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THE UPTAKE OF AMINO ACIDS BY PARTICULATE FRACTIONS FROM BRAIN

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SUMMARY

1. Crude nuclear and mitochondrial fractions from rat brain, upon incubation in appropriate media, take up amino acids against a concentration gradient, with equilibrium reached in most cases within 60 min.

2. The various amino acids are taken up to different degrees; those that are high in the living brain, such as glutamate, γ-aminobutyric acid, and glycine, are taken up by the particulate fractions to higher levels than the amino acids at lower levels in the living brain.

3. Uptake seems to be energy dependent: CN^- and 2,4-dinitrophenol strongly, although not completely, inhibit uptake; and concentrative uptake is absent at 0° for most amino acids.

4. Uptake is dependent on the composition of the medium. Ouabain inhibits uptake; low Na^+ concentration in the medium, which reduces particulate Na^+ and K^+ concentration, is also inhibitory. Ca^{2+} is not an absolute requirement, but it increases uptake.

5. Glutamate uptake is inhibited by aspartic acid, but not significantly by other amino acids. The uptake of other amino acids also shows some substrate specificity.

6. In mitochondrial subfractions from brain, nerve-ending particles are the most active in uptake; but under suitable conditions purified mitochondria also show uptake against a concentration gradient.

7. The uptake by particulate fractions seems to be fairly specific for brain; particulate fractions from newborn and adult brain showed uptake against a concentration gradient, but those from the other organs tried: liver, muscle and kidney, did not.

INTRODUCTION

Properties of the mechanisms that participate in the passage of amino acids into and out of the brain have been investigated in our laboratories, and in those of others[1–5]. Most of these experiments were done on living animals or on brain slices, and very few studies used particulate fractions from brain[6–8] or from other tissues[9].

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The various particles within the cell differ in their structure and in their metabolic function. The metabolite content also is characteristic for each particulate fraction, which represents not only structural elements but also metabolic compartments within the cell surrounded by specific membranes.

It is therefore of interest to establish whether transport mechanisms for amino acids like those described for brain slices can be found in cerebral particulate fractions.

Our interest was 2-fold: (1) since purified particulate fractions are less complex morphologically than brain slices, such preparations may help to elucidate details of cerebral transport mechanisms; and (2) since particles of the synaptic region were recently postulated to inactivate the liberated neurotransmitters by transport into vesicles, the possibility exists that the various particles contain different transport mechanisms, specific for some metabolites and some particles.

The present paper reports some of the properties of uptake of amino acids against a concentration gradient in crude cerebral nuclear and mitochondrial fractions. A preliminary note about this study has been published[10].

METHODS AND MATERIALS

Homogenization of brain

Wistar rats of 200–300 g body wt. were anesthetized by putting them in a chamber containing CO_2 for 3 min, and then were decapitated. The whole brain was promptly removed, weighed, and homogenized at 0° in 0.32 M sucrose for 1 min with a glass homogenizer. The homogenate was diluted to 10% in 0.32 M sucrose and fractionated by the method of HEBB AND WHITTAKER[11].

The total homogenate was centrifuged at 700 × *g* for 10 min, and the debris was removed. The nuclear fraction was obtained by centrifuging the supernatant fluid at 900 × *g* for 15 min, and the mitochondria were isolated by centrifuging the resulting supernatant fluid at 14500 × *g* for 20 min. The debris and the nuclear and mitochondrial fractions were each washed by suspension in 2 vol. of 0.32 M sucrose and recentrifugation, and the supernatant fractions were combined. For large-scale preparations pig brains were used; they were obtained a few minutes after the death of the animal and were worked up in the laboratory within 1 h in the same fashion as rat brain.

Subfractionation of crude mitochondria

Mitochondria were subfractionated in a discontinuous gradient of sucrose of 1.4, 1.2, 1.0, and 0.8 M (ref. 12). This gradient was allowed to equilibrate for 4 h at room temperature and 1 h at 0° before being used. The mitochondrial fraction (M) was dispersed in 0.32 M sucrose at 33% of the original tissue weight, layered on top of the density gradient, and then centrifuged at 2 h at 63500 × *g* in a SW-25.1 Spinco rotor. Five subfractions (A, B, C, D, and E) were obtained and were removed with Pasteur pipettes. Each fraction was diluted with 0.15 M sucrose and centrifuged at 100000 × *g* for 30 min.

