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SELECTIVE INHIBITION BY HEAT OF AMINO ACID TRANSPORT IN SLICES OF MOUSE BRAIN

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SUMMARY

The effect of heat treatment on the transport of amino acids by slices of mouse brain was studied:

1. Brief incubation of slices at 47 °C (up to 30 min) decreased uptake; increasing the temperature further (10 min at 50 °C) abolished transport.

2. Heat sensitivity was different for the various amino acids, and the pattern indicated the existence of transport classes similar to those established in studies of substrate specificity of transport. In general the heat sensitivity of acidic amino acids and ω-amino acids was high, of small neutral and basic amino acids moderate, of the large neutrals low. Valine, with high sensitivity, was an exception.

The pattern of heat inhibition of these classes was similar in influx (5–15 min) and in steady-state (60 min) experiments.

3. There was no difference in the heat sensitivity between L- and D-isomers.

4. The properties of the portion of transport remaining after heat treatment were similar to that in untreated slices. Uptake requires Na^+ and the availability of energy. The greater effect of lowered Na^+ on acidic amino acid uptake, and the greater effect of weak metabolic inhibitors after heat treatment may be due to lowering of the ATP levels of the slices by heat treatment itself.

5. The effect of heat treatment was irreversible: further incubation at 37 °C did not restore uptake.

6. There were some differences in heat sensitivity of uptake during development, although slices from newborn behaved similarly to those from adult brain.

7. The heat sensitivity of exchange seemed to be lower than that of uptake — final evaluation of this needs further study of the effects of altered energy, ions, and amino acid concentration.

8. Because there are differences in the heat sensitivity of the various metabolite transport systems, heat treatment seems to be a suitable method for studying overlapping substrate specificity of transport classes

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INTRODUCTION

Brain slices when incubated in media take up amino acids against a concentration gradient. Various aspects of the transport mechanisms have been studied. Accumulation is dependent on available energy and on Na^+, since metabolic inhibitors or the absence of Na^+ inhibits the uptake of all amino acids[1-5]. In many of its properties cerebral amino acid transport is similar to the metabolite transport observed in other systems.

Studies, especially of competitive inhibition by amino acid analogs, support the existence in brain slices[6-8] and in the living brain[9,10] of multiple carrier systems of amino acid transport with substrate specificities similar to those studied by Christensen and coworkers[11-13] in Ehrlich ascites cells. In addition to multiple carrier systems, the amino acids have multiple affinities to the various carriers, making a quantitative evaluation of amino acid movements difficult.

In the present paper partial denaturation of amino acid carriers by heat treatment of cerebral slices will be reported. Heat treatment affects the uptake of various amino acids to different degrees, and the differences in rates of heat denaturation of the various carriers make a preparation possible in which, because some systems are selectively heat inactivated, the properties of the other carriers can be better measured. It is hoped that a study of the various properties of amino acid uptake after heat treatment may help to delineate differences in the properties of the various transport systems. In addition, such studies may also aid quantitative estimation of the contribution of particular carriers in the transport of each metabolite.

METHODS AND MATERIALS

Young adult Swiss mice were decapitated, the brain was rapidly removed, the pons medulla and cerebellum were discarded, and the cerebrum was sliced with a McIlwain tissue slicer at 0.42 mm thickness. The preparation and incubation of the tissue and the medium were done as previously described[4,5], with slight modification for heat treatment. Briefly, slices of half a hemisphere weighing about 100–150 mg were placed in a 25 ml Erlenmeyer flask containing medium of the following composition: 128 mM NaCl, 5 mM KCl, 2.7 mM $CaCl_2$, 1.2 mM $MgSO_4$, 5 mM Na_2HPO_4, 100 mM Tris (pH 7.4). After incubation in a shaking water bath at the given temperature and time for heat treatment, flasks were rapidly cooled by immersion in an ice bath, medium was added to bring the glucose level to 10 mM, O_2 was bubbled through for 1 min, and the slices were incubated at 37 °C for 10 min. Following this period, 0.01 μCi of ^{14}C labeled amino acid was added, usually to a level of 2 mM and incubation was continued for the stated time of uptake. For competition studies the amino acid inhibitor was added just before the labelled amino acid. The extraction of slices and the determination of radioactivity and of extracellular (inulin) space were previously described[6,14]. Briefly, slices after the incubation were separated by filtration, frozen in solid CO_2, weighed, and extracted with 3 % $HClO_4$; the label of the extract was determined in a scintillation spectrometer.

