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Uptake and Metabolism of Glutamate and Aspartate by Astroglial and Neuronal Preparations of Rat Cerebellum

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Astrocytes, neuronal perikarya and synaptosomes were prepared from rat cerebellum. Kinetics of high and low affinity uptake systems of glutamate and aspartate, nominal rates of $^{14}CO_2$ production from [U−^{14}C]glutamate, [U−^{14}C]aspartate and [1−^{14}C]glutamate and activities of enzymes of glutamate metabolism were studied in these preparations. The rate of uptake and the nomial rate of production of $^{14}CO_2$ from these amino acids was higher in the astroglia than neuronal perikarya and synaptosomes. Activities of glutamine synthetase and glutamate dehydrogenase were higher in astrocytes than in neuronal perikarya and synaptosomes. Activities of glutaminase and glutamic acid decarboxylase were observed to be highest in neuronal perikarya and synaptosomes respectively. These results are in agreement with the postulates of theory of metabolic compartmentation of glutamate while others (presence of glutaminase in astrocytes and glutamine synthetase in synaptosomes) are not. Results of this study also indicated that (i) at high extracellular concentrations, glutamate/aspartate uptake may be predominantly into astrocytes while at low extracellular concentrations, it would be into neurons (ii) production of α-ketoglutarate from glutamate is chiefly by way of transamination but not by oxidative deamination in these three preparations and (iii) there are topographical differences glutamate metabolism within the neurons.

KEY WORDS: Glutamate; aspartate, astrocytes; neurons; synaptosomes; uptake; CO_2 production.

INTRODUCTION

Studies on the metabolism of glutamate family of amino acids (glutamate, glutamine, aspartate, GABA and alanine) in brain assumed importance as these are (i) the most abundant neurotransmitters of brain, (ii) known to support cerebral energy metabolism directly (by providing keto acids to citric acid cycle) or indirectly (as components of malate-aspartate shuttle), and (iii) closely associated with production and detoxification of ammonia. Studies with several precursors such as glucose, acetate, acetoacetate, β-hydroxybutyrate, leucine etc., revealed that the metabolism of glutamate is compartmentalized in brain (1,2). According to the theory of metabolic compartmentation, glutamate exists in two pools - large (neuronal) pool with a slow turnover rate and a small (glial) pool with a rapid turnover rate (1,2). It has been suggested that neurons metabolize glutamate primarily by oxidative deamination and a small amount of glutamate is released in response to depolarizing stimuli. This glutamate is transported to astrocytes where a major portion is converted to glutamine and the rest is transaminated to α-ketoglutarate (α-KG) and is oxidized in the citric acid cycle. Glutamine, so formed in the glial cells, is transported back to the neurons where it serves as a precursor for the releasable pool of glutamate and GABA.

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This recycling of glutamate carbons between glia and neurons prevents the perpetual loss of glutamate (thus α-KG in the TCA cycle) carbons in neurons (3).

Earlier studies of the compartmentation of glutamate were carried out in heterogenous preparations such as brain slices and in extracts of whole brain. As a consequence, not all the results obtained were unequivocal. Though the advent of primary cell cultures resolved a few of these uncertainties, some of the results (such as presence of glutaminase in astrocytes (4) were not supportive of original postulates of the theory of metabolic compartmentation. In the present study, we have used cerebellar preparations enriched with either astrocytes or neuronal perikarya or synaptosomes of adult rats to study the applicability of the theory of metabolic compartmentation of glutamate. The rationale is using cerebellum was that glutamate family of amino acids are the major, if not exclusive, neurotransmitters in this brain region (5).

Our studies indicate that (i) the Km and Vmax of high and low affinity uptake systems for glutamate and aspartate and (ii) the nominal rates of production of $^{14}CO_2$ from these amino acids were higher in the astrocytes than in the neuronal preparations. Activities of glutamine synthetase (GS) and glutamate dehydrogenase (GDH) were observed to be higher in astrocytes than in other two preparations. Activities of glutaminase and glutamate decarboxylase (GAD) were higher in neuronal perikarya and synaptosomes respectively than in astrocytes. Results of the present study are in partial agreement with the theory of metabolic compartmentation. Comparison of the results of the present study with those of cerebral cortex (6) suggested regional variations in the cellular metabolism of glutamate in brain.

