Characteristics and adaptive regulation of glycine transport in cultured glial cells

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The transport of glycine in C6 glioma cells takes place mainly in a heterogeneous Na⁺-dependent manner which can be resolved into different components. A Na⁺- and Cl⁻-dependent component with high affinity for glycine is pH-sensitive and inhibited by sarcosine, all these characteristics corresponding to System Gly. The low-affinity component of the transport of glycine can be discriminated as two components, namely System A and System ASC. The main proportion of glycine transport through the low-affinity system is carried out by the ASC System, which appears to be constitutively expressed by the cells. The adaptive response of the low-affinity Na⁺-dependent transport of glycine to amino acid deprivation was identified with System A on the basis of its ion-dependency, pH-sensitivity and by inhibition analysis. The possible physiological role of the high- and low-affinity components of the transport system for glycine in glial cells is discussed.

INTRODUCTION

Glycine, besides its numerous metabolic functions (Meister, 1975), is widely held to fulfil most of the criteria for an inhibitory neurotransmitter in the mammalian central nervous system, mainly in the spinal cord (Aprison & Daly, 1978) and probably in some localized areas of the brain such as substantia nigra (Pycock *et al.*, 1981).

The uptake of neutral amino acids such as glycine by animal cells is divided between a few transport systems that have overlapping substrate reactivities (Sershen & Lajtha, 1979; Christensen, 1979; Guidotti *et al.*, 1978). Largely on the basis of the pattern of competitive interactions between neutral amino acids and on the ionic dependency, Oxender & Christensen (1963) (see also Christensen *et al.*, 1973) proposed the existence of different transport systems for glycine in various animal cell types: the Na⁺-dependent System Gly, specific for this amino acid and its *N*-methylated derivative sarcosine (Christensen & Handlogten, 1981); and System A, which is effective for amino acids having short, polar or linear side chains, such as alanine glycine and the non-metabolizable analogue 2-aminoisobutyric acid and its *N*-methylated derivative 2-(methylamino)isobutyric acid (MeAIB). Transport by System A is Na⁺-dependent, and it has been shown to be much decreased at lowered extracellular pH. A third Na⁺-dependent transport system for glycine has also been characterized in animal cells. This system, able to transport alanine, serine and cysteine, is designated System ASC. The System L is most effective for branched-chain and aromatic amino acids; it has been described in a wide variety of animal cell types and is Na⁺-independent (for reviews see Shotwell & Oxender, 1983; White, 1985).

In previous studies from our laboratory, we have reported the existence of two efficient uptake systems for

glycine in glioma cells on the basis of the ionic dependency and the kinetic features (Zafra & Giménez, 1986a,b). One system is Na⁺- and Cl⁻-dependent with high affinity for the substrate, and the other is Na⁺-dependent but Cl⁻-independent and with low affinity for glycine. The former system showed kinetic constants and energetic requirements similar to those found in nerve terminals (Mayor *et al.*, 1981; Kuhar & Zarbin, 1978; Logan & Snyder, 1972).

Although the high-affinity component of the transport system for glycine has been fairly well characterized in glial cells (Zafra & Giménez, 1986a,b) and implicated in the termination of glycine action at post-synaptic receptors (Henn, 1976; Schrier & Thompson, 1974), the low-affinity component has been less well studied and is not well understood. For this reason, we undertook this investigation to characterize in detail the transport of glycine in glial cells and to explore its regulation by amino acid availability.

EXPERIMENTAL

Materials

[U-¹⁴C]Glycine (113 mCi/mmol) was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Dulbecco's modified Eagle's medium (DMEM), Earle's balanced salt solution, horse serum and fetal-calf serum were obtained from Gibco, Paisley, Scotland, U.K. Dishes and multi-well plates for tissue culture were purchased from Costar, Cambridge, MA, U.S.A. All other reagents were of the highest purity available.

Cell culture

C6 glioma cells, cloned originally from a rat glioma (Benda *et al.*, 1968) and obtained from the American Type Culture Collection (Rockville, MD, U.S.A.), were

Abbreviations used: MeAIB, 2-(methylamino)isobutyric acid; DMEM, Dulbecco's modified Eagle's medium; KRP, Krebs–Ringer phosphate medium.

