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A COMPARISON OF INHIBITION OF STEADY STATE, NET TRANSPORT, AND EXCHANGE FLUXES OF AMINO ACIDS IN BRAIN SLICES

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SUMMARY

1. The steady state, net transport, and exchange flux at steady state of four amino acids (aminoisobutyric acid, leucine, lysine and D-glutamic acid) whose transport is primarily mediated by different transport systems in brain slices were compared. The effects of various metabolic inhibitors (1 mM cyanide, 10 μM ouabain) and of sub-optimal incubation conditions (sodium- and glucose-free mediums and incubations at 0°) were studied.

2. Steady-state amino acid levels were reduced to a "new" lower level by the above experimental conditions. The changes in steady state appear to be due primarily to changes in net transport. The maintenance of steady state required the expenditure of metabolic energy.

3. The inhibition of influx varied with the particular amino acid studied. This is consistent with differences in sensitivity to the experimental conditions of the transport systems involved.

4. The exit of amino acids was increased by the experimental conditions. The increased exit is not likely to be explained by an inhibition of re-uptake.

5. Exchange influx at steady state compared to influx from identical medium concentrations was significantly greater for lysine. The inhibition of exchange influx by the various experimental conditions was not always identical to that of influx. Exchange influx but not net transport of aminoisobutyric acid and lysine was sodium independent.

6. Exchange efflux was greater than exit for all four amino acids. The ratio of exchange efflux and exchange influx approximated a 1:1 exchange ratio. The inhibition of exchange efflux was similar to the inhibition of exchange influx; inhibition of re-uptake could not explain all the observed data.

7. Whether the differences between exchange flux and net transport were due to exchange diffusion representing different transport systems or to other factors such as variation of properties of the carriers or membrane changes induced by the experimental conditions can not be answered at this time.

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INTRODUCTION

The term "exchange diffusion" was first used by USSING[1] to describe the process whereby ions rapidly pass across a membrane separating two aqueous phases in a one-to-one exchange for the same ion. Exchange diffusion was differentiated from physical diffusion by the formation of an ion–membrane complex; the membrane constituent called "carrier" mediated passage across the membrane. Since there was no net transport and the aqueous phases were unchanged with respect to ion concentration, USSING considered exchange diffusion to be independent of an energy supply. Subsequently, the process of exchange and heteroexchange (exchange of one compound for another structurally related compound) has been demonstrated for sugars and amino acids in a variety of tissue and cell preparations. In most of the models that deal with exchange diffusion[2–6], exchange is mediated through the same carrier system that mediates net transport, except that the step(s) dependent on the expenditure of energy in active transport is not essential or is circumvented in the exchange process. Recent studies have attempted to demonstrate experimentally that a non-energy-requiring exchange process could be differentiated from active transport[7,8].

The process of exchange and heteroexchange in brain has also been well demonstrated with amino acids in *in vivo* and *in vitro* studies[9–13]. These studies, however, do not permit any conclusion as to whether exchange is an energy-requiring process; moreover, they do not indicate if amino acids utilize the same carrier systems for exchange and for net transport.

In this study we have compared the net transport (influx and efflux) to the flux into and out of brain cells under conditions approximating the steady state (exchange influx and exchange efflux) of four amino acids whose net transport is predominantly mediated by different carrier systems[13–15]. The effects of various experimental conditions and metabolic inhibitors on amino acid movements were compared to determine whether steady-state exchange flux or net transport could be selectively altered, or one altered to a significantly greater degree, and whether an alteration in steady-state levels could be explained in terms of an alteration in exchange flux or net transport.

EXPERIMENTAL

Tissue preparation has been described in detail in previous publications[16,14].

The standard incubation 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–HCl buffered to pH 7.4; ^{14}C-labeled or unlabeled amino acid was added where indicated. The five experimental conditions studied were: use of metabolic inhibitors, (1) 10 μM ouabain and (2) 1 mM NaCN; elimination of components from the incubation medium essential for maximum amino acid flux, in (3) sodium-free medium (NaCl was replaced by 128 mM choline chloride) and (4) glucose-free medium; and (5) change of the incubation temperature to 0°. The amino acids (A grade) were purchased from California Biochemical Corporation, the ^{14}C-labeled amino acids from California Biochemical Corporation and Schwarz Bio Research, Inc., choline chloride (99.7 %) from J. T. Baker Chemical Co. and ouabain USP from Nutritional Biochemical Corporation. The pH of all compounds in solution was adjusted to 7.4 before addition to the incubation medium.

TABLE I

SUMMARY OF EXPERIMENTAL PROCEDURE

Type	*Experiment*	*Description*	
		Preincubation	*Incubation*
I	Maintenance or alteration of steady state	70 min in [^{14}C]amino acid	20 or 70 min in [^{14}C]amino acid (same specific activity and at the final concentration of the preincubation medium) *plus* condition
II	Establishment of steady state	70 min in no amino acid	70 min in [^{14}C]amino acid *plus* condition
III	Influx	70 min in no amino acid	20 min in no amino acid *plus* condition 3 min in [^{14}C]amino acid *plus* condition
IV	Exchange influx	70 min in [^{12}C]amino acid	20 min in [^{12}C]amino acid *plus* condition 3 min in [^{14}C]amino acid *plus* condition
V	Exit	70 min in [^{14}C]amino acid	20 min in no amino acid *plus* condition
VI	Exchange efflux	70 min in [^{14}C]amino acid	20 min in [^{12}C]amino acid *plus* condition

This study utilized six different types of experiments to assess the relationship between steady-state accumulation influx, exchange influx, exit, and exchange efflux under the above five conditions. A summary of the six different types of experiments is given in Table I. The experimental design was structured to make variations in technique minimal.

Steady state (Type I and II in Table I)

The effect of the five experimental conditions on steady-state amino acid levels was approached with two different types of experiments. (1) Pooled slices were preloaded with ^{14}C-labeled amino acid (2 mM in standard medium) and preincubated until steady state was reached (70 min); the slices were then separated from the medium and transferred to six different media (control *plus* five conditions) containing ^{14}C-labeled amino acid of the same concentration and specific activity as the final preincubation medium concentration, and were incubated for periods of 20 and 70 min. In this way the effect of experimental conditions on the maintenance of the steady state was determined. (2) Pooled slices were equilibrated in standard amino acid-free medium for 70 min (preincubation); the slices were then separated from the medium and transferred to five different media (control *plus* four conditions) containing 2 mM ^{14}C-labeled amino acid, and were incubated for a period of 70 min. The effect of the various conditions on the establishment of the steady state was thus determined.