EXPERIMENTAL PROCEDURE

Animals. Inbred albino rats of Wistar strain (175 - 225 g), maintained under 12 hrs light and 12 dark cycles at 20 ± 2 °C, were used in this study. Nutritionally balanced pellet food (Hindustan Lever Ltd., India) and water were provided ad libitum.

Preparation of Astrocytes, Neuronal Perikarya, and Synaptosomes. Astrocytes and neuronal perikarya were prepared from the cerebellum using Ficoll - 400 density gradients by the method of Farooq and Norton (7) as suggested by Rao and Murthy (8,9). Cell viability was determined by dye exclusion method, employing 0.4% trypan blue. Synaptosomes were prepared by the method of Cotman (10). Protein content of astrocytes, neuronal perikarya and synaptosomes was determined by the method of Lowry et al., (11).

Uptake. Low and High affinity uptake of [U − ^{14}C]glutamate and [U − ^{14}C]aspartate was studied by the method of Rao and Murthy (8). Astrocytes or granule cell perikarya or synaptosomes (100 μg protein equivalent) was incubated with different concentrations of [U − ^{14}C]glutamate of [U − ^{14}C]aspartate in a medium containing NaCl 140 mM, KCl 2.5 mM, Na_2HPO_4 10 mM, glucose 10 mM, $MgSO_4$ 1.3 mM, $NaHCO_3$ 20 mM, HEPES 10 mM, buffered at pH 7.4. For high affinity uptake, concentrations of glutamate/aspartate used were 1×10^{-6} to 1×10^{-4} M. For low affinity uptake, 1×10^{-4} to 5×10^{-3} M concentrations of amino acid was used. After 5 min of incubation at 37°C, uptake was terminated by rapid centrifugation at 10,000 *g* at 4 °C. The pellet was washed twice with 2 ml of ice-cold medium having same amount of unlabeled amino acid under study. The final pellet was dissolved to 100 μl of 0.1 N NaOH and radioactivity in the pellet and medium was determined. In all the experiments, non-specific uptake was determined by carrying out parallel incubations at 0 °C and the uptake values were corrected accordingly. Kinetic constants (Km and Vmax values) were calculated from the Eddie-Hoffstee plots generated from the data of saturation isotherms.

$^{14}CO_2$ Production. Nominal rates of $^{14}CO_2$ production from [U − ^{14}C]labeled amino acids was determined by the method of Rao and Murthy (9,12). Reaction mixture used for the studying $^{14}CO_2$ production consists of sodium phosphate (5 mM) − HEPES (10 mM) buffer (pH 7.4), $MgSO_4$ 1.2 mM, $CaCl_2$ 1 mM; NaCl 124 mM; KCl 5 mM, glucose 10 mM, [U − ^{14}C]glutamate or [U − ^{14}C]aspartate or [1 − ^{14}C]glutamate (100 μM, 0.1 μCi) and the final volume was adjusted to 0.5 ml. Reaction was carried out for 30 min (at 37 °C with constant shaking) in vials closed with Kontes rubber stoppers from which center wells with strips of Whatman No. 1 filter paper were suspended. Vials were sealed with parafilm and the reaction was started by injecting 100 μg protein equivalent of cell enriched preparation or synaptosomes. At the end of incubation period, 200 μl of hyamine hydroxide was injected into center well and 200 μl of 3M perchloric acid (PCA) into the medium. Reaction vials were incubated for an additional 60 min at 37°C with shaking to trap $^{14}CO_2$ into hyamine hydroxide. At the end of incubation, the center wells were removed and introduced into scintillation vials contaning scintillation fluid (2 g of 2,5-diphenloxazole; 400 mg of 1,4-bis(5-phenyl-2-oxazolyl)benzene, 20 ml of ethylene glycol, 100 ml of methanol and 1 L of dioxane). Radioactivity in hyamine hydroxide was determined in a Beckman LS-1800 liquid scintillation spectrophotometer. Radioactivity in an aliquot of medium was determined for calculating the specific activity of the added amino acid. The "nominal" rate (13) of $^{14}CO_2$ production (nmol of $^{14}CO_2$ produced/mg protein/hr or fmol of $^{14}CO_2$ produced/cell/hr) was calculated from the radioactivity in hyamine, specific activity of labeled glutamate/aspartate and protein content of added cell or synaptosomal preparation. The term "nominal" is used as the specific activity of amino acid in the medium but not of the intracellular precursor pool was used for the rate calculation. Therefore, nominal rates are the minimal estimates of oxidation of these amino acids. Zero-time controls were routinely included and received PCA at the beginning of incubation. Production of $^{14}CO_2$ in zero-time controls was approximately 10% of the experimental values and due corrections were made.

