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ION DEPENDENCE OF AMINO ACID UPTAKE IN BRAIN SLICES

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SUMMARY

1. The concentrative uptake of amino acids by mouse brain slices was measured after 5 and 90 min of incubation in the presence of varying concentrations of Na^+ and K^+. Lowering the Na^+ concentration in the medium from 148 mM to 10–30 mM decreased the tissue concentration of both Na^+ and K^+, with the Na^+ levels approximating those of the medium. Media containing 148 mM Na^+ and devoid of K^+ led to elevated Na^+ levels in the tissue. Decreasing only Na^+ in the medium led to a greater loss of tissue K^+ than the omission of K^+ from the medium.

2. The Na^+ requirement for transport of individual amino acids was not uniform. Lowering the Na^+ concentration of the medium strongly inhibited the uptake of α-aminoisobutyric acid, L-α,γ-diaminobutyric acid, D-glutamic acid and lysine, whereas the uptake of D- and L-aspartic acids, L-glutamic acid and leucine was inhibited to a lesser degree. Even though the two isomers of glutamate were accumulated to approximately the same extent under control conditions, differences in uptake were observed in Na^+-deficient media.

3. The absence of K^+ from the incubation medium led to a marked inhibition of amino acid uptake (ranging from 64 to 82 %), and the degree of inhibition depended upon the Na^+ concentration as well as on the amino acid studied.

4. There was no correlation between tissue levels of ATP and the degree of inhibition of amino acid uptake in ion-deficient media.

5. Although the data clearly show that accumulation of amino acids by brain slices is ion dependent, the presence of a physiological Na^+ gradient between tissue and medium is not necessary.

INTRODUCTION

Brain slices can accumulate amino acids against a concentration gradient. This uptake is greater in slices from brain than in those from most other tissues, and it occurs through a mediated transport mechanism, the properties of which have been investigated in a number of studies[1–4]. Recent investigations of amino acid transport by brain slices[5–7] showed the pattern of substrate specificity to be similar to that found

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in such systems as Ehrlich ascites tumor cells[8], intestine[9], and kidney[10]. Instead of a distinct "carrier" for each amino acid, several "transport classes" could be distinguished, each specific for a group of amino acids with similar structure, and with considerable overlap between the various classes.

It has been shown in a number of tissues and for several amino acids that active transport is a Na^+-dependent process[11–15]. Although there seems to be an absolute requirement for Na^+ in most cases, Na^+-independent components have been demonstrated with some amino acids, *e.g.*, lysine and histidine[16], valine[17] and methionine[18]. K^+-dependent systems of amino acid transport have also been demonstrated in a number of tissues[16,19,20].

In view of these findings, we determined the effects of Na^+ and K^+ on the uptake of amino acids by mouse brain slices in order to establish whether the effects of these ions are the same on all the postulated "carriers".

MATERIALS AND METHODS

Brain slices (0.4 mm thickness) were prepared from the cerebral hemispheres of young-adult Swiss mice as described previously[21]. Slices (approx. 600 mg) from the brains of two animals were pooled in a 50-ml erlenmeyer flask containing 16 ml of cold medium (4°). The flasks were oxygenated for 1 min, stoppered, and placed in a shaker bath at 37°. Unless otherwise indicated, the medium was decanted after a 10-min preincubation period and replaced by 16 ml of fresh medium of the same composition, previously oxygenated and warmed to 37°, and after 30 min the medium was changed again. The total preincubation time was 1 h. The purpose of these changes during the preincubation period was to achieve ionic equilibrium in the slices and to obtain a medium of known ionic composition. In the case of experiments in K^+-free medium, it was of special importance to obtain a medium as nearly free of this ion as possible since the leakage of K^+ from damaged tissue can result in an appreciable external K^+ concentration in the small volume of medium used[22].

Slices were separated from the preincubation medium by pouring the flask contents on a Buchner funnel and gently shaking for a few seconds to allow for drainage of medium. The tissue remaining on the funnel was divided among four 25-ml erlenmeyer flasks containing 3 ml of oxygenated incubation medium at 37°, and incubation was then carried out at that temperature.