Influx and exchange influx (Type III and IV in Table I)

The effect of the five experimental conditions on influx was examined as follows. Pooled slices, after equilibration in standard amino acid-free medium for 70 min (preincubation), were separated and transferred to six different media (control *plus* five conditions) containing no amino acid and incubated for 20 min; they were again drained of medium, transferred for 3 min incubation in identical media now containing ^{14}C-labeled amino acid at the same concentration as the final preincubation media in the steady-state experiments described above. Uptake of amino acids has been shown to be essentially linear during the first 3 min of incubation, and thus to give a measure

of initial accumulation. Exchange influx was determined similarly. Pooled slices were preloaded with unlabeled amino acid (2 mM in standard medium) until steady state was reached (70 min preincubation); the slices were then separated and portions were incubated for 20 min in six different media (control *plus* five conditions) containing unlabeled amino acid at the final concentration of the preincubation medium, so that the steady state would be approximated. After this they were again separated and transferred to media containing ^{14}C-labeled amino acid at the final concentration of preincubation media. The slices were incubated for an additional 3 min during which the steady state was essentially maintained (no net transport), and the initial flux of labeled amino acid passing into the slices in exchange for unlabeled amino acid passing out of the slices was measured. Exchange influx as measured in this paper is not synonymous with exchange diffusion as it is commonly understood; it is a measure of amino acid flux into the cell under conditions approximating the steady state. Similar considerations apply for the definition of exchange efflux.

Exit and exchange efflux (Type V and VI in Table I)

The effect of the five experimental conditions on exit and exchange efflux was determined as follows. Pooled slices were preloaded with ^{14}C-labeled amino acid (2 mM in standard medium) until steady state was reached (70 min preincubation); the slices were then drained of the medium and separated, a small portion being taken to measure the control steady-state level and the rest being transferred to six different media (control *plus* five conditions), containing no amino acid in the study of exit and containing unlabeled amino acid at the final concentration of the preincubation medium in the study of exchange efflux. The slices were incubated for 20 min in a large volume of medium (30 ml/150 mg tissue), which was changed at 5 and 10 min to avoid re-uptake of ^{14}C-labeled amino acid. The difference between the concentration of amino acid in the tissue after the preincubation period (steady-state level) and its concentration after the incubation in amino acid-free medium gave a measure of the net exit of amino acid from the tissue. The difference between the radioactivity in the tissue after the preincubation period (steady-state level) and that after the additional incubation in a medium containing unlabeled amino acid gave a direct measure of exchange efflux in control experiments, where no change in the amino acid concentration in the tissue occurred during the additional incubation period. However, when changes were introduced in the composition of the medium or in the temperature of incubation, a substantial drop in the concentration of the amino acids in the tissue took place. In these cases exchange efflux could be calculated only indirectly, after subtracting, from the total loss of radioactivity occurring during the experimental period, the loss due to net exit of amino acid as determined in the experiments of Type I (Table I).

Calculations

Our calculation of intracellular amino acid concentration is based on the assumptions that the inulin space is a measure of the extracellular compartment, that the non-inulin space is a measure of the intracellular compartment, and that the difference between wet and dry weights is a measure of total tissue water. We also assume that the amino acid concentration in the tissue extracellular fluid was equal to that in the medium, *i.e.*, that the concentration of labeled amino acid in the exit

and exchange efflux experiments was negligible. The various experimental conditions (ouabain, NaCN, sodium-free and glucose-free medium) have been shown to significantly alter the above compartments; the measured values of extracellular space were used in the calculations[17]. Since there is no change in total tissue water between tissue incubated at 37° and 0° for 20 min, the 37° inulin space was also used as a measure of extracellular space at 0° (ref. 17). The presence of amino acids—α-aminoisobutyric acid, leucine, and lysine at 2 ml concentration—had no significant effect on total tissue water and inulin space[17]. D-Glutamate has been shown to cause marked swelling of brain slices[18], therefore, specific data for D-glutamate and the experimental conditions were obtained[17].

The results are expressed as μmoles amino acid per g tissue wet weight as previously described[16]. In addition, the results are expressed as μmoles amino acid per ml intracellular water. In order to calculate the quantity of amino acid lost from the intracellular compartment during the steady state, exit, and exchange efflux experiments, some of the data were re-calculated and expressed as μmoles amino acid per ml initial intracellular water. This latter calculation was necessary because of the changes that occurred in the tissue compartments during the 20-min incubation period[17]. The following formula was used:

$$\text{Intracellular concentration} = \frac{\text{tissue concentration} - \text{extracellular concentration} \times \text{fraction extracellular space}}{\text{fraction intracellular space}}$$

Initial intracellular concentration was calculated as above except that changes in the intracellular concentration resulting from changes in the intracellular and extracellular space during the experimental period were eliminated by calculating the data back to the initial intracellular volume.

RESULTS

The steady-state experiments were performed using an initial amino acid concentration in the medium of 2 mM. All the other experiments (except exit) were run using medium amino acid concentrations equal to the final concentrations in the steady-state experiments. By definition, at steady state the amino acid concentrations in the tissue and in the medium remain constant, and the movement of amino acid into the tissue is balanced by an equal movement of amino acid out of the tissue. We operationally termed these movements exchange influx and exchange efflux respectively. In our experimental model each of these movements may result from the sum of two different processes, namely balanced net transport into and out of the cell and homoexchange. The terms exchange influx and exchange efflux cannot be equated to exchange diffusion.

Establishment and maintenance of steady-state amino acid levels

Tables II through V present the effects of inhibitors (1 mM ouabain) and the effects of suboptimal conditions of incubation (absence of sodium, absence of glucose, and incubation at 0°) on the steady-state levels of four amino acids (α-aminoisobutyric acid, leucine, lysine, and D-glutamate) in brain slices. Two experimental methods were used: the first determines the effect of the above experimental con-

TABLE II

EFFECTS ON STEADY-STATE LEVEL AND ACCUMULATION OF AMINOISOBUTYRIC ACID

Experimental Type I. In the experiments on steady-state level pooled brain slices were preincubated for 70 min in 2 mM amino[^{14}C]isobutyric acid, 3 ml per half-brain; the slices were separated and incubated for 20 min or 70 min in 0.8 mM amino[^{14}C]isobutyric acid of the same specific activity, 20 ml/150 mg tissue, under the experimental condition listed. Intracellular concentrations were calculated as described in METHODS and are expressed as μmoles/ml intracellular water (I.W.). The 20-min decrease or loss of amino acid is expressed as μmoles/ml initial intracellular water (I.I.W.). *Experimental Type II.* In the accumulation experiments pooled brain slices were preincubated for 70 min in standard amino acid-free medium, 3 ml per half-brain; the slices were separated and incubated for 70 min in 2 mM amino[^{14}C]isobutyric acid, 3 ml/150 mg tissue, under the experimental conditions listed. All values are averages of 4 experiments ± S.D., except those for controls, which are averages of 8 experiments.

