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Biochimica. — *Kinetics of the amino acid uptake by normal and transformed cells.* Nota di TAMILLA EREMENKO [1], TONINO MENNA [2], CATELLO BUONO [2], MARIA FAMÀ [3] e PIETRO VOLPE [1, 3], presentata [*] dal Corrisp. A. RUFFO.

ABSTRACT. - HeLa cells, normal rat kidney (NRK) cells and NRK cells transformed with Rous sarcoma virus (RSV) were used to investigate the incorporation of ^{3}H-leucine, ^{3}H-glutamate and ^{3}H-aspartate. Unlike HeLa cells, which are characterized by uniphasic kinetics of uptake of these amino acids, both normal and virus-tranformed NRK cells show two "waves" of uptake: one rapid, the other slow. These waves change in their quantitative proportion when comparing normal and transformed cells. In transformed cells, while the slow wave of leucine and glutamate uptakes is extremely decreased, the rapid wave of glutamate uptake tends to disappear at all. In general, the level of incorporation of aspartate is very low. Anyway, transformation facilitates the rapid wave of penetration of this amino acid. In HeLa cells, the glutamate uptake is strikingly facilitated by carbon dioxide.

KEY WORDS: Amino acids; Uptake; Transformation.

RIASSUNTO. - *Cinetiche di incorporazione di aminoacidi in cellule normali e trasformate.* Cellule HeLa, cellule normali di rene di ratto (NRK) e cellule NRK trasformate con il virus del sarcoma di Rous (RSV) sono state impiegate per studiare l'internalizzazione di leucina-H^3, glutammato-H^3 e aspartato-H^3. Diversamente dalle cellule HeLa, che sono caratterizzate da una cinetica unifasica di incorporazione di questi aminoacidi, le NRK sia normali che trasformate incorporano leucina, glutammato e aspartato in due tempi: con un'onda rapida e una lenta. Queste onde variano quantitativamente, l'una rispetto all'altra, quando vengono comparate cellule normali e virus-trasformate. Nelle cellule trasformate, mentre si abbassa sensibilmente l'onda lenta di incorporazione sia della leucina che del glutammato, l'onda rapida di penetrazione del glutammato addirittura tende a scomparire. Il livello di incorporazione dell'aspartato in generale è molto basso. Comunque, la trasformazione facilita l'onda rapida di penetrazione di questo aminoacido. Nelle cellule HeLa, l'incorporazione del glutammato è fortemente facilitata dalla carbaria.

INTRODUCTION

The amino acid uptake has been investigated from several points of view. Some information concerns its correlation, on the one hand, with the properties of given

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amino acid classes or even with the characteristics of single amino acids (1-3) and, on the other, with the state of the amino acid internal pool (2-6). Thus, the involvement of specific carriers was taken into consideration (1, 7). Particularly, an interesting research regards the so-called A-, ASC- and L-systems of the amino acid transport (2, 3, 6-8). Other data show how the amino acid uptake is influenced by the cell growth (2, 9), density (2, 6, 9, 10), transformation (9, 11), proliferation (9) or by the hormonal regulation (12).

The present work shows that unequivocally the amino acid uptake depends on the conditions under which the cells are grown, for instance, on the aeration conditions, with or without carbon dioxide. Then, by comparing the behaviour of the HeLa and NRK cells, data are presented showing how the amino acid uptake is correlated with the cell type. Moreover, it is suggested that virus-transformation, for different amino acids, does not lead always to the same variations of uptake. On the basis of these facts and considerations, the paper is also asking whether the rate of the amino acid uptake is able per se to modulate the rate of protein biosynthesis itself (4, 10, 13) or is it translation-independent at all.

Materials and methods

Cell cultivation

HeLa cells were grown as spinner suspension at 37°C in Joklik-modified minimum essential medium, without Ca^{++}, supplemented with 10% calf serum under magnetic stirring and a continuous flow of 5% carbon dioxide in air (14). Fresh medium was replaced every two days, maintaining the culture density at between 2.5 and 5×10^5 cells/ml. NRK and NRK cells transformed with RSV (RSV-NRK cells) were grown as monolayers in Eagle minimum essential medium (with Ca^{++}) supplemented with 10% foetal calf serum under continuous flow of 5% CO_2 in air (15). The medium in this case remained unchanged for about 100 hours during the whole growth cycle, while the density was maintained at between $2.5\text{-}5 \times 10^5$ cells/ml.