The control medium contained 10 mM glucose, 128 mM NaCl, 5 mM KCl, 2.7 mM $CaCl_2$, 1.2 mM $MgSO_4$, 10 mM Na_2HPO_4, and 100 mM Tris buffered to pH 7.4. Incubation media were made 2 mM with respect to the labeled amino acids investigated, with a specific activity of 0.005–0.01 $\mu C/\mu mole$.

The experimental media were either K^+-free, or 10, 20 or 30 mM with respect to Na^+. Choline chloride was added in an amount sufficient to substitute for Na^+ in an isomolar fashion. In the case of 10 mM Na^+ medium, only half the usual concentration of Na_2HPO_4 was used.

After incubation the slices were collected by filtration and frozen in powdered dry ice. The frozen tissue was weighed and then homogenized in 2 ml of 3 % $HClO_4$. The clear supernatant obtained by centrifugation was used for the determination of ion and amino acid concentrations, which are expressed on the basis of intracellular water after correction for the cation concentration of the extracellular compartment.

For analyses of Na^+ and K^+, aliquots were diluted with ion-free double-distilled water and the ion concentration was determined by flame photometry.

The amount of radioactivity in the samples was measured by counting 0.5-ml aliquots of the $HClO_4$ extract in a Packard liquid scintillation spectrometer. Since counting error due to quenching was less than 2%, correction for individual sample counts was not necessary. Amino acid levels in the slice were expressed in terms of concentrative intracellular uptake, that is, level in the intracellular compartment above that in the medium, after correction for the amino acid content of the extracellular compartment. Extracellular space of the slices at the end of the incubation period was measured using [^{14}C]carboxy-inulin under conditions of the uptake experiments.

The proportion of the total radioactivity in the tissue extracts representing unmetabolized amino acid was determined for D- and L-aspartate, D-glutamate, lysine and leucine after separation of the amino acids by high-voltage electrophoresis and paper chromatography. For electrophoresis, $HClO_4$ extracts of incubated tissue were neutralized with KOH and centrifuged, and the supernatant evaporated to dryness. The residue was redissolved in a drop of water and electrophoresed at 0° on Whatman 3 MM paper, with 2% pyridine–1% aqueous acetic acid (pH 5.2), at 30 V/cm for 100 min, or with 2.5% formic acid–7.8% acetic acid (pH 1.85), at 30 V/cm for 240 min. For paper chromatography (butanol–acetic acid–water; 6:1.5:5, v/v/v), and in some instances for electrophoresis, 70% ethanol extracts of tissue were used. The extent of amino acid metabolism was quantitated by determining the portion of the total radioactivity represented by unmetabolized amino acid after electrophoretic or chromatographic separation.

ATP was assayed using the scintillation counter method of TAL, DIKSTEIN AND SULMAN[23]. Samples were prepared by neutralizing 3% $HClO_4$ extracts of tissue with 1 M NaOH and diluting with water.

D-[4-^{14}C]Aspartic acid was prepared by the enzymatic decarboxylation of DL-[4-^{14}C]aspartic acid and separation of the α-alanine formed[24]. The incubation mixture contained 2 μmoles of DL-[4-^{14}C]aspartic acid, 4 μmoles of L-[^{12}C]aspartic acid, 60 μmoles of sodium pyruvate and 30 mg of L-glutamic acid decarboxylase (L-glutamate-1-carboxy-lyase, EC 4.1.1.15, Worthington) in a total vol. of 2.6 ml acetate buffer (pH 4.9). Incubation was carried out at 37° for 45 min using the flasks described by SABA AND DI LUZIO[25]. An additional 2 μmoles of L-[^{12}C]aspartic acid (in 0.5 ml of 0.26 M acetate buffer) were then added, and incubation was continued for a total period of 90 min. The CO_2 evolved was trapped in Hyamine and counted. Experiments using uniformly labeled L-[^{14}C]aspartic acid under the same incubation conditions resulted in recovery of 24.5% of the radioactivity in $^{14}CO_2$, agreeing well with the theoretical yield of 25%. When DL-[4-^{14}C]aspartate was used, 49.3% of the radioactivity was recovered in $^{14}CO_2$. The D-[4-^{14}C]aspartic acid was isolated by chromatography on Dowex 1-acetate[26], and the eluate was evaporated to dryness to remove acetic acid. Reincubation of the isolated D-[4-^{14}C]aspartate with glutamic acid decarboxylase yielded less than 1.2% of its radioactivity as $^{14}CO_2$, and the radiochemical purity of the D-aspartic acid was demonstrated by paper chromatography to be greater than 99%.