Experiment	*Maintenance of steady state*				*Accumulation*
	20 min			*70 min*	*70 min*
	(*μmoles/g slice*)	(*μmoles/ml I.W.*)	*loss* (*μmoles/ml I.I.W.*)	(*μmoles/g slice*)	(*μmoles/g slice*)
Control	12.6 ± 0.1	38.9	—	12.9 ± 0.3	13.2 ± 0.3
Ouabain, 10 μM	9.73 ± 0.60	27.4	9.4	5.68 ± 0.28	4.87 ± 0.09
CN^-, 1 mM	9.67 ± 0.71	25.2	9.5	5.54 ± 0.44	3.70 ± 0.14
Na^+ free	9.80 ± 0.41	37.2	8.4	5.67 ± 0.05	3.27 ± 0.11
Glucose free	11.0 ± 0.3	30.0	5.4	12.1 ± 0.8	10.7 ± 0.2
0°	11.8 ± 0.5	36.4	2.5		

TABLE III

EFFECTS ON STEADY-STATE LEVEL AND ACCUMULATION OF LEUCINE

The experiments on steady-state level were done as in Table II, except preincubation in 2 mM [^{14}C]leucine, 2 ml per half-brain, and incubation in 1.6 mM [^{14}C]leucine of the same specific activity. The accumulation experiments were done as in Table II, except preincubation in 2 ml per half-brain and incubation in 2 mM [^{14}C]leucine, 2 ml/150 mg tissue. All values are averages of 4 experiments ± S.D., except those for controls which are averages of 8 experiments.

Experiment	*Maintenance of steady state*				*Accumulation*
	20 min			*70 min*	*70 min*
	(*μmoles/g slice*)	(*μmoles/ml I.W.*)	*loss* (*μmoles/ml I.I.W.*)	(*μmoles/g slice*)	(*μmoles/g slice*)
Control	3.46 ± 0.09	8.41	0	3.53 ± 0.10	3.28 ± 0.11
Ouabain, 10 μM	2.78 ± 0.06	5.83	2.14	2.45 ± 0.03	2.35 ± 0.03
CN^-, 1 mM	2.83 ± 0.10	5.63	1.85	2.36 ± 0.10	2.19 ± 0.05
Na^+ free	2.39 ± 0.06	5.94	3.53	2.06 ± 0.04	2.04 ± 0.01
Glucose free	3.21 ± 0.07	6.89	0.45	2.46 ± 0.07	2.26 ± 0.08
0°	3.39 ± 0.06	8.19	0.22		

ditions on the maintenance of the steady-state amino acid level after steady state has been established; the second determines the effect of the experimental conditions on amino acid accumulation, that is, on the establishment of steady-state levels.

The steady-state levels of the four amino acids were not maintained under the various experimental conditions studied, and the effects of the various conditions

TABLE IV

EFFECTS ON STEADY-STATE LEVEL AND ACCUMULATION OF LYSINE

The experiments on steady-state level were done as in Table II, except preincubation in 2 mM [^{14}C]lysine, 2 ml per half-brain, and incubation in 1.4 mM [^{14}C]lysine of the same specific activity. The accumulation experiments were done as in Table II, except preincubation in 2 ml per half-brain and incubation in 2 mM [^{14}C]lysine, 2 ml/150 mg tissue. All values are averages of 4 experiments, except those of controls which are averages of 8 experiments.

Experiment	*Maintenance of steady state*				*Accumulation*
	20 min			*70 min*	*70 min*
	(*μmoles/g slice*)	(*μmoles/ml I.W.*)	*loss* (*μmoles/ml I.I.W.*)	(*μmoles/g slice*)	(*μmoles/g slice*)
Control	4.26 ± 0.09	11.3	0	4.43 ± 0.11	4.83 ± 0.20
Ouabain, 10 μM	3.73 ± 0.06	8.91	1.72	3.11 ± 0.09	3.30 ± 0.10
CN^-, 1 mM	3.46 ± 0.05	7.59	2.45	2.36 ± 0.03	2.42 ± 0.14
Na^+ free	3.83 ± 0.06	12.1	1.37	2.93 ± 0.06	2.94 ± 0.06
Glucose free	3.87 ± 0.11	9.03	1.22	3.68 ± 0.07	3.84 ± 0.05
0°	3.82 ± 0.10	9.89	1.4		

TABLE V

EFFECTS ON STEADY-STATE LEVEL AND ACCUMULATION OF D-GLUTAMATE

The experiments on steady-state level were done as in Table II, except preincubation in 2 mM D-[^{14}C]glutamate, 5 ml per half-brain, and incubation in 0.9 mM D-[^{14}C]glutamate of the same specific activity. The accumulation experiments were done as in Table I, except preincubation in 5 ml per half-brain and incubation in 2 mM D-[^{14}C]glutamate, 5 ml/150 mg tissue.

Experiment	*Maintenance of steady state*				*Accumulation*
	20 min			*70 min*	*70 min*
	(*μmoles/g slice*)	(*μmoles/ml I.W.*)	*loss* (*μmoles/ml I.I.W.*)	(*μmoles/g slice*)	(*μmoles/g slice*)
Control	24.4 ± 1.2	56.2	0	25.9 ± 0.9	25.2 ± 1.5
Ouabain, 10 μM	23.3 ± 1.0	51.1	2.8	20.7 ± 0.9	19.6 ± 0.7
CN^-, 1 mM	19.3 ± 0.6	40.7	12.3	11.7 ± 0.7	5.58 ± 0.21
Na^+ free	20.4 ± 0.6	68.6	8.7	11.6 ± 0.8	2.36 ± 0.18
Glucose free	21.1 ± 0.7	45.3	8.1	9.34 ± 0.57	7.33 ± 0.29
0°	18.6 ± 0.7	42.6	13.6		

were not always the same for each amino acid. The data of the experiments for 20 min in Tables II–V are expressed on the basis of the final intracellular space (μmoles/ml intracellular water) and of the initial intracellular space (μmoles/ml initial intracellular water). Significant changes in intracellular fluid volume occurred during incubation with the modifications of the medium that were introduced, and the results were sometimes quite different using the two different types of calculations. This is clearly seen in the case of a sodium-free medium, in which amino acid has been lost from the tissue, but the intracellular concentration remained unchanged or even increased as in the case of D-glutamate (Table V) because of the relative contraction of the intracellular compartment.