Enzyme Assays. Before performing enzyme assays, cell preparations and synaptosomes were subjected to two cycles of freezing (−80 °C) and thawing (4 °C) and Triton X-100 was added to a final concentration of 0.1% (v/v). Optimal concentrations of protein, substrate(s), cofactor(s) and time of incubation were determined separately for each enzyme in each preparation and these were used in subsequent studies. Aspartate and alanine aminotransferases (AAT and ALAT), glutaminase (GLNASE), glutamate dehydrogenase (GDH), glutamine synthetase (GS) glutamic acid decarboxylase (GAD) and GABA-transaminase (GABA-T) were assayed as per the methods described earlier (14-19). Succinate dehydrogenase (SDH), acetyl and butyryl cholinesterases (ACHE and BCHE) were assayed by the methods of Nan-

dakumar et al. (20) and Ellman et al. (21) respectively. [U−^{14}C]glutamate and [U−^{14}C]aspartate (specific activity, 240 mCi/mmole and 235 mCi/mmole respectively) were purchased from Babha Atomic Research Centre, India. [1−^{14}C]glutamate (specific activity, 55 mCi/mmole) was purchased from Amersham International, England. All the other chemical were purchased from Sigma Chemical Co., USA.

Data were reported as Mean ± SD. Statistical analysis of the data was performed by Analysis of Variance (ANOVA) and the p values were corrected by doing post tests by Bonferroni method.

RESULTS

Purity of the Cellular and Synaptosomal Enriched Preparations. Neuronal perikarya isolated in the present study had a small cell body with a prominent nucleus and were devoid of processes such as axons and dendrites. This in in contrast to astrocytes, in which cell processes survived the rigors of isolation procedure. Microscopic appearance of the astrocytes was similar to that of protoplasmic astrocytes. In the cerebellum, the yield of neuronal perikarya was higher than that of astrocytes.

Purity, integrity and cross-contamination of the isolated cell preparations were routinely monitored by phase contrast microscopy and by assaying the activities of the marker enzymes specific for astrocytes (ACHE, BCHE and GS). Activities of ACHE and BCHE in the astrocytes were observed to be 0.50 ± 0.06 and 0.07 ± 0.01 pmol of acetylcholine or butyrycholine hydrolyzed/cell/hr respectively. In neuronal perikarya only 10% of the glial activities of these enzymes were observed. GS activity was observed to be 5 to 6 fold higher in astrocytes than neuronal perikarya. This profile of the markers suggested that the cross-contamination of astrocytes with neurons may not be more than 10%. A similar profile of activities of these markers was observed for cortical astrocytes and neurons (6).