Observed differences in results are considered statistically significant if $P < 0.05$ as determined using a one-tailed t-test.

Compounds labeled with ^{14}C were obtained from the following sources: α-amino-[1-^{14}C]isobutyric acid, D-[1-^{14}C]glutamic acid, 2,3-diaminobutyric acid (U.L.), L-leucine (U.L.) and [^{14}C]carboxy-inulin (*i.e.* inulin labeled with additional [^{14}C]carboxyl groups) from Calbiochem; L-aspartic acid (U.L.) and L-lysine (U.L.) from Schwarz BioResearch; DL-[4-^{14}C]aspartic acid from New England Nuclear Corp.

RESULTS

Inulin space and dry weight

Brain slices undergo considerable swelling upon incubation. Since this may depend on the condition of the medium, it was important to determine water content and extracellular space of the tissue under the experimental conditions. In order to determine the inulin space (extracellular space *plus* adherent medium), brain slices were incubated using the usual experimental conditions, which included a 60-min preincubation with an exchange of medium, followed by 90-min incubation in medium containing [^{14}C]carboxy-inulin and 2 mM unlabeled amino acid.

The percent inulin space representing extracellular fluid of the tissue and adherent medium was calculated as (counts/min [^{14}C]carboxy-inulin per gram tissue wet wt./counts/min [^{14}C]carboxy-inulin per ml medium) × 100. The inulin space in medium containing 148 mM Na^+ (and with or without K^+) ranged from 59 to 63 % in the presence of the various amino acids investigated. There was a slight decrease in the size of the inulin space (to approx. 56 %) at lowered concentrations of Na^+ in the presence of all amino acids except aspartic acid, in which case the inulin space was 51 % of the tissue weight in 10–20 mM Na^+. Lack of K^+ had no effect, and when K^+ was combined with low Na^+, the tissue behaved as in low Na^+ alone.

The dry weight of slices was measured following a 60-min preincubation period and subsequent incubation for 90 min in conditions similar to those used for uptake experiments. The dry weight of the tissue was 14 ± 1 % of the wet weight, and this was not affected by the presence of amino acids in the medium or by changes in Na^+ concentration. Changes in the medium affect not only the size of the extracellular but also that of the intracellular compartment, since only the inulin space was altered depending upon the composition of the medium.

Metabolism of amino acids during incubation

Since the tissue concentration of amino acid is calculated on the basis of tissue radioactivity, it was important to know the extent of amino acid metabolism in these experiments. Under our experimental conditions less than 5 % of the tissue radioactivity was in compounds other than D-aspartic and D-glutamic acids, and L-leucine in studies involving those amino acids (Table I). The metabolic inactivity of α-amino-isobutyric acid and L-α,γ-diaminobutyric acid in mammalian tissues has been demonstrated previously[27]. Significant metabolism of lysine (29 %) was found after 90 min of incubation in 30 mM Na^+ medium, and L-aspartate was actively metabolized in all media, although less in media lower in Na^+.

Of the radioactivity taken up by tissue exposed to L-[^{14}C]glutamic acid in both normal and low-Na^+ media, approximately 90–93 % was in unmetabolized glutamate after 5 min and 72–82 % after 90 min (Table I).

After 5 min incubation less than 3 % of the [^{14}C]aspartate and [^{14}C]glutamate

removed from the medium was metabolized to $^{14}CO_2$. By 90 min this figure rose to 25%, except in the case of [^{14}C]aspartate in low-Na^+ media, which was metabolized only to the extent of 16%. The loss of label as $^{14}CO_2$ is assumed to be minimal in the case of leucine and lysine, since less than 1.5% of the amino acid added to rat brain slices was converted to CO_2 in similar experiments[28].