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maintained in monolayer culture in DMEM containing 10% (v/v) horse serum and 2.5% (v/v) fetal-calf serum in 100 mm-diameter plastic dishes. They were maintained at 37 °C in humidified air with 5% CO₂. Cells were subcultured twice weekly by standard procedures. The methods for harvesting and subculturing have been described previously (Volpe & Marasa, 1975). For glycine-uptake experiments, cells were grown in 24-well tissue-culture plates to reach confluency. For amino acid deprivation experiments, the growth medium of confluent cultures was changed to Earle's balanced salt solution with sera dialysed to remove amino acids.

Uptake experiments

Assays were performed at 37 °C in 200 µl of Krebs–Ringer phosphate medium (KRP) (Ross, 1972), containing the following: 140 mM-NaCl, 5 mM-KCl, 0.75 mM-CaCl₂, 1.3 mM-MgSO₄, 10 mM-Na₂HPO₄, 10 mM-glucose, pH 7.4. All solutions used in the uptake experiments were prepared with distilled water and filtered through Millipore filters (0.45 µm pore size). The osmolarity of all solutions was kept constant during the uptake experiments. The growth medium was removed by aspiration and, after two 0.5 ml washes with KRP (37 °C), cells were preincubated with 0.5 ml of KRP for 30 min. At zero time this medium was removed and 200 µl of KRP containing radioactive glycine (0.1 µCi) was added. Cells were incubated for the indicated time, rinsed with 3 × 0.5 ml of fresh KRP (2–4 °C) within 10 s and then dissolved in 250 µl of 0.2 M-NaOH. A 150 µl sample was placed directly into a scintillation vial and counted for radioactivity after addition of 10 ml of scintillation solution (60 g of naphthalene, 4 g of 2,5-diphenyloxazole, 400 ml of ethylene glycol and 600 ml of toluene).

All incubations were carried out in triplicate. Each experiment was repeated at least three times with different cell cultures. The protein content of each well was measured by the method of Bradford (1976).

Analysis of glycine uptake

The total uptake of glycine by C6 glioma cells was divided into three components: one non-saturable component, and two saturable ones conforming to simple Michaelis–Menten kinetics. The following equation was applied:

$$v = \frac{V_{\max,1} \cdot [S]}{[S] + K_{m1}} + \frac{V_{\max,2} \cdot [S]}{[S] + K_{m2}} + K_D \cdot [S] \quad (1)$$

The proportionality coefficient (K_D) of the non-saturable component was estimated from the linear increase in the total glycine uptake with increasing glycine concentration when the experiments were extended to the concentration range of glycine which saturated the saturable transport systems.

The kinetic constants of the saturable components (K_{m1} , K_{m2} , $V_{\max,1}$, $V_{\max,2}$) were calculated by using a conventional BASIC program based on a modified Newton–Gauss least-squares iterative method that was employed to fit directly v versus $[S]$ to eqn. (1). The initial parameters for this iterative algorithm were estimated from an Eadie–Hofstee plot, according to conventional methods (Magar, 1972).

RESULTS

Fig. 1 shows kinetic studies of the transport of glycine in C6 glioblastoma cells which were performed by measuring the initial rates of uptake of glycine over a wide range of substrate concentrations (10 µM–10 mM). Previous workers who studied transport of amino acids into cells corrected it for non-saturable diffusion (non-specific transport). To make this correction, we used the experimental corrections described previously for other preparations (Kontro & Oja, 1978; Abelle *et al.*, 1983). Kinetic analysis of the data revealed that the total uptake was not saturable and, by using the analytical method described in the Experimental section, the system was resolved into two saturable components: one with a K_m of 95 µM and a $V_{\max}$ of 1.85 nmol/min per mg of protein (referred to as the high-affinity component), and a second component with a K_m of 2.45 mM and a $V_{\max}$ of 13.5 nmol/min per mg of protein (referred to as the low-affinity component). The same experiments demonstrate that both high-affinity and low-affinity systems are highly dependent on the presence of Na⁺, and that the high-affinity system disappeared when Cl[−] ions were absent from the external medium (Fig. 1).