Purity of the synaptosomal enriched preparations and the contamination of these preparations by mitochondria and glial elements was monitored by assaying the enzymes glutamic acid decarboxylase (GAD; marker for synaptosomes), succinate dehydrogenase (SDH; marker for mitochondria) and acetyl and butyryl cholinesterases (ACHE and BCHE; markers for glial cells). Snynaptosomes recorded a very high activity of GAD (324 ± 49.5 nmol of [^{14}C−]GABA formed/mg protein/hr) compared to mitochondrial and astroglial preparations (38 ± 5.9 and 26.8 ± 3.6 nmoles of [^{14}C−]GABA formed/mg protein/hr respectively. Synaptosomal SDH activity (0.39 ± 0.04 μmol succinate oxidized/mg protein/hr) was 11% of mitochondrial SDH activity (3.55 ± 0.32 μmol/mg protein/hr). ACHE activity was observed to be 1.38 ± 0.16 and 0.126 ± 0.01 μmoles of acetylcholine iodide hydrolyzed/mg protein/hr in astrocytes and synaptosomes, respectively. Activity of BCHE was observed to be 0.23 ± 0.04 and 0.024 ± 0.003 mmol of butyrylcholine iodide hydrolyzed/mg protein/hr in astrocytes and synaptosomes, respectively. These studies indicate that purity of synaptosomal preparations was approximately 90%.

High and Low Affinity Uptake of Glutamate and Aspartate. Preliminary studies revealed that the uptake of both glutamate and aspartate into astrocytes, neuronal perikarya and synaptosomes was linear with respect to the added protein (upto 200 μg) and time of incubation (upto 10 minutes). Eddie-Hoffstee plots revealed the presence of two different uptake systems (one with a low affinity and high capacity and the other with a high affinity and low capacity to the amino acid) for both glutamate and aspartate in all the three preparations. The kinetics (Km and Vmax) calculated from the Eddie-Hoffstee plots of uptake in these preparations are given in Figure 1. In all the three preparations, rate of transport (as indicated by Vmax values) of both glutamate and aspartate by the low affinity uptake system was 11 to 19 fold higher than that of high affinity uptake system. Similarly, Km of low affinity uptake system was 2 to 3 orders greater than that of the high affinity uptake sys-

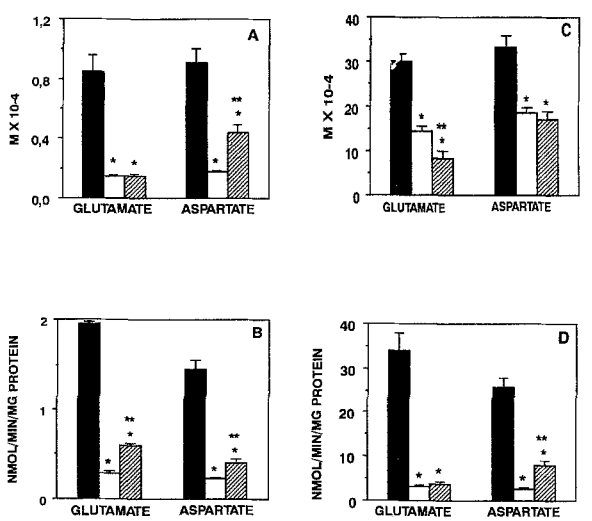

Fig. 1. Kinetic constants for the uptake of glutamate and aspartate into astrocytes (■), neuronal perikarya (□) and synaptosomes (▨) of rat cerebellum. A and B are the Km and Vmax of the high affinity uptake system. C and D are the Km and Vmax of the low affinity uptake system. Km and Vmax values were calculated from the Eddie-Hoffstee plots. Each value is Mean ± SD of five separate experiments done in duplicates.
Statistics: * = $p<0.001$ when compared to astrocytes.
** = $p<0.001$ when compared to neuronal perikarya.

tem. The Vmax and Km of the high and low affinity systems of both glutamate and aspartate were higher in astrocytes than in neuronal perikarya and synaptosomes (Figure 1).

Comparison between neuronal perikarya and synaptosomes revelaed a two fold higher Vmax of the high affinity uptake system of glutamate in synaptosomes than in neuronal perikarya while the Km of this system was equal in both the preparations. However, the Km of the low affinity uptake system in synaptosomes was lower than that of neuronal perikarya (in the case of glutamate) while the Vmax was higher in the former than that of the latter (in the case of aspartate). Both Km and Vmax of the high affinity uptake system of aspartate, were lesser in neuronal perikarya than in synaptosomes. Km of the low affinity uptake system of this amino acid, was more or less same in these two preparations, while the Vmax was three fold lesser in neuronal perikarya than in synaptosomes (Figure 1).