Concentrative uptake of amino acids by brain slices

When incubated in media containing 2 mM amino acid, brain slices reached amino acid levels considerably greater than those of the medium after only 5 min of incubation (Table II). Varying degrees of Na^+ dependence were found (Table III).

TABLE I

THE EFFECT OF ALTERING Na^+ AND K^+ LEVELS ON THE METABOLISM OF GLUTAMATE, ASPARTATE AND LYSINE BY BRAIN SLICES

Incubations and electrophoretic and chromatographic separations were carried out as described in the text. Following incubation with D-aspartic acid, D-glutamic acid and L-leucine the original amino acid accounted for greater than 95% of the radioactivity present in the tissue extract.

Ion concentration in medium (mM)		*Incubation time (min)*	*% of acid-soluble radioactivity present in metabolites*		
Na^+	K^+		L-*Glu*	L-*Asp*	L-*Lys*
148	5	5	7	41	5
148	5	90	28	73	13
10	5	5	10	13	6
10	5	90	18	14	3
20	5	5	9	24	12
20	5	90	23	39	16
30	5	5	7	27	7
30	5	90	26	50	29
148	0	5	—	26	4
148	0	90	—	65	10

TABLE II

THE UPTAKE OF AMINO ACIDS BY MOUSE BRAIN SLICES

The medium contained 10 mM glucose, 128 mM NaCl, 5 mM KCl, 2.7 mM $CaCl_2$, 1.2 mM $MgSO_4$, 10 mM Na_2HPO_4 and 100 mM Tris buffered to pH 7.4. The concentration of the respective amino acids was 2 mM with a specific activity of 0.005–0.01 μC/μmole.

Amino acid	*Concentrative uptake (μmole/ml intracellular water ± S.E.)**	
	5 min incubation	*90 min incubation*
L-Leu	1.78 ± 0.09	3.36 ± 0.17
α-Aminoisobutyric acid	4.67 ± 0.12	26.94 ± 0.90
D-Glu	14.45 ± 0.49	53.65 ± 1.62
L-Glu	17.87 ± 0.34	53.08 ± 1.87
D-Asp	11.77 ± 0.52	55.70 ± 2.41
L-Asp	17.24 ± 0.36	57.61 ± 2.73
L-α,γ-Diaminoisobutyric acid	6.60 ± 0.35	34.95 ± 1.26
L-Lys	0.39 ± 0.11	11.42 ± 0.34

* Concentrative uptake represents the concentration of radioactivity accumulated by the intracellular compartment of the tissue above that in the medium.

The amino acids tested in short-term experiments can be divided into two groups. The uptake of α-aminoisobutyric acid, L-α,γ-diaminoisobutyric acid, lysine and D-glutamate was strongly inhibited by a low Na^+ concentration, whereas the uptake of leucine, L-glutamate, and D- and L-aspartate was affected to a lesser degree by lowering Na^+ in the medium. The pattern of Na^+ dependence following 90 min of incubation was similar to that seen at 5 min. The behavior of D-glutamate was distinct in its greater sensitivity to Na^+ levels; the 90-min concentrative uptake was completely inhibited at 10 mM Na^+, rose to 50 % in 20 mM Na^+, and reached 83 % of control in 30 mM Na^+. The sensitivity of D-glutamate uptake to Na^+ also depended on the length of incubation, in contrast to L-glutamate, which was inhibited much less by low Na^+ in all circumstances.

In the absence of K^+ the accumulation of amino acids by brain slices was decreased to 15–36 % of control values (Table III). However, in short-term experiments with L-α,γ-diaminoisobutyric acid, the tissue retained 67 % of its initial concentrative ability. At 20 mM Na^+, it usually made no difference in short-term experiments whether K^+ was present or absent, except with aspartate. In the longer-term experiments, the absence of K^+ caused further inhibition of uptake in most cases.

No concentrative uptake of amino acids could be demonstrated after 5 min of incubation in the complete absence of Na^+. At 90 min a slight uptake of lysine and leucine (9–13 %) was observed (unpublished observation).