The results unless otherwise stated are expressed as concentrative uptake (uptake above the level in the medium) in units of μmoles per ml intracellular water. For these calculations (see also legend to Table I) the water content of the

tissue is taken as 80% and the extracellular (inulin) space as 52% of tissue water[14].

The method and the preparation of D-[^{14}C]aspartic acid by enzymatic decarboxylation of DL-aspartic acid with L-glutamic acid decarboxylase was described previously[4]. The D-isomer was separated through AG 1-$X4$, 200–400 mesh in the chloride form from Bio-Rad. DL-[^{14}C]Aspartic acid was from New England Nuclear Corp.; decarboxylase was obtained from Worthington Chemical Corp.; ATP was assayed by luciferase in the scintillation counter[5]. Briefly, firefly tails were extracted with an arsenate–Mg^{2+} buffer kept in the cold for 3–4 h until the endogenous ATP was broken down. Before use, the extract was added to neutralized, diluted tissue extract, and 5-s counts were taken repeatedly and compared to an ATP standard (1–0.1 μM).

^{14}C-labeled amino acids were obtained from Calbiochem. Amino acids and analogs were also from Calbiochem; most were analytical grade. Puromycin was from Nutritional Chemical Corp.; cychloheximide, ATP, and firefly tails were from Sigma Chemical Co. The thixotropic gel was a product of Packard Instrument Co.

RESULTS

The effect of heat treatment on the uptake of various amino acids

Incubation of brain slices at temperatures below 37 °C prior to uptake does not affect the uptake of amino acids. Incubation at higher temperatures is inhibitory; 5 min at 60 °C abolishes subsequent concentrative uptake. The rate of heat inactivation is lower at 47 °C, and at this temperature differences could be seen among the amino acids tried (Fig. 1). Of the three amino acids tried (one each of a neutral, basic, and acidic amino acids, representatives of separate amino acid transport

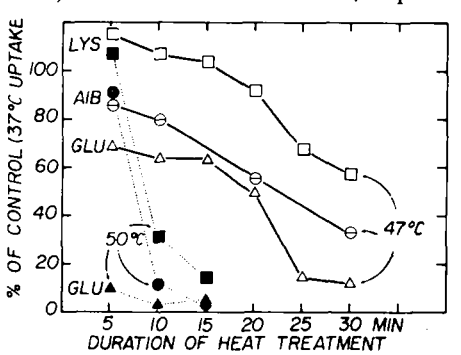

Fig. 1. Effect of heat treatment on the uptake of amino acids by slices of mouse brain. AIB = α-aminoisobutyric acid. Brain cortex slices were first heat treated by incubation in the Krebs–Ringer Tris–phosphate buffer medium (pH 7.4) at the indicated temperature and time; then glucose was added (to 10 mM) and each flask was oxygenated, incubated at 37 °C for 10 min; brought to 2 mM ^{14}C-labeled amino acid with the addition of 0.01 μCi and further incubated for 60 min. Values are expressed as percent of the concentrative uptake of control (37 °C pretreated) slices; control concentrative uptake ± S.E. were the following: AIB = 22.2 ± 2.5; lysine = 7.34 ± 0.60; glutamate = 65.0 ± 2.8 μmoles/ml. Concentrative uptake was calculated according to the following formula

$$C = \frac{(\mu\text{moles/g slice} \times 1.25) - (\mu\text{moles/ml medium} \times 0.52)}{0.48} - \mu\text{moles/ml medium.}$$

This calculation assumes 80% total water in the slices and 52% of this water in extracellular space (inulin space). Averages of 5 experiments are given.

classes[6,8]), glutamate uptake was most sensitive to heat treatment and lysine uptake was the most resistant. At 30 min heat treatment at 47 °C most of glutamate uptake was destroyed, while more than half of lysine uptake remained. For further experiments, therefore, 30-min heat treatment at 47 °C was adopted. For control, brain slices were incubated at 37 °C for 30 min. Changes at 50 °C were too rapid to be reproducible, and were of little use for further studies; but at this temperature too, lysine was the most, glutamate the least, resistant to heat treatment (Fig. 1).