In general, the various experimental conditions altered the steady-state levels of leucine (Table III) and lysine (Table IV) much less than those of aminoisobutyric acid and D-glutamate; this may be partially due to the higher steady-state accumulation levels of aminoisobutyric acid and D-glutamate.

It is interesting to compare the two types of 70-min experiments reported in the last two columns of Tables II–V. It can be seen that under each condition (ouabain, cyanide, *etc.*) all four amino acids tended to reach a "new" steady-state level. In other words the "new" steady-state level of amino acid accumulation in the various experimental conditions could be reached either from tissue preloaded with amino acid at control steady-state levels, or from tissue not previously exposed to amino acid. In the case of leucine and lysine (Tables III and IV respectively) the 70-min tissue levels were almost identical. With aminoisobutyric acid and D-glutamate (Tables II and V respectively) the 70-min steady-state level approached the 70-min accumulation level. Since both aminoisobutyric acid and D-glutamate are accumulated to relatively high levels and their exit is relatively slow, incubation periods greater than 70 min may be needed for complete equilibration.

Effects on influx and exchange influx

Tables VI through IX present the effects of inhibitors and suboptimal incubation conditions on the influx and on the exchange influx of four amino acids. A comparison between control amino acid influx and exchange influx reveals no significant differences with aminoisobutyric acid and leucine (Tables VI and VII respectively). In contrast, the flux of [^{14}C]lysine into tissue preloaded with unlabeled lysine at steady-

TABLE VI

EFFECTS ON INFLUX AND EXCHANGE INFLUX OF AMINOISOBUTYRIC ACID

Experimental Type III (see Table I). In influx experiments pooled brain slices were preincubated for 70 min in standard amino acid-free medium, 3 ml per half-brain; then separated and incubated for 20 min in amino acid-free medium under the experimental conditions listed (except 0°), 5 ml/150 mg tissue; and finally transferred and incubated for 3 min in 0.8 mM amino[^{14}C]isobutyric acid under the same experimental conditions, 5 ml/150 mg tissue. Incubation at 0° was 20 min in ^{14}C-labeled amino acid as above. *Experimental Type IV* (see Table I). In exchange influx experiments pooled brain slices were preincubated for 70 min in 2 mM unlabeled aminoisobutyric acid, 3 ml per half-brain to establish steady-state levels; then the slices were separated and incubated for 20 min in a medium with 0.8 mM unlabeled aminoisobutyric acid (which is the same concentration as the final levels of the preincubation medium) under the same experimental conditions, 5 ml/150 mg tissue. Incubation at 0° was 20 min in ^{14}C-labeled amino acid as above. Flux was calculated from the 3-min (initial) intracellular penetration of the ^{14}C-labeled amino acid and expressed as μmoles/ml intracellular water (I.W.)/min.

Experiment	*Influx*				*Exchange influx*			
	No. of expts.	*μmoles/100 g slice*	*μmoles/ml I.W. per min*	*Per cent of control*	*No. of expts.*	*μmoles/100 g slice*	*μmoles/ml I.W. per min*	*Per cent of control*
Control	16	137 ± 4	1.02	100	6	139 ± 3	1.04	100
Ouabain, 10 μM	8	83.1 ± 1.0	0.424	42	3	93.4 ± 1.1	0.525	51
CN^-, 1 mM	4	75.4 ± 2.5	0.342	34	4	71.0 ± 1.8	0.303	29
Na^+ free	8	94.6 ± 2.6	0.659	65	4	127 ± 4	1.089	105
Glucose free	8	131 ± 3	0.870	85	3	93.4 ± 2.4	0.515	50
0°	4	46.2 ± 0.8	0.008	1	4	40.8 ± 0.9	−0.001	0

TABLE VII

EFFECTS ON INFLUX AND EXCHANGE INFLUX OF LEUCINE

Experimental details and the description of units is the same as in Table VI, except as noted below. In influx experiments preincubation was done in 2 ml per half-brain and second incubation in 1.6 mM [^{14}C]leucine. In exchange influx experiments preincubation was done in 2 mM leucine (unlabeled), 2 ml per half-brain; first incubation in 1.6 mM leucine (unlabeled), and second incubation in 1.6 mM [^{14}C]leucine.

Experiment	*Influx*				*Exchange influx*			
	No. of expts.	*μmoles/100 g slice*	*μmoles/ml I.W. per min*	*Per cent of control*	*No. of expts.*	*μmoles/100 g slice*	*μmoles/ml I.W. per min*	*Per cent of control*
Control	20	176 ± 3	0.994	100	6	184 ± 7	1.08	100
Ouabain, 10 μM	4	155 ± 4	0.738	74	4	145 ± 3	0.640	59
CN^-, 1 mM	4	147 ± 2	0.650	65	4	143 ± 5	0.614	57
Na^+ free	4	140 ± 1	0.667	67	4	146 ± 2	0.747	69
Glucose free	4	154 ± 1	0.721	73	4	147 ± 5	0.655	61
0°	3	110 ± 1	0.044	4	3	120 ± 1	0.060	6

TABLE VIII

EFFECTS ON INFLUX AND EXCHANGE INFLUX OF LYSINE

Experimental details and the description of units is the same as in Table VI, except as noted below. In influx experiments preincubation was done in 2 ml per half-brain and second incubation in 1.4 mM [^{14}C]lysine. In exchange influx experiments preincubation was done in 2 mM lysine (unlabeled), 2 ml per half-brain; first incubation in 1.4 mM lysine (unlabeled), and second incubation in 1.4 mM [^{14}C]lysine.