Studies on $^{14}CO_2$ Production from ^{14}C-Labeled Glutamate and Aspartate. Because of differences in the protein content of astrocytes and neuronal perikarya (Table II), nominal rates of $^{14}CO_2$ production are expressed both as specific and cellular rates. Astrocytes have almost two fold higher protein content than neuronal perikarya. Of this cellular protein, very minute fraction may be contributed by the enzymes involved in this process.

The nominal rate of production of $^{14}CO_2$ from [U−^{14}C]glutamate and [U−^{14}C]aspartate by astrocytes, neuronal perikarya and synaptosomes was linear with respect to the amount of protein added (upto 200 μg) and the time of incubation (upto 45 min).

Irrespective of the mode of expression of rates (per mg protein or per cell), nominal rates of $^{14}CO_2$ production in astrocytes and neuronal perikarya were 2 fold higher with [1−^{14}C]glutamate than with [U−^{14}C]glutamate. In synaptosomes, it was about 4.6 fold higher with [1−^{14}C]glutamate than with [U^{14}C]glutamate. With both the amino acids, the nominal rates of $^{14}CO_2$ production was highest in astrocytes. With [1−^{14}C]glutamate, specific rate (rate/mg protein) of CO_2 production was 3 fold higher in astrocytes than in neuronal perikarya. With [U−^{14}C]glutamate and [U−^{14}C]aspartate as substrates, the rate of $^{14}CO_2$ production was 4 and 6 fold higher in astrocytes than in neuronal perikarya. These differences were amplified when cellular rates (rate/cell) were taken into account. With [U−^{14}C]glutamate, it was 10 fold, with [U−^{14}C]aspartate is was 16 fold and with [1−^{14}C]glutamate is was 6 fold (Table I). The rate of $^{14}CO_2$ production from [U−^{14}]glutamate was higher than that from [U−^{14}C]aspartate in all the three types of preparations.

Distribution of Enzymes of Glutamate Metabolism. Activities of all the enzymes of glutamate metabolism, assayed in the present study, were detectable in all the three preparations viz., astrocytes, neuronal perikarya and synaptosomes. However, their activity levels were different in these preparations. Activities of these enzymes in astrocytes and neuronal perikarya have been expressed either as specific activity (activity/mg protein) or as cellular activity (activity/cell) while for synaptosomes only specific activities are given.

Irrespective of the mode of expression, activities of all the enzymes of glutamate metabolism studied (except GLNASE and GAD) were higher in astrocytes than in neuronal perikarya and synaptosomes. GLNASE and GAD activities were highest in neuronal perikarya and in synaptosomes, respectively. The magnitude of difference between the astrocytes and neuronal perikarya, in the activities of these enzymes, except for GLNASE, was dependent on the mode of expression. When expressed per mg protein, the magnitude of difference in the ac-

Table I. Nominal Rates of [^{14}C] Carbon Dioxide Production from [^{14}C]-Labeled Glutamate and Aspartate by the Cell and Synaptosomal Preparations of Rat Cerebellum

		Astrocytes	Neuronal Perikarya	Synaptosomes
[U-^{14}C[Glu	A.	22.22 ± 2.67 (9)	5.26 ± 0.46 (9)*	3.81 ± 0.30 (13)*
	B.	7.70 ± 1.62 (9)	0.76 ± 0.16 (9)*	
[1-^{14}C]Glu	A.	42.66 ± 6.44 (4)	14.16 ± 1.53 (4)*	17.77 ± 2.56 (4)*
	B.	11.19 ± 0.94 (4)	2.04 ± 0.46 (4)*	
[U-^{14}C]Asp	A.	16.70 ± 1.06 (6)	2.71 ± 0.21 (6)*	3.62 ± 0.28 (8)*
	B.	5.64 ± 0.74 (6)	0.36 ± 0.08 (6)*	

UNITS: A. nmol of $^{14}CO^2$ formed/mg protein/hr. B. fmol of $^{14}CO_2$ formed/cell/hr.
Each value is Mean ± SD. Number in parenthesis indicates the number of experiments done in duplicates. For each experiment, cerebella from six rats were pooled to prepare astrocytes and neuronal perikarya or synaptosomes.
Statistics: * = $p<0.001$ when compared with astrocytes.
** = $p<0.001$ when compared with neuronal perikarya.