It was of importance to determine whether equilibrium was achieved by 90 min of incubation, since ions might affect not only initial rate of uptake but also amino acid efflux. The accumulation of amino acids was measured at 60 min in those cases

TABLE III

THE EFFECT OF Na^+ AND K^+ ON THE RELATIVE UPTAKE OF AMINO ACIDS BY BRAIN SLICES

Amino acid	*Time (min)*	*Concentrative uptake in ion-deficient media (% of control ± S.E.)* *				
		Medium Na^+ (mM): 10	20	30	148	20
		Medium K^+ (mM): 5	5	5	0	0
L-Leu	5	32 ± 4	47 ± 5	80 ± 6	29 ± 5	45 ± 4
	90	56 ± 6	81 ± 4	100 ± 10	36 ± 2	56 ± 8
α-Aminoisobutyric acid	5	0	4 ± 2	22 ± 2	18 ± 3	7 ± 3
	90	15 ± 1	21 ± 1	32 ± 1	15 ± 1	14 ± 1
D-Glu	5	0	9 ± 1	30 ± 2	18 ± 1	5 ± 0
	90	2 ± 0	50 ± 2	83 ± 2	20 ± 3	14 ± 3
L-Glu	5	42 ± 2	76 ± 3	108 ± 3	—	—
	90	87 ± 4	116 ± 8	100 ± 3	—	—
D-Asp	5	38 ± 3	71 ± 2	104 ± 6	—	—
	90	89 ± 2	88 ± 4	105 ± 4	—	—
L-Asp	5	42 ± 4	82 ± 4	91 ± 8	36 ± 3	45 ± 3
	90	90 ± 5	115 ± 8	117 ± 10	21 ± 2	43 ± 3
L-α,γ-Diaminoisobutyric acid	5	6 ± 0	17 ± 3	21 ± 5	67 ± 7	22 ± 3
	90	16 ± 0	21 ± 2	29 ± 1	36 ± 4	25 ± 3
L-Lys	5	0	0	0	0	0
	90	27 ± 0	37 ± 1	50 ± 6	32 ± 3	29 ± 2

* Control values (100 % concentrative uptake) are given in Table II.

in which the inhibition at 5 min was significantly higher than that at 90 min (D-glutamate, 20 mM Na^+; L- and D-aspartate, 10 mM Na^+). In all cases the uptake was similar after 60 or 90 min of incubation, demonstrating that a steady state was achieved.

Intracellular concentration of Na^+ and K^+ following incubation of brain slices

The Na^+ and K^+ content of incubated brain slices was measured after preincubation and incubation without added amino acid (Table IV) and at the end of the experiment (Tables V and VI). The average intracellular Na^+ concentration in brain slices incubated in a control medium (148 mM Na^+, 5 mM K^+) was 110 μequiv/ml

TABLE IV

THE Na^+ AND K^+ CONCENTRATION OF BRAIN SLICES FOLLOWING INCUBATION IN THE ABSENCE OF ADDED AMINO ACID

Ion concn. in medium (mM)		*Ion concn. in brain slices (μequiv/ml intracellular water)*		
		60 min preincubation		*60 min preincubation + 90 min incubation**
Na^+	K^+	Na^+	K^+	K^+
148	5	112	78	51
10	5	13	19	11
20	5	20	28	13
30	5	27	36	17
148	0	152	32	20

* The Na^+ concentration of the tissue remained at preincubation levels.

