

α -Aminoisobutyric Acid Transport into Human Glia and Glioma Cells in Culture

G. RONQUIST,¹ G. ÅGREN,¹ J. PONTEN² AND B. WESTERMARK²

¹ Institute of Medical and Physiological Chemistry, Biomedical Center, Box 575 and ² Division of Cell Biology, The Wallenberg Laboratory, University of Uppsala, Sweden

ABSTRACT The AIB transport into human glia and glioma cells in culture has been studied. Because of the high affinity of AIB to the plastic culture dishes, a special washing technique had to be developed. With this technique, it was possible to perform transport experiments in a single plate containing about one million cells. The cells were viable, intact and adhered to the supporting medium throughout the experiment.

The AIB transport into both types of cells was Na⁺-dependent and showed saturation kinetics when the small component of the transport due to diffusion had been subtracted.

The AIB transport capacity of neoplastic glioma cells was 3.6 times higher than that of glia cells. This difference was related to the V_{max}-values for the two types of cells. The apparent K_m-values were the same.

Inhibition experiments with other amino acids support the view that AIB is transported via System A in both glia and glioma cells.

Sulfhydryl reagents (ethacrynic acid and NEM) and cytochalasin B clearly inhibited the AIB transport into glia cells whereas the effect on glioma cells was minimal.

The amino acid accumulation in brain slices has been studied in detail (Blasberg and Lajtha, '65; Margolis and Lajtha, '68). Brain is suitable for such experiments because accumulation against a concentration gradient is more pronounced here than in slices from most other tissues (Neame, '62). However, the brain slices are not ideal for studying transport behaviour. The transport system A is particularly unsuitable since the cells in the slices do not retain K⁺ or exclude Na⁺ in a normal way (Christensen, '73).

Furthermore, glial cells and neurons are so intimately associated in vivo that studies of intact brain tissue yield no precise information on the biochemistry of each cell. Large scale separation methods exist but give varying cell damage, mutual contaminations by membrane structures and cell processes (Cremer et al., '68; Christensen, '73).

In the present study, we have examined the transport of the non-metabolizable amino acid, α -aminoisobutyric acid (AIB), into cultured glia and glioma cells while still attached to the solid support. In this

way it was possible to study directly the Na⁺-dependent AIB uptake in a defined type of brain cells. The transport of AIB into glia and glioma cells was compared and a considerable increase in the neoplastic cells was noted. This difference was due to an increased V_{max} for transport into the malignant cells while the apparent K_m-values were about the same for normal and malignant cells.

MATERIALS AND METHODS

1. *Materials.* All amino acids and N-ethyl maleimide (NEM) were purchased from Sigma Co, St. Louis, Missouri, U.S.A. Cytochalasin B was obtained from Aldrich Chemical Co., Milwaukee, Wisconsin, U.S.A., and ethacrynic acid was a generous gift from Merck, Sharpe & Dohme through Erik Lindblom & Co. KB, Stockholm. Unlabeled AIB was purchased from Calbiochem., A grade, San Diego, California, and the ¹⁴C-labeled form from New England Nuclear (NEN) Chemicals GmbH, Dreieichenhain, West Germany.

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Unlabeled inulin was obtained from Sigma Co. and ^{14}C -labeled inulin from NEN. Protosol and Aquasol were also from NEN.

2. *A description of cell lines and tissue culture conditions* has been given earlier (Ågren et al., '71). The present experiments were carried out with the glioma cell line U-251 MG and the glia line U-787 CG.

3. *Preparation of cells for incubation experiments.* All preparatory steps and incubations were carried out at 37°C where not otherwise stated. Eagle's medium with 10% calf serum was removed by suction and the cells on the plates were washed twice with an isotonic Krebs-Ringer bicarbonate buffer, either containing 120 mM Na^+ or with all Na^+ replaced by choline ion. The incubation with various concentrations of ^{14}C -labeled AIB (range 0.1–32 mM) was performed in the aforementioned buffers adjusted osmotically to compensate for the AIB added. In some experiments different inhibitors were also added under isotonic conditions. All buffers were gassed with a mixture of 93.5% oxygen and 6.5% carbon dioxide for about 30 minutes just before the experiments.

4. *The incubations* were started immediately after washing the cells using 9 cm standard Nunc (Roskilde, Denmark) plates on which the cells were growing. Total incubation volume was 3 ml. Incubation time ranged from one to five minutes. Generally, the incubation time was three minutes which was found to be convenient for measuring the initial velocity. It was

sufficient to use one plate containing about 4×10^6 glioma or 1×10^6 glia cells, respectively, for the transport studies.