Experiment	*Influx*				*Exchange influx*			
	No. of expts.	*μmoles/100 g slice*	*μmoles/ml I.W. per min*	*Per cent of control*	*No. of expts.*	*μmoles/100 g slice*	*μmoles/ml I.W. per min*	*Per cent of control*
Control	12	106 ± 2	0.360	100	12	122 ± 4	0.530	100
Ouabain, 10 μM	4	91.4 ± 2.7	0.212	59	4	110 ± 2	0.394	74
CN^-, 1 mM	4	86.2 ± 1.7	0.185	51	4	98.5 ± 2.8	0.296	56
Na^+ free	4	94.6 ± 1.0	0.214	59	4	118 ± 2	0.524	99
Glucose free	3	103 ± 2	0.331	92	4	118 ± 1	0.473	89
0°	4	78.1 ± 0.9	0.009	3	4	72.2 ± 0.3	0	0

state conditions was significantly greater than [^{14}C]lysine flux into non-preloaded tissue. The opposite pattern was observed with D-glutamate; control influx was significantly greater than control exchange influx (Table IX). There are two effects of D-glutamate on brain tissue that probably account for this finding; one is the destructive osmotic swelling that the cells undergo while accumulating D-glutamate to high levels[18], and the second is the decrease of intracellular high energy compounds occurring when the tissue is incubated with D-glutamate[19]. An alteration of membrane integrity could also account for the decreased amino acid flux in tissue preloaded with D-glutamate.

The various experimental conditions decrease both amino acid influx and exchange influx. The degree of inhibition varies with respect to the particular amino

TABLE IX

EFFECTS ON INFLUX AND EXCHANGE INFLUX OF D-GLUTAMATE

Experimental details and description of units is the same as in Table VI, except as noted below. In influx experiments preincubation was done in 5 ml per half-brain and second incubation in 0.9 mM D-[^{14}C]glutamate. In exchange influx experiments preincubation was done in 2 mM D-glutamate (unlabeled), 5 ml per half-brain; first incubation in 0.9 mM D-glutamate (unlabeled), and second incubation in 0.9 mM D-[^{14}C]glutamate.

Experiment	*Influx*				*Exchange influx*			
	No. of expts.	*μmoles/100 g slice*	*μmoles/ml I.W. per min*	*Per cent of control*	*No. of expts.*	*μmoles/100 g slice*	*μmoles/ml I.W. per min*	*Per cent of control*
Control	8	262 ± 10	2.29	100	7	119 ± 2	0.634	100
Ouabain, 10 μM	4	159 ± 7	1.12	49	4	100 ± 4	0.474	75
CN^-, 1 mM	4	137 ± 4	0.855	37	4	66.1 ± 1.5	0.222	35
Na^+ free	4	52.2 ± 0.8	0.024	1	4	45.8 ± 0.4	0*	0
Glucose free	3	247 ± 11	1.92	84	4	89.7 ± 1.8	0.394	62
0°	4	45.8 ± 0.6	−0.001	0	4	37.9 ± 0.5	0.001	0

* The calculation of intracellular exchange influx gave a negative number: −0.024.

acid being studied. Furthermore, amino acid influx may be inhibited by the experimental conditions to a considerably different extent than amino acid exchange influx. For example, the inhibition of influx by ouabain was greater than the inhibition of exchange influx in the case of aminoisobutyric acid (Table VI), lysine (Table VII), and D-glutamate (Table VIII), whereas the reverse was true with leucine (Table X).

The inhibitory effect of cyanide was not selective with respect to influx or exchange influx; within the limits of experimental error, both fluxes were inhibited to the same extent (Tables VI–IX).

Influx and exchange influx have different sensitivities to the absence of sodium in the incubation medium in the case of aminoisobutyric acid and lysine but not in the case of leucine; D-glutamate fluxes appear almost totally dependent on the presence of sodium (Tables VI–IX).

The exchange influx of aminoisobutyric acid, leucine, and D-glutamate was more sensitive to the lack of glucose than the influx of these amino acids. This may be due in part to a higher expenditure of cellular energy stores for the maintenance of the non-physiological steady-state level of amino acids in the exchange experiments. Lysine flux, however, was resistant to the effects of glucose deprivation.

Incubation at 0° had the most severe effect on amino acid flux of all the experimental conditions studied. Both influx and exchange influx were essentially abolished in the case of aminoisobutyric acid, lysine, and D-glutamate (Tables VI, VIII, and IX respectively). Leucine appears to retain some degree of mediated (Table VII) penetration at 0°, which is consistent with previous findings[16].

Effects of exit and exchange efflux

Tables X through XIII present the effects of five experimental conditions on the exit of four amino acids into an amino acid-free medium and on the exchange of intracellular ^{14}C-labeled amino acid for unlabeled amino acid in medium under conditions which approximate steady state. The loss of tissue radioactivity in the

TABLE X

EFFECTS ON EXIT AND EXCHANGE EFFLUX OF AMINOISOBUTYRIC ACID

Experimental Type V (Table I). In exit experiments pooled brain slices were preincubated for 70 min in 2 mM amino[^{14}C]isobutyric acid, 3 ml per half-brain, to establish a steady state; the slices were then separated and incubated for 20 min in amino acid-free medium, 30 ml/150 mg tissue, under the experimental conditions listed, with medium changes at 5 min and 10 min. The intracellular loss of amino acid is expressed as μmoles/ml initial intracellular water (I.I.W.). *Experimental Type VI* (Table I). In exchange efflux experiments pooled brain slices were preincubated for 70 min in 2 mM amino[^{14}C]isobutyric acid, 3 ml per half-brain, to establish steady-state levels; then the slices were separated and incubated for 20 min in a medium with 0.8 mM unlabeled aminoisobutyric acid (which is the same concentration as the final levels of the preincubation medium), 30 ml/150 mg tissue, under the experimental conditions listed, with medium changes at 5 min and 10 min. The "^{14}C-tissue pool" indicates the fraction of the original ^{14}C-labeled amino acid pool remaining in the slices after 20 min incubation; it does not indicate tissue amino acid concentration (the latter was present in Tables II–V). The intracellular exchange of amino acid was determined from the difference between the total loss of ^{14}C-labeled amino acid from the tissue measured above and the small net loss of amino acid which occurred under the various conditions as measured in Tables II–V and is expressed as μmoles/ml initial intracellular water (I.I.W.). All values are averages of 4 experiments, except steady-state and control values, which are averages of 8 experiments.