The uptake of 10 µM-glycine in a medium containing Na⁺ and Cl[−] ions was tested for possible inhibition by various amino acids and derivatives, some naturally occurring and others artificial (Table 1). The results of this experiment show that the high-affinity system is highly specific for glycine, being inhibited only in the presence of unlabelled glycine, sarcosine (*N*-methylglycine) or the methyl and ethyl esters of glycine. It is

Fig. 1. Kinetics of glycine uptake in glioblastoma cells

C6 cells grown to confluence were incubated for 7 min in the presence of labelled glycine at concentrations ranging from 10 µM to 10 mM in the following media: KRP control (with 140 mM-NaCl) (●) or KRP in which NaCl has been iso-osmotically replaced by sodium gluconate (○). Kinetic parameters of the two saturable components were calculated as indicated in the Experimental section, and values were replotted as an Eadie–Hofstee plot.

Table 1. Inhibition of Na⁺-dependent glycine uptake in glioblastoma cells by amino acids and amino acid derivatives

C6 glioblastoma cells were incubated for 7 min in KRP medium with 10 μ M-[¹⁴C]glycine and 1 mM inhibitors (A), or in Cl⁻-free KRP medium with 1 mM-[¹⁴C]glycine and 10 mM inhibitors (B). Results in (A) were corrected for diffusion and low-affinity component. Results in (B) were corrected for diffusion. Values are the means $\pm$ S.E.M. of three replicate determinations. Values in parentheses represent % of control values in the absence of competing amino acids. Values were compared with control values by using Student's *t* test: **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

Addition	High-affinity uptake (nmol/7 min per mg of protein) (A)	Low-affinity uptake (nmol/7 min per mg of protein) (B)
None	3.06 $\pm$ 0.14 (100)	55.1 $\pm$ 1.6 (100)
Glycine	0.39 $\pm$ 0.04*** (13)	12.7 $\pm$ 1.1*** (23)
L-Serine	2.62 $\pm$ 0.05 (86)	12.1 $\pm$ 1.7*** (22)
L-Alanine	2.66 $\pm$ 0.05 (97)	12.8 $\pm$ 1.3*** (23)
D-Alanine	2.10 $\pm$ 0.07 (69)	26.4 $\pm$ 1.8** (48)
L-Proline	2.67 $\pm$ 0.13 (87)	24.2 $\pm$ 1.4** (44)
L-Threonine	2.75 $\pm$ 0.08 (90)	20.9 $\pm$ 1.1*** (38)
L-Cysteine	2.01 $\pm$ 0.10* (66)	17.3 $\pm$ 1.2*** (31)
Glycine methyl ester	0.61 $\pm$ 0.08*** (20)	46.8 $\pm$ 2.2 (85)
Glycine ethyl ester	0.92 $\pm$ 0.07** (30)	29.2 $\pm$ 2.1* (53)
Aminomethylphosphonic acid	3.00 $\pm$ 0.11 (98)	40.2 $\pm$ 3.5 (73)
Ethanolamine	2.63 $\pm$ 0.15 (86)	46.7 $\pm$ 1.8 (85)
Glycine amide	3.20 $\pm$ 0.15 (105)	54.5 $\pm$ 1.6 (99)
Sarcosine	0.30 $\pm$ 0.05*** (10)	22.2 $\pm$ 2.7* (53)
<i>NN</i> -Dimethylglycine	2.02 $\pm$ 0.06* (66)	42.4 $\pm$ 1.4 (77)
<i>N</i> -Formylglycine	3.13 $\pm$ 0.10 (102)	57.8 $\pm$ 3.1 (105)
<i>N</i> -Chloroacetyl glycine	3.00 $\pm$ 0.06 (98)	49.6 $\pm$ 2.5 (90)
Hydantoic acid	2.88 $\pm$ 0.15 (94)	60.0 $\pm$ 2.8 (109)
MeAIB	2.75 $\pm$ 0.20 (90)	44.1 $\pm$ 2.1 (80)
α -Aminoisobutyric acid	2.51 $\pm$ 0.17 (82)	14.3 $\pm$ 2.4*** (26)
β -Aminoisobutyric acid	2.84 $\pm$ 0.15 (93)	54.3 $\pm$ 2.5 (98)

noteworthy that this component of glycine transport appears to be entirely MeAIB-insensitive, thus ruling out transport by System A.

The activity of the low-affinity transport system for glycine (1 mM) in the presence of the same amino acids and derivatives is also shown in Table 1. As the high-affinity component is completely saturated at 1 mM-glycine, the uptake of glycine was measured in a Cl⁻-free medium in order to avoid interference by the high-affinity component of the transport system. The results demonstrate that the low-affinity Na⁺-dependent component of glycine transport is more sensitive to the presence of other amino acids, being inhibited by serine, alanine, proline and cysteine. However, it is scarcely inhibited by MeAIB even at very high concentrations up to 40 mM (results not shown).