Table II. Activities of the Enzymes of Glutamate Metabolism in the Cell and Synaptosomal Preparations of Rat Cerebellum

ENZYME		Astrocytes	Neuronal Perikarya	Synaptosomes
AAT	A.	49.96 ± 4.8 (7)	34.24 ± 5.55 (6)*	41.78 ± 2.70 (6)*,**
	B.	14.15 ± 1. 0 (8)	4.34 ± 0.75 (6)*	
AlAT	A.	7.51 ± 0.90 (6)	5.41 ± 0.60 (6)*	2.44 ± 0.27 (6)*,**
	B.	2.30 ± 0.32 (7)	0.56 ± 0.07 (5)*	
GDH	A.	10.86 ± 2.70 (6)	3.26 ± 0.65 (6)*	1.23 ± 0.12 (6)*
	B.	3.69 ± 0.35 (6)	0.29 ± 0.05 (6)*	
GS	A.	59.88 ± 7.36 (8)	10.08 ± 2.03 (8)*	12.36 ± 1.23 (7)*
	A.	15.94 ± 2.60 (8)	1.39 ± 0.17 (8)*	
GLNASE	A.	4.30 ± 1.49 (7)	10.23 ± 2.42 (7)*	3.03 ± 0.19 (7)**
	B.	0.96 ± 0.13 (5)	2.29 ± 0.30 (5)*	
GAD	A.	26.78 ± 3.60 (7)	74.32 ± 7.30 (8)*	324.0 ± 49.5 (8)*,**
	B.	5.99 ± 0.70 (6)	13.96 ± 1.19 (6)*	
GABA-T	A.	10.16 ± 0.94 (7)	6.89 ± 0.87 (5)*	6.73 ± 1.13 (6)*
	B.	2.83 ± 0.31 (6)	0.75 ± 0.09 (5)*	
Protein		324 ± 67 (27)	145 ± 29 (27)*	

AAT: Aspartate aminotransferase; ALAT: Alanine aminotransferase; GDH: Glutamate dehydrogenase; GLNASE: Glutaminase; GS: Glutamine synthetase; GAD: Glutamate decarboxylase; GABA-T: GABA-transaminase. Each value is Mean ± SD. Number in parenthesis indicates the number of experiments done in duplicates. For each experiment, cerebella from 6 rats were used. UNITS: A: Activities/mg protein/hr. B: Activities/cell/hr. AAT, AlAT, GLNASE AND GDH: A: μmoles and B: pmoles of NADH oxidized. GS: A: μmol and B: pmoles of γ-glutamyl hydroxamate formed. GABA-T: A: μmol and B: pmoles of succinic semialdehyde formed. GAD: A: nmol and B: Fmoles of GABA formed. Protein: pgms protein/cell.
Statistics: * = $p<0.05$ when compared with astrocytes.
** = $p<0.05$ when compared with neuronal perikarya.

tivities of aminotransferases, GDH, GS and GABA-T were about 1.5, 3.3, 5.9, and 1.5 fold higher in astrocytes than neuronal perikarya. When cellular activity was taken into account, this difference was amplified to 12 fold for GDH and GS, 3.5 fold for aminotransferases and 3.8 fold for GABA-T. Irrespective of the mode of expression, GLNASE activity was 2.5 fold higher in neuronal perikarya than in astrocytes. Astrocytes recorded 1.2, 3.1, 8.8, 4.8, and 1.5 fold higher activities of AAT, ALAT, GDH, GS, and GABA-T respectively than synaptosomes. Synaptosommal GAD activity was 12.1 fold higher than that of astrocytes.

