acid is more efficient than any hetero-exchange. Furthermore, preloading with L-Dopa, very similar to L-Tyr, also yielded a high level of L-Tyr accumulation.

Representative amino acids transported by system L, such as L-Leu and L-Ile, produced a rapid initial L-Tyr uptake, but the transport reached an early saturation and the level of L-Tyr accumulated inside cells was relatively low (Fig. 5). Preloading with L-Pro, a representative amino acid transported by system A, produced a very low rate of L-Tyr uptake (even lower than the uptake by melanoma cells depleted of amino acids), but this uptake remained linear for about 30 min. The level of L-Tyr accumulated after 1 h was comparable to the one reached in melanoma cells preloaded by L-Leu and L-Ile (Fig. 5).

Preloading with representative amino acids transported by system ASC, such as L-Ala and L-Ser, produced a low initial rate of L-Tyr uptake, but the transport was linear for 30 min, and the level of L-Tyr accumulated inside the cell after 1 h was high (Fig. 6) and similar to the one produced by L-Dopa and L-Trp. L-Thr can also be included in this group since it is a good substrate for the system ASC. In addition, the contribution of system L to the transport of this amino acid is also significant (Shotwell *et al.* 1981) and probably that is the reason why the initial uptake of L-Tyr by cells preloaded with L-Thr was higher than those obtained for L-Ala and L-Ser, but equilibrium was reached earlier.

Finally, Fig. 7 shows the profiles for melanoma cell preloaded with the model amino acids AIBA, MeAIBA and BCH. BCH is a specific substrate for system L, and the profile obtained for this amino acid was very similar to those of other amino acids transported by this system, such as L-Leu and L-Ile. The equilibrium is obtained quite

rapidly. MeAIBA is a specific substrate for system A. As expected, the initial rate of L-Tyr uptake after preloading with this amino acid was low, and the time course similar to that of L-Pro. The results obtained for AIBA preloading indicate that this model amino acid is probably a substrate for both systems A and L, in accordance with the results reported for other cell types (Shotwell *et al.* 1981).

The uptake of L-Phe by malignant melanocytes

It has been reported that the aromatic amino acids L-Trp and L-Tyr are transported into erythrocytes by a specific transport system named T-system (Rosenberg *et al.* 1980).

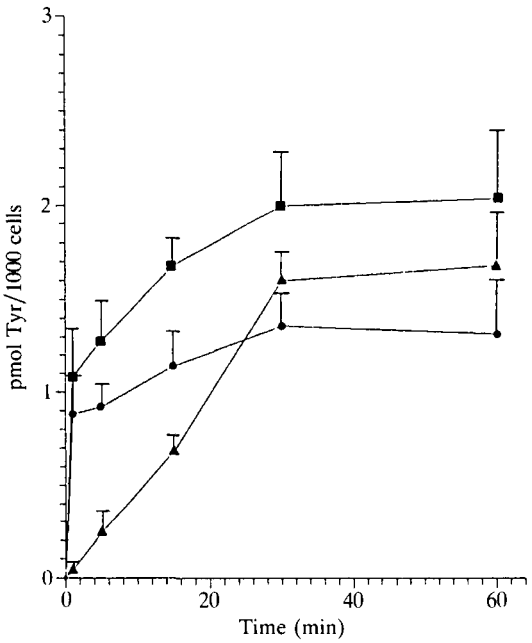

Fig. 5. Time courses of uptake of 0.1 mM L-Tyr by melanoma cells preloaded with (■) L-Ile, (●) L-Leu or (▲) L-Pro. Other details as Fig. 2.

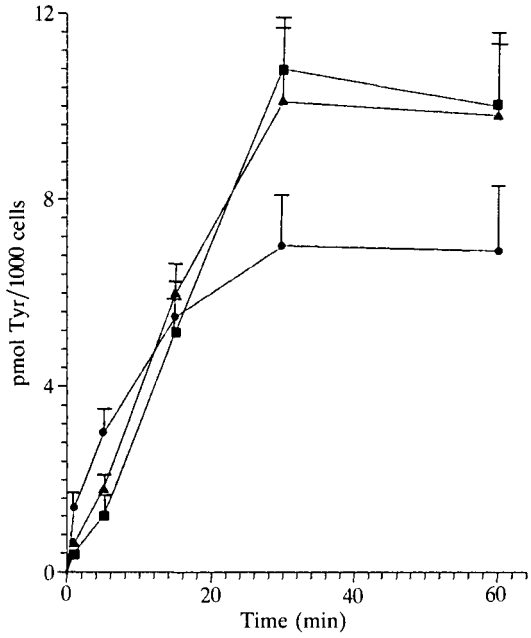

Fig. 6. Time courses of uptake of 0.1 mM L-Tyr by melanoma cells preloaded with (■) L-Ala, (▲) L-Ser or (● L-Thr. Other details as Fig. 2.