One characteristic that seems to be specifically different between Systems A and ASC is the adaptive response of System A to amino acid deprivation in the culture medium. The experiment presented in Table 2, with cells maintained for 20 h in Earle's balanced salt solution (without amino acids), shows the effect of amino acid deprivation on the low-affinity transport system for glycine. Amino acid deprivation markedly enhanced the uptake of glycine. The induced component was inhibited when the transport assay was carried out in the presence of 20 mM-MeAIB, which suppresses specifically the operation of System A. The component of transport which was MeAIB-non-inhibitable scarcely changed on amino acid deprivation. Furthermore, since the uptake of glycine in the absence of Na⁺ is negligible (thus ruling out any substantial contribution of Na⁺-independent

systems to its total uptake), the Na⁺-dependent MeAIB-non-inhibitable fraction is likely to reflect the activity of System ASC. The activity of System A for glycine in the controls (normal culture medium) can be estimated by the difference between total and MeAIB-non-inhibitable uptake, and represents about 15 % of total uptake.

Table 2. Effect of amino acid deprivation in culture on the low-affinity glycine uptake

C6-cell cultures grown to confluence in DMEM medium plus 10 % horse serum and 2.5 % fetal-calf serum were incubated for 20 h in DMEM with sera (medium with full complement of amino acids) (non-deprived cells) or in Earle's balanced salt solution with dialysed sera (medium without amino acids) (20 h-deprived cells). Transport activity was measured for 15 min in a modified KRP medium (without Cl⁻) containing 1 mM-[¹⁴C]glycine and the indicated additions. Values represent means $\pm$ S.E.M. of triplicate determinations in a representative experiment. Values were compared with control values in non-deprived cells in the absence of MeAIB by using Student's *t* test: ****P* < 0.001.

Cultures	Addition	Uptake (nmol/7 min per mg of protein)
Non-deprived	None	58.2 $\pm$ 4.5
	20 mM-MeAIB	49.5 $\pm$ 3.0
20 h-deprived	None	145.2 $\pm$ 7.2***
	20 mM-MeAIB	51.3 $\pm$ 2.5

Fig. 2. Effect of amino acid deprivation in culture on the kinetics of low-affinity glycine transport

C6-cell cultures grown to confluence were incubated for 16 h in Earle's balanced salt solution with dialysed sera. Transport assays were carried out for 7 min in a modified KRP medium (without Cl^-) in the presence of the indicated concentrations of $[^{14}\text{C}]$ glycine and in the absence (●) or presence (○) of a 25-fold excess of MeAIB. Diffusion was estimated in the absence of Na^+ (replaced by choline chloride) (■).

Fig. 2 presents a kinetic study of the MeAIB-non-inhibitable and the MeAIB-inhibitable components of the low-affinity uptake of glycine in cells previously maintained in amino acid-free culture medium. Analysis of the kinetic data by the Eadie-Hofstee method gave a K_m of 1.9 mM and $V_{\max}$ of 13.6 nmol/min per mg of protein for System A, and a K_m of 2.4 mM and a $V_{\max}$ of 10.4 nmol/min per mg of protein for System ASC (Fig. 3).

Fig. 4 shows the pH-sensitivity of the high- and low-

Fig. 3. Eadie-Hofstee plot of initial rates of specific low-affinity glycine transport and the effect of amino acid deprivation in culture

The experimental conditions and symbol definitions are as described in the legend to Fig. 2. Transport was corrected for diffusion as indicated in the legend to Fig. 2.

Fig. 4. Effect of pH of the extracellular medium on glycine uptake

C6 cells grown to confluence were incubated for 8 h in DMEM with 10% horse serum and 2.5% fetal-calf serum (○, □) or in Earle's balanced salt solution containing dialysed sera (●). Transport activity was determined for 15 min under the following conditions: 10 μM - $[^{14}\text{C}]$ glycine in KRP medium (□) or 1 mM- $[^{14}\text{C}]$ glycine in modified KRP medium without Cl^- (●, ○). The buffer was varied between pH 5.5 and 8.0. Each point represents the mean ($\pm$ S.E.M.) of triplicate determinations in a representative experiment. Note the difference in scales.