5. *Termination of reaction* was accomplished by decanting the incubation medium from the plates. The cells were then immediately washed four times with 3 ml of ice-cold Krebs-Ringer bicarbonate buffer also containing 48 mM glycylglycine. This procedure was performed with the cells still firmly attached to the plastic plates. The cells were treated with 3 ml of 3% sulfosalicylic acid (60°C) (Christensen and Handlogten, '68). The addition of glycylglycine was necessary to remove labeled AIB unspecifically adsorbed to the plastic surface. In a series of experiments four washings were found to be sufficient to obtain a low background radioactivity.

Table 1 shows the inhibitory effect of various amino acids on the transport of ^{14}C AIB. It is apparent *inter alia* that glycine in the incubation medium together with ^{14}C AIB caused a partial inhibition of the AIB uptake. The failure of glycylglycine to induce a similar effect indicates that dipeptidase activity was absent from the cell surface and the incubation buffer medium. The presence of glycylglycine in the washing fluid does not thus interfere with the transport assay.

6. *Radiometric determination of ^{14}C -labeled AIB.* The warm sulfosalicylic acid extract was transferred to tubes, heated at 100°C for five minutes and centrifuged before the radiometric determinations. Light microscopy of the plates revealed that

TABLE 1

Percentage inhibition of ^{14}C -AIB transport into glia and glioma cell in the presence of another unlabeled amino acid

Amino acid added	Percentage inhibition (averages of 2 experiments)	
	Glia cells	Glioma cells
L-leucine	57	50
L-norleucine	55	63
L-isoleucine	38	44
N-methylglycine (sarcosine)	13	12
L-alanine	57	71
L-methionine	66	78
L-proline	33	4
Glycine	53	30
Glycylglycine	+ 28	+ 7 (stimulation)

^{14}C -AIB concentration was 8 mM. All amino acids were added in the concentration of 8 mM. Incubation time was three minutes at 37°C .

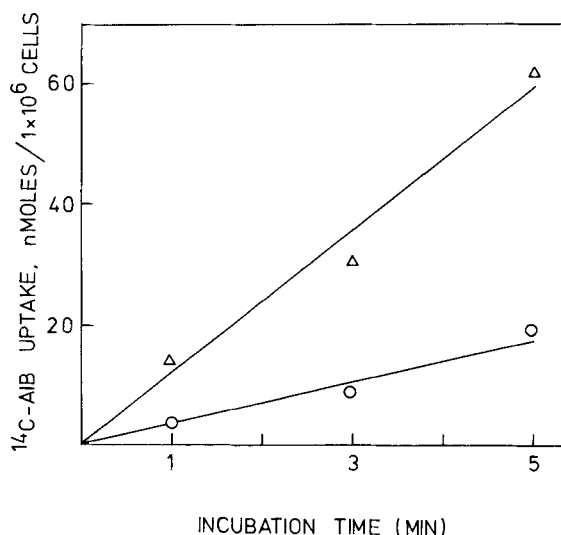


Fig. 1 Time dependency of AIB uptake by glia (circles) and glioma (triangles) cells. The incubation medium contained 4 mM ^{14}C AIB in 120 mM Na at 37°C . For other details, see text.

the cell residues had not been removed by the warm sulfosalicylic acid treatment. These cell residues were then scraped off with a razor-blade in the presence of cold sulfosalicylic acid and centrifuged. This pellet was solubilized in Protosol for radiometric determination. The residual radioactivity of the cell fragments on the plates was in most experiments about 5%—occasionally reaching 10% of the radioactivity of the sulfosalicylic acid extract. The sum of the two values was used for the calculation of the AIB transport. The ^{14}C -labeled AIB was determined with a liquid scintillation counter (Unilux II, Nuclear Chicago).

7. *The inulin spaces* of the monolayered cells on the plates were measured using ^{14}C -labeled carboxyinulin (Rosenberg et al., '62) under the conditions given for the uptake experiments.

This space was calculated from ten individual experiments to be 1.01% of the intracellular volume of the glia cells. The corresponding figure for glioma cells was 0.82%. The cellular volumes have been calculated as given in a previous paper (Ågren et al., '74).

RESULTS

Transport of AIB into glia and glioma cells

Time dependency. Figure 1 illustrates

the linear relationship for the AIB transport into glia and glioma cells during the first five minutes of incubation at 37°C with 120 mM Na^+ in the medium.

Na^+ -dependency of AIB uptake. The AIB transport into glia and glioma cells was Na^+ -dependent. The amino acid uptake was small and linear when all Na^+ was replaced by choline and this component of the AIB uptake was considered to be due to diffusion. A saturation kinetic curve is obtained with AIB and sodium in the medium when the component due to diffusion is subtracted (fig. 2).