This system could also transport L-Phe, but the main route for this last amino acid is the L system. Taking into account the lower uptake of L-Tyr by melanoma cells preloaded with L-Phe in comparison to L-Tyr and L-Trp (Table 2), we carried out some experiments to compare the transport of L-Phe and L-Tyr by our cells. Fig. 8 shows the results obtained for melanoma cells either depleted of amino acids or preloaded with an amino acid representative of the L system, L-Leu. It can be observed that both the initial rate of L-Phe uptake (the means and standard

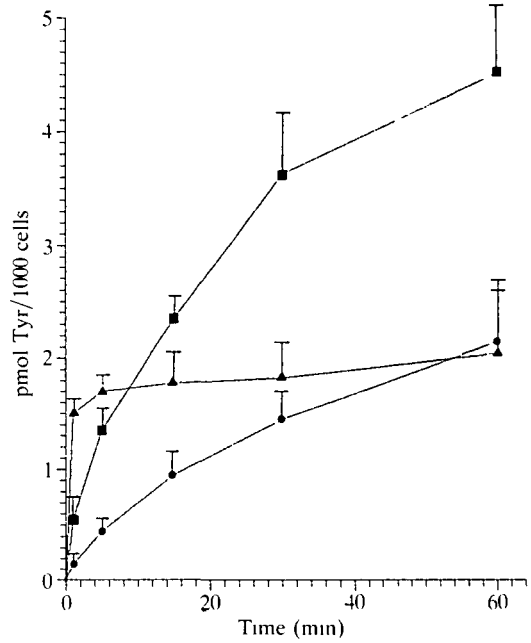

Fig. 7. Time courses of uptake of 0.1 mM L-Tyr by melanoma cells preloaded with (■) AIBA, (●) MeAIBA or (▲) BCH. Other details as Fig. 2.

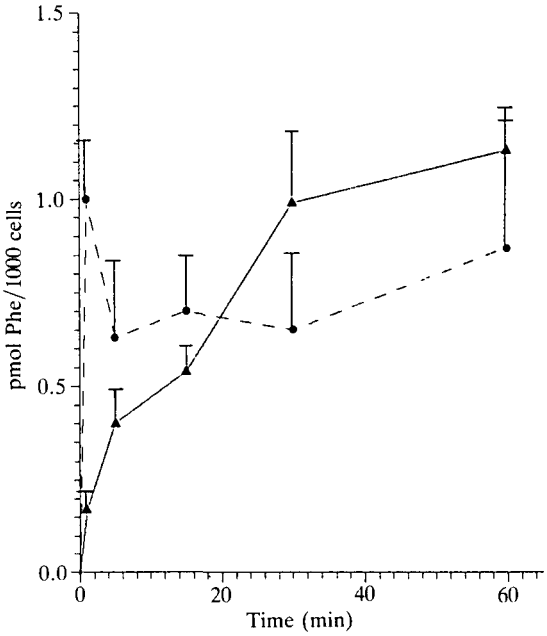

Fig. 8. Time courses of uptake of 0.1 mM L-Phe by melanoma cells depleted of amino acids (▲) or preloaded with L-Leu (●). The experimental procedures were identical to those used to study L-Tyr transport but the tracer was L-[U-^{14}C]Phe isotopically diluted with unlabelled L-Phe.

deviations of three measurements were 0.17±0.04 and 1.01±0.15 pmol Phe/10^3 cells, min^{-1} in depleted and L-Leu preloaded cells respectively) and the profiles of the time course curves were very similar to those obtained for L-Tyr uptake. However, a significant difference was obtained in cells preloaded with L-Leu. The maximal level of L-Phe inside the cells was reached very rapidly (1 min or less); after this overshoot a slight decrease was observed, and the amino acid level remained almost constant for 1 h. This behaviour could suggest that L-Phe is indeed a better substrate for the system L than L-Tyr, so that the exchange with L-Leu is quicker in the first case than in the second.