Experiment	*Exit*				*Exchange efflux*			
	Tissue level		*Intracellular loss*		*^{14}C-tissue pool*		*Intracellular exchange*	
	μmoles/g slice	*μmoles/ml I.W.*	*μmoles/ml I.I.W.*	*Per cent of control*	*μmoles/g slice*	*μmoles/ml I.W.*	*μmoles/ml I.I.W.*	*Per cent of control*
Steady state*	11.6 ± 0.4	35.7			11.6 ± 0.4	35.7		
Control	9.47 ± 0.52	30.2	5.5	100	7.10 ± 0.32	22.7	13.0	100
Ouabain, 10 μM	7.92 ± 0.72	23.3	10.7	195	5.48 ± 0.26	16.1	9.0	69
CN^-, 1 mM	7.94 ± 0.58	21.5	10.6	193	6.52 ± 0.82	17.7	5.6	43
Na^+ free	8.12 ± 0.40	32.3	9.2	167	4.88 ± 0.59	19.4	11.4	88
Glucose free	9.00 ± 0.49	25.5	7.2	131	6.07 ± 0.27	17.2	11.1	85
0°	11.0 ± 0.5	35.1	0.6	11	10.9 ± 0.4	34.8	0.9	7

* Preincubation steady-state level at the beginning of exit and exchange efflux experiments.

TABLE XI

EFFECTS ON EXIT AND EXCHANGE EFFLUX OF LEUCINE

Experimental details and the description of units is the same as in the legend to Table X, except as noted below. For exit experiments preincubation was done in 2 mM [^{14}C]leucine, 2 ml per half-brain. In exchange efflux experiments preincubation was done in 2 mM [^{14}C]leucine, 2 ml per half-brain, and incubation in 1.6 mM leucine (unlabeled). All values are averages of 4 experiments, except steady-state and control values, which are averages of 8 experiments.

Experiment	*Exit*				*Exchange efflux*			
	Tissue level		*Intracellular loss*		*^{14}C-tissue pool*		*Intracellular exchange*	
	μmoles/g slice	*μmoles/ml I.W.*	*μmoles/ml I.I.W.*	*Per cent of control*	*μmoles/g slice*	*μmoles/ml I.W.*	*μmoles/ml I.I.W.*	*Per cent of control*
Steady state	3.43 ± 0.07	8.32			3.43 ± 0.07	8.32		
Control	0.806 ± 0.031	2.57	5.75	100	0.559 ± 0.023	1.79	6.53	100
Ouabain, 10 μM	0.664 ± 0.019	1.95	6.22	108	0.487 ± 0.030	1.43	4.64	71
CN^-, 1 mM	0.731 ± 0.046	1.98	6.01	105	0.476 ± 0.013	1.29	4.97	76
Na^+ free	0.639 ± 0.018	2.54	6.23	108	0.358 ± 0.019	1.42	3.62	55
Glucose free	0.762 ± 0.034	2.16	5.91	103	0.461 ± 0.019	1.30	6.42	98
0°	2.46 ± 0.07	7.86	0.46	8	2.16 ± 0.07	6.90	1.20	18

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TABLE XII

EFFECTS ON EXIT AND EXCHANGE EFFLUX OF LYSINE

Experimental details and the description of units is the same as in the legend to Table X, except as noted below. In exit experiments preincubation was done in 2 mM [^{14}C]lysine, 2 ml per half-brain. In exchange efflux experiments preincubation was done in 2 mM [^{14}C]lysine, 2 ml per half-brain, and incubation in 1.4 mM lysine (unlabeled). All values are averages of 4 experiments, except steady-state and control values, which are averages of 8 experiments.

Experiment	*Exit*				*Exchange efflux*			
	Tissue level		*Intracellular loss*		*^{14}C-tissue pool*		*Intracellular exchange*	
	μmoles/g slice	*μmoles/ml I.W.*	*μmoles/ml I.I.W.*	*Per cent of control*	*μmoles/g slice*	*μmoles/ml I.W.*	*μmoles/ml I.I.W.*	*Per cent of control*
Steady state	4.11 ± 0.08	10.8			4.11 ± 0.08	10.8		
Control	1.82 ± 0.10	5.81	4.99	100	1.14 ± 0.09	3.64	7.16	100
Ouabain, 10 μM	1.29 ± 0.08	3.79	6.73	135	0.982 ± 0.081	2.89	5.97	83
CN^-, 1 mM	1.27 ± 0.01	3.43	6.80	136	0.928 ± 0.098	2.51	5.42	76
Na^+ free	1.72 ± 0.06	6.83	5.19	104	1.03 ± 0.07	4.09	6.07	85
Glucose free	1.39 ± 0.04	3.93	6.41	128	0.926 ± 0.079	2.60	6.68	93
0°	3.23 ± 0.04	10.3	0.50	10	3.29 ± 0.04	10.5	0.30	4

TABLE XIII

EFFECTS ON EXIT AND EXCHANGE EFFLUX OF D-GLUTAMATE

Experimental details and the description of units is the same as in the legend to Table X, except as noted below. In exit experiments preincubation was done in 2 mM D-[^{14}C]glutamate, 5 ml per half-brain. In exchange efflux experiments preincubation was done in 2 mM D-[^{14}C]glutamate, 5 ml per half-brain, and incubation in 0.9 mM D-glutamate (unlabeled). All values are averages of 4 experiments, except values for steady-state and control values, which are averages of 8 experiments.

Experiment	*Exit*				*Exchange efflux*			
	Tissue level		*Intracellular loss*		^{14}*C-tissue pool*		*Intracellular exchange*	
	μmoles/g slice	*μmoles/ml I.W.*	*μmoles/ml I.I.W.*	*Per cent of control*	*μmoles/g slice*	*μmoles/ml I.W.*	*μmoles/ml I.I.W.*	*Per cent of control*
Steady state	26.0 ± 0.7	59.9			26.0 ± 0.7	59.9		
Control	21.8 ± 0.3	51.0	8.9	100	20.7 ± 0.6	48.4	11.5	100
Ouabain, 10 μM	21.1 ± 0.5	47.0	10.8	121	19.6 ± 0.4	43.6	11.5	100
CN^-, 1 mM	20.7 ± 0.7	44.4	12.0	135	18.5 ± 0.4	39.7	4.8	42
Na^+ free	20.7 ± 0.5	71.3	10.5	118	19.9 ± 0.8	68.6	3.7	32
Glucose free	21.1 ± 0.6	46.1	11.0	124	17.8 ± 0.4	38.9	10.5	91
0°	17.4 ± 0.4	40.7	19.2	216	18.3 ± 0.4	42.8	3.5	30

control exchange efflux experiments was due only to exchange of tissue ^{14}C-labeled amino acid for unlabeled amino acid in the medium since there was no net change in amino acid concentration of the medium or tissue during the 20-min experimental period. The same reasoning does not strictly apply under the various modifications in media and incubation conditions; over the experimental period there was a small but significant net loss of amino acid from the tissue. Therefore the measured decrease of the ^{14}C-labeled amino acid pool in the exchange efflux experiments can be assumed to be the sum of two components: (1) amino acid exchange efflux and (2) decrease in total intracellular amino acid which occurs under the experimental conditions over the 20-min incubation period (Tables VI and V). The exchange efflux fraction can be calculated by subtracting the decrease in intracellular amino acid measured in the 20-min experiments of Tables II–V from the decrease in the ^{14}C-labeled amino acid pool shown in Tables X–XIII, correcting for changes in intracellular volume. This calculation involves the difference between two different sets of experiments and lends itself to rather large errors, even though the daily variations in our experiments were small. This is the only calculation, however, by which exchange efflux could be estimated under the various experimental conditions. Initial rates of exit and exchange efflux could not be reliably calculated because the loss of labeled amino acid was not linear over the experimental period[13].