Incubation

Unless otherwise noted in the tables, the following were the standard conditions for incubation: Crude nuclei, mitochondria, and subfractions of the mitochondrial

fraction were suspended in 3 ml of incubation medium in a 25-ml erlenmeyer flask; the medium was 10 mM glucose, 128 mM NaCl, 5 mM KCl, 2.7 mM $CaCl_2$, 1.2 mM $MgSO_4$, 5 mM Na_2HPO_4, 50 mM Tris, buffered at pH 7.4, and 1 mM ^{14}C-labeled amino acid. The flasks were incubated at 37° with shaking for 40 min (ref. 13).

To obtain a pellet, the incubation mixture was centrifuged at 14500 × *g* for 1 min at room temperature. Keeping the temperature at 37° during this 1-min centrifugation did not alter the results, indicating that no loss of the amino acids from the particles occurred because of separation at room temperature. The pellet was quickly frozen in dry ice, weighed, homogenized in 2 ml of 3 % $HClO_4$, and centrifuged; 0.5 ml of the clear supernatant fluid was added to 16 ml of modified Prockop–Ebert scintillation mixture for counting in a Packard Tri-carb liquid scintillation counter[14].

RESULTS

Uptake

Amino acids were rapidly taken up by the cerebral nuclear and mitochondrial fractions, and the intraparticulate levels were already above those of the medium at the shortest time period that was practical to measure (3 min). Since studies with brain slices[15,16] showed that, as with amino acids in other tissues[17], several transport classes can be distinguished in brain, representatives of different classes, a neutral (α-DL-aminoisobutyrate), a basic (L-lysine), and an acidic (D-glutamate) amino acid, were used. Each accumulated rapidly and seemed to reach equilibrium in 40 to 60 min

TABLE I

THE TIME-COURSE OF UPTAKE OF AMINO ACIDS BY PARTICULATE PREPARATIONS FROM RAT BRAIN

The particulate preparations from adult rat brain were incubated in standard media for the various times, then were centrifuged for 1 min, frozen, weighed and extracted with $HClO_4$ as described in the experimental section. Intraparticulate concentration was calculated according to the following formula: $(S-I \times M)/(1-I)$ where S = level of amino acid in the pellet (μmoles/g); M = level of amino acid in the medium (μmoles/ml) and I = inulin space (nuclei = 0.65, mitochondria = 0.57). Averages of 6 experiments are given.

Amino acid	*Concn. (mM)*	*Amino acid (μmoles/ml intraparticulate water)*						
		3 min	*5 min*	*10 min*	*20 min*	*40 min*	*60 min*	*90 min*
Nuclei								
DL-Aminoisobutyric acid	0.5			1.21	1.44	1.59		1.73
	1.0	1.55	1.84	2.14	2.61	2.93	3.23	3.43
L-Lysine	0.5			0.82	1.37			
	1.0	1.27	1.23	1.33	2.20	2.53		2.70
D-Glutamic acid	0.5			1.41	1.98			
	1.0	2.16	2.31	2.77	3.22	4.22		4.51
Mitochondria								
DL-Aminoisobutyric acid	0.5	1.02	1.16	1.24				
	1.0	1.65	2.09	2.20	2.80	3.38	3.68	3.74
	3.0	3.05		4.18	5.45	7.57		7.30
L-Lysine	0.5	0.69	0.77	0.86	1.27			
	1.0	1.32	1.46	1.58	2.11	2.23	2.52	2.72
	3.0	2.99		4.23		5.15		
D-Glutamic acid	1.0	1.97	2.83	3.26	4.81	5.94	6.24	6.01
	3.0		3.18	5.40	9.04			

(Table I). The same time (40 min) was used for most subsequent experiments since prolonged incubation gave more variable results because of partial damage to the particles. Uptake in each case was dependent on the concentration of the amino acid in the incubation medium. To have the uptake considerably exceed the endogenous pool levels, 1 mM medium was used in most experiments. The level of amino acid was expressed in units in intraparticulate water by correcting the total found in the pellet for the amino acid contained in adhering medium. The extraparticulate space of the pellet was estimated by measuring the inulin content of the pellet after 40 min incubation of the particulate fractions in the standard medium containing [^{14}C]inulin. This correction assumes that inulin does not penetrate the intraparticulate water. The 57 % inulin space of the mitochondrial pellet agrees well with the approx. 60 % space found for extraparticulate space of mitochondrial pellet from other organs, using sucrose as marker[18]. Of the amino acids measured, D-glutamate was accumulated to the highest extent in the nuclear and mitochondrial fractions.