The pattern of heat inhibition when a number of amino acids were tested seemed to confirm differences in the heat sensitivity of the various amino acid transport classes (Table I). The least sensitive are large neutral amino acids with a side chain (leucine, phenylalanine, tyrosine) followed by the basic amino acid group (diaminobutyric acid, arginine, ornithine, lysine), while the most sensitive group is represented by the acidic amino acids (glutamic, aspartic acid) and γ-aminobutyric acid and β-alanine. Heat sensitivity is similar within a transport group; only valine, with a high sensitivity to heat, seems to differ from related compounds. The pattern of heat sensitivity of the various groups was similar whether initial rate (5–15-min uptake) or steady state (60-min uptake) was measured. In order to measure net

TABLE I

EFFECT OF HEAT TREATMENT ON INFLUX AND STEADY-STATE UPTAKE.

The level of amino acids in the medium at the beginning of the experiment was, unless given, 2 mM. Influx times were varied to yield significant concentrative uptake by the brain slices. Heat treatment was 30 min at 47 °C followed by 10 min at 37 °C before addition of the amino acid, as described in Methods and Materials. For calculating concentrative uptake see legend to Fig. 1. Incubation time for steady-state experiments was 60 min. Averages of 5 experiments ± S.E. are given. DAB, L-2,4-diaminobyturic acid; GABA, γ-aminobutyric acid; AIB, α-aminoisobutyric acid.

Amino acid	*Incubation time (min)*	*Concentrative uptake of amino acid*			
		Initial rate		*Steady state*	
		Control (μmoles/ml)	*Heat treated (% of control)*	*Control (μmoles/ml)*	*Heat treated (% of control)*
Glyc	5	8.97 ± 1.09	23	43.7 ± 3.9	38
L-Ala	5	6.97 ± 0.50	43	25.9 ± 1.6	57
β-Ala	15	23.6 ± 2.5	14	56.9 ± 1.8	13
GABA	10	11.9 ± 1.6	12	43.1 ± 3.3	11
AIB	10	6.23 ± 0.62	22	22.7 ± 1.5	33
D-Val	15	4.59 ± 0.58	11	6.88 ± 0.65	17
D-Leu	10	2.93 ± 0.56	72	4.95 ± 0.41	69
D-Leu (0.2 mM)	10	0.56 ± 0.07	46		
L-Phe	10	2.30 ± 0.12	107	2.78 ± 0.18	70
L-Tyr	5	2.33 ± 0.22	58	11.6 ± 0.4	55
DAB	5	6.00 ± 0.80	41	21.4 ± 0.9	47
L-Orn	10	1.08 ± 0.10	35	5.18 ± 0.15	54
L-Lys	10	1.18 ± 0.26	62	6.48 ± 0.33	58
L-Lys (0.2 mM)	10	0.25 ± 0.01	32		
L-Arg	15	2.08 ± 0.05	35	6.85 ± 0.37	33
D-Glu	5	9.77 ± 1.06	7.2	64.3 ± 5.2	9.4
D-Glu	10	16.1 ± 0.9	20		
D-Asp	5	15.67 ± 3.2	24	89.6 ± 6.2	19
D-Asp	10	21.9 ± 1.4	29		

uptake not influenced to a significant degree by exchange and metabolism, and to have levels in the medium near the K_m of the uptake of amino acids, most compounds were 2 mM in the medium. When D-leucine and L-lysine were lowered to 0.2 mM the heat sensitivity increased (Table I). This raises the possibility that the pattern of sensitivity is altered at lower amino acid concentrations.