Cell radiolabelling

Before the incubation with the labelled amino acids, HeLa, NRK and RSV-NRK cells were withdrawn from the corresponding suspension cultures when their density reached the level of 5×10^5 cells/ml. The cells were washed twice in Hanks salt solution, resuspended in this same solution at the concentration of 1×10^6 cells/ml and then subdivided into the needed aliquots for detection of the uptake kinetic curves, as specified in the legends to the figures. For cell labelling, the ^3H-amino acids were used at the same final radioactive concentration, i.e. 0.5 μCi/ml $\times 10^{-6}$ cells.

Cell disruption

At various times after radio-labelling, the cells were fractionated at 10% trichloroacetic acid to obtain the TCA-precipitable fraction, containing the newly synthesized proteins, and the TCA-soluble fraction, containing the amino acids transported through the membranes, as non-incorporated precursors.

Materials

The Joklik-modified and the Eagle minimum essential media as well as adult calf and foetal calf sera were purchased from Grand Island Biological Company, New York, USA. Before use, the sera were heated for 2 hours at 56°C and filtered through Seitz EKS II. Trypsin was diluted in TD-solution. The radioactive amino acids were furnished by NEN chemicals GmbH Research Products, Dreieichenhain, West Germany: (^{3}H-4,5)L-leucine, 35.5 Ci/mmol; (^{3}H-2,3)L-glutamic acid, 17.5 Ci/mmol; (^{3}H-2,3)L-aspartic acid, 16.9 Ci/mmol.

RESULTS

Kinetics of uptake of protein precursors by HeLa cells under different experimental conditions

Without carbon dioxide, the total uptake of leucine is slightly higher than that of glutamate, while the amount of aspartate entering the cell membrane is at least ten times less than that of leucine and glutamate (fig. 1A). For all three amino acids, the maximal point of uptake occurs approximately at the 2nd hour, whereas the material detected free in the pool during the whole 6-hour uptake follows patterns which are different for leucine and for the two acidic compounds. The free leucine concentration is very low during the first hour of uptake and then increases to a peak at the 2nd hour. The curves of the free glutamic and aspartic acid concentrations follow the curves of the corresponding uptakes. These patterns complement those of incorporation of the three amino acids into proteins (shaded areas). Leucine incorporation into proteins is very rapid during the first hour of uptake, while the incorporation of glutamic and aspartic acids into proteins begins slowly.

When the amino acid uptake is measured under a continuous flow of 5% carbon dioxide in air, dramatic changes occur (fig. 1B) – in general, it is increased particularly for glutamic acid. The behaviour of the individual amino acids is, however, differential. Penetration of leucine through the cell membrane does not decrease until the 6th hour of uptake except in the absence of carbon dioxide. Concentration of nonincorporated leucine parallels its total uptake during the first 30 min, but over longer periods it is sharply reduced. Correspondingly, at these times there is a rather high incorporation of leucine into proteins. Although with strong quantitative differences, glutamate and aspartate behave with some similarity (the pool-free concentrations of these amino acids parallel their total uptake). For both, penetration