Exchange efflux was greater than exit itself for each of the amino acids studied. For aminoisobutyric acid and lysine, exchange efflux was 2.4 and 1.4 times that of exit respectively (Tables X and XII). For leucine and D-glutamate the ratio of exchange efflux to exit was 1.1 and 1.3 respectively (Tables XI and XIII).

Amino acid exit was enhanced by the presence of inhibitors and by modifications in the medium except incubation at 0°. In contrast, amino acid exchange exit was inhibited by the above conditions. Incubation at 0° had the most significant effect. Both exit and exchanges were strongly inhibited in the case of aminoisobutyric acid, lysine, and leucine; D-glutamate exit is enhanced at 0°, again demonstrating the effects of destructive swelling on membrane integrity and subsequent diffusion of amino acid out of the cell.

The increase in amino acid exit resulting from the various experimental conditions varied with the different amino acids, being maximum with aminoisobutyric acid and minimum with leucine. The fact that leucine exit could be increased only slightly (Table XI) may be partially due to the extremely rapid exit of this amino acid from brain slices even in optimal conditions[13].

DISCUSSION

The factors that determine steady-state metabolite levels within cells and the mechanisms by which they are altered are many and complex; intracellular synthesis, utilization, degradation, compartmentation, and binding may play a significant part. In the present study we were concerned with amino acid flux; metabolism was minimized by using non-metabolized or slowly metabolized amino acids and by working at higher than physiologic intracellular concentrations[16]. Mouse brain slices comprise a heterogeneous cell population and the data reflect a composite average from many cell types.

Steady state

The experiments in Tables II–V demonstrate that steady-state levels of amino acids are altered by inhibitors that alter energy stores in brain slices[19], and by sub-optimal incubation conditions, which has been known for some time. The above conditions were not totally disruptive of membrane function, since a "new" tissue level lower than control steady state but higher than medium concentrations was established in each case. A similar tissue level was reached in brain slices not previously pre-incubated with amino acid (Columns 4 and 5 in Tables II–V). These data suggest that a new equilibrium level is established which depends on the degree amino acid flux and membrane integrity is altered.

The presence of cyanide had the greatest effect on the steady-state level of all four amino acids. Cyanide has a marked depressant effect on the ATP levels in brain slices, to less than 5 % of control[19]. The absence of glucose, the only oxidizable substrate present in our incubation medium, had a small effect on the steady-state levels except in the case of D-glutamate, suggesting that transport processes have lower energy requirements or can be preferentially supported by endogenous catabolism. The absence of glucose reduces cell ATP levels to approx. 15 % of control; in the presence of D-glutamate but not of other amino acids ATP is further reduced to less than 10 % of control[19]. This explains why D-glutamate transport is more sensitive to glucose deprivation than is the transport of other amino acids. D-Glutamate transport is relatively insensitive to ouabain (10 μM) but very sensitive to the absence of sodium, suggesting that although its transport requires sodium ion it is not completely dependent on a ouabain-sensitive, sodium-dependent ATPase. The accumulation of acidic amino acids is coupled to sodium[20], which is in part responsible for the decrease in resting membrane potential and excitatory effect of these amino acids on discharging cerebral neurons[21].

Net transport

It has not been clearly demonstrated whether amino acids utilize the same system for mediated passage both into and out of cells. In some models exit is considered to be a non-saturable first-order process. This is not the case in brain, *Escherichia coli*, and Ehrlich ascites cells; mediated exit shows a tendency towards saturation and analogue inhibition[13,22,23]. Previous studies in brain slices have demonstrated many similarities between the substrate specificity of amino acid influx and exit[13,14], suggesting that the transport sites responsible for the net transfer of amino acids into and out of the cells may have similar properties. The present data seem to confirm these findings in that, for each amino acid, the order of effect of the various conditions tested on influx and efflux was generally similar. It has to be noted that the results are not strictly comparable, since in influx experiments lasting 3 min a linear flux was measured, whereas in efflux experiments lasting 20 min the flux was not linear with time.

In most cases the experimental conditions strongly inhibitory of influx increased the exit of amino acids over the control exit values. The increased exit of D-glutamate at 0° is of interest: in fact at 0° both influx and exit of other amino acids were inhibited. Whether the increased exit of amino acids is due to the inability of the tissue to maintain high intracellular amino acid levels, or to increased physical diffusion through a more porous or otherwise altered membrane is not known. Certain com-

pounds, including tetraethyl tin, phlorizin, and morphine, have been shown to inhibit exit, whereas most other compounds studied increased exit[25]. CHRISTENSEN AND HANDLOGTEN[23] have suggested that the increased exit observed when Ehrlich cells are incubated in the presence of metabolic inhibitors is due to an inhibition of re-uptake and that this artifact can be demonstrated by increasing the medium-to-cell volume ratio.

Our medium-to-tissue volume ratio (about 300:1) *plus* two medium changes after 5 and 10 min of incubation kept medium amino acid to insignificant levels. If the increase in exit were the result of an inhibition of re-uptake, a closer correlation than that observed would be expected between the stimulation of exit and the inhibition of influx and/or exchange influx.

The four amino acids chosen for this study have been shown to be predominantly transported by separate systems at the concentrations used[13,15], therefore the differences in the inhibitory effect of the various experimental conditions on transport most likely reflect differences in the sensitivity of the transport systems to the absence of glucose or sodium and to metabolic inhibitors. The net transport of amino acids into brain cells is dependent on energy and on sodium ion. The "L" (leucine) transport system in Ehrlich cells is independent of sodium[24,26]. The comparable leucine or large neutral amino acid transport system in brain appears to be at least partially dependent on sodium[27,28].