In order to measure the effects of heat treatment on the transport rather than the metabolism of the amino acids, in a number of cases the D-isomers were used. Although there is some stereospecificity in the uptake of amino acids by brain slices[2,9], the properties of uptake of the two isomers are similar[4,9], and the two isomers of an amino acid seem to belong to the same transport class[15]. There did not seem to be a significant difference in the effect of heat treatment on the two isomers of an amino acid among those tried Uptake of L- and D-glutamate and valine was strongly inhibited while that of L- and D-leucine and lysine was inhibited to a much lower degree by the heat treatment (Table II).

TABLE II

DIFFERENCE IN HEAT SENSITIVITY IN THE UPTAKE OF STEREOISOMERS

Averages of 6 experiments ± S.E. are shown.

Amino acid	*Incubation time (min)*	*Concentrative uptake of amino acids*			
		D-*isomer*		L-*isomer*	
		Control (μmoles/ml)	*Heat treated (% of control)*	*Control (μmoles/ml)*	*Heat treated (% of control)*
Val	15	5.20 ± 0.53	17	6.69 ± 0.30	28
Leu	10	2.64 ± 0.13	65	2.62 ± 0.14	58
Lys	20	3.95 ± 0.40	79	4.19 ± 0.53	84
Glu	10	25.44 ± 1.23	21	20.30 ± 1.15	25

Although there was clearly a difference in the heat sensitivity of the various transport classes, the pattern of substrate specificity of transport did not change greatly in the instances tried. The greater inhibition of lysine uptake by leucine after heat treatment (Table III) would indicate a significant transport of lysine by a carrier for neutral and a carrier for basic amino acids. Since the carrier for basic amino acids is more heat sensitive, after heat treatment a correspondingly larger portion of lysine is transported by the carrier for neutral amino acids and is in turn inhibited more by leucine. In most other cases the pattern of inhibition changed little after heat treatment, as would be expected if one carrier is the major contributor for the uptake of an amino acid (Table III). It must be emphasized that short intervals for uptake, especially in heat inactivated slices, result in relatively small uptake of amino acids with larger experimental errors of measurements.

Inhibitory conditions

Na^+ is required for concentrative uptake of amino acids in brain slices. There is no significant uptake in the absence of Na^+, and decreasing Na^+ results in lower uptake, to various degrees dependent on the amino acid[2,4] used. Na^+ remains a

TABLE III

INHIBITION OF UPTAKE BY AMINO ACID ANALOGS

For the absolute values of uptake in the absence of inhibitor see Table I. Incubation was for 10 min in 2 mM amino acid and 20 mM inhibitor. Averages of at least 4 experiments are given. For abbreviations see legend of Table I.

Inhibitor	*Treatment* (°C)	*Relative uptake (uptake in absence of inhibitor = 100)*				
		AIB	*GABA*	D-*Leu*	L-*Lys*	D-*Glu*
AIB	37				54	109
	47				83	88
L-Leu	37	67	104		42	107
	47	48	90		0	89
L-Ile	37	44		48		
	47	51		66		
DAB	37		39		26	113
	47		37		61	94
L-Orn	37				42	
	47				65	
L-Lys	37	121	98			
	47	111	117			
L-Arg	37			86	0	117
	47			76	0	97
D-Glu	37	58		87		
	47	133		96		
D-Asp	37		121			0
	47		117			0

requirement in heat-treated slices (Table IV). Decreasing Na^+ levels from 128 to 12.8 mM reduced leucine and lysine uptake in control and heat-treated slices to the same degree. The uptake of the acidic amino acids became more sensitive to alterations in Na^+ after heat treatment, as shown by greater inhibition of low Na^+ in heat-treated slices both in influx and in steady-state uptake experiments (Table IV). In these experiments L-glutamate and D-aspartate were used, since in previous experiments L-glutamate and L- and D-aspartate showed similar sensitivity to low Na^+ whereas D-glutamate showed higher sensitivity than L-glutamate[4].

The dependence of amino acid uptake on the availability of metabolic energy did not change; the "heat-resistant" portion of uptake was inhibited by metabolic inhibitors (Table V). Strong inhibitors of uptake in control slices inhibited uptake also strongly in heat-treated slices. With a weaker inhibitor (dinitrophenol) inhibition was greater in heat-treated as compared to control slices; this again may be due to the lowered levels of available energy after heat treatment. Inhibitors of protein synthesis, puromycin at 10 μg/ml and cycloheximide at 1 μg/ml, had no effect on the uptake of the five amino acids tested in Table V by control or heat treated slices; *N*-ethyl maleimide had a somewhat greater inhibitory effect in heat-treated slices.