affinity uptake of glycine in order to distinguish between the possible contribution of Systems A and ASC to the total uptake. The high-affinity component of the transport system is moderately pH-sensitive. The low-affinity component shows a different pH-dependency from that previously reported for the A and ASC Systems in other preparations (Christensen & Handlogten, 1981),

Table 3. Effect of cycloheximide and actinomycin D on the enhancement of glycine uptake by amino acid deprivation of C6 glioma cells

C6 cultures grown to confluence were incubated for 6 h in DMEM medium with 10% horse serum and 2.5% fetal calf serum (controls), or in Earle's balanced salt solution and dialysed sera (deprived) with the indicated additions. Transport assays were carried out for 5 min in a modified KRP medium (without Cl^-) containing 1 mM- $[^{14}\text{C}]$ glycine. Values represent means $\pm$ S.E.M. of triplicate determinations in a representative experiment. Values were compared with control values in non-deprived cells in the absence of additions by using Student's *t* test: ** $P < 0.01$.

Cultures	Addition	Uptake (nmol/5 min per mg of protein)
Non-deprived	None	38.0 $\pm$ 0.8
6 h-deprived	None	59.2 $\pm$ 1.1**
Non-deprived	Cycloheximide (50 μM)	32.1 $\pm$ 1.9
6 h-deprived	Cycloheximide (50 μM)	34.4 $\pm$ 2.5
Non-deprived	Actinomycin D (5 $\mu\text{g/ml}$)	36.8 $\pm$ 3.0
6 h-deprived	Actinomycin D (5 $\mu\text{g/ml}$)	40.8 $\pm$ 1.2

Table 4. Reversal of the enhancement by amino acid deprivation of low-affinity glycine transport

C6 glioblastoma cells grown for 3 days in DMEM plus 10% horse serum and 2.5% fetal calf serum were transferred to Earle's salt solution plus 10% dialysed serum for 19 h. Preincubation was continued for 5 h in the Earle's medium (24 h-deprived) and the indicated additions in the absence or presence of 50 μ M-cycloheximide. Transport assays were carried out for 7 min in a modified KRP medium (without Cl^-) containing 1 mM- ^{14}C glycine. Values represent the means $\pm$ S.E.M. of three replicate determinations. They were compared with the control value (19 h-deprived cells) by using Student's *t* test: **P* < 0.05; ***P* < 0.01. Values in parentheses show values as % of control values.

Conditions	Uptake (nmol/7 min mg of protein)	
	No cycloheximide	+ cycloheximide
Non-deprived (DMEM medium)	57.0 $\pm$ 1.0	—
19 h-deprived (control)	156.6 $\pm$ 0.5 (100)	—
24 h-deprived	177.3 $\pm$ 4.9* (113)	159.6 $\pm$ 9.1 (102)
19 h-deprived + 5 h 10 mM-glycine	98.6 $\pm$ 1.3* (63)	142.5 $\pm$ 3.1 (91)
19 h-deprived + 5 h 10 mM-L-alanine	94.6 $\pm$ 3.2* (60)	158.5 $\pm$ 7.0 (101)
19 h-deprived + 5 h 10 mM- γ -aminobutyric acid	185.2 $\pm$ 5.0* (118)	153.9 $\pm$ 6.3 (98)
19 h-deprived + 5 h DMEM	68.4 $\pm$ 2.1** (44)	143.9 $\pm$ 5.6 (92)

but nevertheless the ASC System in glioma cells shows a marked pH-sensitivity (Fig. 4).

The requirement for macromolecular synthesis in the adaptive response of System A was tested by exploring the effect of actinomycin D and cycloheximide, inhibitors of transcription and translation respectively, on the amino acid-deprivation-induced transport of glycine (Table 3). After incubation with actinomycin D or cycloheximide for periods not exceeding 6 h, in order to avoid cell damage, this experiment demonstrates that the enhancement of System A activity by deprivation of amino acids apparently requires synthesis of both RNA and protein.