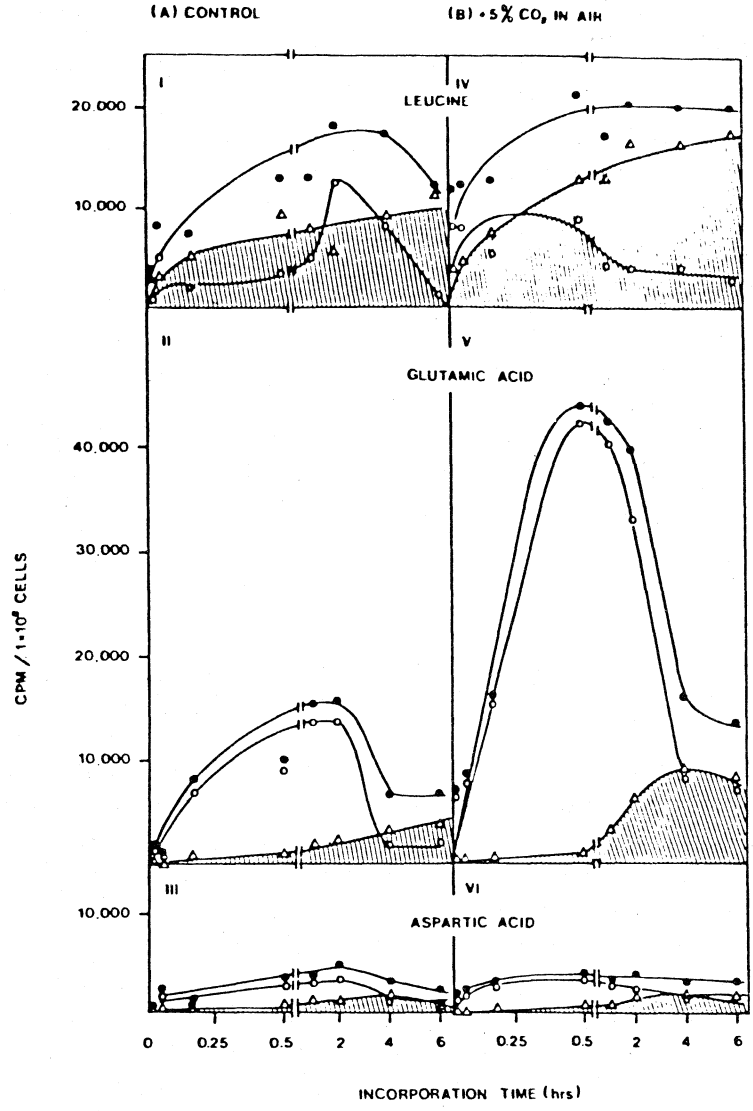

Fig. 1. - *Kinetics of incorporation of protein precursors by HeLa cells.*

For each amino acid (^{3}H-leucine, ^{3}H-glutamic acid and ^{3}H-aspartic acid), 20×10^6 cells were withdrawn from the culture suspension, resuspended in 18 ml of Hanks and mixed with 2 ml of the solution containing 10μCi of radioactive material. The cell suspension (0.5μCi/1 $\times$ 10^6 cells/ml) was then incubated for 6 hours at 37°C under continuous shaking. To stop the incorporation at the indicated times during 6 hours, 2 ml of experimental suspension (with 2×10^6 labelled cells) were withdrawn and quickly replaced in a centrifuge tube containing 5 ml of chilled Hanks. Cell harvesting was achieved in 6 min at 2,000 rev./min. The cell pellet was then washed twice in 5 ml of chilled Hans, recentrifuged under the same conditions and treated for 30 min in the cold with 1 ml 20% trichloroacetic acid. The TCA-lysate was fractionated by centrifuging for 6 min at 2,000 rev./min. The supernatant was replaced directly in a vial containing 10 ml Insta-gel, while the precipitate was washed with 8 drops of water which, after centrifuging again for 6 min at 2,000 rev./min, was added to the same vial to test the TCA-soluble radioactivity yielded. The TCA-precipitable fraction was dissolved in 10 ml Insta-gel and also tested for radioactivity. (A) On the left there are the control kinetics of incorporation of the three amino acids as measured in the absence of CO_2; (B) On the right there are the same kinetics measured under a continuous flow of 5% CO_2 in air. The values represent the mean of three experiments. (•——•) Total radioactivity transported into the cell; (o——o) TCA-soluble radioactivity; (Δ——Δ) TCA-precipitable radioactivity (dashed areas).

through the cell membrane occurs early, while incorporation into proteins is maximal at the 4th hour of uptake. Finally, protein synthesis does not consume all the glutamic and aspartic acids which have penetrated into the cell.