Since the level of amino acids was determined by measuring the radioactivity in the particulate fractions, even with the relatively stable compounds chosen it was important to determine the degree of metabolism of the amino acids. Chromatographic analysis of the extracts showed that 80–90 % of the label in the particulate fractions at the end of the incubation was present as the unchanged amino acid and no correction for metabolism was necessary. Uptake was pH-dependent, with a pH optimum

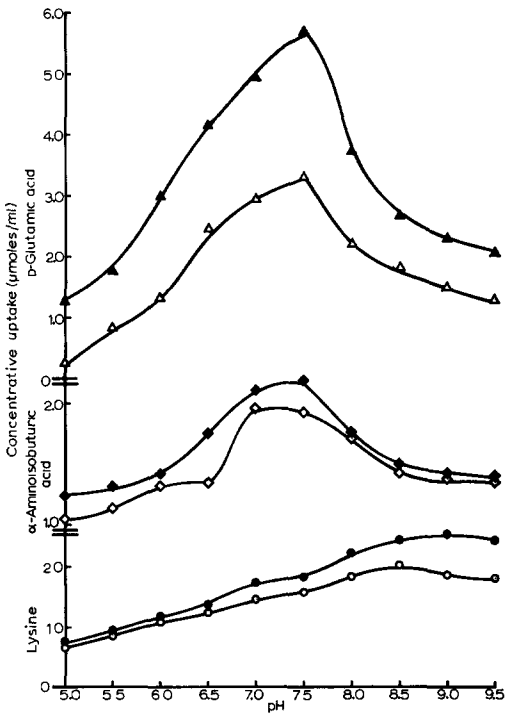

Fig. 1. pH dependence of amino acid uptake. The particulate fractions were incubated under standard conditions for 40 min. Averages of 3 experiments are shown. L-Lysine: ○, nuclei; ●, mitochondria. DL-Aminoisobutyric acid: □, nuclei; ■, mitochondria. D-Glutamic acid: △, nuclei; ▲, mitochondria.

of about 7.5 for α-aminoisobutyric acid and glutamate, and highest uptake around pH 9.0 for lysine, in both the nuclear and mitochondrial fractions (Fig. 1). Incubations reported in this paper were done at pH 7.4.

Inhibition of uptake

Concentrative uptake (uptake in the intraparticulate water accumulated above medium levels) seemed to depend on available energy. CN^- and dinitrophenol inhibited uptake, the inhibition by dinitrophenol being less than that of CN^-. A significant portion of uptake remained after CN^- treatment (Table II). In these experiments the particulate fractions were preincubated with the inhibitor. Without such

TABLE II

EFFECT OF INHIBITORS ON AMINO ACID UPTAKE

Concentrative uptake (uptake above medium levels = intraparticulate level, medium level at the end of incubation). Per cent inhibition. ((control − inhibited)/control) × 100. Ouabain 0.01 mM, NaCN 1 mM, dinitrophenol: 0.05 mM. The particulate fraction was incubated for 10 min either with the inhibitor or under control conditions; then 1 mM amino acid was added to the medium, and the incubation was continued for another 40 min. Averages of 8 experiments are given.

Amino acid	*Concentrative uptake (μmoles/ml)*				*Inhibition of concentrative uptake (%)*		
	Control	*Ouabain*	*NaCN*	*Dinitrophenol*	*Ouabain*	*NaCN*	*Dinitrophenol*
Nuclei							
Aminoisobutyric acid	2.34	0 90	1 40		62	40	
L-Leucine	1.04	0 25	0 45		76	57	
L-Lysine	1.79	0.83	0.91	1 36	54	49	24
D-Glutamic acid	3.68	0.40	1.40	2 17	89	62	41
Mitochondria							
Aminoisobutyric acid	2.55	0 67	0.96		74	62	
L-Leucine	0.84	0 31	0.61		63	27	
L-Lysine	1.62	0 76	0.81	1.36	53	50	16
D-Glutamic acid	6 54	0.79	1.17	2 63	88	82	60

preincubation the inhibition was less. Ouabain inhibited uptake more strongly than CN^- did. This is in contrast to the effect of these inhibitors on amino acid uptake by brain slices, where CN^- was a considerably stronger inhibitor than ouabain, and where most of the concentrative uptake was abolished by CN^- (ref. 19). The dependence on energy for concentrative uptake by particulate fractions was further supported by the absence of most of such uptake at 0° (Table III). It is of interest that leucine, as in brain slices[20], has a component that shows concentrative uptake at 0°. The sensitivity to lowering of temperature was highest for glutamate uptake and lowest for leucine.

The inhibition by ouabain indicated that amino acid accumulation in brain particulates requires Na^+. Lowering Na^+ concentration in the incubation medium inhibited amino acid uptake strongly; omission of K^+ was not as effective (Table IV). D-Glutamate uptake was again the most sensitive; it was completely inhibited in

TABLE III

TEMPERATURE DEPENDENCE OF UPTAKE

Incubation was for 40 min under standard conditions. Averages of 4 experiments ± S.D. are given.