When neuronal perikarya and synaptosomes were compared, activities of ALAT and GLNASE were 2.3 and 3.4 fold higher in neuronal perikarya than in synaptosomes, while activities of GAD and AAT were 4.4 and 1.2 fold higher in synaptosomes than neuronal perikarya. There were no statistically significant differences in the activities of GDH, GS and GABA-T between neuronal perikarya and synaptosomes.

DISCUSSION

Results of the present study indicates that both glutamate and aspartate are transported into astrocytes, neucronal perikarya and synaptosomes by high and low affinity uptake systems. This observation is in agreement with the results of previous studies (8, 22, 23). It must, however, be mentioned that homo− (glutamate - glutamate) and hetero− (glutamate - aspartate) exchange of glutamate and aspartate might also account for the transport of these amino acids to some extent. Such an exchange results in apparent but not the net (chemical) transport of amino acid. Though the magnitude of contribution of this exchange (diffusion) in the cells is not known presently, results from brain slices indicated that this may be about 10 to 15% of the total transport (24). Hence, the observed Vmax value may have an overestimate to this extent.

Observed Vmax values indicated a rapid and substantially higher uptake of these two amino acids into astrocytes than into neuronal preparations. The Km values of both high and low affinity system were lower in neuronal perikarya and synaptosomes than in astrocytes. This indicated that neurons might be more efficient in removing glutamate and aspartate at low concentrations of these amino acids in the extracellular compartment. Transport into astrocytes might be predominant at higher concentrations of glutamate and aspartate (25, 26). An intense uptake into astrocytes, observed presently, was also in agreement with previous reports (8, 25 - 27).

Results of the present study also indicated that astrocytic and neuronal preparations have the capacity to oxidize the carbons of both glutamate and aspartate. Nominal rates of CO_2 production were higher in astrocytes than in neuronal preparations. Similar observations

were made in primary cultures of astrocytes (13, 28). Differences in the rates of glutamate oxidation (and also of transport) in neurons and astrocytes could be due to several factors, some of which may be real while others may be experimental artifacts. Under the latter category are differences (i) in the loss of cell processes (such as axons and dendrites in neurons), (ii) in the viability of neurons and astrocytes during isolation and incubation assays, and (iii) in the loss/modifications of the transport proteins during cell isolation. However, similar differences in the rates of transport and oxidation of these amino acids in primary cell cultures suggest that the differences observed in the present study might not be experimental artifacts (13, 27, 28, 29).

Prior to their entry into TCA cycle and further oxidative metabolism, carbons of glutamate and aspartate are converted to their respective keto acids (α-ketoglutarate and oxaloacetate). Production of α-ketoglutarate from glutamate occurs either (a) by oxidative deamination of glutamate (glutamate ---> α-ketoglutarate + ammonia) mediated by GDH or (b) by transamination (α-keto acid + glutamate ---> α-amino acid + α-ketoglutarate). Contributions made by these reactions to the production of α-ketoglutarate from glutamate has been a subject of controversy. It was earlier predicted that the equilibrium of GDH reaction favours glutamate synthesis under physiological conditions. However, Benjamin and Quastel (3) indicated that the equilibrium of this reaction, at least in the large compartment (neuronal) of glutamate, might be in the direction of oxidative deamination, while Berl (30) suggested that GDH might be involved in glutamate synthesis in astrocytes. However, Yu et al. (29) reported an intense oxidation of glutamate carbons in primary cultures of astrocytes, and this process might be due to oxidative deamination mediated by glutamate dehydrogenase. Results of Lai et al. (13) and Farinelli and Nicklas (31) using similar cultures of astrocytes and of Yudkoff et al., (32) in nerve terminals do not support the above suggestion. We have recently reported that in bulk preparations of astrocytes, neuronal perikarya and synaptosomes, $^{14}CO_2$ production from [U−^{14}C]glutamate was suppressed by AOAA but not by glutamic acid diethyl ester (a GDH inhibtor) (9,12). This suggests that at least in cells and nerve terminals prepared from adult rats, transamination reactions might be the major, if not exclusive, reactions for the production of α-ketoglutarate from glutamate. There are no such controversies in the production of oxaloacetate from aspartate as this is mediated to a large extent by AAT. Though fumarate can be formed from aspartate through alternate reactions (purine nucleotide cycle and arginine biosynthetic pathway), precise rates of these reactions are yet to be determined.