The greater sensitivity of uptake in heat-treated slices to metabolic inhibitors may be caused by a diminished supply of energy, the heat treatment interfering also with energy metabolism. The ATP content of heat-treated slices was greatly dimi-

TABLE IV

EFFECT OF LOW Na^+ CONCENTRATION ON AMINO ACID UPTAKE BY HEAT-TREATED BRAIN SLICES

High Na^+: 128 mM; low Na^+: 12.9 mM. Brain slices were incubated in high or low Na^+ medium at 37 °C for 30 min, then washed with the same medium, transferred into freshly prepared Na^+ medium, and kept at either 37 or 47 °C for 30 min. Glucose was added, to 10 mM, and the slices were incubated for 10 min at 37 °C after oxygenation; then ^{14}C-labeled amino acid was added, to 2 mM, and the slices were incubated for 10 min for influx or 60 min for steady state. Choline chloride was substituted for Na^+ in the 12.8 mM media. Averages of 5 experiments ± S.E. are given.

Amino acid	*Concentrative uptake of amino acids*							
	Influx (10 min uptake)				*Steady state (60 min uptake)*			
	Uptake with high Na^+ (μmoles/ml)		*Uptake with low Na^+ (% of uptake with high Na^+)*		*Uptake with Na^+ (μmoles/ml)*		*Uptake with low Na^+ (% of uptake with high Na^+)*	
	Control	*Heat treated*	*Control*	*Heat treated*	*Control*	*Heat treated*	*Control*	*Heat treated*
D-Leu	2.26 ± 0.41	0.73 ± 0.09	66	46	4.21 ± 0.19	1.13 ± 0.31	84	75
L-Lys	0.84 ± 0.11	0.49 ± 0.10	37	32	5.48 ± 0.32	1.35 ± 0.27	60	70
L-Glu	20.4 ± 2.7	4.70 ± 0.52	38	13	52.4 ± 4.6	6.17 ± 0.55	68	25
D-Asp	23.2 ± 0.9	4.64 ± 0.31	59	18	74.5 ± 1.9	8.97 ± 0.76	89	18

TABLE V

EFFECT OF METABOLIC INHIBITORS ON AMINO ACID UPTAKE DURING 10 min

Inhibitors were present during heat treatment and the subsequent incubations. After heat treatment slices were incubated with 10 mM glucose for 10 min and then with 2 mM ^{14}C-labeled amino acid for 10 min. 0 indicates no concentrative uptake. Data are expressed as percentage of the concentrative uptake without inhibitors; 37 °C = control; 47 °C = heat treatment. Values are the average of at least 4 experiments ± S.E. IAA, iodoacetic acid; DNP, 2,4-dinitrophenol; AIB, α-aminoisobutyric acid; GABA, γ-aminobutyric acid.

Amino acid	*Concentrative uptake (μmoles/ml)*		*Uptake as % of uptake in the absence of inhibitor*							
			IAA 1 mM		*DNP 0.2 mM*		*NaCN 1 mM*		*N-Ethylmaleimide 0.1 mM*	
	37 °C	*47 °C*	*37 °C*	*47 °C*	*37 °C*	*47 °C*	*37 °C*	*47 °C*	*37 °C*	*47 °C*
AIB	6.77 ± 0.99	1.96 ± 0.47	19	0	13	17	6.0	11	90	68
D-Leu	2.48 ± 0.19	2.32 ± 0.17	18	2	34	13	9.1	5.5	68	27
L-Lys	0.82 ± 0.12	0.46 ± 0.06	39	1	30	26	0	0	102	98
D-Glu	18.3 ± 0.5	2.78 ± 0.45	2	0	43	20	0	0	63	46
GABA	12.5 ± 1.4	2.42 ± 0.17	13	0	16	9	0	0	71	16

TABLE VI

THE LEVEL OF ATP IN HEAT-TREATED BRAIN SLICES

o time = after 30-min treatment (control 37 °C, heat treated 47 °C). 10,60 min = 30-min heat treatment, 10 min incubation with glucose and 10 or 60 min further incubation at 37 °C. These conditions were similar to those of the influx or steady-state amino acid uptake experiments. Averages of 4 experiments ± S.E. are given.