Amino acid	*Concentrative uptake (μmoles/ml)*		
	0°	*22°*	*37°*
Nuclei			
Aminoisobutyric acid	0	1.54 ± 0.12	2.20 ± 0.33
L-Leucine	0.45 ± 0.11	0.77 ± 0.10	0.92 ± 0.19
L-Lysine	0.17 ± 0.09	0.89 ± 0.07	1.51 ± 0.21
D-Glutamic acid	0	2.19 ± 0.39	4.05 ± 0.75
Mitochondria			
Aminoisobutyric acid	0	2.12 ± 0.19	2.87 ± 0.27
L-Leucine	0.70 ± 0.04	0.83 ± 0.13	1.08 ± 0.14
L-Lysine	0.30 ± 0.07	1.27 ± 0.11	1.67 ± 0.16
D-Glutamic acid	0	3.81 ± 0.47	7.78 ± 0.83

TABLE IV

EFFECT OF Na^+ AND K^+ LEVELS IN THE MEDIUM ON AMINO ACID UPTAKE

The preparation of the particulate fraction was the same as described in the previous table. The ionic composition of the incubation medium was varied as indicated. Incubation was for 40 min in the medium containing 1 mM [^{14}C]amino acid. Averages of 6 experiments are given. Lys = L-lysine; Glu = D-glutamic acid.

Concn. of ions in the medium	*Concentrative uptake (μmoles/ml)*			*Inhibition of concentrative uptake (%)*		
	DL-*Amino-isobutyric acid*	*Lys*	*Glu*	DL-*Amino-isobutyric acid*	*Lys*	*Glu*
Nuclei						
128 mM Na^+	1.65	1.25	2.70			
64 mM Na^+	0.84	0.89	0.41	49	29	85
32 mM Na^+	0.53	0.79	0	68	37	100
16 mM Na^+	0.31	0.70	0	81	44	100
5 mM K^+	1.53	1.40	2.62			
2.5 mM K^+	1.67	1.47	3.17	9	5	21
No K^+	1.33	1.20	1.21	13	14	54
Mitochondria						
128 mM Na^+	2.33	1.64	6.05			
64 mM Na^+	1.36	1.28	1.88	42	22	62
32 mM Na^+	0.90	0.90	0.45	61	45	92
16 mM Na^+	0.67	0.78	0.07	71	52	99
5 mM K^+	2.55	1.62	7.45			
2.5 mM K^+	2.36	1.24	6.59	7	23	11
No K^+	2 62	1.62	4.59	0	0	38

media containing 16 mM Na^+ and was significantly inhibited by lack of K^+. L-Lysine uptake was less sensitive to the ionic composition of the medium. The greater sensitivity of uptake of D-glutamate than that of lysine to low Na^+ levels was also found in brain slices; but in contrast to the relative insensitivity of particulate fractions to the absence of K^+, the uptake of most amino acids by brain slices was inhibited by the absence of K^+ from the incubating medium[21]. One of the reasons for relative insensitivity to the lack of K^+ may be that the particles retain K^+ even in K^+-free media (Table V). The particulate Na^+ was close to that of the medium in media high

TABLE V

Na^+ AND K^+ LEVELS IN THE PARTICULATE FRACTIONS

Ion content (μmoles/ml water) of the particulates was determined from the experiments measuring amino acid uptake described in Table IV. Averages of 6 experiments are given.

Medium		*Intraparticulate*			
		Nuclei		*Mitochondria*	
Na^+	K^+	Na^+	K^+	Na^+	K^+
128	5	114	48	120	53
16	5	7	14	8	14
128	0	141	14	142	15

TABLE VI

THE EFFECT OF Ca^{2+} AND PO_4^{3-} LEVELS IN THE MEDIUM ON AMINO ACID UPTAKE

Standard conditions were used for incubation, except that the Ca^{2+} and PO_4^{3-} content of the medium was varied. Averages of 4 experiments are given.