A survey on the activity of enzymes of glutamate metabolism indicated the presence of all the enzymes of glutamate metabolism in isolated cells and in synaptosomes. In all these preparations, AAT activity was higher than that of GDH. This supports the tenet that transamination might be more involved in the production of α-ketoglutarate from glutamate than oxidative deamination (31). This difference might be further amplified if the activities of all the transaminases are taken into account. Some of the α-ketoglutarate produced in astrocytes might be transported out of these cells and serve as a precursor of the neurotransmitter pool of glutamate in the neurons (33-35). Peng et al., (35) recently showed that ALAT, *in vivo*, not only operating for the production of alanine but also produces some of the releasable pool of glutamate in granule neurons.

Though all the enzymes of glutamate metabolism were present in the three preparations, their activities were different in different preparations. The observed higher activities of all the enzymes of glutamate metabolism, except glutaminase and GAD, in astrocytes than in neuronal preparations is in agreement with the postulates of the theory of metabolic compartmentation (1-3) and with some of the immunohistochemical studies (36, 37). Presence of equal activities of glutaminase in astrocytes and nerve terminals was, however, not in agreement with the postulates of the theory of metabolic compartmentation and the reports of other investigators (38). Synaptosomal contamination of astrocytes might be ruled out as (i) synaptosomes do not survive the isolation procedures adopted for the isolation of astrocytes and their buoyant densities are also different and (ii) GAD (a marker for the nerve endings) activity in astrocytes was only 8% of synaptosomal activity indicating that contamination of astroglia with synaptosomes might not be more than 10%. Such high activities of glutaminase were also reported in astrocytes in primary cultures (4, 39) suggesting that the present observation might not be an experimental artifact. Presence of both GS and glutaminase in the astrocytes might result in futile cycle of glutamine synthesis and hydrolysis but this may be prevented by product inhibition of glutaminase (39). Though the physiological role of the glial glutaminase is not known at present, it is interesting to note that Murthy and Hertz (40) reported that the glutamate formed by the hydrolysis of glutamine supports the operation of malate-aspartate shuttle in astrocytes.

Observed presence of GS in synaptosomes was also contrary to the postulates of theory of metabolic com-

partmentation and results of immunohistochemical studies (36). This may not be due to gliosomal contamination of synaptosomes, as pseudocholinesterase (astrocytic marker) activity in the latter preparation was less than 10% of astrocytic activity. Presence of GS in cortical synaptosomes (6, 41 & 42) and the synthesis of [^{15}N]glutamine from $^{15}NH_3$, albeit in small amounts, has been reported recently (32). Physiological function and the metabolic fate of glutamine synthesized endogenously in synaptosomes is not known at present.

Another interesting observation of the present study is differences in the rates of transport of glutamate and aspartate and in the activities of some of the enzymes of glutamate metabolism in the neuronal perikarya and synaptosomes. These differences are suggestive of topographical heterogeneity of glutamate metabolism within the same cell and also, suggests the differences in glutamate metabolism between the sub compartments of large compartment. In addition to this, differences were also observed in the activities of these enzymes in the cellular preparationsand in synaptosomes prepared from cerebral cortex (6), which is suggestive of regional differences in the glutamate metabolism even in the same cell type.

Results of the present study clearly demonstrated that the metabolism (as indicated by the activities of the enzymes and the rate of $^{14}CO_2$ production) and the transport of glutamate and aspartate are different and compartmentalized between neurons and astrocytes in the cerebellum. Astrocytes metabolize these amino acids more rapidly than neurons. The rate of transport was also greater into astrocytes than neurons for both the amino acids.

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