TABLE V

THE Na^+ CONCENTRATION OF BRAIN SLICES FOLLOWING INCUBATION IN THE PRESENCE OF ADDED AMINO ACID*

Amino acid	*Time (min)*	*μequiv Na^+/ml intracellular water ± S.E.*					
		Medium Na^+: 148	10	20	30	148	20
		Medium K^+: 5	5	5	5	0	0
L-Leu	5	107 ± 5	11 ± 1	20 ± 2	29 ± 5	143 ± 7**	20 ± 1
	90	127 ± 6*	11 ± 3	22 ± 2	35 ± 4	155 ± 13**	23 ± 1
α-Aminoisobutyric acid	5	96 ± 6	13 ± 2	15 ± 1	29 ± 4	126 ± 9**	18 ± 1
	90	110 ± 7	9 ± 1	16 ± 1	26 ± 4	128 ± 11	24 ± 1
D-Glu	5	110 ± 6	12 ± 3	18 ± 1	23 ± 1	120 ± 15	18 ± 1
	90	103 ± 7	8 ± 2	15 ± 1	20 ± 2*	139 ± 14**	21 ± 2
L-Asp	5	116 ± 8	13 ± 2	23 ± 3	25 ± 2	166 ± 19**	24 ± 2
	90	94 ± 5*	8 ± 1	17 ± 2	19 ± 1*	173 ± 15**	21 ± 2
L-α,γ-Diaminoisobutyric acid	5	108 ± 4	14 ± 1	20 ± 2	24 ± 1	151 ± 7**	24 ± 2
	90	124 ± 5*	8 ± 2	18 ± 1	24 ± 1	136 ± 5	22 ± 1
L-Lys	5	114 ± 11	14 ± 2	18 ± 2	29 ± 3	127 ± 9	20 ± 1
	90	126 ± 6*	8 ± 1	18 ± 2	28 ± 3	182 ± 12**	25 ± 3

* Values are significantly different ($P < 0.05$) from those obtained using medium with the same ion concentration but without added amino acid (see Table IV).

** Values significantly different from control (148 mM Na^+, 5 mM K^+).

water after the 60-min preincubation period (Table IV) and after a 5-min incubation period in the presence of amino acid (Table V). After 90 min there was a small but significant decrease of tissue Na^+ with aspartic acid and an increase with the basic amino acids and with leucine.

When the Na^+ in the medium was lowered to 10–30 mM the Na^+ level in the tissue was in each case close to that of the medium. The Na^+-depleting action of aspartate was again evident in medium containing 30 mM Na^+. In the absence of K^+ there was usually an increase in tissue Na^+ by the end of the preincubation period.

The level of K^+ in the slices varied with the composition of the medium with respect to Na^+ as well as K^+ (Table VI). At the end of the preincubation period in

TABLE VI

THE K^+ CONCENTRATION OF BRAIN SLICES FOLLOWING INCUBATION IN THE PRESENCE OF ADDED AMINO ACID*

Amino acid	*Time (min)*	*μequiv K^+/ml intracellular water ± S.E.*					
		Medium Na^+: 148	10	20	30	148	20
		Medium K^+: 5	5	5	5	0	0
L-Leu	5	75 ± 2	16 ± 1	25 ± 2	36 ± 2	29 ± 1	10 ± 1
	90	60 ± 2*	11 ± 1	15 ± 1	21 ± 1	16 ± 2	5 ± 0
α-Aminoiso-butyric acid	5	82 ± 2	16 ± 1	22 ± 3	37 ± 1	28 ± 4	10 ± 2
	90	72 ± 3*	11 ± 1	15 ± 1	21 ± 1	18 ± 2	6 ± 1
D-Glu	5	77 ± 2	18 ± 1	23 ± 2	32 ± 1	26 ± 2	9 ± 1
	90	67 ± 3*	9 ± 1	17 ± 1*	26 ± 1*	17 ± 2	6 ± 1
L-Asp	5	82 ± 3	16 ± 0	26 ± 3	36 ± 2	35 ± 5	10 ± 2
	90	76 ± 3*	17 ± 1*	23 ± 2*	32 ± 2*	22 ± 4	5 ± 0
L-α,γ-Diaminoiso-butyric acid	5	67 ± 2*	17 ± 1	26 ± 1	27 ± 3*	31 ± 2	13 ± 1
	90	47 ± 2	8 ± 1	13 ± 1	16 ± 1	15 ± 1	—
L-Lys	5	70 ± 3	17 ± 1	17 ± 2*	30 ± 2	21 ± 2*	7 ± 1
	90	58 ± 2	9 ± 1	11 ± 1	17 ± 1	13 ± 1*	7 ± 1

* Values are significantly different ($P < 0.05$) from those obtained using medium with the same ion concentration but without added amino acid (see Table IV).