Concn. (mM) in medium		*Concentrative uptake (μmoles/ml)*			*Per cent change in concentrative uptake*		
Ca^{2+}	PO_4^{3-}	*α-Aminoiso-butyric acid*	*Lys*	*Glu*	*α-Aminoiso-butyric acid*	*Lys*	*Glu*
Nuclei							
0	0	1.26	1.05	1.60	−19	+ 8	−32
0	5.0	1.40	0.91	1.88	−10	− 6	−20
1.0	5.0	1.64	1.10	2.09	+ 6	+13	−11
1.5	5.0	2.36	1.44	2.51	+52	+48	+ 7
2.7	0.0	1.52	0.81	2.19	− 2	−17	− 7
2.7	5.0	1.55	0.97	2.35	0	0	0
8 1	0	1.63	0.75	2.39	+ 5	−23	+ 2
8 1	5.0	2.09	0.83	3.50	+35	−14	+49
Mitochondria							
0	0	2.74	1.83	3.56	+15	+ 4	−29
0	5 0	2.55	2.20	5.27	+ 7	+25	+ 5
1.0	5.0	2 92	2 36	3 97	+23	+34	−21
1.5	5.0	3.55	2.22	5.22	+49	+26	+ 4
2.7	0	2 3	1.62	5 17	− 3	− 8	+ 3
2 7	5 0	2.38	1.76	5.02	0	0	0
8.1	0	2 66	1.83	5.62	+12	+ 4	+12
8.1	5.0	3.38	1.90	6.38	+42	+ 8	+27

in Na^+, significantly below that of the medium in media low in Na^+, and above that of the medium when K^+ was absent. In control (5 mM) media, K^+ was accumulated by the particles to 10-fold above the medium; lowering either Na^+ or K^+ in the medium resulted in lowering of particulate K^+. All these changes were independent of the amino acid content of the medium.

The effect of Ca^{2+} was variable, with lack of Ca^{2+} inhibiting glutamate uptake mostly; lack of PO_4^{3-} had no significant effect (Table VI). It seemed that for optimal glutamate uptake considerably more Ca^{2+} was needed than for the optimal uptake of lysine, while the uptake of α-aminoisobutyric acid was increased both at lower and at higher Ca^{2+} levels. For most amino acids 1.5 mM Ca^{2+} seemed to be optimal; for glutamate no difference was found between media of 1.5 or 2.7 mM in respect to Ca^{2+}. This was also true for glutamate uptake by purified mitochondrial fractions (Fraction E, see below).

Specificity

All the amino acids that were tried were accumulated against a concentration gradient by the nuclear and mitochondrial fractions (Table VII). The ratio of intraparticulate to medium levels at the end of the incubation depended on the concentration of the amino acid in the medium and was higher in 0.1 than in 1 mM media, indicating approaching saturation of uptake at higher medium level. Not all amino

TABLE VII

UPTAKE OF VARIOUS AMINO ACIDS BY PARTICULATE FRACTIONS

Incubation was for 40 min under standard conditions. Averages of 6 experiments ± S.D. are given Ratio of intraparticulate to medium levels is given at the end of incubation when medium levels are slightly decreased due to uptake by particulates.

Amino acid	*Concn. (mM)*	*Amino acid (μmoles/ml intraparticulate water)*		*Ratio of intraparticulate concentrate to medium concentrate*	
		Nuclei	*Mitochondria*	*Nuclei*	*Mitochondria*
Glycine	1	6.35 ± 0.54	5.78 ± 0.47	6.7	6.1
	0.1	1.79 ± 0.19	1.32 ± 0.14	21	15
Aminoisobutyrate	1	3.66 ± 0.49	4.19 ± 0.35	3.9	4.4
	0.1	0.62 ± 0.05	0.42 ± 0.06	6.6	4.4
Valine	1	2.29 ± 0.31	2.53 ± 0.22	2.4	2.6
	0 1	0 38 ± 0.05	0.44 ± 0.02	4 1	4 7
Leucine	1	1.84 ± 0.12	2 11 ± 0 14	1.9	2.2
	0.1	0.33 ± 0.02	0.31 ± 0.04	3.6	3.2
Phenylalanine	1	1.95 ± 0.22	1.68 ± 0.20	2.1	1.7
	0.1	0.47 ± 0.07	0.30 ± 0.04	5.0	3.0
Proline	1	3.98 ± 0.43	4.28 ± 0.61	4.2	4.5
	0.1	0 82 ± 0.09	0.93 ± 0.11	8.7	10
Histidine	1	4.38 ± 0.61	4.22 ± 0.39	4.6	4.4
	0 1	0.61 ± 0.08	0.50 ± 0.04	6.5	5.2
Diaminobutyrate	1	3.09 ± 0.45	1.68 ± 0 25	3.3	1.7
	0.1	0.34 ± 0.05	0.21 ± 0.03	3.6	2.2
Lysine	1	2.41 ± 0.49	2.48 ± 0.17	2.5	2.6
	0.1	0.35 ± 0.09	0.33 ± 0.03	3.7	3.4
γ-Aminobutyrate	1	7.32 ± 0.89	7.15 ± 0.97	7.7	7.7
D-Glutamate	1	4.95 ± 0.90	7.21 ± 0.68	5.2	7.8

acids were accumulated to the same degree. It is of interest that the order of degree of uptake in rat-brain particulates was the same as that found previously in mouse-brain slices[2]: glutamate and γ-aminobutyric acid were the highest, followed by glycine and histidine, then by α-aminoisobutyric acid and proline, then by lysine, and finally by leucine and phenylalanine. The order of magnitude of uptake, while not identical, was fairly similar in the nuclear and mitochondrial fractions.