Comparison of the kinetics of the amino acid uptake by NRK and HeLa cells

There are similarities and differences of uptake if one compares HeLa and NRK cells. As with HeLa cells labelled in the absence of carbon dioxide (fig. 1A), NRK cells incorporate more leucine than glutamate or aspartate, while the incorporation of aspartate is about ten times less than for glutamate (fig. 2A). Unlike HeLa cells, where the uptake curves show one peak (fig. 1A), NRK cells show two waves of amino acid transport and incorporation into proteins: one rapid, the other slow (fig. 2A). The waves of uptake of the three amino acids change in their quantitative proportion or temporal sequence, while there is, in general, an inverse correlation between the free amino acid concentration in the pool and their incorporation into proteins. For leucine only, this incorporation continuously increases up to the 6th hour of uptake (fig. 2, I), as for HeLa cells (fig. 1, I).

Kinetics of uptake of protein precursors by NRK and RSV-NRK cells

RSV-transformed NRK cells, as well as NRK (fig. 2A) and HeLa (fig. 1A) cells, incorporate more leucine than glutamate and a very small amount of aspartate (fig. 2B). However, whereas the transport of leucine and glutamate in normal NRK cells is higher than that observed in RSV-transformed NRK cells, the transport of aspartate in normal cells is lower than that observed in transformed cells. As regards leucine particularly, the uptake by RSV-NRK cells reaches its maximal value in 30 min and then remains at a more or less constant level for up to 6 hours (fig. 2, IV). Compared with NRK cells, RSV-NRK cells show a less pronounced increase in the incorporation of leucine into proteins in the earlier stages. This increase slowly develops up to the 6th hour, whereas during the first 30 min the concentration of the pool-free leucine is higher than that observed in NRK cells. As regards the acidic amino acids, the incorporation of glutamate into proteins is low in viral-transformed cells, whereas that of aspartate is relatively high. The differential beheviour between NRK and RSV-NRK cells can be judged, moreover, on the basis of the quantitative proportion of the peaks of uptake and incorporation of amino acids into proteins.

DISCUSSION

This investigation analyses the kinetics of the amino acid uptake by considering: (I) different conditions of aeration of a culture; (II) two types of cells; (III) virus-transformation. Leucine represents the L-system (2). Glutamate and aspartate were chosen because information about the uptake of the acidic class of amino acids was almost inexistent (13).