TABLE VII

THE EFFECT OF Na^+ AND K^+ ON THE ATP CONCENTRATION OF BRAIN SLICES FOLLOWING INCUBATION

Ion concentration in medium (mM)		*Relative ATP concentration in brain slices (% of control ± S.E.)**	
Na^+	*K^+*	*5 min incubation*	*90 min incubation*
148	5	100	100
10	5	94 ± 6	65 ± 5
20	5	109 ± 5	86 ± 5
30	5	143 ± 3	123 ± 7
148	0	63 ± 4	35 ± 2
20	0	69 ± 4	23 ± 2

* The average concentration of ATP was found to vary only with changes in ion concentration in the medium, but was not affected by the presence of added amino acid.

control media, tissue K^+ was 78 mM; when Na^+ was lowered in the medium, tissue K^+ levels approximated the Na^+ concentration of the medium. Further incubation in the absence of amino acid resulted in lowered tissue K^+ (Table IV). This loss of K^+ upon prolonged incubation was prevented by most amino acids except lysine and L-α,γ-diaminoisobutyric acid, with aspartate having the greatest effect. It is of interest that the absence of K^+ was not as effective in lowering tissue K^+ as was a decrease in Na^+ in the medium.

ATP content of brain slices after incubation

The ATP content of incubated brain slices was not influenced by the presence of different amino acids in the medium. ATP levels were significantly elevated in media containing 30 mM Na^+ (Table VII). Further lowering of Na^+ had only a small effect and this insensitivity of ATP levels to lack of Na^+ may reflect an impaired utilization of ATP. Omission of K^+ significantly decreased tissue ATP. Although the absolute level of ATP varied considerably, the percent change from control was similar in each preparation. Therefore, the data are given as percent change relative to control levels.

DISCUSSION

The requirement for Na^+ in the transport of metabolites such as amino acids and sugars has been well documented in several tissues, and the mechanisms that have been proposed to explain this phenomenon have been discussed[9,29–33]. The present investigation has focussed on the effect of decreased Na^+ levels, rather than the complete absence of Na^+ which has been studied by other workers. Our data confirm previous reports that the active transport of amino acids is also an Na^+-dependent process in the brain. It has been observed that the ion dependence of amino acid transport varies in different tissues. For example, although lysine uptake was shown to be Na^+ dependent in brain, this is not the case in kidney[15], and the inhibition of α-aminoisobutyric acid uptake in the absence of K^+ was strong in brain but weak in reticulocytes and diaphragm[19,20].

Shifts in the Na^+ and K^+ concentration of cerebral tissue occur after slicing and prior to incubation[34–36] although partial restoration of the original tissue electrolyte levels takes place during the course of incubation[22,36,37]. In agreement with the findings of BACHELARD, CAMPBELL AND MCILWAIN[36] and KEESEY, WALLGREN AND MCILWAIN[22], our data show that the maximum attainable restoration of Na^+ takes place during the preincubation period, after which the levels of Na^+ remain stable (Table IV). However, there is some leakage of K^+ as incubation progresses. The loss of K^+ which occurs in media low in Na^+ has been noted previously[36].

In the present studies, ion effects were noted in both short- and long-term uptake experiments. After 5 min of incubation the dominant process is amino acid influx which gives a measure of the initial rate of accumulation. By 90 min, a steady state of amino acid accumulation against a concentration gradient is reached. This steady state represents an equilibrium between influx, efflux, and the metabolism of the compound being measured. In our experiments, metabolites represented only a small portion of the tissue radioactivity at five min, and most amino acids tested were not significantly metabolized in the long-term experiments. Moreover, amino acid

metabolism did not appear to have a major effect on uptake, since the effect of ions on the accumulation of L-aspartate was not different from their effect on the uptake of the unmetabolized D-isomer.