Uptake of the various amino acids was not independent of the presence of other amino acids, which was inhibitory in most cases. The pattern of inhibition seemed to be compatible with several classes of uptake mechanisms, with a considerable

TABLE VIII

INHIBITION OF UPTAKE OF AMINO ACIDS BY OTHER AMINO ACIDS

Amino acid, 1 mM; inhibitor, 10 mM. Incubation was for 40 min under standard conditions. Averages of 4 experiments are given.

Amino acid	*Inhibitor*	*Concentrative uptake (μmoles/ml)*		*Per cent inhibition of concentrative uptake*	
		Nuclei	*Mitochondria*	*Nuclei*	*Mitochondria*
Aminoisobutyrate		1.88	2.83		
	Glycine	0.39	1.19	79	58
	Leucine	0.98	0.85	48	70
	Histidine	0.24	0.48	87	83
	Diaminobutyrate	0.79	1.19	58	58
	Lysine	1.43	2.12	24	25
	Arginine	1.11	1.58	41	44
	Aspartate	1.49	2.07	21	27
	Glutamate	0.77	1 42	59	50
Leucine		0.62	1.12		
	Valine	0.14	0.32	77	71
	Histidine	0 23	0.39	63	65
	Diaminobutyrate	0.29	0.67	54	40
	Lysine	0.33	0.69	46	38
	Arginine	0.30	0 58	52	48
	Aspartate	0.47	0 85	25	24
	Glutamate	0 42	0.85	32	24
Lysine		1.25	1 37		
	Aminoisobutyrate	0.59	1.12	53	18
	Valine	0.25	0.84	80	39
	Leucine	0.28	0.42	78	69
	Histidine	0.40	0.62	68	55
	Diaminobutyrate	0.48	0 67	62	51
	Arginine	0.60	0 84	52	39
	Aspartate	0.58	0 78	54	43
	Glutamate	0.78	1.47	38	0
Glutamate		5.12	8.21		
	Valine	4.92	6.73	4	18
	Leucine	4 04	7 80	21	5
	Histidine	4.71	7.88	8	4
	Diaminobutyrate	4.30	8.31	16	0
	Lysine	5 21	6 90	0	16
	Arginine	5.11	8.19	0	0
	Aspartate	0	0	100	100

overlap among the classes (Table VIII). The inhibition pattern depended somewhat on the particular amino acid, but was fairly similar in the two particulate fractions.

Glutamate uptake showed the highest degree of specificity; it was strongly inhibited by aspartate but was not affected significantly by most other amino acids tried. Leucine and aminoisobutyrate showed some specificity of uptake; they were inhibited to a greater degree by the neutral amino acids, although acidic and basic amino acids also had inhibitory effects. The specificity of uptake seems to be similar to that established in brain slices[14–16], with somewhat greater sensitivity to inhibition by analogs in the particulate preparation. Because of greater reproducibility these experiments were performed at 40-min incubations, measuring effects on steady-state distribution rather than initial rates of uptake.

Uptake in other preparations

When the cerebral particulate fractions were further purified, most of the concentrative uptake was in the fractions containing nerve-ending particles, especially those rich in synaptic vesicles (Table IX). In most cases, the degree of uptake was the same in the nerve-ending particle fraction as in the crude nuclear and mitochondrial fractions, with most amino acids taken up somewhat less by the nerve-ending particle fractions.

TABLE IX

AMINO ACID UPTAKE BY CEREBRAL MITOCHONDRIAL SUBFRACTIONS

Incubation was for 40 min under standard conditions. Subfractions were prepared according to DEROBERTIS *et al.*[12]. A, myelin fragments; B, complex containing membranes, vesicles and nerve ending fragments, C, pinched-off nerve endings containing numerous synaptic vesicles; D, the purest fraction of nerve endings; E, mitochondria. Averages of 6 experiments are given.