Comparison between glia and glioma cells. Figure 2 also shows that the transport capacity into glioma cells was about four times higher than into glia cells. When Na^+ was replaced by choline, a small linear uptake due to diffusion was noted both in glia and glioma cells.

Figure 3 illustrates the Lineweaver-Burk plots for the AIB uptake into glia and glioma cells after correction for diffusion. The apparent K_m -values for the uptake were 1.38 mM for glia cells and 1.45 mM for glioma cells and were thus quite similar. The V_{\max} -values differed significantly being 4.8 nmoles per 1×10^6 cells per minute for glia cells and 17.4 nmoles per 1×10^6 cells per minute for glioma cells.

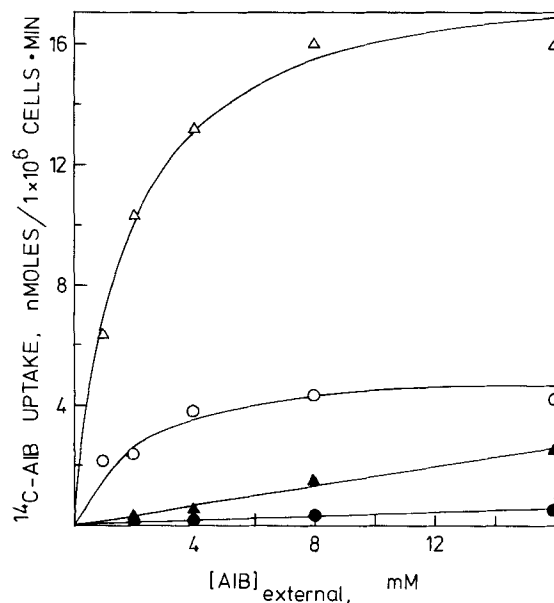


Fig. 2 Calculated AIB transport into glioma (circles) and glioma (triangles) cells in the presence of 120 mM Na (open symbols) after withdrawal of values obtained with choline. Filled symbols indicate AIB transport when all Na was replaced by choline. Details concerning the preparation of cells and the incubation procedure have been given in text.

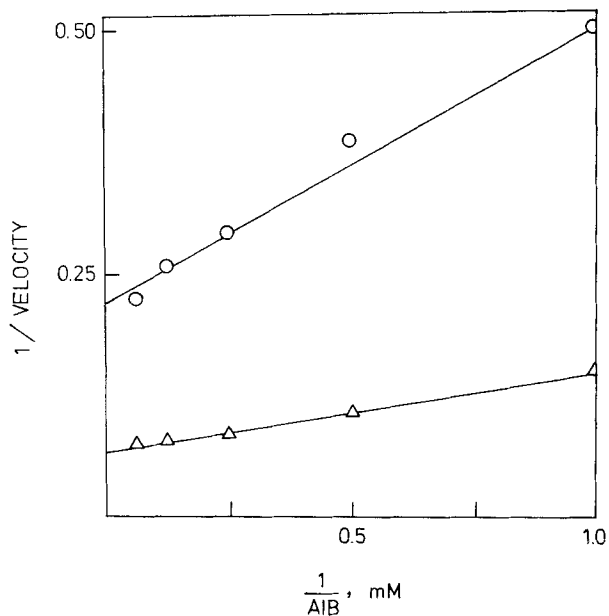


Fig. 3 Lineweaver-Burk plot of AIB transport into glioma (circles) and glioma (triangles) cells. Incubation time: three minutes at 37°C. For other details, see text.

Effect of different inhibitors on AIB transport into glia and glioma cells

Amino acids. Table 1 shows the effect of different amino acids and glycylglycine on the AIB transport into glia and glioma cells. The inhibitor concentration and ^{14}C AIB concentration were the same, 8 mM. Alanine, a typical system A substrate (Christensen, '70), displayed, as expected, a relatively high inhibition of the AIB transport into both cell types. Methionine also had a relatively high inhibitory effect. Glycine seemed to have a more pronounced effect on the AIB transport into glia cells than into glioma under the present conditions. Sarcosine, on the other hand, had little effect on either cell types. The effect of leucine seemed to be more pronounced than that of isoleucine. This could be attributable to the β -methyl group of isoleucine which impairs the surface binding of this amino acid to the cells (*cf.* Yu, '73).