Incubation time after heat treatment:	*μmoles ATP per ml intracellular water*		
	o	*10 min*	*60 min*
Control	1.47 ± 0.16	2.12 ± 0.12	2.07 ± 0.07
Heat treated	0.38 ± 0.03	0.34 ± 0.04	0.28 ± 0.02

nished. In control slices, upon incubation ATP was resynthesized to close to physiological levels; in the heat-treated slices no recovery of ATP levels was observed upon further incubation (Table VI). These lower levels of ATP may be so low as to account for much of the observed inhibition, since it was found that reducing ATP greatly was in most (but not all) cases accompanied by a similar reduction of amino acid uptake in brain slices[5].

Irreversibility of inhibition

Further incubation of heat-treated slices at 37 °C to allow for possible reversal of inhibition was not successful. Untreated brain slices incubated at 37 °C even for prolonged periods do not lose their ability to accumulate amino acids. When slices after heat treatment were further incubated at 37 °C, uptake decreased rather than recovered (Table VII). This effect is clearly seen with D-leucine: incubation for 180 min at 37 °C did not diminish uptake by control slices, but abolished uptake by heat-treated slices.

TABLE VII

POSSIBLE RECOVERY OF INFLUX AFTER HEAT DENATURATION BY LONGER PREINCUBATION

Brain cortex slices were preincubated after heat treatment for 10, 60, or 180 min in Krebs–Ringer–Tris-phosphate buffer (pH 7.4) with 10 mM glucose after oxygenation; then 2 mM ^{14}C-labeled amino acid was added to each flask and incubated for 10 min. Data shown are percent of concentrative uptake of value of 10 min preincubation. Averages of at least 4 experiments with S.E. are shown. See legend of Table I for abbreviatious.

Preincubation time:	*Concentrative uptake*					
	10 min		*60 min*		*180 min*	
	37 °C (μmoles/ml)	*47 °C (% of control)*	*37 °C (% of control)*	*47 °C (% of control)*	*37 °C (% of (control*	*47 °C (% of control)*
AIB	6.70 ± 0.80	36	99	24	—	—
D-Leu	2.57 ± 0.26	76	91	71	91	3
L-Orn	0.75 ± 0.11	25	74	0	63	0
L-Lys	1.31 ± 0.03	62	96	0	0	—
D-Glu	20.2 ± 0.61	23	82	4	72	0
GABA	14.2 ± 0.87	32	90	18		—

TABLE VIII

EFFECT OF HEAT TREATMENT ON AMINO ACID EXCHANGE IN BRAIN SLICES

For intracellular 60-min levels, slices were incubated with ^{14}C-labeled amino acid for 60 min. For exchange, slices were incubated with ^{14}C-labeled amino acid for 60 min then with ^{14}C-labeled amino acid of the same concentration for 5 min. Averages of at least four experiments ± S.E. are given. See legend of Table I for abbreviations.

Amino acid	*μmoles amino acid per ml*							
	Control				*Heat treated*			
	Medium	*Intracellular at 60 min*	*Exchanged in 5 min*	*% Exchanged*	*Medium*	*Intracellular at 60 min*	*Exchanged in 5 min*	*% Exchanged*
AIB	2	24.5 ± 1.4	4.62 ± 0.41	18.9	2	9.27 ± 1.06	1.09 ± 0.19	11.8
AIB					8	21.9 ± 1.0	2.51 ± 0.32	11.5
D-Leu	2	6.42 ± 0.79	2.32 ± 0.28	36.1	2	4.95 ± 0.64	1.84 ± 0.18	37.2
D-Lys	2	8.63 ± 0.31	1.87 ± 0.18	21.7	2	5.67 ± 0.17	1.19 ± 0.06	21.0
L-Lys					4	11.2 ± 0.2	2.30 ± 0.56	20.5
L-Arg	2	8.77 ± 0.40	2.60 ± 0.03	29.6	2	4.23 ± 0.34	0.97 ± 0.08	22.9
D-Glu	2	70.0 ± 6.8	11.3 ± 0.4	16.1	2	8.87 ± 1.16	2.75 ± 0.60	31.0
D-Glu	0.4	11.0 ± 1.0	4.51 ± 0.23	41.0				
D-Asp	2	90.4 ± 6.2	11.4 ± 0.9	12.6	2	18.8 ± 2.1	4.59 ± 0.11	24.4
GABA	2	44.5 ± 3.4	4.80 ± 0.28	10.8	2	6.66 ± 0.55	0.94 ± 0.08	14.1