Amino acid	*Medium (mM)*	*Amino acid (μmoles/ml pellet water)*				
		A	*B*	*C*	*D*	*E*
Glycine	1.0	1.08	3.20	3.03	1.27	1.02
α-Aminoisobutyric acid	0.1	0.096	0.257	0.311	0.165	0.103
Leucine	0.1	0.075	0.232	0.192	0.121	
Phenylalanine	1.0	1.17	1.98	1.46	1.38	
Phenylalanine	0 1	0.116	0.274	0.226	0.182	
Histidine	0.1	0.098	0.269	0.179	0.219	0.101
α,γ-Diaminobutyric acid	1.0	1 33	3.45	1.88	2.54	
Lysine	1.0	0.85	1.57	2.06	1.36	1.10
Lysine	0 1	0.102	0.264	0.208	0.100	0.115
γ-Aminobutyric acid	1.0	0.91	2.97	3.42	1.44	1.04
D-Glutamate	1.0	0.92	3.02	3.04	1 57	1.12

In these experimental conditions the purified mitochondrial fraction showed very little concentrative uptake. This may have been due to three factors: (1) damage to the mitochondria in the purification process, (2) unfavorable experimental conditions, and (3) no concentrative capacity. The most likely explanation is a combination of (1) and (2), since purified mitochondria from pig brain showed concentrative uptake of those amino acids that were tried (Table X). As will be shown in a subsequent paper, the optimal conditions for uptake in purified cerebral mitochondrial

fractions are different from those of the crude fractions and vary with the amino acid tested, and are also dependent on time, substrates present, concentration of amino acid measured, *etc.* Short-term experiments are necessary to show significant uptake of glutamate, while longer experiments are needed for lysine; aminoisobutyrate uptake is weak in these preparations (Table X).

Under the conditions of incubation suitable for showing concentrative uptake by the crude cerebral particulate fractions, other tissue preparations did not show such uptake. In adult and newborn brain, particulate fractions took up amino acids against a concentration gradient, while fractions from the other tissues tested: liver muscle, and kidney, reached the level of the medium, with liver slightly above and muscle slightly below the medium (Table XI). The fractions from newborn brain took

TABLE X

UPTAKE IN MITOCHONDRIAL SUBFRACTIONS FROM PIG BRAIN

Incubation conditions and the method of separation were the same as described in the previous table except instead of rat-brain, pig-brain homogenates were used. Averages of 3 experiments are given.

Fraction	*Time (min)*	*Concentrative uptake (μmoles amino acid per ml interparticulate water)*		
		Amino-isobutyrate	L-*Lysine*	D-*Glutamate*
C	15	0.87	0.87	4.82
	40	2.22	1.67	5.62
D	15	0.50	0.32	4.25
	40	1.07	1.07	2 80
E	15	0.12	0.32	1.77
	40	0.55	0.87	0.45

TABLE XI

AMINO ACID UPTAKE BY PARTICULATE FRACTIONS FROM VARIOUS TISSUES

Amino acid level in the medium was 1 mM; incubation was for 40 min. Inulin space newborn brain nuclei, 58%; mitochondria, 57%, adult liver nuclei, 61%; mitochondria, 58%. Averages of 3 experiments are given.

Amino acid	*Amino acid (μmoles/ml water)*					
	Brain (newborn)		*Liver*		*Muscle pellet*	*Kidney pellet*
	Pellet	*Intra-particulate*	*Pellet*	*Intra-particulate*		
Nuclei						
α-Aminoisobutyric acid	4.09	8.43	1.00	1.03	0.87	0.90
L-Lysine	1.80	2.97	1.08	1.24	0.76	0.92
D-Glutamate	2.76	5.26	0.98	0.98	0.87	0 91
Mitochondria						
α-Aminoisobutyric acid	4.78	9.86	1.04	1.12	0.82	0.98
L-Lysine	2.22	3.90	1.10	1.27	0.78	0.94
D-Glutamate	2 99	5.69	1.03	1.10	0.86	0.94

up amino acids to a greater level than fractions from adult brain, as a comparison of Tables I and XI shows. The difference was especially great with α-aminoisobutyric acid, where preparations from newborn accumulated the amino acid to more than twice the level in adult particulates. Purified mitochondria (E-fraction) from liver were equally inactive in accumulating amino acids against a concentration gradient under conditions where such preparations from brain were active; adding pyruvate and malate instead of glucose as substrate did not change such uptake.