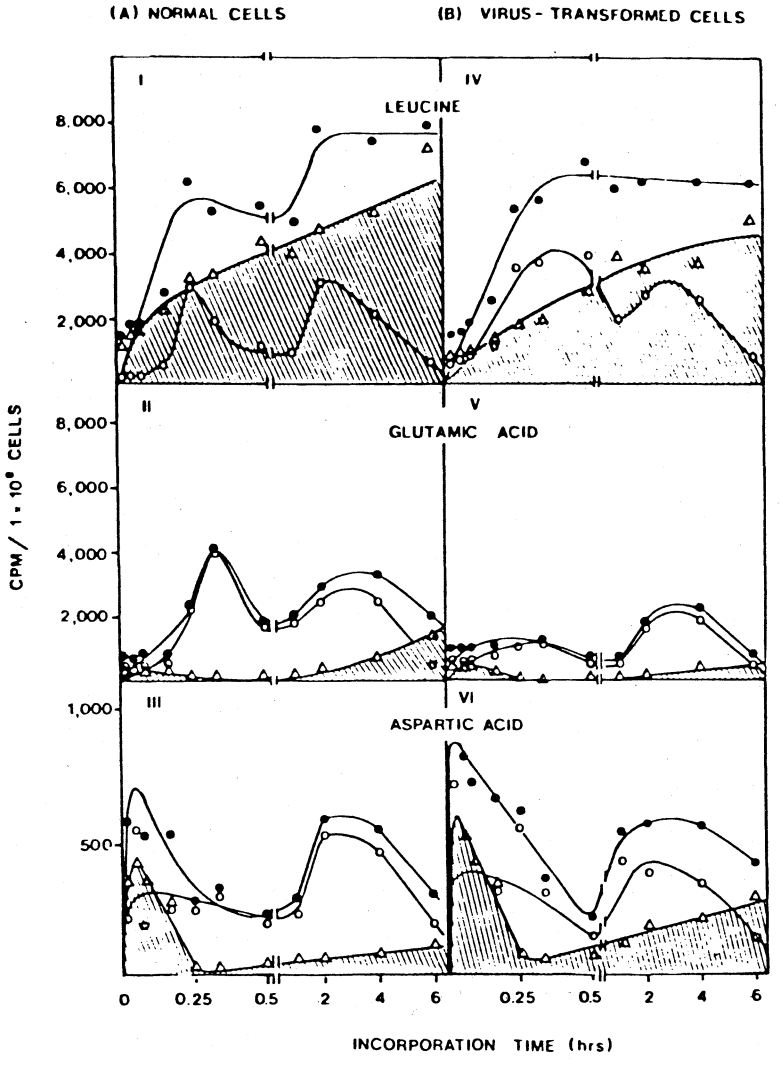

Fig. 2. - *Kinetics of incorporation of protein precursors by NRK and RSV-transformed NRK cells.* The uptake curves for all three amino acids (^{3}H-leucine, ^{3}H-glutamic acid and ^{3}H-aspartic acid) were followed, both in normal (A) and virus-trasformed (B) cells, using the same procedure described in the legend to figure 1. However, the incorporation of the radioactive material was only allowed to occur in the absence of CO_2. The values are the mean of three experiments. (●——●) Total radioactivity transported into the cells; (○——○) TCA-soluble radioactivity; (△——△) TCA-precipitable radioactivity (dashed areas).

Carbon dioxide influences the amino acid uptake by HeLa cells. Particularly, it leads them to increase the glutamate uptake. This suggests the existence of a specific, undescribed hitherto, system for transport of glutamate, sensitive to CO_2. The experiments with NRK and RSV-NRK cells were performed, therefore, in the absence of this air component in order to detect clearly any net variation of the uptake processes. Thus, the bi-phasic kinetics of the amino acid uptake appear to be a real characteristics of the NRK cells. Such kinetics are conserved, in fact, by these cells after their virus-transformation. The difference of the kinetics of amino acid uptake, observed for two cell types which, besides, belong to different evolutive species, in view of the large number of tissues existing in eukaryotes, may explain why among the different cells in them the distribution of the nutrients is highly heterogeneous, as known. Virus-transformation, alternatively, leads the NRK cells to a slightly decreased uptake of leucine and glutamate and to a slightly increased uptake of aspartate (the two acidic amino acids behave in different ways). As for RNA precursors (16), the increased uptake of aspartate by RSV-NRK cells corresponds fairly well to a similar increased uptake of several amino acids by various virus-transformed cells (2,17-19). The decreased uptake of leucine and glutamate by RSV-NRK cells corresponds to the decreased uptake of the α-aminobutyric acid by transformed cells (11,20-22). These differences might be interpreted in terms of a specific interaction which probably continues to exist between the virus-transformed cell membrane and a given amino acid, although with an inverted tendency.

Anyway, while the experiments with virus-transformed cells appear to suggest that the variations of amino acid uptake are able to exert some pressure on the rate of protein synthesis, as a rule, other observations suggest that the incorporation of the amino acid precursors into proteins is, in a given range, independent of their uptake or intracellular pool-free concentration. In fact, in HeLa cells, in the presence of CO_2, while leucine uptake is lower than glutamate uptake, vice versa the incorporation of leucine into proteins is greater than that of glutamate and asperatate. Moreover, there are time-shifts between the maximal rates of amino acid uptake and protein labelling: in HeLa (either in the absence or presence of CO_2), and in NRK cells (either normal or virus-transformed). In this frame, of course, it is apparent that not all amino acids which enter the cytosol are used for building peptides. Hence, some differences could be explained by an efflux of the amino acids or by their utilization for other biochemical events.

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