It was hoped that with protection by the presence of substrate the selectivity of inactivation could be increased, if all transport systems except the protected one were inactivated. Lysine was found not to protect from inactivation by heat. If during the heat treatment (47 °C for 30 min) 2 or 5 mM lysine was present in the medium, and subsequently the slices were drained, transferred to fresh medium, and incubated with 2 mM ^{14}C-labeled amino acid, the heat inactivation of lysine or glutamate uptake was not significantly changed. Having glutamate present during heat treatment caused greater inhibition, rather than protection, of the subsequent uptake of D-glutamate or L-lysine.

The influx of amino acids was also inhibited at steady-state conditions (exchange) by heat treatment, although the inhibition was somewhat less than that of the initial rate of uptake. For these experiments control and heat-treated slices were first incubated with unlabeled amino acids to reach steady-state levels; then the slices were transferred into a medium that was identical except that it contained the amino acid in the labeled form. Influx was thus measured under conditions where intracellular levels of the amino acid did not change. Since incubation in 2 mM amino acid did not give the same steady-state levels in control and heat-treated slices, to make intracellular levels more comparable, in some experiments the level of the amino acid in the medium was increased with heat-treated slices or was decreased with control slices. The flux at equilibrium in most cases was decreased by heat treatment (Table VIII), although at comparable intracellular levels steady-state uptake (exchange flux) was inhibited less than influx (compare Tables I and VIII), indicating less heat sensitivity of uptake at steady state as compared to initial rate of uptake.

Newborn

The uptake of amino acids by slices from newborn mouse brain was also inhibited by heat treatment, but the pattern of heat inhibition was somewhat different from that of slices of adult brain. Lysine uptake is somewhat higher and glutamate uptake is lower in newborn slices as compared to adults[2,16], but the uptake of lysine

TABLE IX

SENSITIVITY OF AMINO ACID UPTAKE BY SLICES OF NEWBORN MOUSE BRAIN TO HEAT TREATMENT

Slices from six hemispheres of newborn mice (less than 24 h old) with a total weight of approx. 100 mg were combined for each incubation flask. The sequence of incubations was 30 min for heat treatment (37 °C for control or 47 °C for the heat treated group), 10 min with glucose, and then 10 min (for influx) or 60 min (for steady state) with 2 mM ^{14}C-labeled amino acid. Averages of six experiments ± S.E. are given. AIB, α-aminoisobutyric acid.

Amino acid	*Concentrative uptake of amino acids*			
	Influx		*Steady state*	
	Control (μmoles/ml)	*Heat treated (% of control)*	*Control (μmoles/ml)*	*Heat treated (% of control)*
AIB	4.86 ± 0.26	16	20.29 ± 0.52	20
L-Lys	2.10 ± 0.42	0	8.82 ± 0.63	29
D-Glu	7.74 ± 0.92	0	34.39 ± 0.27	53

was more inhibited and that of glutamate less inhibited by heat treatment in newborn slices, indicating some differences in heat sensitivity of uptake in different developmental stages (Table IX).