DISCUSSION

The results show that nuclear and mitochondrial fractions from brain have the capacity to accumulate amino acids against a concentration gradient. Since for incubation in an amino acid-containing medium, the levels used were near or above endogenous levels present in brain cells under physiological conditions, such accumulation may play an important role in the living brain. The initial rate of accumulation was high, and after 3 min of incubation, levels in the particulate fraction were well above that of the media. Accumulation continued for a longer time at a lower rate, and equilibrium was reached, in most cases, in less than 60 min of incubation. There may be a slow component of uptake that does not reach equilibrium by that time; this is difficult to evaluate because prolonged incubation produces damage to particles, as also shown for measuring uptake in particles that have been preincubated for various periods. The uptake was independent of the metabolic fate of the amino acid, since both compounds that are metabolized in the brain and stable compounds were accumulated. The rate of metabolism of amino acids such as leucine and aspartic acid is considerably lower in brain particulate fractions than in whole brain[26].

Concentrative uptake by the particulate fractions requires energy, as shown by the inhibition of uptake by most metabolic inhibitors tried, and by incubation at 0°. There seems to be some difference among the various amino acids, for example, the lack of complete inhibition of leucine uptake at 0°. Inhibition of transport by metabolic inhibitors has been shown in numerous systems; of more particular pertinence is the inhibition in brain slices[19,21], where in numerous instances the level of ATP in the slices and the degree of uptake was parallel[22], indicating that the available energy is one of the rate-limiting factors in transport. The levels of ATP in particulates were found to be much lower (less than 1/10) than in brain slices[27], which may partially explain the lower accumulation by particulates. Adding nucleotides to the incubation medium did not increase particulate ATP levels or particulate amino acid uptake. It is important to emphasize that changes in energy levels may affect metabolite transport indirectly; for example, metabolic inhibitors may, in many systems, influence transport by affecting membrane structure, swelling, or other factors. Particulates were relatively resistant to inhibition by CN^-, which very strongly inhibits the uptake of most amino acids in brain slices[19]. The average inhibition of uptake of amino acids by slices was between 80 and 90 %, which shows that a small portion of amino acid uptake may be CN^--resistant in brain slices also. The properties of this CN^--resistant portion of transport are under investigation in our laboratory[28]. Dinitrophenol was a weaker inhibitor than CN^-, as was shown in other systems as well, and high levels of this compound were necessary to show inhibition. A more

detailed knowledge of the energy metabolism of the particulates under the experimental conditions is necessary to evaluate the effect of metabolic inhibitors. Ouabain seemed to strongly inhibit amino acid transport in particulates as well as in brain slices, indicating a requirement for Na^+ for amino acid transport in particulates. A requirement for Na^+ in the movement of amino acids in thymus nuclei has been shown previously[9]. A Na^+-dependent binding of γ-aminobutyric acid by brain particulate fractions was shown in a set of studies that established the properties of this binding in careful detail[7,8]. The mechanism for γ-aminobutyric acid transport in particulates has been proposed[8]. It is of interest that the binding of γ-aminobutyric acid was not inhibited at 0° (ref. 23). In brain slices, as in other tissues, Na^+ is an absolute requirement for concentrative uptake of amino acids[3,24]. The lowering of Na^+ affected different amino acids to a different degree, both in slices[21] and in particulates (Table IV). The effect of ions may be important under physiological conditions in increasing the substrate specificity of amino acid movements.

Ions may affect different particles to different degrees. For example, brain mitochondria were shown to be much more resistant to lowering of respiration by Ca^{2+} than were liver mitochondria[25].

In most of its characteristic properties the uptake by particulates is similar to that by brain slices, including its dependence on available energy and on the ion composition of the medium. The similarity includes closer details of behavior, such as the small components of active uptake characteristic of leucine, but not of other amino acids, at 0°. Further similarity is that amino acids that are accumulated by brain slices to high levels are also accumulated by brain particulates to high levels. This is of particular interest because the amino acids at high level physiologically are the ones that are accumulated in the preparations *in vitro* to a high level, indicating that the accumulating capacities of the transport mechanisms revealed in the study of the preparations *in vitro* play an important role in determining the physiological levels of cerebral metabolites.

The substrate specificity of amino acid transport in the particulate fractions also seems to be similar to that in other brain preparations, although it may have somewhat broader specificity in the particulates. This may be due to a greater overlap of the carrier sites or to the more exposed condition of the membranes.

Although partial damage to membranes may explain all the observed differences between the transport properties of brain slices and the various particulate preparation, some genuine differences in properties may exist in the permeability properties of the various structural elements of nervous tissue that account for the heterogeneous distribution and movements of cerebral substrates. The capacity of particulate fractions for metabolite uptake emphasizes that the heterogeneous distribution of metabolites within cells is controlled by mechanisms of transport as well as metabolism.

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