Discussion

It is well known that the type of medium used to culture cells may affect the characteristics of amino acid transport. Moreover, cells from different tissues can express different transport systems. In spite of this, the patterns of the time courses of L-Tyr uptake in melanoma cells preloaded with certain amino acids are remarkably similar to the time courses described for the direct uptake of these amino acids in other types of cells. Thus, the low level of saturation for the transport of L-Leu and L-Val was already observed in Ehrlich cells by Oxender and Christensen (1963). In addition, Shotwell *et al.* (1981) have reported a rapid initial uptake of L-Leu but a low level of saturation in CHO cells. This is in comparison to the uptake of L-Ala by the same cells, which is linear for a longer period of time and reaches a higher level of accumulation. Our data for preaccumulation of these amino acids in melanoma cells show a very similar pattern for the L-Tyr uptake, suggesting that the process is an exchange between the amino acid preloaded inside the cells and the L-Tyr outside. Thus, the time course of the L-Tyr uptake would be governed by the time courses of the release of the amino acid accumulated in melanoma cells rather than by the L-Tyr uptake itself.

Weissbach *et al.* (1982) described the existence of two different systems L in rat hepatocytes, L1 and L2. They classified the amino acids in different groups according to their abilities to inhibit L-His and L-Leu uptake. L-Met, L-His, L-Trp and L-Tyr represented a unique group which inhibited L-His uptake substantially more than L-Leu uptake, and these amino acids were tentatively assigned to the L1-system. According to our results, these amino acids yielded the highest initial rates of L-Tyr uptake by malignant melanocytes (see Table 2). L-Dopa and L-NorLeu also exchange efficiently with L-Tyr, but they were not tested by Weissbach *et al.* (1982). Since L-Dopa is structurally very similar to L-Tyr and L-NorLeu is a non-branched amino acid very similar to L-Met, their pattern is not surprising. Therefore, it is likely that B16/F10 malignant melanocytes contain a transport system very similar to the L1 system described in rat hepatocytes. This system should be the most important for L-Tyr uptake by melanoma cells.

The results presented show that establishing the initial state of the melanoma cells (depletion of intracellular amino acids or preloading of certain amino acids) is an obligatory step if subsequent measurements of L-Tyr uptake are to be correctly interpreted. According to our results, it is suggested that L-Tyr could be transported in B16/F10 melanoma cells by the L1 and ASC systems. The bulk of the L-Tyr uptake into the cells can be accounted for by an exchange mechanism with the intracellular amino acids. Thus, the use of the L1 or ASC transport systems would depend on the nature of the amino acid to be exchanged with L-Tyr, but both systems seem to be susceptible to trans-stimulation. Moreover, and bearing in mind that the system A is susceptible to trans-inhibition, the preloading of B16/F10 malignant melanocytes with L-Pro produced a very low uptake of L-Tyr, even lower than the rate of uptake by cells depleted of amino acids.

The existence of specific systems for the uptake of L-Tyr has not been detected, in spite of the specialized function of L-Tyr as melanin precursor within melanocytes. Moreover, the rate of L-Tyr uptake is poorly affected by exposure of melanoma cells to α-MSH (α-melanocyte stimulating hormone) during 24 h (data not shown). As melanin polymerization occurs in the melanosomes (Wick *et al.* 1986) but not in the cytosol, the possible specialized mechanisms for transport of L-Tyr, if they exist, might be in the melasonome instead of the cytosolic membrane.

On the other hand, it has been reported that pretreatment with non-radioactive L-Dopa greatly enhanced the uptake of subsequently administered radioactive L-Dopa by Harding-Passey melanomas in tumor-bearing mice (Berjian *et al.* 1986). The treatment of malignant melanoma with radioactive L-Dopa could be important for therapy, since L-Dopa can act as a substrate for tyrosinase and therefore melanin synthesis. Thus, L-Dopa and related compounds have been tested as melanoma-specific drugs (Wick *et al.* 1977; Morrison *et al.* 1985). The increase in the uptake of radioactive L-Dopa by pretreatment with non-radioactive L-Dopa was not explained, but it is very probably related to the stimulation of L-Tyr uptake by the preloading treatment of malignant melanocytes herein reported, although it is unclear whether or not L-Tyr and L-Dopa in melanoma cells share a common transport system. Our results and some competition studies (Nutt and Fellman, 1984) suggest that L-Dopa shares the carrier-mediated transport with L-Tyr, but it has also been reported that both amino acids are transported into melanoma cells by systems showing different sensitivity against uptake inhibitors (McEwan and Parsons, 1987). This point is being further investigated in our laboratory.

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