Cell membrane active substances. Three compounds were studied. Cytochalasin B alters the morphology of cultured nerve cells (Daniels, '72) and glia cells (Westermarck, '73). It has been claimed that it interacts with the microfilaments of brain plasma membranes (Hemminki, '73). NEM and ethacrynic acid are both known to interact with sulfhydryl groups on the cell surface (Ronquist and Ågren, '75). The AIB transport into glioma cells was not affected by any of the three cell membrane active substances. By contrast, these compounds strongly inhibited the transport process into glia cells (table 2).

Neuro-transmitters. Two substances, γ -amino-butyric acid (GABA) and taurine were studied. The inhibitory neuro-transmitter

TABLE 3

Percentage inhibition of ^{14}C -AIB transport into glia and glioma cells in the presence of neurotransmitters

Substance added	Percentage inhibition (averages of 2 experiments)	
	Glia cells	Glioma cells
Taurine	35	12
GABA	28	12

^{14}C -AIB concentration was 8 mM. GABA and taurine were added to a final concentration of 8 mM as well. Incubation time: three minutes at 37°C.

GABA is especially interesting because its accumulation in nervous tissue is Na^+ -dependent (Levi, '72). Furthermore, GABA is also transported against a high concentration gradient into glia cells (Henn and Hamberger, '71; Neal and Iversen, '72; Boveri and Brown, '72). GABA as well as taurine in relatively high concentrations had readily observed effects on the AIB transport into glia cells while the effect on the tumor cells was weak (table 3).

DISCUSSION

The strictly Na^+ -dependency of the AIB uptake into both glia and glioma cells together with the finding of a saturation kinetic curve for the transport favor the view of a carrier mediated transport. An amino acid carrier with such characteristics was defined as system A for other types of cells by Oxender and Christensen, ('63). Furthermore, the competitive experiments with amino acids reported here, corroborate earlier findings (Christensen, '70), namely that AIB is mainly transported via system A. The capacity for AIB transport into cultivated human glioma cells was 3.6 times higher than into glia cells.

TABLE 2

Percentage inhibition of ^{14}C -AIB transport into glia and glioma cells in the presence of sulfhydryl group inhibitors and cytochalasin B

Substance added	Percentage inhibition (averages of 2 experiments)	
	Glia cells	Glioma cells
Cytochalasin B	43	4
N-ethylmaleimide (NEM)	43	2
Ethacrynic acid	54	4

^{14}C -AIB concentration was 8 mM. The concentration of NEM and ethacrynic acid was 0.74 mM and 1 mM respectively. Cytochalasin B was used in the concentration of 5 $\mu\text{g}/\text{ml}$. Incubation time: three minutes at 37°C.

The accelerated uptake of AIB into the tumor cells is not unexpected in the light of the earlier experiments by Christensen and Henderson ('52) who concluded that the increased amino acid accumulation in tumor cells could be a significant factor in the growth and multiplication of these cells. This conclusion has more recently been reformulated and expanded by Holley ('72), who took into account the possible role of certain critical nutrients, which normally limit growth. According to him, these nutrients would be available at higher concentrations inside the tumor cells because changes in the cell membrane involving, e.g. transport proteins took place at transformation into a malignant cell. Therefore, malignant growth might result from an increase in either the amount or the affinity of the carrier protein. Our finding of a distinct increase in V_{\max} but evidently similar apparent K_m -values in glioma compared to glia cells favours the former alternative. This may at least partly be a secondary effect of the irregular and therefore probably larger average surface of the glioma cell (Pontén, '75). The interpretation agrees with those obtained with virus transformed cells (Foster and Pardee, '69; Isselbacher, '72). However, the increased AIB transport into rat lymphocytes activated by concanavalin A was also due to a change in V_{\max} not accompanied by any alteration in apparent K_m (Van den Berg, '73). This finding is noteworthy, because a change in surface area of the cell is not expected in this instance. The results with certain inhibitors of AIB transport suggest that K_m -values probably do not reveal all differences in the function of carrier proteins. Providing amino acids were used, glia and glioma cells reacted similarly. However, when compounds known to interact with the cell membrane were used, inhibition was only detected in the normal cells. Morphological evidence has been accounted indicating that a normal glia cell modifies parts of its plasma membrane according to the requirements of the local environment (Pontén, '75). In glioma cells this mechanism seems to be impaired. In normal glia cells, all entry points for amino acids may be well organized and confined to defined parts of the membrane, where-

as in glioma cells the membrane may be composed of a heterogenous mosaic of partially autonomous stretches with different dynamic properties as, for example, the transport of amino acids. It is possible that an essential aspect of malignancy is an incapacity to coordinate cell membrane function. The effects of GABA and taurine on the AIB transport into glia cells is worthy of note and may be explained by the fact that these neuro-transmitters are transported into glia cells by a route which is not shared by AIB.

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