Exchange

The interpretation of attempts to measure exchange diffusion is often difficult. The difference between flux into tissue preloaded with substrate and flux into untreated tissue is taken as the measure of exchange diffusion, on the basis of coupled transport or flow-driving-counter-flow. We could not measure an "exchanging fraction" in our studies because there was no difference, except with lysine, between net transport and transport into tissue preloaded with amino acid under steady-state conditions. There were significant differences in flux measured with and without preloading in the presence of metabolic inhibitors and suboptimal incubation conditions. Therefore we have operationally used the term "exchange flux" to represent the total flux (balanced net flux *plus* any possible exchange diffusion) of the amino acid moving across the cell membrane under conditions approaching a steady state. It is interesting that in our studies of heteroexchange, exchange diffusion (influx of one amino acid increased by preloading with another) could be shown—a very significant increase in lysine and in leucine influx driven by histidine exit, for example[29].

A comparison of efflux and exchange efflux demonstrates the latter to be significantly greater for all four amino acids (Tables X–XIII). It is not clear why exchange efflux and not exchange influx (except in the case of lysine) was significantly greater than the corresponding net flux. The relatively low value for exchange influx measured could be due to the presence of unlabeled amino acid in the extracellular space competing with ^{14}C-labeled amino acid for the transport site. This possibility was tested by increasing the concentration of tissue unlabeled amino acid and lowering the concentration of ^{14}C-labeled amino acid in the medium to non-steady-state conditions during which there is a net exit of amino acid. The exchange influx to influx ratio was reduced only in the case of leucine. Since leucine has a very rapid exit[13], its extracellular concentration may be high enough to compete for the transport site

with the [^{14}C]leucine present. However, according to recent observations of BELKHODE AND SCHOLEFIELD[30], the intracellular amino acid may inhibit the uptake of the extracellular amino acid even without having moved into the extracellular space.

Several studies have been interpreted as supporting a common site for net transport and exchange diffusion[31,32]. More recently the studies of CHRISTENSEN AND HANDLOGTEN in the Ehrlich cell have suggested that the "L" system predominantly mediates neutral amino acid exchange; the differences observed between small and large neutral amino acids are then explained by the relative affinity of the amino acid to the "A" and the "L" systems[24,26]. Additional studies[33,30] support this mechanism.

Difference between net flux and exchange flux is shown in the sodium dependence of net flux and sodium-independent exchange flux of aminoisobutyric acid and lysine. Methionine exchange but not influx was also independent of sodium[34]. Whether the differences between unidirectional and exchange flux are due to different properties of two separate transport systems, one for unidirectional flux and one for exchange, or are due to changes in membrane function induced by preloading the tissue to high amino acid levels in the exchange flux experiments (as is likely with D-glutamate) cannot be answered at this time.

Energy dependence of exchange

Exchange diffusion is usually distinguished from active transport by assuming that it does not require the expenditure of energy. Several publications have appeared in support of this interpretation[7,8]. Other studies have presented contrary evidence[35,36]. In the one-to-one exchange process, for every molecule passing the membrane in one direction another passes in the opposite direction. Conditions altering exchange should affect the flux in both directions to the same extent, and should not alter steady-state levels. Any variation in steady-state level must therefore depend on the alteration of processes other than exchange.

Most of the metabolic inhibitors and suboptimal conditions of incubation tested inhibited the exchange flux of amino acids in both directions. In most cases the effects obtained on exchange influx and on exchange efflux were very similar. A better correspondence could not be expected, in view of the difference in the experimental procedure in the two sets of experiments, and of the possibility of errors in the calculation of exchange efflux experiments.

The measured movements of amino acids at steady-state conditions may be the result of net transport and exchange diffusion. If the various inhibitors and suboptimal conditions tested only affected net transport, the inhibition of exchange influx would be lower, or at best equal to the inhibition of influx, and exchange efflux would be increased when exit is increased. However, in several cases, exchange influx was inhibited more than influx, and exchange efflux was never increased by the conditions tested.

In spite of this suggestive evidence, the uncertainties in the interpretation of our experimental model do not allow a conclusion about the energy requirements of exchange diffusion in brain slices.

Relation between steady-state accumulation and fluxes

Table XIV compares the relative effects of the various experimental conditions studied on the steady-state amino acid concentrations with the effects on net transport

TABLE XIV

COMPARISON OF EFFECTS ON STEADY STATE AND NET FLUXES

S.S.: steady-state experiments, Tables II–V, μmoles/ml initial intracellular water (I.I.W.) lost during 20 min incubation. *E*—*I*: undirectional flux experiments, Tables VI–XIII. *E* is exit (μmoles/ml initial intracellular water over 20 min); *I* is influx (μmoles/ml intracellular water per min × 20 min × correction factor). The correction factor was calculated by (control exit per 20 min)/(control influx/min × 20 min) to correct the difference between calculated net influx and observed exit, assuming the ratio applies for the experimental conditions as well.

Experiment	*μmoles/ml I.I.W.*				*Amino acid lost*			
	Amino-isobutyric acid		*Leucine*		*Lysine*		D-*Glutamate*	
	S.S.	*E*—*I*	*S.S.*	*E*—*I*	*S.S.*	*E*—*I*	*S.S.*	*E*—*I*
Ouabain, 10 μM	9.4	8.4	2.1	2.0	1.7	3.6	2.8	6.4
CN^-, 1 mM	9.5	8.8	1.9	2.3	2.5	4.2	12.3	8.7
Na^+ free	8.4	6.1	3.5	3.4	1.4	2.2	8.7	10.4
Glucose free	5.4	3.0	0.5	1.7	1.2	1.8	8.1	3.5
0°	2.5	0.6	0.2	0.2	1.4	0.4	13.6	19.2

(influx and efflux); the limitations of this type of comparison have already been discussed. There is good agreement between effects on steady state and on net fluxes. Poor correlation is obtained when the effects on steady state and on the exchange fluxes are compared.

In previous studies we concluded that the net fluxes (influx and efflux) are the main processes by which steady-state levels are established in brain slices[13,16] and other processes such as metabolism play only a minor role. Kinetic constants for amino acid influx and efflux in Ehrlich cells are in a range which is consistent with the steady-state intracellular-to-medium ratios observed[23]. From this study it seems that any decrease in the steady-state level caused by an inhibitor is the result of different effects on influx and efflux. The maintenance of steady state is energy dependent in the measure that influx and efflux are energy dependent. Exchange might be an independent energy-requiring process and might act in addition to net transport.

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