The sensitivity of uptake to lowered Na^+ levels varied with the amino acid tested. The uptake of L-α,γ-diaminoisobutyric acid, α-aminoisobutyric acid and lysine was strongly dependent upon the presence of Na^+. In some instances these amino acids also appeared to promote Na^+ retention and K^+ loss by the tissues that concentrate them. The dependence of leucine on Na^+ was intermediate, being less than that of L-α,γ-diaminoisobutyric acid, α-aminoisobutyric acid, and lysine, and greater than that of the acidic amino acids. A decrease in the efflux of leucine may have occurred, since in the steady state control levels were attained at a lower Na^+ concentration than in the shorter incubation periods.

Brain slices retained their ability to concentrate L-glutamate and D- and L-aspartate in media with low Na^+ levels. The greater inhibition of the initial rate of uptake (especially in 10 mM Na^+) indicates either a decreased efflux of amino acid, or that the influx of amino acid is more sensitive to low Na^+ concentrations than is efflux. The similar response of glutamate and aspartate uptake to increasing Na^+ concentration was compatible with a common transport mechanism for these amino acids.

Some differences between stereoisomers were observed at low Na^+ levels, where D-glutamate uptake was inhibited to a much greater extent than that of L-glutamate (Table III). The tissue uptake of D- and L-glutamate under control conditions was similar. The different effects of decreased Na^+ on the uptake of the two isomers cannot be ascribed solely to metabolism since L-glutamate was metabolized to only a very small extent in 5 min of incubation. It is therefore possible that the interaction of the carrier with the region of the α-carbon of glutamic acid was modified by the presence or absence of Na^+.

The effect of the complete absence of K^+ varied, depending upon the Na^+ concentration and the amino acid studied. The decrease in amino acid uptake produced by the complete absence of K^+ was not due to nonspecific tissue damage, since it was possible to restore uptake by replacement of K^+ (unpublished observations). It would not appear that the effect of K^+ ions is necessarily related to concomitant alterations in Na^+ levels, since it was found in short-term experiments that L-α,γ-diaminoisobutyric acid, while quite sensitive to Na^+, was least affected by changes in K^+. On the other hand, aspartate was less sensitive to lack of Na^+ and more affected by changes in K^+. These two amino acids also differed in that aspartate promoted retention of K^+ in the tissue whereas L-α,γ-diaminoisobutyric acid led to K^+ depletion. A possible role for K^+ in amino acid transport is also suggested by long-term experiments in low-Na^+ media (20 mM) where the absence of K^+ resulted in additional inhibition of the uptake of D-glutamate, L-aspartate and leucine. However, the tissue ATP was also significantly depleted under these conditions.

According to present views, Na^+ might affect the affinity of postulated carriers for nonelectrolyte transport[15,29,30], possibly through the formation of an $(Na^+)_x$-carrier complex[11,31,32], where x is variable depending on the amino acid being transported. While it is possible that K^+ only modifies the reaction of Na^+ with the carrier and thus acts indirectly, a $(K^+)_x$- or $(Na^+)_x$-$(K^+)_x$-carrier complex must also be considered in view of the present data.

Some workers have postulated that an Na^+ gradient ($Na^+_{out} > Na^+_{in}$) is necessary for amino acid transport, probably connected with energy production[11,30,33]. However, our findings support the conclusion of WHEELER AND CHRISTENSEN[13] and SCHAFER AND JACQUEZ[32] that such an Na^+ gradient is not a prerequisite for transport, although some type of electrochemical potential gradient may be required for the accumulation of amino acids. In the absence of K^+, Na^+_{in} is greater than Na^+_{out} before incubation, yet brain slices are able to concentrate amino acids; and uptake also occurs when Na^+_{in} approximately equals Na^+_{out} in low-Na^+ media. The relationship between energy production and ion gradients is still obscure. While active transport is by definition an energy-requiring process, in agreement with VIDAVER[11] and EDDY, MULCAHY AND THOMSON[33], in our study of the effects of inhibitors of energy utilization on amino acid uptake in brain slices[38] we found no consistent correlation between the tissue levels of ATP and the extent of concentrative uptake of individual amino acids. However, it is possible that the nearly uniform effect of K^+-deficiency on transport is related to the depressed ATP levels. Clearly, the available energy in the tissue is important. However, energy sources other than ATP may be involved, or very small amounts of ATP might be sufficient; also, the various amino acids may have widely differing energy requirements for their transport.

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