The deprivation-enhanced activity of low-affinity transport of glycine (System A) is reversed by amino acid repletion. As shown in Table 4, after a 19 h incubation in an amino acid-free medium, the low-affinity transport of glycine was increased 3-fold above the values in cells incubated in DMEM medium. This increase was augmented when the incubation was continued for a further 5 h. This additional increase was blocked when cycloheximide was present during the 5 h incubation. When glioblastoma cells subjected to a 19 h de-repression were transferred to a medium containing a full complement of amino acids (DMEM medium), the activity of transport System A decreased. The addition of cycloheximide to this repressive medium prevented the decrease in transport activity. Repression of the amino acid-deprivation-induced transport component was also observed when either 10 mM-glycine or 10 mM-alanine was added for 5 h to the 19 h-deprived cells. Again, addition of cycloheximide prevented this amino acid-mediated repression. γ -Aminobutyric acid was ineffective in repression of the deprivation-induced transport component.

DISCUSSION

Previous results from our laboratory and those included in the present paper clearly show that the transport of glycine in C6 glioma cells take place mainly in a heterogeneous Na^+ -dependent manner, which can be resolved into different components. The Na^+ -dependent

system accounts for practically all of the transport of glycine in glioma cells at physiologically relevant substrate concentrations, and there was a minor Na^+ -insensitive component which was not saturated at substrate concentrations up to 15 mM.

The Na^+ - and Cl^- -dependent component of the transport system shows high affinity for glycine, is pH-sensitive, and is very specific for the substrate, only recognizing glycine, its *N*-methylated derivative sarcosine, or the methyl and ethyl esters. All these characteristics correspond to the System Gly described by Christensen and co-workers in pigeon erythrocytes (Christensen & Handlogten, 1981), rabbit reticulocytes (Eavenson & Christensen, 1967), and rat hepatocytes (Christensen & Handlogten, 1981). This system shows kinetic features and ionic dependency very similar to those of the high-affinity system for glycine in nerve terminals (Mayor *et al.*, 1981), and it is thought to participate in the inactivation of neuronally released glycine. The existence of this transport system located in glial cells supports the hypothesis that glial cells may participate actively in the modulation of neuronal excitability by controlling the concentrations of neuroactive substances in the extracellular milieu of neurons.

On the other hand, the low-affinity component of the transport of glycine in glial cells can be discriminated as two components, namely System A and System ASC, by using the general criteria given by Christensen and his associates to differentiate between transport systems for amino acids in intact cells (Christensen, 1982, 1984). On the basis of sensitivity to inhibition by MeAIB and substrate specificity, it can be demonstrated that the main proportion of glycine transport through the low-affinity system is carried out by the ASC System, which appears to be constitutively expressed by the cell (Shotwell *et al.*, 1983). Nevertheless, the system showed a different pH-sensitivity in glial cells from that described for the System ASC in other preparations (Christensen & Handlogten, 1981). The adaptive response of the low-affinity Na^+ -dependent transport of glycine to amino acid deprivation was identified with System A on the basis of its Na^+ -dependency, pH-sensitivity and by

inhibition analysis. This deprivation-induced transport was accompanied by an increase in the $V_{\max}$ of uptake of glycine by System A, with no significant change in the K_m . These results are consistent with an increase in the number of active System A carriers. Because the increased transport activity could still be observed in cells depleted of amino acids before the uptake measurements, release from *trans*-inhibition was ruled out as a basis for the adaptive response to amino acid deprivation. The reversal of the deprivation-induced increase in the low-affinity transport by amino acid repletion is a process mediated by System A-reactive substrates (glycine and alanine, but not γ -aminobutyric acid), and requires protein synthesis. The regulation of System A transport activity by amino acid supply has now been described in many tissues and cell types (Heaton & Gelehrter, 1977; Guidotti *et al.*, 1978; Dall'Asta *et al.*, 1978; Kelley & Potter, 1979; Shotwell *et al.*, 1981, 1983; Schenerman & Kilberg, 1986), including cells in culture, but never before in cells from the nervous system. This phenomenon, as demonstrated by the present results and previously shown by Guidotti *et al.* (1978) in fibroblasts, requires synthesis of both RNA and protein, and it has been suggested that the System A transport activity is regulated by a repression/derepression mechanism acting at the transcriptional level (Gazzola *et al.*, 1981).

The possible contribution of the low-affinity uptake systems to the removal of glycine from the synaptic cleft is still a matter of conjecture. This system may co-operate with high-affinity uptake in the elimination of glycine shortly after its release, when transmitter concentration is at its greatest. On the other hand, low-affinity high-capacity transport systems for amino acid neurotransmitters in glial cells could serve general metabolic functions similar to those proposed in neurons (Johnston & Iversen, 1971).

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