DISCUSSION

Knowledge of substrate specificity of amino acid transport is crucial to elucidate the role transport mechanisms play in the metabolism of the organism, and to understand pathological changes affecting metabolite distribution. Several transport classes, each consisting of a number of amino acids with related structure, could be distinguished on the basis of their net ionic charge (acidic, neutral, basic) and size[6,8,17]. In bacterial systems carriers with greater specificity (affinity to a single amino acid only) have also been described[18,19]. There may be differences in the substrate specificity of uptake, exit, and exchange[7,15,20]. In brain as in other organs considerable overlap was found among these transport classes. An example in brain slices is that leucine inhibits lysine uptake but lysine does not affect leucine uptake (leucine and lysine belong to different classes, with leucine having some affinity to the lysine carrier)[6,15].

The differences in substrate specificity of amino acid transport between various organs are not as well worked out. Little if any acidic amino acid transport was found in the intestine, and in brain and sarcoma differences could be found between short chain and long chain basic amino acid transport, but no difference in intestinal mucosa and kidney. (For a recent survey of tissue differences, see ref. 8.) It is likely that specific systems exist in various tissues according to specific needs of the particular tissue, and thus other systems are not necessarily good models for the brain.

Changes in substrate specificity were found during development in brain in the relative amount of the carriers and in the affinity of one amino acid (γ-aminobutyrate) to the carriers[21]. A number of changes occur in the brain during development, in amino acid transport and in the composition of the amino acid pool[16,22-24]. This behavior is to be expected if transport mechanisms participate in the determination of the composition and supply of the intracellular metabolite pool. Another example of developmental changes in a cell is the rabbit reticulocyte, which upon maturation to an erythrocyte retains only one (L-type) of three neutral amino acid transport systems initially present[25]. Changes during development in cerebral neurotransmitter amine (norepinephrine) transport have been recently reported[26].

Of particular interest are recent findings in neural tissue elements of transport systems with possible neurotransmitter function. Snyder and collaborators[27] found in addition to a regular also a specific high affinity transport system for glutamic acid in brain synaptosomes (pinched-off nerve endings) and could separate a population of synaptosomes that selectively accumulated acidic amino acids. Glycine was also recently found to be taken up by two different transport systems in slices from the central nervous system, one with low affinity, predominantly in cortex cerebellum and midbrain, and one with high affinity, predominantly in spinal cord medulla and pons. The low affinity system was shared by other small neutral amino acids; the high affinity system was specific for glycine[28]. For most putative neurotransmitters, where their rate of metabolism is not high enough to explain activity of very short duration, release and reuptake were suggested for activation and inactivation. In

this especially the transport and specific binding of γ-aminobutyric acid were studied, most recently for taurine[29-31].

It is possible that transport systems for other metabolites overlap with amino acid transport systems. The possible overlap of hexose and amino acid transport was studied in some detail[3,33]; evidence for such overlap in the brain is lacking. A transport system was found[34] in brain for diamines (putrescine, cadaverine) which is different from the transport system for related amino acids.

The differences in heat inactivation indicate differences in the properties of the various carriers. Heat treatment is unlikely to have a uniform effect in all parts of the nervous system. Differences in amino acid levels and transport have been found between various areas of the nervous system[35]; there were differences between particulate fractions, nuclei, mitochondria, and synaptosomes[36] and between neuronal and glial fractions[37]. This heterogeneity in transport is in accord with the complex structure of the nervous system.

It has to be emphasized that heat treatment may also inhibit transport indirectly. The lowering of ATP levels in heat-treated slices (Table VI) indicates such an indirect effect through decreasing available energy. Lowering ATP levels in slices resulted in lower uptake of amino acids[5], with differences in sensitivity to lower ATP among the various amino acids. The inhibition of uptake by lowered ATP also depended on the agent used to lower ATP[5]. For these reasons it is not clear how much reduction of the supply of energy contributed to the inhibition of heat treatment of transport.

The smaller inhibition of exchange as compared to uptake (Table IX) is of interest. This result is in accord with the finding that uptake and exchange do not occur through the same carrier for each amino acid[17,38]. Homo-exchange and hetero-exchange of amino acids have been shown to occur in the brain[39,40] and have properties similar to those described in other systems.

It is clear that transport processes in the brain occur through multiple carriers and in multiple structures. The inhibition by heat treatment described here points out one possibility for at least partially separating some of the mechanisms involved. Such separation is necessary if we want to fully understand metabolite transport in organs where genetic selection cannot be used as an experimental tool.

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